Detection of Indicator and Pathogenic Bacteria in Raw Vegetables and Fruits Sold in Fresh Markets and Supermarkets, Bangkok, Thailand

Miss Jutanat Srisamran

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Veterinary Public Health
Department of Veterinary Public Health
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University
Academic Year 2020

การตรวจหาแบคทีเรียชี้วัดและแบคทีเรียก่อโรคในผักและผลไม้สดจากตลาดสดและซูเปอร์มาร์เก็ตในเขตกรุงเทพมหานคร ประเทศไทย

น.ส.จุฑานาถ ศรีสำราญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตวแพทยสาธารณสุข ภาควิชาสัตวแพทยสาธารณสุข
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

Thesis Title	Detection of Indicator and Pathogenic Bacteria in Raw Vegetables and Fruits Sold in Fresh Markets and Supermarkets, Bangkok, Thailand
By	Miss Jutanat Srisamran
Field of Study	Veterinary Public Health
Thesis Advisor	Professor Dr. RUNGTIP CHUANCHUEN, D.V.M.
Thesis Co Advisor	Dr. SAHARUETAI JEAMSRIPONG, D.V.M.

Accepted by the FACULTY OF VETERINARY SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.... Dean of the FACULTY OF VETERINARY SCIENCE
(Professor Dr. ROONGROJE THANAWONGNUWECH, D.V.M.)

THESIS COMMITTEE

.... Chairman
(Professor Dr. ALONGKORN AMONSIN, D.V.M.)

.... Thesis Advisor
(Professor Dr. RUNGTIP CHUANCHUEN, D.V.M.)

.... Thesis Co-Advisor
(Dr. SAHARUETAI JEAMSRIPONG, D.V.M.)

.... Examiner
(Dr. TARADON LUANGTONGKUM, D.V.M.)

.... External Examiner
(Assoc.Prof. Dr. Sunpeth Angkittitrakul, D.V.M.)

จุฑานาถ ศรีสำราญ : การตรวจหาแบคทีเรียชี้วัดและแบคทีเรียก่อโรคในผักและผลไม้สดจากตลาดสดและซูเปอร์มาร์เก็ตในเขตกรุงเทพมหานคร ประเทศไทย. (Detection of Indicator and Pathogenic Bacteria in Raw Vegetables and Fruits Sold in Fresh Markets and Supermarkets, Bangkok, Thailand) อ.ที่ปรึกษาหลัก : ศ. ดร.รุ่งทิพย์ ชวนชื่น, อ.ที่ปรึกษาร่วม : ดร.สหฤทัย เจียมศรีพงษ์

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบปริมาณฟิคัลโคลิฟอร์มและเอสเชอริเชีย โคไลและการปนเปื้อนแบคทีเรียก่อโรคซาลโมเนลลา ชิเกลลา อี โคไล สายพันธุ์ O157: H7 และลิสทีเรีย โมโนไซโตจีเนส ในผักและผลไม้สด ที่เก็บจากตลาดสดและซูเปอร์มาร์เก็ต จากกรุงเทพมหานคร ประเทศไทย ตัวอย่างผักและผลไม้สดจำนวนทั้งหมด 405 ตัวอย่าง ประกอบด้วย โหระพา ต้นหอม ผักชี กะหล่ำปลี ผักกาดหอม แตงกวาและมะเขือเทศ สุ่มเก็บจาก 7 เขตในกรุงเทพมหานคร จากตลาดสด 7 แห่ง (n=203) และ ซูเปอร์มาร์เก็ต 29 แห่ง (n=202) ตั้งแต่เดือนมิถุนายน ปี พ.ศ. 2561 ถึง เดือนกรกฎาคม ปี พ.ศ. 2562 การวิเคราะห์ทางสถิติด้วยวิธี T-test และการวิเคราะห์ความแปรปรวนทางเดียว (one-way ANOVA) นำมาใช้ในการทดสอบความแตกต่างของปริมาณฟิคัลโคลิฟอร์มและอี โคไลระหว่างผักในตลาดสดและซูเปอร์มาร์เก็ต และระหว่างตัวอย่างผักและผลไม้แต่ละชนิด Chi-square นำมาใช้ทดสอบความแตกต่างของการปนเปื้อนแบคทีเรียก่อโรคระหว่างผักในตลาดสดและซูเปอร์มาร์เก็ต และระหว่างตัวอย่างแต่ละชนิดด้วย โดยรวมปริมาณฟิคัลโคลิฟอร์มและอี โคไลเฉลี่ย มีค่าเท่ากับ 2.97 (±2.03) $\log_{10}$MPN ต่อกรัม และ 2.49 (±1.98) $\log_{10}$MPN ต่อกรัม และพบการปนเปื้อนซาลโมเนลลา 7.16% จากตัวอย่างทั้งหมด แต่ไม่พบการปนเปื้อนชิเกลลา อี โคไล สายพันธุ์ O157: H7 และลิสทีเรีย โมโนไซโตจีเนส ปริมาณฟิคัลโคลิฟอร์มและอี โคไลเฉลี่ยในตัวอย่างจากตลาดสด (3.85 and 3.41 $\log_{10}$MPN ต่อกรัม) มีค่าสูงกว่าตัวอย่างจากซูเปอร์มาร์เก็ต (2.08 and 1.57 $\log_{10}$MPN ต่อกรัม) อย่างมีนัยสำคัญทางสถิติ ($P<0.001$) ความชุกของการปนเปื้อนซาลโมเนลลาในตัวอย่างจากตลาดสด (9.36%) มีค่าสูงกว่าตัวอย่างจากซูเปอร์มาร์เก็ต (4.95%) ($P=0.085$) นอกจากนี้ยังพบว่า โหระพา ผักชีและผักกาดหอม มีปริมาณฟิคัลโคลิฟอร์มและอี โคไล สูงกว่าต้นหอม กะหล่ำปลี แตงกวาและมะเขือเทศอย่างมีนัยสำคัญ ($P<0.05$) การปนเปื้อนที่พบมากในโหระพา ผักชีและผักกาดหอมอาจเกิดจากผักดังกล่าวซึ่งเป็นผักใบนั้นมักเพาะปลูกและเก็บเกี่ยวใกล้กับดิน ในขณะที่แตงกวาและมะเขือเทศนั้นจัดอยู่ในกลุ่มไม้เลื้อยซึ่งมักเก็บเกี่ยวห่างจากดินที่ใช้เพาะปลูก และกะหล่ำปลีจะประกอบไปด้วยใบที่ซ้อนทับกันแน่นหลายชั้น ซึ่งอาจเป็นปัจจัยหนึ่งที่ช่วยป้องกันการปนเปื้อนของแบคทีเรียได้ ผลจากการทดสอบบ่งชี้ว่าตัวอย่างผักและผลไม้สดจากซูเปอร์มาร์เก็ตมีสุขลักษณะที่ดีกว่าและผ่านเกณฑ์คุณภาพทางจุลชีววิทยาของอาหารมากกว่าตัวอย่างจากตลาดสด ดังนั้นวิธีการควบคุมการปนเปื้อนแบคทีเรียอย่างมีประสิทธิภาพในผักและผลไม้สดจึงมีความจำเป็นเพื่อให้อยู่ในเกณฑ์คุณภาพทางจุลชีววิทยาของอาหารและให้เกิดความปลอดภัยแก่ผู้บริโภค

สาขาวิชา	สัตวแพทยสาธารณสุข	ลายมือชื่อนิสิต ....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

6075322131 : MAJOR VETERINARY PUBLIC HEALTH
KEYWORD:

Jutanat Srisamran : Detection of Indicator and Pathogenic Bacteria in Raw Vegetables and Fruits Sold in Fresh Markets and Supermarkets, Bangkok, Thailand . Advisor: Prof. Dr. RUNGTIP CHUANCHUEN, D.V.M. Co-advisor: Dr. SAHARUETAI JEAMSRIPONG, D.V.M.

This study aimed to compare the concentrations of fecal coliforms and *Escherichia coli*, and the contamination of *Salmonella*, *Shigella*, *E. coli* O157: H7 and *Listeria monocytogenes* in raw vegetables and fruits between fresh markets and supermarkets in Bangkok, Thailand. A total of 405 produce samples, consisting of sweet basil, spring onion, coriander, cabbage, lettuce, cucumber and tomato, were randomly collected from seven fresh markets (n=203) and 29 supermarkets (n=202) in seven districts of Bangkok between June 2018 and July 2019. T-test and one-way ANOVA were used to compare the concentrations of fecal coliforms and *E. coli* (log_{10}MPN/g) between fresh markets and supermarkets, and among different types of samples. Chi-square test was used to compare the prevalence of pathogenic bacteria between fresh markets and supermarkets, and among different types of samples. Overall, the average concentrations (±S.D.) of fecal coliforms and *E. coli* were 2.97 (±2.03), and 2.49 (±1.98) log_{10}MPN/g. The prevalence of *Salmonella* was 7.16%, while *Shigella*, *E. coli* O157: H7 and *L. monocytogenes* were not detected in the samples. The concentrations of fecal coliforms and *E. coli* were significantly higher in fresh markets (3.85 and 3.41 log_{10}MPN/g) than in supermarkets (2.08 and 1.57 log_{10}MPN/g) ($P<0.001$). The prevalence of *Salmonella* in fresh markets (9.36%) was higher than those in supermarkets (4.95%) ($P=0.085$). The average concentrations of fecal coliforms and *E. coli* of sweet basil, coriander, and lettuce were significantly higher than spring onion, cabbage, cucumber and tomato. This maybe because sweet basil, coriander, and lettuce are leafy vegetables normally grown and harvested in proximity with the ground, while cucumber and tomato are vining crops, which are harvested off the ground. Cabbage consists of thick and compact leaves which could prevent bacterial contamination. This result indicated that the produce collected from supermarkets had better sanitation and met microbiological standard than fresh markets. Therefore, effective control measures should be implemented to meet microbiological standards and secure the safety of raw produce consumption in Thailand.

Field of Study:	Veterinary Public Health	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....
		Co-advisor's Signature ....

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest appreciation to my advisor, Prof. Dr. Rungtip Chuanchuen, and co-advisor, Dr. Saharuetai Jeamsripong, the Department of Veterinary Public Health, the Faculty of Veterinary Science, Chulalongkorn University, for their immeasurable advice, guidance and encouragement that they have provided throughout my study.

I would like to convey my gratitude to the committee chair, Prof. Dr. Alongkorn Amonsin, the Department of Veterinary Public Health, the Faculty of Veterinary Science, Chulalongkorn University, for his counsel and valuable suggestions. I also would like to show my profound appreciation to the committee members, Dr. Taradon Luangtongkum, and an external examiner, Assoc. Prof. Dr. Sunpeth Angkittitrakul, for their constructive advice and recommendations.

I would very much like to express my utmost respect and sincere appreciation to my family, friends and the staff from the Department of Veterinary Public Health, the Faculty of Veterinary Science, Chulalongkorn University, for their unfathomable guidance and moral support. Without their encouragement and assistance, I would not have achieved my goals.

Finally, my research would not have been successful without the support by "Chulalongkorn University Graduate Scholarship to Commemorate the 72nd Anniversary of His Majesty King Bhumibol Adulyadej", and the grant by The University of California Davis (A18-1020S001).

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et al.	et alii, and others
etc.	et cetera, and the other things
g	gram
hr	hour
i.e.	id est, that is
min	minute
ml	milliliter
No.	number
spp.	species
°C	degree Celsius

CHAPTER I

INTRODUCTION

One health is the concept of collaborating among several professionals across multiple sectors to solve complex public health issues concerning human, animal and environmental health. Animals have been a crucial vehicle that can transfer zoonotic pathogens impacting on human health worldwide. A number of research focusing on the contamination of bacteria in food animals have been reported in several countries. However, among diverse food commodities, raw vegetables and fruits have also been implicated in various zoonotic foodborne outbreaks.

The world trend of vegetable and fruit consumption per capita has been increasing since 1961 (GCDL, 2017). Fresh vegetables and fruits provide essential vitamins and minerals, maintain body function, and potentially prevent some diseases. The consumption of vegetables and fruits is associated with risk reduction of non-communicable diseases, such as cancer and cardiovascular disease (Aune et al., 2017). In Thailand, raw or partially-cooked vegetables and fruits, including sweet basil, spring onion, coriander, cabbage, lettuce, cucumber and tomato are generally served in local Thai dishes. Therefore, these vegetables and fruits could serve as a source of bacterial contamination that may threaten Thai people's health condition.

