

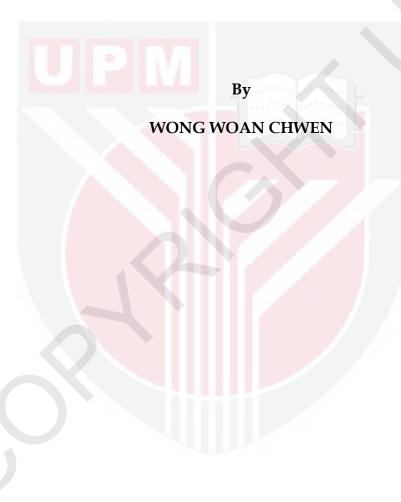
# **UNIVERSITI PUTRA MALAYSIA**

# SURVIVAL OF *LISTERIA MONOCYTOGENES*IN FROZEN BURGER PATTIES

**WONG WOAN CHWEN** 

**FSTM 2011 25** 

# SURVIVAL OF LISTERIA MONOCYTOGENES IN FROZEN BURGER PATTIES



Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science



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

SURVIVAL OF LISTERIA MONOCYTOGENES IN FROZEN BURGER PATTIES

By

**WONG WOAN CHWEN** 

June 2011

Chairman: Professor Son Radu, PhD

Faculty: Food Science and Technology

Listeria monocytogenes is a foodborne pathogen which has caused outbreaks in several nations in which processed meats were the vehicle. The purpose of this study were to determine the prevalence of *L. monocytogenes* from frozen burger patties, assess the characteristics of the *L. monocytogenes* strains isolated from burger patties, and determine the effect of different cooking time in decontamination of *L. monocytogenes* in chicken burger patties.

A total of 220 samples were purchased from hypermarkets and retail shops in Malaysia from June to October 2009. Prevalence of *L. monocytogenes* in burger patties from this study was found to be 15.9%, in which the prevalence of *L. monocytogenes* in meat-based burger patties (22.3%) is

iii

significantly higher than vegetarian burger patties (9.3%) at P<0.05. *L. monocytogenes* was found to be most frequently detected in chicken patties (33.3%), followed by beef patties (22.9%), and fish patties (10.5%). By using MPN-PCR method, 15.9% of the samples were found to be positive for *L. monocytogenes*. MPN plating and direct plating method can only detected 7.7% and 7.3%, respectively. The density of *L. monocytogenes* detected in burger patties was ranged from 0 to 1,100 MPN/g.

Forty-one isolates of *L. monocytogenes* recovered from raw burger patties were characterized based on their antibiotic resistance and RAPD banding pattern. In particular, 31.7% of isolates were susceptible to 11 antibiotics tested. Result showed that, resistance to tetracycline was most common (46.3%), followed by erythromycin (36.6%), amikacin (31.7%), and SMZ-TMP (17.1%). All *L. monocytogenes* strains were sensitive towards imipenem and gentamicin. On the other hand, 31 out of 41 isolates in current study were typabled by RAPD-PCR with primer OPA 10. As observed from the dendrogram, these *L. monocytogenes* strains were classified into 3 clusters. These clusters were occupied by the strains recovered from all types of burger patties.

A simulation study was conducted to determine sufficient cooking time to reduce the number of *L. monocytogenes* present in chicken burger patties to

safe level which is fit for human consumption. Artificially contaminated burger patties were cooked for 0, 2, 4, 5, 8, and 10 min to determine survival of L. monocytogenes. Results demonstrated a linear correlation ( $R^2 = 0.87$ ) between mean log reduction of L. monocytogenes and cooking time. As a result from this study, it is suggested that a cooking time of 6 min or more is sufficient to decontaminate the burger patties, without control of temperatures of internal burger patties and cooking environment.

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

SURVIVAL LISTERIA MONOCYTOGENES DALAM DAGING BURGER SEJUKBEKU

Oleh

**WONG WOAN CHWEN** 

Jun 2011

Chairman: Professor Son Radu, PhD

Faculty: Sains dan Teknologi Makanan

Listeria monocytogenes ialah patogen bawaan makanan yang telah menyebabkan wabak di beberapa negara, di mana daging terproses adalah medium untuk patogen ini. Matlamat kajian ini adalah untuk menentukan kekerapan L. monocytogenes dalam daging burger sejukbeku, mengenalpasti ciri-ciri pencilan L. monocytogenes yang dipencil dari daging burger, dan mengkaji kesan masa memasak yang berbeza terhadap penyahkontaminasi L. monocytogenes dalam daging burger ayam.

