



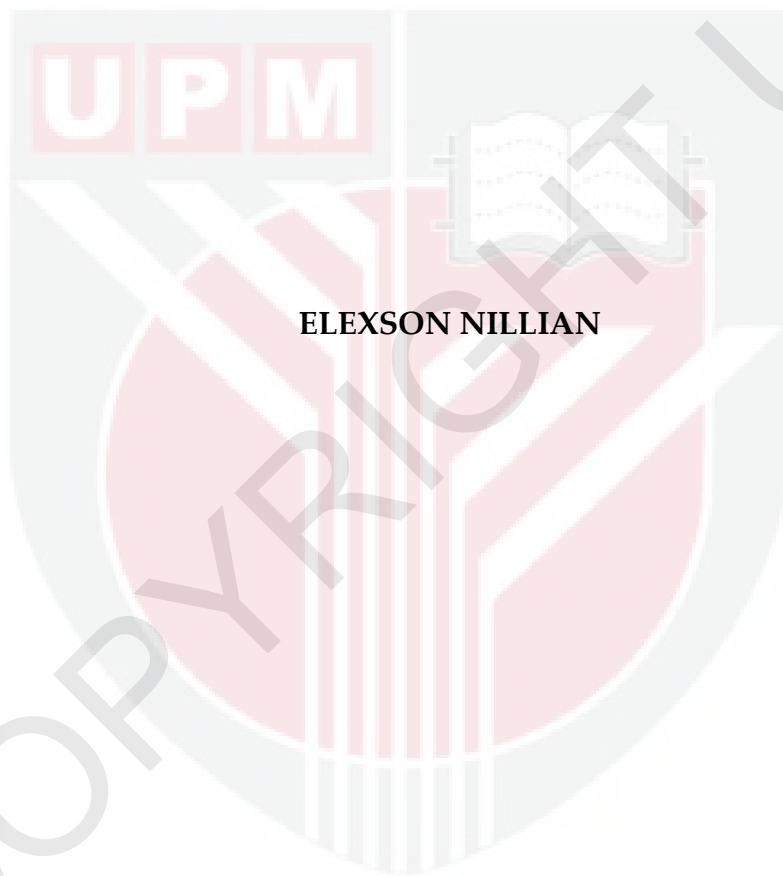
UNIVERSITI PUTRA MALAYSIA

BIOSAFETY OF *SALMONELLA* IN RAW VEGETABLES

ELEXSON NILLIAN

FSTM 2011 19

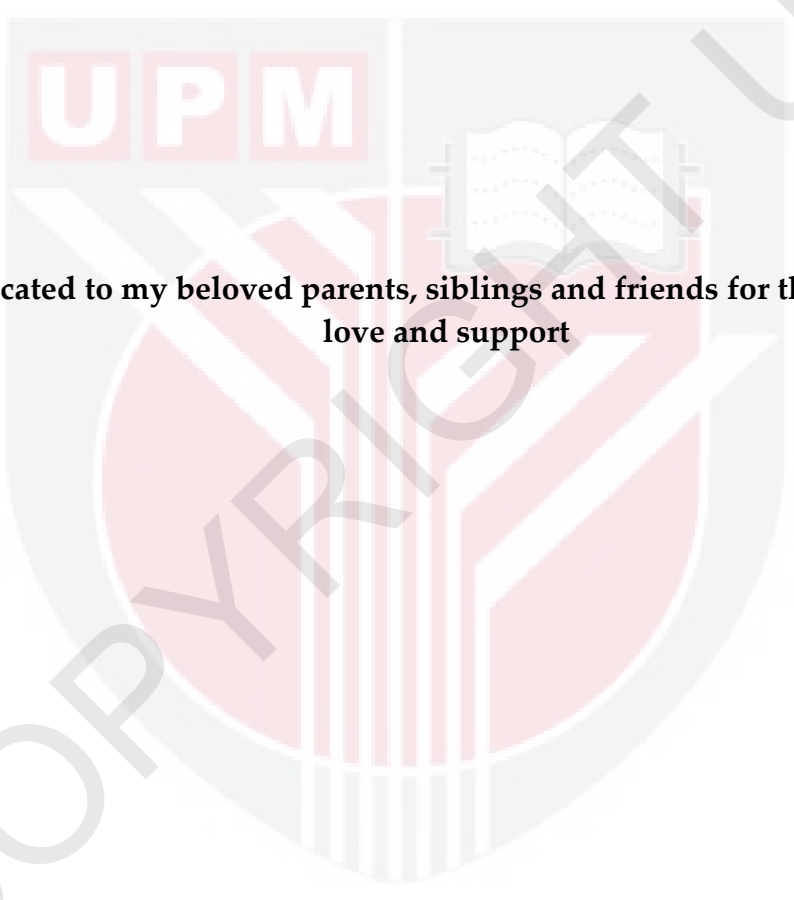
BIOSAFETY OF SALMONELLA IN RAW VEGETABLES



ELEXSON NILLIAN

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2011

The image features a large, semi-transparent watermark of the Universiti Putra Malaysia (UPM) logo in the background. The logo is a shield-shaped emblem with a red and white color scheme. At the top left of the shield, the letters 'UPM' are written in white on a red rectangular background. The central part of the shield contains a stylized white 'U' shape with a red book icon inside it. Below the 'U' are several vertical white lines of varying heights, and at the bottom, there are several horizontal white lines. The entire logo is set against a light grey background.