According to the World Health Organization (WHO), the global burden of foodborne illnesses was estimated that 600 million people are affected with more than 400,000 deaths annually (WHO, 2020). Several causative agents, including *Salmonella*, pathogenic *Escherichia coli*, and *Listeria* were identified among previous foodborne diseases. According to National Outbreak Reporting System (NORS), a total of 228 outbreaks attributed to fresh produce contaminated with *Salmonella*, *E. coli*, and *L. monocytogenes* resulted in more than 4,500 illnesses, 895 hospitalizations, and 55 deaths in the United States during 2010-2017 (Carstens et al., 2019). Additionally, raw produce-associated outbreaks also affect European countries, such as Austria, Finland, Sweden, Denmark and the United Kingdom (EFSA, 2018). Approximately 198 outbreaks were reported during 2004-2012 with a wide range of etiological pathogens, such as *E. coli*, *Salmonella*, and *Shigella* (Callejón et al., 2015). During 2008-2014, raw vegetable and fruits were linked to 30 outbreaks in Brazil, which reportedly resulted in almost 3,000 illnesses and 347 hospitalizations without any deaths, and the most predominant agent was *Salmonella* which accounted for 30% of the outbreaks (Elias et al., 2018).

Several outbreaks associated with raw produce have been recognized among European countries and the U.S. Nevertheless, the outbreak among Asian countries have not been widely reported. According to Foodborne Disease Outbreak Surveillance System (FDOSS), a total of 127 outbreaks were related to vegetables and fruits causing almost one thousand illnesses with 379 hospitalizations and one death in China during 2011-2016 (Wu et al., 2018). In Korea, 45 vegetable-related outbreaks associated with pathogenic *E. coli* and *Salmonella* affected almost 50,000 people with more than 6,000 illnesses during 2007-2012 (Moon et al., 2014).

Bacterial contamination in raw vegetables and fruits can occur from pre-harvest (improper usage of organic fertilizers, poor soil and water management) to post-harvest processes (inappropriate storage and transportation) (Rajwar et al., 2015). Several studies indicated that inadequately composted animal manure and contaminated water irrigation may harbor *E. coli*, *Salmonella*, and *Listeria* (Maffei et al., 2016; Sharma and Reynnells, 2016). Similarly, lack of appropriate temperature control during transportation and storage could facilitate the growth and survival of bacteria. Personal hygiene of food handlers and sanitation at retail level plays a major role in a food production chain (Hassan and Purwani, 2016).

In Thailand, fresh markets and supermarkets are two important retail establishments that are the closest spot to consumers. Fresh markets are normally an open-air traditional place where local commodities are provided with lower prices. On the other hand, supermarkets are perceived as a modern place with better management. Both of them are the most common places where consumers choose to purchase raw vegetables and fruits. According to the microbiological standard limits for food and contact surfaces of the Department of Medical Sciences (DMSC), the Ministry of Public Health, Thailand, the acceptable level of concentration of *E. coli* and the presence of pathogenic bacteria, such as *Salmonella*, and *L. monocytogenes* in ready-to-eat vegetables and fruits are also settled (MOPH, 2017). Even though the trend of fresh produce consumption has been rising in Thailand, the study of bacterial contamination in raw vegetables and fruits is limited. Therefore, it is necessary to estimate the bacterial contamination in raw vegetables and fruits at retail level that could threaten human health.

Objectives of the study

1. To determine the level of fecal coliforms and *E. coli* in fresh vegetables and fruits among fresh markets and supermarkets
2. To detect the contamination of *Salmonella*, *Shigella*, *E. coli* O157:H7 and *L. monocytogenes* in fresh vegetables and fruits among fresh markets and supermarkets

CHAPTER II
LITERATURE REVIEW

General Characteristics and Distribution of Fecal Coliforms, *E. coli* and *E. coli* O157:H7 in Fresh Vegetables and Fruits

Fecal coliforms and *E. coli* are facultative anaerobic, non-sporulating, Gram negative, rod-shape bacteria, which belong to family *Enterobacteriaceae*. These bacteria can grow in bile salt, ferment lactose, and produce gas at 44±0.5°C within 48 hr. Fecal coliforms and *E. coli* generally present in feces and colonize in digestive tracts of humans and warm-blooded animals, so they are normally used as bacterial indicators of fecal contamination in food and water (Feng et al., 2002). However, a group of fecal coliforms contains some species of *Klebsiella* spp. that are not necessarily from fecal-origin (Feng et al., 2002). Therefore, *E. coli* is considered as a better bacterial indicator for fecal contamination and can be served as a good indicator for other pathogenic bacterial contamination (Pan et al., 2015).

In Thailand, Most Probable Number (MPN) is currently used as a method to estimate the concentration of bacteria in a wide range of food, such as raw, ready-to-eat and fermented food. According to the Bureau of Quality and Safety of Food (BQSF), Department of Medical Sciences (DMSC), Ministry of Public Health, Thailand, more than half of *E. coli* positive vegetable samples were detected over 100 MPN/g, which was higher than the standard (Chungsamanukool et al., 2010). Furthermore, a study of bacterial contamination in ready-to-served vegetables among 14 hospitals in Thailand illustrated that 40.9% and 23.5% of the samples were contaminated with more than 1,100 MPN/g of fecal coliforms and 10 MPN/g of *E. coli*, respectively (Dhiraputra et al., 2005).

Although most of *E. coli* is non-pathogenic, some strains of *E. coli* can cause enteric or extra-intestinal illnesses in humans, including enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Kaper et al., 2004). EHEC, known as verocytotoxin-producing *E. coli* (VTEC) or shiga toxin-producing *E. coli* (STEC), is an important pathogenic *E. coli* that can cause severe illnesses in humans. The incubation period of STEC can vary from three to eight days (WHO, 2018). The infection of STEC can result in hemorrhagic colitis, bloody diarrhea and hemolytic uremic syndrome (HUS) due to Shiga toxin (Gyles, 2007).

E. coli O157:H7 is the most commonly reported serotype from patients of foodborne illnesses in many countries, such as the U.S., Japan and the U.K. (Lim et al., 2010). The infection of *E. coli* O157:H7 in humans can occur by fecal-oral route due to the consumption of contaminated food and water. Food products derived from cattle and food contaminated with animal manure, such as fresh vegetables and fruits are also important vehicles for the bacterial contamination. *E. coli* O157:H7 can be introduced to crops, colonize on the internal part of plants, capable of surviving in harsh environment, and carry throughout food production chain to humans (Chekabab et al., 2013). Although *E. coli* O157 infection is more related to the consumption of beef, some foodborne outbreaks associated with fresh produce have been identified (Berger et al., 2010). According to the Center for Disease Control and Prevention (CDC), fresh spinach contaminated with *E. coli* O157:H7 caused 199 illnesses with over 30 HUS cases, and three deaths in the U.S. in 2006 (CDC, 2006). The recent outbreaks associated with the contamination of romaine lettuce was indicated in the U.S., and the number of illnesses reached up to 210 persons, and 27 developed HUS with five deaths (CDC, 2018).

Apart from the U.S., the outbreaks associated with *E. coli* O157:H7 contamination in fresh produce have also been reported. In 2005, a large outbreak of *E. coli* O157 related to lettuce were reported with a total of 135 illnesses and 11 cases of HUS in Sweden (Söderström et al., 2008). During 2001-2009, six produce-associated outbreaks of *E. coli* O157:H7 caused almost 300 illnesses in Canada, and identified sources of the outbreaks consisted of spinach, lettuce, and onion (Kozak et al., 2013). Recently, the outbreak associated with mixed salad leaves occurred in the U.K. causing 165 cases, with 66 hospitalizations and nine HUS cases (Gobin et al., 2018). Even though the outbreak of *E. coli* O157:H7 has never been reported in Thailand, this pathogen was detected in bovine feces and retail beef, and antibody against *E. coli* O157 was detected in healthy people from southern Thailand (Vuddhakul et al., 2000; Sukhumungoon et al., 2011).

General Characteristics and Epidemiology of *Salmonella* spp.

1. General Characteristics of *Salmonella* and Salmonellosis

Salmonella is a facultative anaerobic, Gram negative, non-spore forming and rod-shaped bacterium that belongs to family *Enterobacteriaceae*. Most of *Salmonella* are motile by peritrichous flagella (Ryan et al., 2017). This pathogen is frequently found in the intestinal tracts of humans and some animals, such as farm animals, birds, and reptiles (Hanning et al., 2009). *Salmonella* consists of two species which are *S. enterica* and *S. bongori*. *S. enterica* can be divided into six subspecies, including, *S. enterica* subspecies *enterica*, *S. enterica* subspecies *salamae*, *S. enterica* subspecies *arizonae*, *S. enterica* subspecies *diarizonae*, *S. enterica* subspecies *houtenae* and *S. enterca* subspecies *indica* (Grimont and Weill, 2007). While *S. enterica* subspecies *enterica* is mainly isolated from warm-blooded animals and humans, the other five subspecies and *S. bongori* are more commonly found in cold-blooded animals (Desai et al., 2013). According to Kauffman and White scheme, *Salmonella* is classified based on serological identification of three antigenic determinants, including somatic (O), flagellar (H) and capsular (K) antigen. O antigen is oligosaccharide component on the outer membrane, H antigen is found in the flagella, and K antigen is the least common antigen located on capsular surface (Eng et al., 2015). More than 2,500 serovars have been identified and most of them belong to *S. enterica* subspecies *enterica* (Grimont and Weill, 2007).

Salmonellosis is an important foodborne disease in humans that can occur by ingestion of contaminated water and food or direct contact with animals. The patients can develop abdominal cramps, diarrhea, and fever within 12-72 hr, and the illnesses often last 4-7 days (Crum-Cianflone, 2008). *Salmonella* can also be divided into typhoidal and non-typhoidal which result in different ranges of human illnesses. Non-typhoidal *Salmonella* consists of several serovars except Typhi, Paratyphi A, B, and C. It can be found in humans and animals. Non-typhoidal *Salmonella* causes acute gastroenteritis that is primarily self-limiting. The symptoms include fever, vomiting, abdominal pain and diarrhea with or without blood. However, some strains, such as Enteritidis and Typhimurium might be invasive and cause systemic illnesses without the occurrence of diarrhea.

In contrast, typhoidal *Salmonella* comprises of a few serovars, including Typhi, Paratyphi A, B, and C, and humans are the specific reservoir. It is a causative agent of enteric fever, also known as typhoid (Typhi) and paratyphoid fever (Paratyphi), which is different from gastroenteritis. Typhoidal *Salmonella* is more frequently reported in many countries due to poor sanitation and lack of clean water. The patients associated with typhoidal salmonellosis often show persistent fever, headache, nausea, liver and spleen enlargement, rash, abdominal pain, diarrhea and constipation (Gal-Mor et al., 2014; Kurtz et al., 2017). According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), invasive non-typhoidal *Salmonella* and typhoidal *Salmonella* were responsible for approximately 535,000 cases and 14.3 million cases during 1990-2017, respectively (Stanaway et al., 2019).

2. Occurrence and Epidemiology of *Salmonella* in Fresh Vegetables and Fruits

The most common food sources leading to the infection of *Salmonella* in humans consist of eggs, poultry, beef and dairy products (Laufer et al., 2015; Khan et al., 2018). Nevertheless, raw vegetables and fruits have been increasingly identified as an important source of *Salmonella* infection in human. According to CDC, a total of 972 raw produce outbreaks associated with *Salmonella* were reported during 1998-2013 in the U.S. (Bennett et al., 2018). The outbreaks of *S. enterica* accounted for 161 outbreaks, causing over 10,000 illnesses, and more than 1,200 hospitalizations with 15 deaths (Bennett et al., 2018). Among these outbreaks, the most commonly recognized sources of contamination were fruits, seeded vegetables, sprouts and vegetable row crops (Bennett et al., 2018). During 2001-2009, *Salmonella* was identified as the most common bacteria that linked to half of the outbreaks associated with fresh vegetables and fruits in Canada (Kozak et al., 2013). A total number of 178 illnesses were reported due to foodborne illnesses, and food vehicles identified among these outbreaks included sprouts, cantaloupe, cucumber and tomato (Kozak et al., 2013). Likewise, *S.* Typhimurium, *S.* Saintpaul, and *S.* Virchow were mainly identified among 778 foodborne outbreaks in Australia with 15,000 illnesses and 48 deaths (Ford et al., 2018). Additionally, the prevalence of *Salmonella* contaminated in raw vegetables and fruits have been reported in Mexico, Brazil, Indian, Korea, and Japan (Quiroz-Santiago et al., 2009; Seo et al., 2010; Sant'Ana et al., 2011; Hara-Kudo et al., 2013; Mritunjay and Kumar, 2017).

In Thailand, the occurrence of *Salmonella* has been commonly reported in pork, chicken, and eggs rather than raw vegetables and fruits. According to the Ministry of Public Health of Thailand, *Salmonella* was responsible for 27.6% of vegetable samples that were collected from fresh markets in Bangkok and Nonthaburi (Chungsamanukool et al., 2010). More than 10% of vegetables were *Salmonella* positive in the northeastern part of Thailand, and the sources of contamination were identified in lettuce, coriander, sweet basil and cucumber (Woranetsudatip et al., 2013).