Sejumlah 220 sampel dibeli dari pasaraya dan kedai runcit di Malaysia dari Jun hingga Oktober 2009. Kekerapan *L. monocytogenes* dalam daging burger dari kajian ini adalah sebanyak 15.9%, di mana kekerapan *L. monocytogenes* dalam daging burger (22.3%) adalah lebih tinggi daripada burger

vi

vegetarian (9.3%), signifikan pada tahap P<0.05. *L. monocytogenes* didapati paling kerap dikesan dalam daging burger ayam (33.3%), diikuti dengan daging burger lembu (22.9%), dan daging burger ikan (10.5%). Dengan menggunakan kaedah MPN-PCR, sebanyak 15.9% sampel adalah positif bagi *L. monocytogenes*. Manakala, kaedah plating MPN dan plating langsung hanya dapat mengesan 7.7% dan 7.3%, masing-masing. Kepadatan *L. monocytogenes* yang dikesan dalam daging burger berada dalam julat 0 hingga 1,100 MPN/g.

Empat puluh satu pencilan *L. monocytogenes* yang dipencil dari daging burger mentah telah dicirikan berdasarkan kerintangan antibiotik dan pola pita RAPD. Khususnya, 31.7% pencilan *L. monocytogenes* adalah sensitif terhadap 11 antibiotik yang diuji. Keputusan kajian menunjukkan bahawa kerintangan *L. monocytogenes* terhadap tetrasiklin adalah paling umum (46.3%), diikuti eritromisin (36.6%), amikasin (31.7%), dan SMZ-TMP (17.1%). Kesemua pencilan *L. monocytogenes* adalah sensitif terhadap imipenem dan gentamisin. Sebaliknya, sebanyak 31 daripada 41 pencilan dalam kajian ini dapat ditaip dengan RAPD-PCR oleh primer OPA 10. Seperti yang diperhatikan dari dendrogram, pencilan-pencilan *L. monocytogenes* telah diklasifikasi kepada 3 kelompok. Kesemua kelompok telah dihuni oleh pencilan-pencilan yang dipencil dari semua jenis daging burger.

Suatu kajian simulasi turut dijalankan untuk menentukan masa memasak yang mencukupi untuk mengurangkan kuantiti L. monocytogenes yang hadir dalam daging burger ayam ke tahap yang selamat dan sesuai untuk dimakan oleh manusia. Daging burger yang dikontaminasi telah dimasak selama 0, 2, 4, 6, 8, 10 minit untuk menentukan kemandirian L. monocytogenes dan keputusan menunjukkan korelasi lelurus ( $R^2 = 0.87$ ) di antara pengurangan purata  $\log L$ . monocytogenes dan masa memasak. Keputusan kajian ini mencadangkan bahawa masa memasak 6 minit atau lebih adalah mencukupi untuk menyahkontaminasi daging burger, tanpa mengawal suhu dalaman daging burger dan suhu persekitaran memasak.

#### **ACKNOWLEDGEMENTS**

First and foremost, I would like to express my heartfelt gratitude to Professor Dr. Son Radu, the chairman of my supervisory committee for the invaluable guidance and support throughout my study. Thank you very much for the advice and encouragement which help me to finish my study.

My deepest appreciation also goes to my co-supervisors, Dr. Farinazleen Mohamad Ghazali (Faculty of Food Science and Technology) and Associate Professor Dr. Cheah Yoke Kqueen (Faculty of Medicine and Health Sciences). Thank you so much for the trust, love and kindness from both of you.

Million thanks to all my dearest laboratory mates, (Dr. Chai, Dr. John, Jeya, Natasha, kak Noorlis, Tuan, Chai Fung, Tunung, Sandra, Li Fen, Elex, Jeshveen, Hidayah, Petrus, Ubong and Marian). You all are awesome! The laboratory is just too dull without your presence. I appreciate you and all moments that we went through together will remain in my heart forever.