**Dedicated to my beloved parents, siblings and friends for their endless
love and support**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

BIOSAFETY OF *SALMONELLA* FROM RAW VEGETABLES

By

ELEXSON NILLIAN

November 2011

Chairman: Professor Son Radu, PhD

Faculty: Food Science and Technology

Foodborne infectious diseases have emerged as an important public health problem in many countries in the last decade. The global appearance of enteric pathogens such as *Salmonella* which is similar foodborne diseases may challenge many countries at once. The main objective of this study is to determine the level of contamination by *Salmonella* spp, *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw vegetables.

Most Probable Number (MPN) and Multiplex Polymerase chain reaction (MPCR) have been applied as combination method to enumerate the density of *Salmonella* presence in the food samples and to rapidly detect multiple microorganisms in a single reaction. The MPN method is capable

to measure only live and active organism. This technique results in more uniform recovery of a microbial population and the detection of the organism. Three specific primers pairs were use is ST11 and ST15, Fli15 and Typ04, Sef A-1 and Sef A-2 acts as a detection tool to genus level. The result showed that the primers amplified at 429 bp, 620 bp and 330 bp fragment respectively. This multiplex PCR represents a major of advance in terms of speed, sensitivity and specificity of diagnostic tools.

A total of hundred fifty samples (n=150) were collected randomly from hypermarket and wet market in Serdang, Selangor including cabbage lettuce, cucumber and carrot which is twenty five samples (n=25) per each type. *Salmonella* spp was detected is 40.67%, *Salmonella* Enteritidis and *Salmonella* Typhimurium is 16.67% and 10.67% respectively in all the samples. Lettuce shows the highest contamination by *Salmonella* Enteritidis (40%) and *Salmonella* Typhimurium (28%). However, 40% of *Salmonella* spp was detected in vegetarian burger. The density of cell concentration of *Salmonella* detected in all the samples were in the ranged <3 to>53 MPN/g. Most of the samples were in the minimum ranged of < 3 MPN/g.

The survival of *Salmonella* Enteritidis studies in this research was tested in egg sandwiches with different temperatures and initial microbial loads with 10^1 CFU/ml and 10^3 CFU/ml. Room temperature and $4\text{ }^\circ\text{C}$ were selected to determine the growth of *Salmonella* in different interval of incubation time from 0 hour to six hours. The initial microbial load with 10^3 and 10^1 CFU/ml was loaded in raw vegetables namely are cucumber and lettuce after there are washed with distilled water. It was found that there are slow kinetics growths of *Salmonella* Enteritidis in the survival of *Salmonella* Enteritidis in egg sandwiches. *Salmonella* is able to grow even at temperature of $4\text{ }^\circ\text{C}$. Microbial although was loaded in small amount for the initial stage. Initial microbial load 10^1 CFU/ml show the growth of *Salmonella* is slow and their rapidly grow in room temperature with 10^1 to 10^3 CFU/ml. The highest growth of *Salmonella* is from 10^3 CFU/ml to 10^4 CFU/ml within 6 hr incubation in both temperature and initial microbial load.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

BIO-KESELAMATAN *SALMONELLA* DARI SAYURAN MENTAH

Oleh

ELEXSON NILLIAN

November 2011

Chairman: Professor Son Radu, PhD

Faculty: Sains dan Teknologi Makanan

Jangkitan penyakit bawaan makanan telah muncul sebagai satu masalah kesihatan awam yang penting di kebanyakan negara pada beberapa dekad yang lalu. Kemunculan global patogen secara enterik seperti *Salmonella* bermakna penyakit bawaan makanan yang sama menjadi cabaran kepada kebanyakan negara pada masa yang sama. Objektif utama penyelidikan ini adalah untuk menentukan aras kontaminasi makanan oleh *Salmonella* spp., *Salmonella* Enteritidis dan *Salmonella* Typhimurium di dalam sayuran mentah.