General Characteristics and Epidemiology of *Shigella*

1. General Characteristic of *Shigella* and Shigellosis

Shigella is a facultatively anaerobic, Gram negative, non-sporulating, non-motile and rod-shaped bacterium that belongs to family *Enterobacteriaceae*. This bacterium is closely related to *E. coli*. Even though both *E. coli* and *Shigella* have similar biochemical characteristics and lipopolysaccharide O antigens, they are considered different entities due to epidemiology and clinical features (Chattaway et al., 2017). *Shigella* is comprised of four serogroups, including *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). Each species of subgroup A, B, C, and D is serologically subdivided into distinct serotypes based on O antigen of lipopolysaccharide on cell membrane, which consist of 15 serotypes, 18 serotypes, 20 serotypes and one serotype, respectively (Wu et al., 2019).

Shigellosis is a foodborne disease causing bacillary dysentery that usually affects children in developing countries with poor resources and sanitation (Talaat et al., 2019). Fecal-oral route and person-to-person contact are major routes of bacterial infection. After the consumption of *Shigella*-contaminated food, the pathogen can pass through gastrointestinal tracts, predominantly invade colon epithelial cells, and spread from cell to cell, resulting in inflammatory colitis and diarrhea (Killackey et al., 2016). After 1-4 days of infection, the common symptoms, such as diarrhea with or without blood and mucus, abdominal pain, tenesmus and fever can be found. In general, shigellosis is a self-limiting disease that most patients can recover within one week. Nevertheless, severe complications may occur in some cases, especially in children, the elderly and the immunocompromised persons. The complications of intestinal perforation, rectal prolapse, megacolon, HUS, sepsis, metabolic abnormalities, and neurologic abnormalities were reported (Baker and The, 2018).

Shigella can produce Shiga toxin, inclusing *Shigella* enterotoxin 1 and 2. Shiga toxin is mainly produced by *S. dysenteriae* serotype 1 and STEC *E. coli*. However, recent studies reported that *S. flexneri* and *S. sonnei* have been recognized as Shiga toxin-producing organisms (Gray et al., 2014; Lamba et al., 2016). Shiga toxin is associated with life-threatening manifestation because the toxin involves hemorrhagic colitis and HUS (O'Loughlin and Robins-Browne, 2001).

According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) from 1990 to 2016, *Shigella* is responsible for 212,438 cases and 63,713 deaths in children younger than five years old (Khalil et al., 2018). The distribution of *Shigella* species varies based on geographical location and socioeconomic condition. Since *S. dysenteriae* and *S. boydii* are much less common, *S. flexneri* and *S. sonnei* have become the main burden of shigellosis worldwide (Baker and The, 2018). *S. flexneri* prevails in developing countries, while *S. sonnei* is more likely to occur in developed countries (Anderson et al., 2016). Additionally, *S. boydii* and *S. dysenteriae* are more limited to Southeast Asia and Sub-Saharan Africa (Killackey et al., 2016). Each species of *Shigella* is responsible for different severity of illnesses. *S. sonnei* and *S. boydii* typically cause only mild symptoms, whereas *S. dysenteriae* type 1 shows severe illnesses with high case fatality rates (Williams and Berkley, 2018).

2. Occurrence and Epidemiology of *Shigella* in Fresh Vegetables and Fruits

According to the Foodborne Disease Outbreak Surveillance System (FDOSS) in the U.S., a total of 120 foodborne outbreaks related to *Shigella* infection resulted in 6,208 illnesses, 197 hospitalizations and one death from 1998 to 2008 (Nygren et al., 2013). Lettuce-based salad was identified as one of the important implicated food vehicle of *Shigella* outbreaks (Nygren et al., 2013). In the U.S., *S. flexneri* contaminated in tomatoes was reported in restaurants, and it caused almost 900 illnesses in 2006 (Reller et al., 2006). In Canada, *Shigella* accounted for 17% of foodborne outbreaks which resulted in more than 700 illness cases, and lettuce, carrot, and Greek pasta salad were identified as main sources of the contamination (Kozak et al., 2013). Additionally, *Shigella* infection due to raw produce also affects European countries, such as Norway, Denmark and Australia (ECDC, 2016). Imported baby corn was indicated as a possible source of *Shigella* contamination that caused 215 cases in Denmark and 12 cases in Australia in 2007 (Lewis et al., 2009). In Norway, the outbreak of *Shigella*-contaminated sugar pea in 2009 and basil in 2011 caused 20 and 46 illnesses, respectively (Heier et al., 2009; Guzman-Herrador et al., 2011).

In Thailand, studies related to *Shigella* contamination in raw vegetables and fruits are scarce. Between 1996-2000, *Shigella* was isolated among clinical isolates, and the predominant species were *S. sonnei*, followed by *S. flexneri* and *S. boydii* (Hiranrattana et al., 2005). *S. sonnei* and *S. flexneri* were identified among 1,913 isolates retrieved from hospitals throughout Thailand between 2001-2005 (Pulsrikarn et al., 2009). Additionally, baby corn exported from Thailand was reported as a potential cause of shigellosis in Denmark and Australia due to poor sanitation of workers and inappropriate usage of disinfectants (Tikhamram et al., 2009).

General Characteristics and Epidemiology of *Listeria monocytogenes*

1. General Characteristic of *L. monocytogenes* and Listeriosis in Human

L. monocytogenes is Gram positive, intracellular, motile, rod-shaped, facultatively anaerobic pathogen that can cause listeriosis. A total of 17 species of *Listeria* has been recognized, consisting of *L. monocytogenes, L. ivanovii, L. welshimeri, L. seeligeri, L. fleischmannii, L. booriae, L. innocua, L. floridensis, L. cornellensis, L. marthii, L. grandensis, L. aquatica, L. riparia, L. weihenstephanensis, L. grayi, L. newyorkensis* and *L. rocourtiae.* However, only *L. monocytogenes* and *L. ivanovii* are considered as pathogenic bacteria (Orsi and Wiedmann, 2016). *L. monocytogenes is well-adapted and can* be found in water, soil, plant, intestinal tracts of animals and humans, and food. This pathogen *has the* ability to survive and grow in poor conditions, such as bile acid, low temperature, dry and acidic condition (Gahan and Hill, 2014). Some animals, such as cattle, goat, sheep, deer, moose, otter, and raccoon can carry *L. monocytogenes*, so they can shed the pathogen in their feces to the environment and feedstuff (Nightingale et al., 2004; Lyautey et al., 2007). Due to the ability to persist and thrive in harsh environment, *L. monocytogenes* can be easily transferred throughout food chain to consumers and cause public health risk (Jemmi and Stephan, 2006).

According to the global burden analysis of listeriosis between 1990-2012, listeriosis caused approximately 23,150 illnesses and 5,463 deaths worldwide (de Noordhout et al., 2014). The severity of the infection can vary from mild to fetal conditions, which results in two different forms; non-invasive and invasive form. Non-invasive listeriosis is usually self-limiting among healthy people. Infected patients can develop diarrhea, fever, headache, and muscle pain. On the contrary, people at high risk, including infants, pregnant women, immunosuppressed patients and the elderly, are more likely to develop an invasive listeriosis. The mortality rate of patients infected with the invasive form is higher than the non-invasive form. The symptoms of invasive form are severe, including fever, muscle pain, abortion, stillborn, septicemia, meningitis, and encephalitis (Pohl et al., 2019). The incubation period of

L. monocytogenes infection varies depending on clinical form of the disease. For example, among 37 invasive listeriosis cases, pregnancy-associated cases had prolonged incubation period up to 67 days comparing to patients with central nervous system signs (1-14 days) and bacteremia (1-12 days) (Goulet et al., 2013).

2. Occurrence and Epidemiology of *L. monocytogenes* in Fresh Vegetables and Fruits

Meat, poultry, seafood, and dairy products are mainly associated with the contamination of *L. monocytogenes*. In addition, raw vegetables and fruits also play an important role of *L. monocytogenes* transmission (Gómez et al., 2015; Tahoun et al., 2017). One large outbreak of *L. monocytogenes* in 2011 was reported across 28 states in the U.S. with 147 infected patients, 143 hospitalizations, and subsequently 33 deaths (CDC, 2012). According to European Food Safety Authority and European Center for Disease Prevention and Control, *L. monocytogenes* contamination in frozen corn, spinach and green bean, has been linked to the outbreaks in Austria, Finland, Sweden, Denmark and the U.K., causing 47 cases with nine deaths (EFSA, 2018).

The prevalence of *L. monocytogenes* has been reported in cabbage (28.3%) and cucumber (23.4%) from local markets in Nigeria, and frozen vegetable salad (25.4%) from supermarkets in Chile (Cordano and Jacquet, 2009; Ajayeoba et al., 2016). The prevalence of *L. monocytogenes* associated with raw mixed vegetables in Estonia was responsible for almost 20% of all contaminated food samples (Toomas et al., 2013). Among Asian countries, the low prevalence of *L. monocytogenes* implicated with produce was reported in China (5.7%) and Korea (0.3%), while the occurrence of *L. monocytogenes* was 22.5% in Malaysia (Ponniah et al., 2010; Seo et al., 2010; Wu et al., 2015). In Thailand, the contamination of *Listeria* was accounted for 16.8% in raw meat and vegetables (Stonsaovapak and Boonyaratanakornkit, 2010). According to the Ministry of Public Health of Thailand, around 48.4% (47 samples) of fresh vegetables and fruits were contaminated with *Listeria* spp., while only 2.1% (2 samples) were identified *L. monocytogenes* (Chungsamanukool et al., 2010).

Fresh Markets and Supermarkets in Thailand and their Possible Bacterial Contamination in Produce

According to the Ministerial Regulation on the Hygienic Practices of the Market A.D. 2008 by the Department of Health, the Ministry of Public Health, Thailand, fresh markets are categorized as type one and type two based on building structures (MOPH, 2008). Briefly, type one fresh markets shall have permanent buildings and market stalls for market vendor with separate areas of loading and parking lot. On the contrary, for type two fresh markets, permanent buildings are not a requirement, but the area must at least be well-built and market stalls can be temporary. In spite of different building structures, either type of fresh markets must be maintained hygienically clean on a daily basis. Mandatory facilities must also be provided, such as, water supply, waste disposal area, bathroom and toilets (MOPH, 2008).

The occurrence of bacterial contamination at retails mainly depend on sanitation and hygiene practices during handling, packaging, and processing. In fresh markets, different types of raw vegetables and fruits are regularly displayed on stall without any packaging under ambient air temperature. Therefore, direct contact of sellers and customers constantly happen most of the time, and it may introduce bacterial cross-contamination to the consumers. Additionally, bacterial loads harbored on the surface of produce can be transferred from one another with uncontrolled temperature facilitating the bacterial growth and survival (Gil et al., 2015). In supermarkets, basic sanitation and hygiene practices are implemented. Nevertheless, cross contamination among fresh vegetables and fruits can appear during produce processing, such as cutting, trimming and washing. Fresh produce sold in supermarkets must be processed and packed separately before selling, hence unclean equipment and contaminated sources of water could lead to bacterial contamination (Hassan and Purwani, 2016).

CHAPTER III
MATERIALS AND METHODS

This study was divided into 2 phases; phase 1: Sample collection, and phase 2: Bacterial isolation and confirmation (Figure 1). The contamination among different types of vegetables and fruits were then compared.

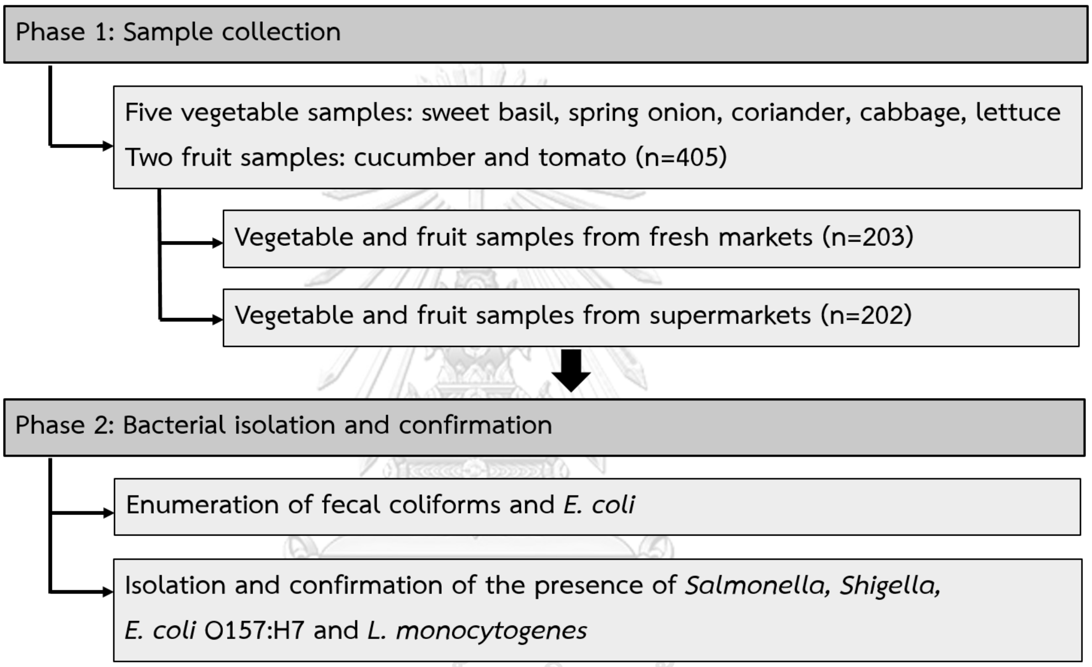

Figure 1. Conceptual framework

Phase1: Sample Collection

Five types of vegetables and two types of fruits were collected. Five vegetables consisted of sweet basil (*Ocimum basilicum*), spring onion (*Alliumcepa* var. *aggregatum*), coriander (*Coriandrum sativum*), cabbage (*Brassica oleracea* var. *capitata*), and lettuce (*Lactuca sativa*), whereas two fruits included cucumber (*Cucumis sativus*) and tomato (*Lycopersicon esculentum* Mill.). These vegetables and fruits were selected because they are commonly consumed raw or minimally cooked in Thai cuisine. The sample size (n=405) was calculated based on 50% expected prevalence, 5% error, and 95% confidence interval. Therefore, a total of 405 samples were collected between June 2018 and July 2019 from fresh markets (n=203) and supermarkets (n=202). Seven districts in Bangkok were randomly selected based on fresh market distribution in Bangkok, according to the Department of City Planning and Urban Development, Bangkok Metropolitan Administrator, Thailand. The seven districts consisted of Pathumwan, Chatuchak, Bangkok noi, Bang Khun Thian, Khlong Toei, Lat Phrao, and Bang Khae. One fresh market and four to five supermarkets were selected in each district. Hence, a total of seven fresh markets and 29 supermarkets were randomly chosen. In each fresh market, four to five vendors were selected. All samples were collected separately in a sterile plastic bag, transported in containers with ice packs to maintain the temperature. The samples were processed within 24 hr in the Department of Veterinary Public Health, the Faculty of Veterinary Science, Chulalongkorn university.

Phase 2: Bacterial Isolation and Confirmation

1. Sample Preparation

Approximately 25 g of vegetable and 200 g of fruit samples were weighed and placed in plastic bags using aseptic technique. A total of 225 ml of proper enrichment media was added to vegetables samples, while 200 ml of the enrichment media were added to fruit samples and performed a ten-fold dilution. Subsequently, all prepared samples were shaken by hand for two min. Fresh vegetable and fruit samples were enumerated for the concentrations (MPN/g) of indicator bacteria (fecal coliforms and *E. coli*) and *Salmonella*, *Shigella*, *E. coli* O157:H7, and *L. monocytogenes* were indicated as presence or absence.

2. The Enumeration of Fecal coliforms and *E. coli*

The concentrations of fecal coliforms and *E. coli* were analyzed according to Bacteriological Analytic Manual (BAM), the U.S. Food and Drug Administration (US-FDA) (Feng et al., 2002). Approximately, 25 g of vegetable samples and 200 g of fruit samples were added in 225 ml and 200 ml buffered peptone water (BPW) (Difco, MD, USA) to make a 10-fold serial dilution (10^{-1} to 10^{-7}). One ml from each dilution of BPW was inoculated into three 9-ml lactose broth tubes (LB) (Difco), as for three-tube MPN calculation and incubated at 35±0.5 °C for 24±2 hr. One loopful suspension of positive gassing tubes was transferred to 9-ml EC broth (Difco) and incubated in waterbath at 45.5 °C for 24±2 hr.

The positive gassing tubes of EC broth were used to calculate most probable number (MPN) of fecal coliforms. One loopful from positive gassing tubes of EC was streaked on levine-methylene blue agar (L-EMB agar) (Difco) and incubated at 35±0.5°C for 18-24 hr. Suspected colonies of *E. coli* from L-EMB agar plates, which are flat dark colonies with or without green metallic sheen were selected and streaked on plate count agar (PCA) (Difco), incubated at 35±0.5 °C for 18-24 hr, and used for further biochemical test.

Indole production test was biochemically used for *E. coli* confirmation. A single colony of suspected *E. coli* was inoculated in 5-ml tryptone broth (Difco) and incubated at 35±0.5 °C for 24±2 hr. After the incubation, approximately 0.2-0.3 ml of Kovacs' reagent (Sigma-Aldrich, Steinheim, Germany) was added. Positive reaction of *E. coli* provides distinct red color in upper layer of the inoculum due to the ability to produce indole. The concentration of *E. coli* was calculated and recorded. Three colonies of *E. coli* from each sample were collected in 20% glycerol and stored at -80 °C freezer.

3. The Detection and Serotyping of *Salmonella* spp.

The detection of *Salmonella* spp. was performed according to International Organization for Standardization, horizontal method for the detection, enumeration and serotyping of *Salmonella* spp. (ISO, 6579:2017). Briefly, 25 g of vegetable samples and 200 g of fruit samples were enriched in 225 ml and 200 ml of BPW (Difco) as 10-fold dilution and incubated at 37±1 °C for 18±24 hr. After that, 0.1 ml from BPW solution were transferred to modified semi-solid Rappaport Vassiliadis agar (MSRV) (Difco). MSRV agar plates were incubated at 42±0.5 °C overnight, and suspected *Salmonella* is greyish swarming zone. After the incubation, one loopful from positive MSRV plates were streaked onto xylose lysine deoxycholate agar (XLD agar) (Difco) and hektoen enteric agar (HE agar) (Difco) and incubated at 37±1 °C for 24±3 hr. Colonies of *Salmonella* on XLD agar are red typically with black center due to lysine decarboxylation and hydrogen sulfide production. *Salmonella* on HE agar are blue-green colonies with black center.

Three to four suspected colonies of *Salmonella* from each sample on XLD and HE agar plates were confirmed by using triple sugar iron agar (TSI Agar) (Difco). Inoculated TSI tubes were incubated at 37±1 °C for 24±3 hr to observe the usage of lactose, sucrose and glucose. Typical colonies of *Salmonella* show alkaline (red) slant and acid (yellow) butt due to the degradation of glucose. Bubbles and blackening of the agar are also observed due to the capability of gas and hydrogen sulfide formation. Then, typical *Salmonella* cultures were streaked on PCA and incubated at 35±0.5 °C for 18-24 hr. Three to four colonies of *Salmonella* from each sample were collected in 20% glycerol, stored at -80°C freezer and used for serological test.

Slide agglutination test was used to determine the serovars of *Salmonella* by detecting O (somatic) and H (flagella) antigens according to Kauffman and White scheme (Grimont and Weill, 2007). *Salmonella* isolates were serotyped using available antisera (S&A Reagents Lab, Bangkok, Thailand). The isolates were grown on nutrient agar (NA) (Difco) and incubated at 35±0.5 °C for 18-24 hr. The isolate was tested with polyvalent O antisera by placing one drop of the antisera on a glass slide and adding a single colony of *Salmonella*. After mixing thoroughly, tilt the slide gently and observe the agglutination. When the agglutination occurred, monovalent O antisera were further used to determine O antigen. After the identification of O antigen, the isolates were grown on swarm agar at 37 °C for 18-24 hr and tested for H antigen which is divided into two phases. Phase 1, the isolates were tested with polyvalent H antisera when the agglutination occurred, monovalent H antisera were used to identify H antigen. After the first H antigen was determined, phase 2 or inversion phase was carried out. After O, H phase 1 and H phase 2 antigen were identified, the serovars of *Salmonella* were specified.

4. The Detection and Confirmation of *Shigella spp.*

The detection of *Shigella* was carried out according to International Organization for Standardization, horizontal method for the detection of *Shigella* spp. (ISO, 21567:2004). Briefly, 25 g of vegetable samples and 200 g of fruit samples were transferred into 225 ml and 200 ml Shigella broth base (Himedia, Mumbai, India) and incubated at 41.5±1 °C for 16-20 hr. One loopful from the broth was then streaked onto MacConkey agar (Difco), XLD agar and HE agar. Then, the plates were incubated at 37±1 °C for 20-24 hr. Typical colonies of *Shigella* are colorless, red and green with no black center on MacConkey, XLD and HE agar, respectively.

Suspected colonies of *Shigella* were transferred to TSI agar, and incubated at 37±1 °C for 24±3 hr to observe the degradation of lactose, sucrose and glucose. Similar to *Salmonella*, *Shigella* cultures can use only glucose, hence alkaline (red) slant and acid (yellow) butt are observed without gas or hydrogen sulfide formation. The suspected colonies of *Shigella* were then streaked on PCA and incubated at 35±0.5 °C for 18-24 hr. Three to four colonies were collected in 20% glycerol and stored at -80°C freezer for further serological test.

Slide agglutination test was undertaken for *Shigella* confirmation and serotyping using *Shigella* Polyvalent antisera (S&A Reagents Lab) at the Research Unit in Microbial Food Safety and Antimicrobial Resistance, the Department of Veterinary Public Health, the Faculty of Veterinary Science, Chulalongkorn University. O (somatic) antigens are utilized to determine the serotype of *Shigella*. Briefly, suspected *Shigella* colonies were grown on NA plate, and incubated at 35±0.5 °C overnight. A single drop of polyvalent antisera was placed on a glass slide with a suspected *Shigella* colony to observed agglutination. Finally, the species of *Shigella* (*S. dysenteriae*, *S. flexneri*, *S. sonnei* and *S. boydii*) were identified based on the agglutination that appeared with a specific polyvalent antiserum.

5. The Detection of *E. coli* O157:H7

The detection of *E. coli* O157:H7 was performed according to International Organization for Standardization, horizontal method for the detection *Escherichia coli* O157:H7 with modification (ISO, 16654:2001). Twenty-five g of vegetable samples and 200 g of fruit samples were prepared and added to 225 ml and 200 ml of modified tryptone-soy broth with novobiocin (mTSB+n) (Merck, Darmstadt, Germany) and incubated at 41.5±1 °C for 18 hr. After the incubation, one loopful suspension was transferred onto sorbitol MacConkey agar plates with supplemented cefixime and tellurite (CT-SMAC) (Himedia) and CHROMagar^{TM}O157 agar plates (Sharlau, Paris, France). The plates were then incubated at 37 °C for 24 hr. *E. coli* O157 do not ferment sorbitol, consequently the colonies appear colorless, while the other bacteria fermenting sorbitol show pink colonies on CT-SMAC agar. On CHROMagar^{TM}O157 agar, typical colonies of *E. coli* O157 appear mauve, while other coliforms show metallic blue colonies.

6. The Detection of *Listeria monocytogenes*

The detection of *L. monocytogenes* was conducted according to International Organization for Standardization, horizontal method for the detection and enumeration of *L. monocytogenes* and of *Listeria* spp. with modification (Jamali et al., 2013; ISO, 11290-1:2017). An enrichment step was performed by adding 25 g of vegetable samples and 200 g of fruit samples in 225 ml and 200 ml of half-fraser broth (Sharlau). All prepared samples were incubated at 30 °C for 24-26 hr. One loopful of enrichment broth was streaked on CHROMagar^{TM} Listeria (Sharlau) plates and incubated at 37 °C for 24 hr. Suspected *L. monocytogenes* colonies typically exhibit blue colonies with a diameter less than three mm with white halo zone.

Statistical Analysis

Descriptive statistics was used to illustrate the concentrations of fecal coliforms and *E. coli* (MPN/g), and prevalence of pathogenic bacteria among vegetable and fruit samples from fresh markets (n=203) and supermarkets (n=202). The concentrations of fecal coliforms and *E. coli* (log_{10}MPN/g) between fresh market and supermarket were compared by using T-test whereas the concentrations of fecal coliforms and *E. coli* (log_{10}MPN/g) among different types of samples were compared by using one-way Analysis of Variance (one-way ANOVA) and Bonferroni. Chi-square was used to compare the prevalence of pathogenic bacteria between fresh market and supermarket, and compare the prevalence of pathogenic bacteria among different types of samples. All statistical analyses were performed in SPSS Software (Version 22). *P*-value<0.05 was considered statistical significance.

CHAPTER IV
RESULTS

A total of 405 samples were collected from seven fresh markets (n=203) and 29 supermarkets (n=202) from seven districts in Bangkok (Table 1 and 2). One fresh market and four to five supermarkets were selected in each district. Four to five vendors were selected in each fresh market (Table 2). Fifty-eight samples were collected in each type of vegetables and fruits (Table 3).