Most Probable Number (MPN) dan Multiplex Polymerase Chain Reaction telah diaplikasikan sebagai pengabungan kaedah untuk menentukan

kepadatan kehadiran *Salmonella* di dalam sampel makanan dan untuk mengenalpasti dengan pantas beberapa mikroorganisma di dalam satu reaksi. MPN mampu mengukur organism yang hidup dan aktif sahaja. Teknik ini menunjukkan keputusan yang lebih jitu untuk pemulihan populasi mikroorganisma dan pengesanan organisma. Tiga pasang primer telah digunakan seperti ST11 dan ST15, Fli15 dan Typ04, Sef A-1 dan Sef A-2 sebagai alat untuk mengesan aras genus. Keputusan kajian telah menunjukkan bahawa primer telah diampikasi pada fragment 429 bp, 620 bp dan 330 bp. Multiplex PCR menunjukkan kemajuan besar dari segi kelajuan masa, kesensitifan, dan pengkhususan dalam kaedah diagnostik.

Sebanyak seratus lima puluh (n=150) sampel dikumpulkan secara rawak dari hypermarket dan pasar borong di Serdang, Selangor termasuk tomato, kobis, lettuce, timun dan lobak iaitu 25 sampel bagi setiap satu jenis (n=25). *Salmonella* spp., telah dikesan sebanyak 40.67%, *Salmonella* Enteritidis dan *Salmonella* Typhimurium adalah 16.67% dan 10.67% dari semua sampel.

Sebagai keputusan, salad menunjukkan kontaminasi yang tinggi disebabkan oleh *Salmonella* Enteritidis (40%) dan *Salmonella* Typhimurium(24%). Kepekatan sel *Salmonella* yang dikesan di dalam

sampel adalah didalam lingkungan <3 MPN/g ke >53 MPN/g. Kebanyakan daripada sampel berada di dalam julat minimum iaitu <3 MPN/g.

Kehidupan *Salmonella* Enteritidis didalam penyelidikan ini telah di uji di dalam 'sandwich' telur dengan suhu dan jumlah mikrob yang berbeza. Suhu bilik dan suhu 4 °C, telah di uji untuk menentukan pertumbuhan *Salmonella* di dalam selang masa inkubasi yang berbeza di mana dari satu jam hingga ke enam jam. Peletakan mikrobial pada permulaan dengan jumlah 10^3 dan 10^1 CFU/ml, telah diletakkan ke dalam sayuran mentah iaitu timun dan salad selepas ia di cuci dengan air suling. Didapati bahawa pertumbuhan kinetik *Salmonella* Enteritidis agak lambat di dalam kelangsungan hidup *Salmonella* Enteritidis pada 'sandwich' telur. *Salmonella* mampu hidup walaupun di dalam suhu 4 °C. Peletakan microbial sebanyak 10^1 CFU/ml menunjukkan walaupun pertumbuhan *Salmonella* lambat tetapi tumbuh dengan cepat di dalam suhu bilik dengan 10^1 ke 10^3 CFU/ml. Pertumbuhan *Salmonella* tertinggi adalah dari 10^3 CFU/ml ke 10^4 CFU/ml selepas enam jam inkubasi bagi kedua-dua suhu dan peletakan mikrobial.

ACKNOWLEDGEMENTS

I want to thank God for the wisdom that He has given to me and through His guidance and mercy He had granted me with unconditional love that He had shown me. Although my name appears on the cover of this thesis as the author, those closest to me know that it never would have been written without the extraordinary godly men and women who invested in me over the years. Without all this, I never have the perseverance to pursue and complete Degree of Master Science.

I would like to dedicate my heartfelt thanks to Supervisory Committee Chairman, Professor Dr. Son Radu, who had contributed tremendously of his time and expertise in this research. His encouragement motivation, guidance has always made me a visionary person.

My gratitude also goes to Dr. Farinazleen Mohamad Ghazhali, my Supervisory Committee for her comments, suggestion and support as well as reviewing my thesis. A million thanks to Dr. Chai Lay Ching, for the continuous guidance, support, time, energy and opportunity that's she give to me throughout the year. Thank you so much for her patience and for always believing in me and seeing my potential in this field.

I would like to express my sincere appreciation to Tunung Robin, Ubong Anyi, Tuan Zainazul, Noorlis Ahmad, Nor Hidayah, Pui Chai Fung, Wong for sharing your experience and life with me in the Centre of Excellence. Thank you very much for your time, advice, guidance to me when I was is your friend.

My deepest appreciation with forever loves to my family: my wonderful mum, dad, brothers and sisters, uncle and auntie who really trust, caring and love me. Thanks so much when the world against me, you are always there to support me. I proud to have your in my life.

A special appreciation goes to my members at Lake City. Pak Kenny, Brother Jhawn, Brother Mervin, my lovely brother Koko Wagner and wife, and my good buddies who teach me about life. My Relevant Youth Community, you are the inspirational, vision, passion and journey in my life to be History and Impact maker. You are awesome.