Table 1. The number of samples stratified by sampling location (seven districts in Bangkok, Thailand)

Location	No. of samples		
	Fresh market (n=203)	Supermarket (n=202)	Total (n=405)
Bang Khae	35	28	63
Bang Khun Thian	28	28	56
Bangkok Noi	28	28	56
Chatuchak	28	28	56
Khlong Toei	28	34	62
Lat Phrao	28	28	56
Pathumwan	28	28	56
Total	203	202	405

Table 2. The number of markets stratified by sampling location (seven districts in Bangkok, Thailand)

Location	No. of markets		
	Fresh market (n=7)	Supermarket (n=29)	Total (n=36)
Bang Khae	1 market (5 vendors)	4 markets	5 markets
Bang Khun Thian	1 market (4 vendors)	4 markets	5 markets
Bangkok Noi	1 market (4 vendors)	4 markets	5 markets
Chatuchak	1 market (4 vendors)	4 markets	5 markets
Khlong Toei	1 market (4 vendors)	5 markets	6 markets
Lat Phrao	1 market (4 vendors)	4 markets	5 markets
Pathumwan	1 market (4 vendors)	4 markets	5 markets
Total	7 markets (29 vendors)	29 markets	36 markets

Table 3. The number of samples stratified by the types of vegetables and fruits

Type of sample (n)	No. of samples		
	Fresh market (n=203)	Supermarket (n=202)	Total (n=405)
Sweet basil (n=58)	29	29	58
Spring onion (n=58)	29	29	58
Coriander (n=58)	29	29	58
Cabbage (n=58)	29	29	58
Lettuce (n=57)	29	28	57
Cucumber (n=58)	29	29	58
Tomato (n=58)	29	29	58
Total (n=405)	203	202	405

Prevalence and Concentrations of Fecal Coliforms

The overall prevalence of fecal coliforms was 81.98%. The highest prevalence was detected in sweet basil (100%) and lettuce (100%), followed by spring onion (98.28%) and coriander (98.28%), cucumber (75.86%), cabbage (55.17%), and tomato (46.55%), respectively (Table 4). The prevalence of fecal coliforms in fresh markets was 90.64% while the prevalence of fecal coliforms in supermarkets was 73.27% (Table 4).

The prevalence of fecal coliforms ranged from 58.62% to 100% in fresh markets while the prevalence of fecal coliforms was from 20.69% to 100% in supermarkets (Table 4). Sweet basil, spring onion, coriander, and lettuce in fresh markets were 100% positive for fecal coliforms. Likewise, sweet basil and lettuce purchased from supermarkets were also 100% contaminated with fecal coliforms. However, the lowest prevalence of fecal coliforms from fresh markets was found in tomato (58.62%) and supermarkets were observed in cabbage (20.69%) (Table 4).

Table 4. Prevalence of fecal coliforms stratified by the types of vegetables and fruits in fresh markets (n=203) and supermarkets (n=202)

Type of sample (n)		Prevalence of fecal coliforms (%)		
		Fresh market (n=203)	**Supermarket (n=202)**	**Total (n=405)**
Sweet basil	(n=58)	29/29 (100%)	29/29 (100%)	58/58 (100%)
Spring onion	(n=58)	29/29 (100%)	28/29 (96.55%)	57/58 (98.28%)
Coriander	(n=58)	29/29 (100%)	28/29 (96.55%)	57/58 (98.28%)
Cabbage	(n=58)	26/29 (89.66%)	6/29 (20.69%)	32/58 (55.17%)
Lettuce	(n=57)	29/29 (100%)	28/28 (100%)	57/57 (100%)
Cucumber	(n=58)	25/29 (86.21%)	19/29 (65.52%)	44/58 (75.86%)
Tomato	(n=58)	17/29 (58.62%)	10/29 (34.48%)	27/58 (46.55%)
Total	(n=405)	184/203 (90.64%)	148/202 (73.27%)	332/405 (81.98%)

Average concentrations (±standard deviation) of fecal coliforms of each market were displayed in Table 5. Overall, the average concentration of fecal coliform was 2.97 (±2.03) log_{10}MPN/g, and the highest concentration was found in sweet basil, followed by lettuce, coriander, spring onion, cucumber, cabbage and tomato, respectively (Table 6). In fresh markets, lettuce had the highest concentration of fecal coliforms, followed by sweet basil, and coriander. However, different trends were observed among the samples from supermarkets. Sweet basil harbored the highest concentration of fecal coliforms, followed by lettuce. The lowest concentrations of fecal coliforms were found in tomato from fresh markets, and in cabbage from supermarkets (Table 6).

Table 5. Average concentrations (S.D.) (log_{10}MPN/g) of fecal coliforms stratified by fresh markets (n=203) and supermarkets (n=202)

Type of market	Average concentrations (±S.D.) (log_{10}MPN/g)
Fresh market 1	4.20 (±1.62)
Fresh market 2	4.35 (±1.80)
Fresh market 3	3.44 (±2.02)
Fresh market 4	3.86 (±1.57)
Fresh market 5	4.22 (±1.83)
Fresh market 6	4.26 (±2.13)
Fresh market 7	2.84 (±2.46)
Supermarket 1	3.05 (±0.93)
Supermarket 2	2.58 (±1.50)
Supermarket 3	2.27 (±1.35)
Supermarket 4	2.92 (±1.86)
Supermarket 5	3.32 (±2.38)
Supermarket 6	2.13 (±1.46)
Supermarket 7	1.87 (±1.34)
Supermarket 8	2.38 (±2.05)

Table 5. Average concentrations (S.D.) (log_{10}MPN/g) of fecal coliforms stratified by fresh markets (n=203) and supermarkets (n=202) (continue)

Type of market	Average concentrations (±S.D.) (log_{10}MPN/g)
Supermarket 9	2.47 (±1.90)
Supermarket 10	2.83 (±2.14)
Supermarket 11	2.05 (±2.15)
Supermarket 12	1.09 (±0.88)
Supermarket 13	1.44 (±1.29)
Supermarket 14	2.08 (±1.73)
Supermarket 15	2.27 (±1.89)
Supermarket 16	2.40 (±1.50)
Supermarket 17	1.73 (±1.77)
Supermarket 18	2.08 (±1.97)
Supermarket 19	2.00 (±1.39)
Supermarket 20	1.65 (±1.47)
Supermarket 21	1.96 (±1.57)
Supermarket 22	1.77 (±1.47)
Supermarket 23	2.31 (±1.85)
Supermarket 24	1.02 (±1.33)
Supermarket 25	1.43 (±1.49)
Supermarket 26	1.78 (±1.43)
Supermarket 27	2.28 (±1.76)
Supermarket 28	1.53 (±1.49)
Supermarket 29	1.72 (±1.55)

Table 6. Average concentrations (S.D.) (log_{10}MPN/g) of fecal coliforms in fresh markets (n=203) and supermarkets (n=202)

Type of sample (n)		**Average concentrations (±S.D.) (log_{10}MPN/g)**		
		Fresh market (n=203)	**Supermarket (n=202)**	**Total (n=405)**
Sweet basil	(n=58)	5.83 (±0.76)	3.76 (±0.83)	4.80 (±1.31)
Spring onion	(n=58)	3.25 (±0.87)	2.93 (±1.08)	3.09 (±0.98)
Coriander	(n=58)	5.39 (±1.07)	2.93 (±1.05)	4.16 (±1.63)
Cabbage	(n=58)	2.60 (±1.35)	0.27 (±0.63)	1.43 (±1.57)
Lettuce	(n=57)	5.87 (±0.87)	3.22 (±1.04)	4.57 (±1.64)
Cucumber	(n=58)	2.49 (±1.28)	0.99 (±0.97)	1.74 (±1.35)
Tomato	(n=58)	1.48 (±1.37)	0.52 (±0.85)	1.00 (±1.23)
Total	(n=405)	3.85 (±2.01)	2.08 (±1.62)	2.97 (±2.03)

The average concentration of fecal coliforms from fresh markets (3.85 (±2.01) log_{10}MPN/g) was statistically different from the samples from supermarkets (2.08 (±1.62) log_{10}MPN/g) (P<0.001) (Figure 2). Most of the samples purchased from fresh markets had statistically significant higher concentrations of fecal coliforms (P<0.05) except spring onion (P=0.21).

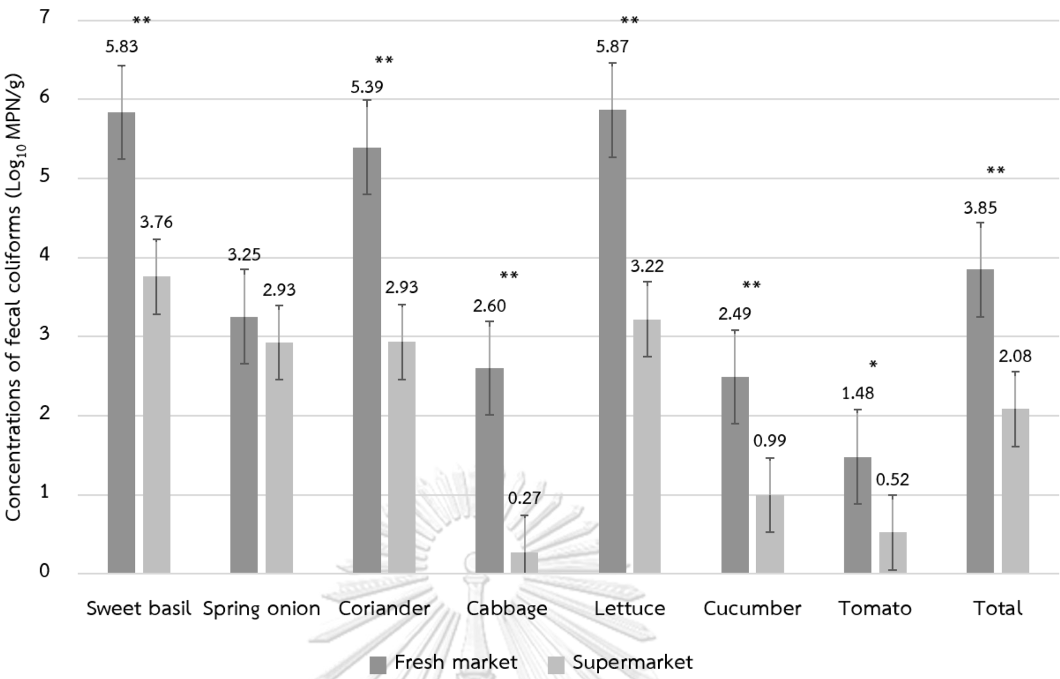

Figure 2. Concentrations of fecal coliforms (log_{10}MPN/g) of vegetables and fruits from fresh markets (n=203) and supermarkets (n=202)

** represents the significant difference between the samples from fresh market and supermarket at *P*<0.001

* represents the significant difference between the samples from fresh market and supermarket at *P*<0.05

The average concentrations of fecal coliforms (log_{10}MPN/g) were statistically significant among different types of samples according to one-way ANOVA and Bonferroni test (*P*<0.001). The overall average concentration of fecal coliforms of sweet basil, coriander, and lettuce significantly differed from spring onion, cabbage, cucumber and tomato (*P*<0.05) (Table 7). Likewise, the average concentration of spring onion was also significantly different from cabbage, cucumber and tomato (*P*<0.05) (Table 7).

Table 7. The mean comparisons of the average fecal coliform concentrations (log_{10}MPN/g) among the seven types of vegetables and fruits (n=405)

Sample 1	Sample 2	Mean Difference	S.E.	95% C.I.	*P*-value
Sweet basil	Spring onion	1.70648	0.26091	0.90868 to 2.50428	<0.001
	Cabbage	3.36144	0.26091	2.56364 to 4.15925	<0.001
	Cucumber	3.05497	0.26091	2.25717 to 3.85277	<0.001
	Tomato	3.79430	0.26091	2.99650 to 4.59210	<0.001
Coriander	Spring onion	1.07424	0.26091	0.27644 to 1.87204	0.001
	Cabbage	2.72920	0.26091	1.93140 to 3.52700	<0.001
	Cucumber	2.42273	0.26091	1.62493 to 3.22053	<0.001
	Tomato	3.16206	0.26091	2.36425 to 3.95986	<0.001
Lettuce	Spring onion	1.47729	0.26206	0.67600 to 2.27858	<0.001
	Cabbage	3.13225	0.26206	2.33096 to 3.93355	<0.001
	Cucumber	2.82578	0.26206	2.02449 to 3.62707	<0.001
	Tomato	3.56511	0.26206	2.76381 to 4.36640	<0.001
Spring onion	Cabbage	1.65496	0.26091	0.85716 to 2.45276	<0.001
	Cucumber	1.34849	0.26091	0.55069 to 2.14629	<0.001
	Tomato	2.08782	0.26091	1.29002 to 2.88562	<0.001

S.E.: standard error; C.I.: confidence interval

Prevalence and Concentrations of *E. coli*

The overall prevalence of *E. coli* was 73.33% (Table 8). Sweet basil (100%) showed the highest contamination of *E. coli*, followed by lettuce (96.49%), coriander (94.83%), spring onion (77.59%), cucumber (62.07%), cabbage (51.72%) and tomato (31.03%). The prevalence of *E. coli* in fresh markets was 85.71%, while the prevalence in supermarkets was 60.89% (Table 8).

The prevalence of *E. coli* in spring onion, coriander, cabbage, lettuce, cucumber and tomato in fresh markets was higher than those in supermarkets (Table 8). In fresh markets, the prevalence of *E. coli* ranged between 37.93% and 100%. The highest contamination of *E. coli* was in sweet basil (100%), coriander (100%) and lettuce (100%), whereas the lowest contamination of *E. coli* was detected in tomato (37.93%). In supermarkets, the prevalence of *E. coli* varied between 17.24% and 100%. The highest prevalence of *E. coli* was detected in sweet basil (100%), while the lowest contamination was observed in cabbage (17.24%) (Table 8).

Table 8. Prevalence of *E. coli* stratified by the types of vegetables and fruits in fresh markets (n=203) and supermarkets (n=202)

Type of sample (n)		**Prevalence of *E. coli* (%)**		
		Fresh market (n=203)	**Supermarket (n=202)**	**Total (n=405)**
Sweet basil	(n=58)	29/29 (100%)	29/29 (100%)	58/58 (100%)
Spring onion	(n=58)	27/29 (93.1%)	18/29 (62.07%)	45/58 (77.59%)
Coriander	(n=58)	29/29 (100%)	26/29 (89.66%)	55/58 (94.83%)
Cabbage	(n=58)	25/29 (86.21%)	5/29 (17.24%)	30/58 (51.72%)
Lettuce	(n=57)	29/29 (100%)	26/28 (92.86%)	55/57 (96.49%)
Cucumber	(n=58)	24/29 (82.76%)	12/29 (41.38%)	36/58 (62.07%)
Tomato	(n=58)	11/29 (37.93%)	7/29 (24.14%)	18/58 (31.03%)
Total	(n=405)	174/203 (85.71%)	123/202 (60.89%)	297/405 (73.33%)

Average concentrations (±standard deviation) of *E. coli* stratified by markets were illustrated in Table 9. The average concentration of *E. coli* was 2.49 (±1.98) log_{10}MPN/g. The highest concentration of *E. coli* was detected in sweet basil, followed by lettuce, coriander, spring onion, cucumber, cabbage and tomato (Table 10). The highest average concentration of *E. coli* among the samples from fresh markets was detected in sweet basil, followed by lettuce and coriander (Table 10). Similarly, sweet basil purchased from supermarkets also contained the highest concentration of *E. coli*, followed by lettuce and coriander. On the other hand, the lowest concentration of *E. coli* in fresh markets and supermarkets were observed in tomato and cabbage, respectively (Table 10).

Table 9. Average concentrations (S.D.) (log_{10}MPN/g) of *E. coli* stratified by fresh markets (n=203) and supermarkets (n=202)

Type of market	Average concentrations (±S.D.) (log_{10}MPN/g)
Fresh market 1	3.61 (±1.80)
Fresh market 2	3.73 (±1.70)
Fresh market 3	3.37 (±1.96)
Fresh market 4	3.72 (±1.71)
Fresh market 5	3.14 (±2.20)
Fresh market 6	3.74 (±2.03)
Fresh market 7	2.72 (±2.46)
Supermarket 1	2.13 (±0.79)
Supermarket 2	2.15 (±1.15)
Supermarket 3	1.59 (±1.27)
Supermarket 4	2.46 (±1.52)
Supermarket 5	2.38 (±1.68)
Supermarket 6	1.84 (±1.26)
Supermarket 7	1.24 (±1.22)
Supermarket 8	1.76 (±1.84)

Table 9. Average concentrations (S.D.) (log_{10}MPN/g) of *E. coli* stratified by fresh markets (n=203) and supermarkets (n=202) (continue)

Type of market	Average concentrations (±S.D.) (log_{10}MPN/g)
Supermarket 9	1.14 (±1.52)
Supermarket 10	1.32 (±1.70)
Supermarket 11	0.93 (±1.45)
Supermarket 12	0.59 (±0.85)
Supermarket 13	0.71 (±1.24)
Supermarket 14	1.78 (±1.34)
Supermarket 15	1.59 (±1.27)
Supermarket 16	1.93 (±1.07)
Supermarket 17	1.50 (±1.89)
Supermarket 18	1.94 (±1.83)
Supermarket 19	1.61 (±1.61)
Supermarket 20	0.89 (±1.11)
Supermarket 21	1.68 (±1.36)
Supermarket 22	1.23 (±1.66)
Supermarket 23	2.11 (±2.03)
Supermarket 24	1.02 (±1.33)
Supermarket 25	1.23 (±1.59)
Supermarket 26	1.69 (±1.32)
Supermarket 27	1.79 (±1.70)
Supermarket 28	1.53 (±1.49)
Supermarket 29	1.68 (±1.53)

Table 10. Average concentrations (S.D.) (log_{10}MPN/g) of *E. coli* in fresh markets (n=203) and supermarkets (n=202)

Type of sample (n)		Average concentrations (±S.D.) (log_{10}MPN/g)		
		Fresh market (n=203)	**Supermarket (n=202)**	**Total (n=405)**
Sweet basil	(n=58)	5.51 (±0.77)	3.20 (±0.68)	4.35 (±1.37)
Spring onion	(n=58)	2.78 (±1.19)	1.71 (±1.44)	2.24 (±1.42)
Coriander	(n=58)	4.89 (±0.95)	2.29 (±1.00)	3.59 (±1.63)
Cabbage	(n=58)	2.18 (±1.26)	0.22 (±0.59)	1.20 (±1.39)
Lettuce	(n=57)	5.41 (±0.87)	2.65 (±0.97)	4.05 (±1.67)
Cucumber	(n=58)	2.12 (±1.24)	0.60 (±0.86)	1.36 (±1.31)
Tomato	(n=58)	0.98 (±1.33)	0.37 (±0.70)	0.67 (±1.10)
Total	(n=405)	3.41 (±2.02)	1.57 (±1.44)	2.49 (±1.98)

The average concentrations of *E. coli* among the samples from fresh markets (3.41 (±2.02) log_{10}MPN/g) were significantly higher than supermarkets (1.57 (±1.44) log_{10}MPN/g) (P<0.001) (Figure 3). In addition, the average concentrations of *E. coli* of each sample type purchased from fresh markets was significantly different from supermarkets (P<0.05) (Figure 3).

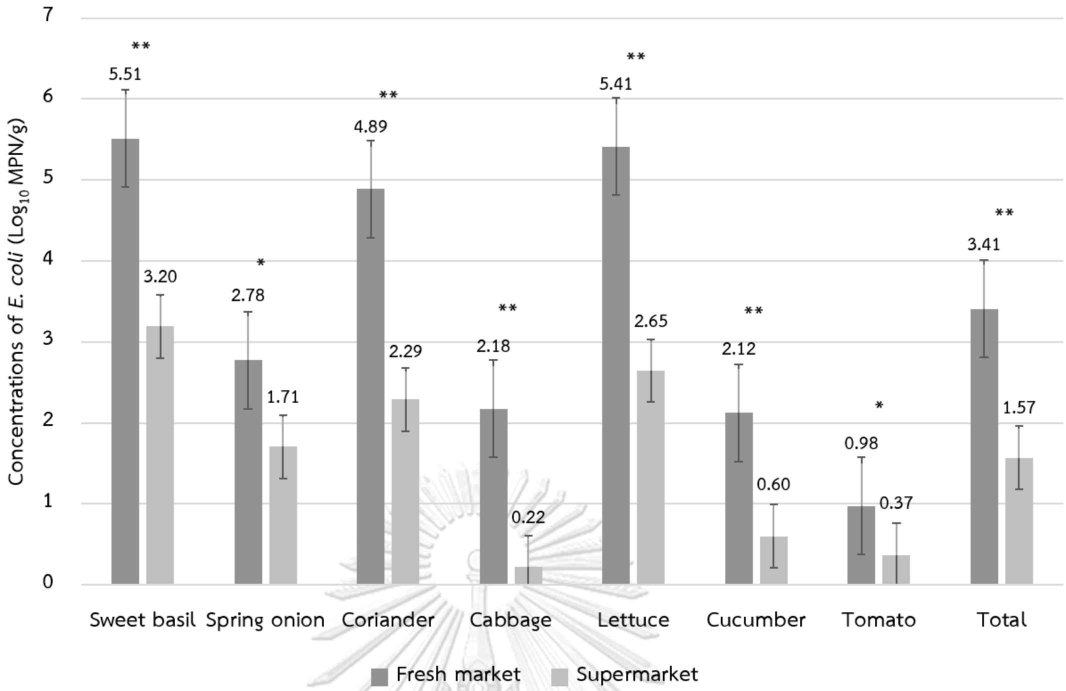

Figure 3. Concentrations of *E. coli* (log_{10}MPN/g) of vegetables and fruits from fresh markets (n=203) and supermarkets (n=202)

** represents the significant difference between the samples from fresh market and supermarket at P<0.001

* represents the significant difference between the samples from fresh market and supermarket at P<0.05

The average concentrations of *E. coli* (log_{10}MPN/g) were significantly different among different types of vegetables and fruits according to one-way ANOVA and Bonferroni test ($P<0.001$). The average concentration of *E. coli* of sweet basil, coriander, and lettuce significantly differed from spring onion, cabbage, cucumber and tomato ($P<0.001$) (Table 11). Similarly, the average concentration of spring onion was also significantly different from cabbage, cucumber and tomato ($P<0.05$) (Table 11).

Table 11. The mean comparisons of the average *E. coli* concentrations (log_{10}MPN/g) among seven types of vegetables and fruits (n=405)

Sample 1	Sample 2	Mean Difference	S.E.	95% C.I.	*P*-value
Sweet basil	Spring onion	2.11368	0.26405	1.30628 to 2.92107	<0.001
	Cabbage	3.15681	0.26405	2.34941 to 3.96420	<0.001
	Cucumber	2.99228	0.26405	2.18489 to 3.79968	<0.001
	Tomato	3.68131	0.26405	2.87391 to 4.48871	<0.001
Coriander	Spring onion	1.35000	0.26405	0.54260 to 2.15739	<0.001
	Cabbage	2.39312	0.26405	1.58573 to 3.20052	<0.001
	Cucumber	2.22860	0.26405	1.42120 to 3.03600	<0.001
	Tomato	2.91763	0.26405	2.11023 to 3.72502	<0.001
Lettuce	Spring onion	1.81336	0.26521	1.00243 to 2.62429	<0.001
	Cabbage	2.85648	0.26521	2.04555 to 3.66741	<0.001
	Cucumber	2.69196	0.26521	1.88103 to 3.50289	<0.001
	Tomato	3.38099	0.26521	2.57006 to 4.19192	<0.001
Spring onion	Cabbage	1.04313	0.26405	0.23573 to 1.85052	0.002
	Cucumber	0.87860	0.26405	0.07121 to 1.68600	0.020
	Tomato	1.56763	0.26405	0.76024 to 2.37503	<0.001

S.E.: standard error; C.I.: confidence interval

Prevalence of *Salmonella* and Detection of *Salmonella* Serovars

The overall prevalence of *Salmonella* in the samples was 7.16%, and sweet basil (24.14%) was the most contaminated vegetable, followed by lettuce (12.28%), and coriander (6.9%) (Table 12). Cabbage and tomato were absent of *Salmonella*. In fresh markets, the highest contamination of *Salmonella* was in sweet basil (27.59%), followed by lettuce (20.69%), and coriander (10.34%). In supermarkets, the highest contamination of *Salmonella* was detected in sweet basil (20.69%), followed by spring onion (6.9%), and lettuce (3.57%) (Table 12).

Table 12. Prevalence of *Salmonella* stratified by the types of vegetables and fruits in fresh markets (n=203) and supermarkets (n=202)

Type of sample (n)		Prevalence of *Salmonella* (%)		
		Fresh market (n=203)	Supermarket (n=202)	Total (n=405)
Sweet basil	(n=58)	8/29 (27.59%)	6/29 (20.69%)	14 (24.14%)
Spring onion	(n=58)	1/29 (3.45%)	2/29 (6.9%)	3 (5.17%)
Coriander	(n=58)	3/29 (10.34%)	1/29 (3.45%)	4 (6.9%)
Cabbage	(n=58)	0	0	0
Lettuce	(n=57)	6/29 (20.69%)	1/28 (3.57%)	7 (12.28%)
Cucumber	(n=58)	1/29 (3.45%)	0	1 (1.72%)
Tomato	(n=58)	0	0	0
Total	(n=405)	19/203 (9.36%)	10/202 (4.95%)	29 (7.16%)

The overall prevalence of *Salmonella* in fresh markets (9.36%) was higher than those in supermarkets (4.95%) (*P*=0.085). No statistically significant difference of *Salmonella* contamination was observed between fresh markets and supermarkets (*P*>0.05), except lettuce (*P*=0.049). In addition, sweet basil, coriander and lettuce showed statistically significant difference of the *Salmonella* prevalence comparing to other types of the samples (*P*<0.05) (Table 13).