I would like to express my loving thanks to wonderful and great people in my life who really make my life more beautiful: Mami Anis and Brother JJ,

Love you all and you are blessing for me. Your great support through life investment and prayer, determined who I'm now.

Finally, to Sagau and Sara who always be my inspirational and role model to be a great and visionary person in life. Both of you make my dream come true. Thank you so much for investment of your time, love, energy to me.



I certify that a Thesis Examination Committee has met on 8 November 2011 to conduct the final examination of Elexson Nillian on his thesis entitled “Biosafety of *Salmonella* from raw vegetables “ in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

Lasekan Olu, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Nor Ainy bt. Mahyudin, PhD

Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Alfi Khatib, PhD

Senior Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

I. Karunasagar, PhD

Professor
Animal Fisheries Sciences University
India
(External Examiner)

SEOW HENG FONG, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Son Radu, Ph.D

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Farinazleen Mohamad Ghazali, Ph.D

Senior Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is nor concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



ELEXSON NILLIAN

Date: 8 November 2011

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	Ix
APPROVAL	Xii
DECLARATION	Xiv
LIST OF TABLES	Xix
LIST OF FIGURES	Xx
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1
1.1 General introduction	1
1.2 Objectives	5
2 LITERATURE REVIEW	6
2.1 <i>Salmonella</i>	6
2.1.1 History background	5
2.1.2 Morphology of <i>Salmonella</i>	7
2.1.3 Taxonomy of <i>Salmonella</i>	8
2.1.4 Characteristics of <i>Salmonella</i>	10
2.1.5 Pathogenicity of <i>Salmonella</i>	11
2.2 Disease Manifestation	13
2.3 Epidemiology and Transmission of <i>Salmonella</i>	16
2.3.1 Ecology of <i>Salmonella</i>	16
2.3.1.1 <i>Salmonella</i> from Poultry	18
2.3.1.2 <i>Salmonella</i> from Livestock	19
2.3.1.3 <i>Salmonella</i> Enteritidis contamination the Eggs	21
2.4 Surveillance study of <i>Salmonella</i> by regions	30
2.5 Isolation and identification of <i>Salmonella</i>	32
2.5.1 Sampling	32
2.6 Sample preparation	33
2.6.1 Attribute sampling plans	37
2.6.2 Variables Sampling plans	41
2.7 Total plate count	43
2.8 <i>Salmonella</i> Culture	44
2.9 Enumeration protocol	46
2.9.1 Most Probable Number	46

	2.9.2 Rapid detection method	48
	2.9.2.1 Polymerase chain reaction	48
	2.9.2.2 Multiplex Polymerase Chain Reaction	50
	2.10 Food safety	52
3	EVALUATION OF A MULTIPLEX PCR ASSAY FOR SIMULTANEOUS IDENTIFICATION OF <i>SALMONELLA</i> SPP, <i>SALMONELLA</i> ENTERITIDIS AND <i>SALMONELLA</i> TYPHIMURIUM	54
	3.1 Introduction	54
	3.2 Materials and Methods	56
	3.2.1 Bacterial strains and culture condition	56
	3.2.2 PCR Primers	57
	3.2.3 DNA Isolation	58
	3.2.4 PCR assay	58
	3.3 Results and discussions	60
	3.4 Conclusion	68
4	PREVALENCE OF <i>SALMONELLA</i> SPP., <i>SALMONELLA</i> ENTERITIDIS AND <i>SALMONELLA</i> TYPHIMURIUM IN RAW VEGETABLES by MPN-MULTIPLEX PCR	74
	4.1 Introduction	74
	4.2 Materials and Methods	76
	4.2.1 Samples collection and preparation	76
	4.2.2 Most Probable Number	73
	4.2.3 Multiplex Polymerase Chain Reaction	78
	4.3 Results and discussion	75
	4.4 Conclusion	88
5	SURVIVAL STUDY OF <i>SALMONELLA</i> ENTERITIDIS IN EGG SANDWICHES USING DIFFERENT CONDITION WITH DIFFERENT INITIAL MICROBIAL LOAD	92
	5.0 Introduction	92
	5.1 Materials and Methods	95
	5.1.1 Sampling	95
	5.1.2 Bacterial culture	95
	5.1.3 Inoculums preparation	96

5.1.4	Raw vegetables samples preparation	96
5.1.5	Inoculums and counting of <i>Salmonella</i> Enteritidis	97
5.1.6	Growth modelling	98
5.7	Results and discussions	98
5.8	Conclusion	108
6	GENERAL DISCUSSIONS AND CONCLUSIONS	113
	REFERENCES / BIBLIOGRAPHY	114
	APPENDIX A	140
	APPENDIX B	145
	BIODATA OF STUDENT	146
	LIST OF PUBLICATION	147