Table 13. The comparison of *Salmonella* prevalence among seven types of vegetables and fruits (n=405)

Sample 1	Sample 2	*P*-value
Sweet basil	Spring onion	0.004
	Coriander	0.01
	Cabbage	<0.001
	Cucumber	<0.001
	Tomato	<0.001
Coriander	Cabbage	0.042
	Tomato	0.042
Lettuce	Cabbage	0.006
	Cucumber	0.026
	Tomato	0.006

Twenty-eight serotypes of *Salmonella* were detected in 109 *Salmonella* isolates (Table 14). The common serotypes were Stanley (29/109; 26.6%), Chester (6/109; 5.5%) and Hvittingfoss (5/109; 4.6%). The main sources of *Salmonella* Stanley were sweet basil and lettuce (Table 12). While the contamination of *Salmonella* Chester was commonly found in spring onion and lettuce, and *Salmonella* Hvittingfoss was in sweet basil and cucumber.

Table 14. The serovars of *Salmonella* isolates (n=109)

Serovars	No. of isolates (%)							
	SB	SO	CO	CA	LE	CU	TO	Total
Stanley	17 (58.6)	-	2 (6.9)	-	10 (34.5)	-	-	29 (26.6)
Chester	-	3 (50)	-	-	3 (50)	-	-	6 (5.50)
Hvittingfoss	3 (60)	-	-	-	-	2 (40)	-	5 (4.6)
Bareilly	1 (25)	3 (75)	-	-	-	-	-	4 (3.7)
Biafra	-	-	4 (100)	-	-	-	-	4 (3.7)
Derby	-	4 (100)	-	-	-	-	-	4 (3.7)
Djugu	4 (100)	-	-	-	-	-	-	4 (3.7)
Heidelberg	-	-	-	-	4 (100)	-	-	4 (3.7)
Huettwilen	-	-	4 (100)	-	-	-	-	4 (3.7)
Mountpleasant	-	-	4 (100)	-	-	-	-	4 (3.7)
Newport	4 (100)	-	-	-	-	-	-	4 (3.7)
Thies	4 (100)	-	-	-	-	-	-	4 (3.7)
Typhimurium	4 (100)	-	-	-	-	-	-	4 (3.7)
Weltevreden	3 (75)	1 (25)	-	-	-	-	-	4 (3.7)
Uganda	4 (100)	-	-	-	-	-	-	4 (3.7)
Braenderup	3 (100)	-	-	-	-	-	-	3 (2.8)
Brunei	-	-	-	-	2 (66.7)	1 (33.3)	-	3 (2.8)
Kaapstad	3 (100)	-	-	-	-	-	-	3 (2.8)
Vegesack	1 (50)	-	-	-	1 (50)	-	-	2 (1.8)
Yoruba	-	-	-	-	2 (100)	-	-	2 (1.8)
Aarhus	-	-	1 (100)	-	-	-	-	1 (0.9)
Aberdeen	-	-	-	-	1 (100)	-	-	1 (0.9)
Abony	-	-	-	-	1 (100)	-	-	1 (0.9)
Augustenborg	1 (100)	-	-	-	-	-	-	1 (0.9)
Krefeld	1 (100)	-	-	-	-	-	-	1 (0.9)
Saphra	-	-	-	-	-	1 (100)	-	1 (0.9)
Shamba	-	-	-	-	1 (100)	-	-	1 (0.9)
Virchow	-	-	-	-	1 (100)	-	-	1 (0.9)
Total	53 (48.6)	11 (10.1)	15 (13.8)	-	26 (23.9)	4 (3.7)	-	109 (100)

SB: Sweet basil; SO: Spring onion; CO: Coriander; CA: Cabbage; LE: Lettuce; CU: Cucumber; TO: Tomato

Prevalence of *Shigella*, *E. coli* O157:H7, *L. monocytogenes*

Shigella, *E. coli* O157:H7, and *L. monocytogenes* were not detected in any vegetable and fruit samples purchased from fresh markets (n=203) and supermarkets (n=202).

CHAPTER V
DISCUSSION

Prevalence and Concentrations of Fecal Coliforms and *E. coli* Contaminated in Vegetables and Fruits from Fresh Markets and Supermarkets

The results of this study showed that the prevalence of fecal coliforms and *E. coli* was more observed in fresh markets (90.64% and 85.71%) than in supermarkets (73.27% and 60.89%). The consistent results were reported in another study conducted in Thailand where the prevalence of fecal coliforms and *E. coli* in vegetable samples purchased from fresh markets (89.00% and 33.00%) was higher than those from supermarkets (60.00% and 25.00%) (Ananchaipattana et al., 2012). Similarly, the prevalence of *E. coli* in local produce was higher in fresh markets (55.20%) than in supermarkets (30.80%) in Thailand (Chungsamanukool et al., 2010). In Mexico, prevalence of fecal coliforms in ready-to-eat salads and sprouts collected from open-air stalls was 65.00%, while in supermarkets was only 36.00% (Cerna-Cortes et al., 2015). This could be a result from better sanitation and good management of raw produce in supermarkets. In northern California, prevalence of fecal coliforms was found in all vegetable samples sold in farmer's markets, whereas only 20.00% of the samples were *E. coli* positive (Pan et al., 2015).

In this study, fecal coliforms and *E. coli* were more detected in leafy vegetables, including sweet basil, lettuce, and coriander, than in cucumber, cabbage and tomato. The finding was similar to another study that *E. coli* was high in leafy vegetables, including spinach (27.10%) and lettuce (18.00%), while none of *E. coli* was reported in tomato purchased from farmer's markets in Alberta, Canada (Bohaychuk et al., 2009). Additionally, the prevalence of *E. coli* was observed 23.33% in lettuce, 13.33% in tomato, and 11.67% in cabbage purchased from markets in the Philippines (Vital et al., 2014). However, cabbage, tomato, lettuce, and onion collected in Ghana (96.70%), and tomato collected from local markets in Bangladesh

(46.00%) showed elevated prevalence of *E. coli* comparing to our study (Abakari et al., 2018; Harris et al., 2018)

According to the microbiological standard limits for food and contact surfaces of the Department of Medical Sciences (DMSC), the Ministry of Public Health of Thailand, the concentrations of *E. coli* in ready-to-eat vegetables and fruits must not exceed 100 MPN/g (MOPH, 2017). In this study, approximately 59.75% (242/405 samples) of total samples exceeded the acceptable concentration of *E. coli*. The samples exceeding the standard limit were detected in fresh markets (75.86%; 154/203 samples) more than in supermarkets (43.56%; 88/202 samples). Moreover, *E. coli* in the produce that exceeded the limit was sweet basil (96.55%; 56/58 samples), followed by lettuce (92.98%; 53/57 samples), and coriander (82.75%; 48/58 samples), while less number of the unhygienic samples were observed in tomato (15.52%; 9/58 samples), cucumber (32.76%; 19/58 samples) and cabbage (34.48%; 20/58 samples). Similarly, the study in Nakorn Ratchasima, Thailand, also revealed that more than half of the local produce (n=30) collected from a fresh market harbored more than 100 MPN/g of *E. coli* (Poompak et al., 2019). However, only 23.71% of produce samples exceeded the acceptable concentration of *E. coli* were reported in another study (Chungsamanukool et al., 2010).

In this study, the average concentrations of fecal coliforms and *E. coli* were significantly higher in fresh markets (3.85 and 3.41 log_{10}MPN/g) than in supermarkets (2.08 and 1.57 log_{10}MPN/g) (P<0.001). In Mexico, high fecal coliform concentrations were observed in ready-to-eat produce purchased from street vendors (1.37 log_{10}MPN/g) than in supermarkets (0.52 log_{10}MPN/g) (Cerna-Cortes et al., 2015). In the present study, sweet basil, coriander, and lettuce showed statistically significant more concentrations of fecal coliforms and *E. coli* comparing to the other types of samples (P<0.05). A study in Cameroon also displayed higher fecal coliform concentrations in lettuce (2.22 log_{10}MPN/g) than in cucumber (2.12 log_{10}MPN/g) and cabbage (1.73 log_{10}MPN/g) (Akoachere et al., 2018). Comparatively, a study in Canada illustrated that *E. coli* concentrations were also elevated in leafy vegetables, such as spinach (1.54 log_{10}MPN/g) and lettuce (1.25 log_{10}MPN/g), while *E. coli* was not detected in tomato (Bohaychuk et al., 2009). Likewise, higher concentrations of *E. coli* were found in lettuce (3.09-3.15 log_{10}CFU/g) than cabbage (1.00-2.88 log_{10}CFU/g) collected from supermarkets in the Philippines (Vital et al., 2014). Similar results were reported in northern California that lower concentrations of fecal coliforms and *E. coli* were observed in vining crops, including yard-long beans (3.82 and 0.31 log_{10}CFU/g) and bitter squash (3.94 and 0.36 log_{10}CFU/g), while leafy vegetables, including cilantro (4.16 and 1.13 log_{10}CFU/g) and squash stem and leaves (4.02 and 0.94 log_{10}CFU/g) showed higher contamination of the bacteria (Pan et al., 2015).

In our study, spring onion contained significantly higher fecal coliform and *E. coli* concentrations than tomato (P<0.05). Similarly, elevated *E. coli* concentration was detected in green onion (1.43 log_{10}MPN/g) comparing to tomato (not detected) (Bohaychuk et al., 2009). Another study at retail reported that *E. coli* was not detected in tomato, whereas *E. coli* concentrations in parsley, cilantro, and scallion exceeded 3.00 log_{10}CFU/g (Arthur et al., 2007). Furthermore, our study illustrated low average *E. coli* concentrations in tomato in fresh markets (0.98 log_{10}MPN/g), comparing to a study in local markets in Kano (3.20 log_{10} MPN/g), Kaduna (2.70 log_{10} MPN/g), and Katsina (2.00 log_{10}MPN/g) in northwest Nigeria (Shenge et al., 2015). Nonetheless, average *E. coli* concentrations in overall vegetable samples at retail markets (2.18 log_{10}MPN/g) in Bangladesh were lower than our study (2.49 log_{10}MPN/g) (Harris et al., 2018).

The Contamination of *Salmonella* in Vegetables and Fruits from Fresh Markets and Supermarkets

According to the microbiological standard limits for food and contact surfaces, MOPH, Thailand, the prevalence of *Salmonella* in ready-to-eat vegetables and fruits must not be presented in 25 g of samples (MOPH, 2017). In this study, the overall prevalence of *Salmonella* was 7.16%. Slightly lower prevalence was reported among vegetable and fruit samples purchased from local markets and supermarkets in the U.S. (6.6%), Mexico (5.7%), India (4.0%), and Turkey (3.8%) (Quiroz-Santiago et al., 2009; Buyukunal et al., 2015; Pan et al., 2015; Mritunjay and Kumar, 2017). In addition, low prevalence of *Salmonella* was observed in ready-to-eat vegetables in some countries, such as, Brazil (0.4%), Canada (0.03%), and Finland (0.02%) (Sant'Ana et al., 2011; Denis et al., 2016; Nousiainen et al., 2016). Even though *Salmonella* contamination at retail level is generally low in our study (7.16%), a study in Ghana, and Nigeria showed high prevalence of *Salmonella* (73.30% and 31.54%) in a wide range of local produce and mixed salad, such as cucumber, tomato, carrot, cabbage, lettuce, etc. (Lennox et al., 2015; Abakari et al., 2018).

Although there was no statistical difference of *Salmonella* contamination between fresh markets and supermarkets, the prevalence of *Salmonella* in the samples purchased from fresh markets (9.36%) was higher than those from supermarkets (4.95%). Consistent results were reported in a previous study carried out in Thailand that *Salmonella* was found in 16.5% of vegetable samples, and the contamination was observed only among the samples sold in fresh markets (27.6%) (Chungsamanukool et al., 2010). Similarly, cabbage, cucumber, tomato, lettuce, and carrot, collected from fresh markets (4.4%) in Malaysia showed slightly higher prevalence of *Salmonella* when comparing with supermarkets (3.0%) (Saw et al., 2019).

This study showed that the prevalence of *Salmonella* was significantly higher in leafy vegetables, (i.e. sweet basil, coriander, and lettuce), than in cabbage, cucumber and tomato. Consistently, lettuce (25.0%) harbored high *Salmonella* contamination, comparing to cabbage (20.0%) and tomato (16.7%) in the Philippines (Vital et al., 2014). In Dhanbad city, India, similar result was observed in leafy vegetables (20.0%), which contained high *Salmonella* contamination (Kumar, 2012). Another study at local markets in India also showed high *Salmonella* contamination in spinach (11.7%), while cucumber (5.0%), tomato (5.0%), and cabbage (0%) contained less *Salmonella* (Mritunjay and Kumar, 2017). Likewise, a study conducted in supermarkets in Turkey reported that *Salmonella* contamination was more detected in lettuce (17.39%) than in cucumber (0%) and tomato (0%) (Buyukunal et al., 2015). Similarly, a study conducted in Mexico found that high prevalence of *Salmonella* was detected in leafy vegetable samples collected from supply chain markets (n=1,700), such as parsley (12.0%), cilantro (11.0%), and lettuce (10.2%), whereas cabbage (1.0%) harbored less *Salmonella* (Quiroz-Santiago et al., 2009). *Salmonella* was commonly found in leafy vegetables, such as squash stem and leaves (27.60%) and cilantro (22.20%) than in vining plants, including yard-long bean (2.90%) and bitter squash (0%) (Pan et al., 2015). A surveillance in Canada reported that 0.04% of leafy vegetables and herbs (n=17,427) was *Salmonella* contaminated while *Salmonella* was not detected in tomato (Denis et al., 2016).

Among 109 *Salmonella* isolates, 28 serotypes were identified in this study, and the most common serotypes were Stanley (26.6%), followed by Chester (5.5%) and Hvittingfoss (4.6%). Another study in Thailand demonstrated inconsistent results that serotype Panama (25.0%) was the most detected among *Salmonella*-positive lettuce samples (8.0%), followed by Stanley (12.0%), Hvittingfoss (12.0%) and Weltevreden (12.0%) (Niyomdecha et al., 2016). Additionally, *Salmonella* Typhimurium (33.30%) was commonly observed among vegetables in the south of Thailand, followed by Hvittingfoss (23.30%) and Weltevreden (20.0%) (Lertworapreecha et al., 2012). Similarly, a study in raw produce sold at retail level in

Mexico and Malaysia showed that Typhimurium (23.9% and 66.7%) was the most common serovars (Quiroz-Santiago et al., 2009; Saw et al., 2019).

Salmonella associated with outbreaks of raw-produce and other foods in the Australia, U.S., and Canada were mainly caused by Typhimurium and Enteritidis, which were contrasted with our study (Kozak et al., 2013; Bennett et al., 2018; Ford et al., 2018). Even though, *Salmonella* Stanley is uncommon in Europe, it is more common in southeast Asia. It was the seventh most common serovars (3.80%) among 44,087 *Salmonella* isolates in human during 1993-2002. Moreover, Stanley was the second most common serovars collected from patients (12.9%) and retailed foods (10.9%) during 2001-2006 in Thailand (Bangtrakulnonth et al., 2004; Sirichote et al., 2010). In addition, *S.* Stanley was also observed in frozen duck meat (10.4%), pigs (6.6%) and pork (20.8%) in slaughterhouses and retail outlets in Thailand (Bangtrakulnonth et al., 2004; Pulsrikarn et al., 2012; Phongaran et al., 2019). Therefore, the contamination of Stanley might be a result of cross-contamination from other food products, and that the contamination of Stanley in raw produce may be a source of human salmonellosis.

Even though Chester is not commonly reported, it was associated with outbreaks in several countries, such as the U.S., Canada, Australia, France, Belgium, the Netherlands, Spain, Denmark, Sweden and Japan (Fonteneau et al., 2017). In addition, Chester was previously reported in frozen duck meat (6.4%) in Thailand (Bangtrakulnonth et al., 2004). From 2007 to 2011, Heidelberg, Newport and Braenderup caused a number of outbreaks associated with fresh produce, chicken, and pork in the U.S. (Andino and Hanning, 2015). Thus, the contamination of these serovars (i.e. Chester, Heidelberg, Newport and Braenderup) observed in this study could serve as an origin of salmonellosis in human. Moreover, Hvittingfoss (4.6%), Derby (3.7%), Typhimurium (3.7%), Weltevreden (3.7%), Krefeld (0.9%), and Virchow (0.9%) observed in this study were commonly isolated from human and food products in Thailand (Bangtrakulnonth et al., 2004). Therefore, raw vegetables and fruits could be an important vehicle of cross-contamination and human salmonellosis.

The Contamination of *Shigella*, *E. coli* O157: H7, and *L. monocytogenes* in Vegetables and Fruits from Fresh Markets and Supermarkets

Even though *Shigella*, *E. coli* O157: H7, and *L. monocytogenes* were implicated in some raw produce-associated outbreaks, they were not detected in any samples in this study. A consistent result was reported that *Shigella* was not observed among local produce at retail outlets in Thailand (Chungsamanukool et al., 2010). Similarly, none of the produce in Canada and Italy, were contaminated with *Shigella* (Cardamone et al., 2015; Denis et al., 2016). However, *Shigella* (8.9%) was reported in cucumber (14.6%) and tomato (7.9%) in India (Jatmane, 2016). Approximately 15.9% and 76.7% of raw produce samples sold in local markets in Nigeria and Ghana were *Shigella* positive (Lennox et al., 2015; Abakari et al., 2018).

None of *E. coli* O157: H7 was detected in vegetable and fruit samples purchased from supermarkets in Spain and Iran, and from retail markets in Italy (Abadias et al., 2008; Jeddi et al., 2014; Cardamone et al., 2015). Likewise, in the U.S. and Canada, leafy vegetables and herbs, tomato, green onion, berries, cantaloupe contained no *E. coli* O157: H7 (Pan et al., 2015; Denis et al., 2016). Nevertheless, *E. coli* O157: H7 was previously reported in India (1.3%), and spinach (6.7%) and beetroot (3.3%) were the main sources of contamination (Mritunjay and Kumar, 2017).

Although no *L. monocytogenes* was detected in this study, less than 5% of *L. monocytogenes* was detected in produce samples at retail outlets in Thailand (Chungsamanukool et al., 2010; Stonsaovapak and Boonyaratanakornkit, 2010). In India, *L. monocytogenes* was detected in 3.5% of raw produce samples, such as spinach (13.3%), tomato (6.7%), and cucumber (5.0%) (Mritunjay and Kumar, 2017). A study in northern Germany reported lower prevalence of *L. monocytogenes* (1%) in mixed salad leaves at retail establishments (Fiedler et al., 2017). Additionally, *L. monocytogenes* was detected in only 0.32% out of 4,435 leafy vegetables, such as lettuce, and spinach collected at retail in Canada (Denis et al., 2016). Therefore, *Shigella*, *E. coli* O157: H7, and *L. monocytogenes* are uncommon in raw vegetables and fruits in fresh markets and supermarkets in Thailand.

Bacterial Contamination in Vegetables and Fruits at Different Retail Levels (fresh market and supermarket)

Bacterial contamination in fresh produce can occur from pre-harvest to post-harvest. Sources of bacterial contamination during pre-harvest include improper use of organic fertilizers, in appropriate soil amendment and contaminated water irrigation. Even though chemical fertilizers have been widely used in Thai agriculture, organic fertilizers could also provide efficient crop production with lower cost (Sankaew, 2015). Organic fertilizers available in Thailand consist of animal manure, compost, green manure and liquid organic fertilizers. Animal manure, such as poultry, cattle and swine, is commonly used in organic agriculture since chemical fertilizers are prohibited (ACFS, 2014). Several studies have reported potentially higher bacterial contamination in organic produce, such as *E. coli*, *Salmonella* and *L. monocytogenes* (Maffei et al., 2016). Therefore, animal manure might harbor pathogenic bacteria that can be contaminated in crop production and may cause foodborne diseases in human.

Bacterial contamination during post-harvest can occur due to improper handling and unsanitary equipment during processing, storage and transportation. After harvesting, raw vegetables and fruits are processed either on the farm or processing plants before being distributed to retail establishments. Fecal coliform and *E coli* are generally found in intestinal tracts of human and animals. Thus, personal hygiene of raw-produce handlers plays an important is mandatory to reduce fresh produce contamination along the food chain. In addition, high temperature during transportation and storage might have a positive effect on bacterial contamination. Therefore, using low temperature is recommended to decrease bacterial growth (FAO, 2008).

Fresh markets and supermarkets are important sources where bacterial pathogens can be transferred from raw vegetables and fruits to consumers. At retail level as fresh markets and supermarkets, bacterial contamination can occur due to improper hygiene practices, contaminated washing water, and inappropriate storage and handling (Gil et al., 2015). In this study, fecal coliforms, *E. coli*, and *Salmonella* in vegetables and fruits purchased from fresh markets may result from poor sanitation and inappropriate management. Fresh markets in Thailand are an open area that do not provide a separate area for produce processing, such as cutting, trimming, and washing. Unlike supermarkets, packaging and wrapping are not used to separate each type of raw produce in fresh markets. Consequently, dirt and debris remaining on the stall after processing can be directly contaminated in ready-for-sale produce. Therefore, sanitation and personal hygiene guidelines should be implemented for good quality control to prevent the occurrence of cross-contamination among raw vegetables and fruits, and direct contact of personnel and customers (MOPH, 2006). Appropriate storage temperature is also essential for raw produce preservation. Storage produce in low temperature is a recommended measure to assist prevent produce deterioration and delay bacterial growth (Gil et al., 2015). Raw vegetables and fruits in fresh markets are processed and sold under ambient air, whereas cooling facilities are utilized during processing, storage, and display to keep raw produce fresh and delay bacterial proliferation. Thus, bacterial contamination might be less detected in supermarkets than in fresh markets.

Bacterial Contamination in Different Types of Vegetables and Fruits

Leafy vegetables, such as basil, coriander, cabbage, and lettuce, are ranked the first commodity of public health concerns due to implication in foodborne disease outbreaks. While spring onion and tomato are ranked the second priority, and cucumber is ranked the third priority (FAO, 2008). In this study, the contamination of fecal coliforms, *E. coli*, and *Salmonella* were higher in sweet basil, coriander and lettuce comparing to spring onion, cabbage, cucumber and tomato. Sweet basil, coriander and lettuce are categorized as leafy vegetables and their leaves are mainly the edible part. They are usually grown and harvested in close proximity to the soil. Thus, these plants are directly exposed to contaminated soil (manure) and water irrigation, which may be important sources of pathogenic and non-pathogenic bacteria. Cabbage is also considered as a leafy vegetable and grown directly from the ground like sweet basil, coriander and lettuce. Nevertheless, cabbage contains several layers of compact leaves that grow inward and might help prevent bacterial contamination from the environment (Mritunjay and Kumar, 2017). The outer cabbage leaves are peeled off before selling. Therefore, the bacteria mostly attached to the outer leaves might also be removed. Cucumber and tomato are grown differently from sweet basil, coriander, cabbage, and lettuce since they are vining crops. Trellis, strings, or other metal and wooden structures are designed and utilized to support the vines that spread out. During harvesting, cucumber and tomato are collected far off the ground. Therefore, less contamination of bacteria could be observed in cucumber and tomato (Pan et al., 2015).

CONCLUSION AND SUGGESTIONS

General knowledge and suggestions

1. The contamination of indicator and pathogenic bacteria of raw vegetables and fruits purchased from supermarket was lower than fresh markets. This indicated appropriate handling and better sanitation in supermarket than fresh market.

2. Consumers should be well-aware of possible bacterial contamination in raw vegetables and fruits, especially when consuming raw leafy vegetables, such as sweet basil, coriander and lettuce.

3. National policies aiming for better sanitation in fresh markets, such as separate processing area, refrigerator and pest control measures, should be established to reduce bacterial contamination in raw vegetables and fruits.

4. Personal hygiene of workers, and fresh produce storage and packaging should be closely investigated in fresh market to minimize the chance of bacterial cross-contamination due to direct contact of sellers and customers.

5. Surveillance on *E. coli* concentration and pathogenic bacterial contamination in fresh vegetables and fruits should be implemented to ensure fresh produce safety for consumers.

6. Appropriate cleaning methods before consumption, such as using running water are still mandatory for vegetables and fruits to reduce biological hazards to public health.

Application of knowledge and further study

1. The results of this study can be used for further biological risk assessment and antimicrobial susceptibility test.

2. *Salmonella* serotyping among vegetables and fruits derived from wholesale sources should be conducted to determine the possible source of contamination

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VITA

NAME	Jutanat Srisamran
DATE OF BIRTH	26 January 1992
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Veterinary Public Health, Faculty of Veterinary Science
HOME ADDRESS	Number 9, Pracha-uthit Road, Pracha-uthit 83, Thung Khru District, Bangkok, Thailand