My sincere gratitude is extended to all my best friends, especially Chai Fung, Cindy, Vicky, Se Vern, Albert, Siew Yoke, Ai Chen, Jiun Ting and Ching Hui who supported me morally and mentally. Thank you for always be

there whenever I need help. Not forgetting to acknowledge all staffs of Faculty of Food Science and Technology who helped me a lot during my study life.

Last but not least, I like to express my thousand heartfelt thanks to my father, mother, brothers and sister. Thanks so much for your loves and encouragements. I will never give up until I achieve my mission!

I certify that a Thesis Examination Committee has met on 16<sup>th</sup> June 2011 to conduct the final examination of Wong Woan Chwen on her thesis entitled "Survival of *Listeria monocytogenes* in frozen burger patties" 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:

# Jinap binti Selamat, PhD

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

# Muhajir bin Hamid, PhD

Associate Professor Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Internal Examiner)

# Fatimah binti Abu Bakar, PhD

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

# Jaime Martinez-Urtaza, PhD

Senior Lecturer
University de Santiago de Compostela
Spain
(External Examiner)

#### NORITAH OMAR, PhD

Associate Professor and Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date: 23 August 2011

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, PhD

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

# Cheah Yoke Kqueen, PhD

Associate Professor Faculty of Medical and Health Sciences Universiti Putra Malaysia (Member)

# Farinazleen Mohamad Ghazali, PhD

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

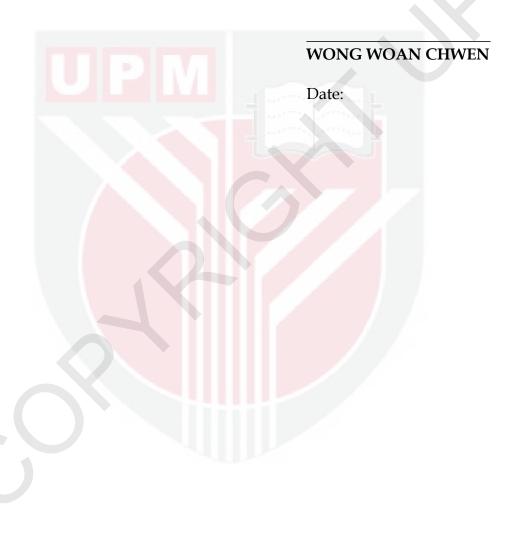
# HASANAH MOHD GHAZALI, PhD

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.



# TABLE OF CONTENTS

AB AC AP DE LIS	PROVECLAR ST OF ST OF	CT K WLED <sup>(</sup> AL ATIO) TABL) FIGUE	ES	Page ii iii vi ix xi xii xv xiix
CH	IAPTE	R		
1	INT	RODU	J <mark>CTION</mark>	
	1.1	Gene	ral introduction	1
	1.2	Objec	tives	5
_				
2			URE REVIEW	
	2.1		ia monocytogenes	6
			Classification of the genus Listeria	8
			Virulence factors involved in infectious process	
			Foodborne Listeriosis	11
			Outbreaks of listeriosis caused by foods	14
			Treatment for listeriosis	17
	2.2		Antibiotic resistance of Listeria monocytogenes	18
	2.2		ia monocytogenes in environment and foods  Association with various foods	19 20
		2.2.2	Prevalence of <i>Listeria monocytogenes</i> in different foods	20
		222		20
		2.2.3	3 8	22
		224	processing environments	23
		2.2.4	Factors affecting reduction of <i>Listeria monocytog</i> in foods	
	2 2	Dotos		26 31
	2.3		ction of <i>Listeria monocytogenes</i> in foods	32
		2.3.1 2.3.2	Culture dependent methods	38
		2.3.2	Rapid methods Combined MPN-PCR method	39
	2.4		acterization of Listeria monocytogenes	40
	∠. <del>'1</del>	2.4.1	Antimicrobial susceptibility testing	40
		2.4.1		41

		2.4.3	<i>7</i> 1 <i>0</i>		4.0					
	2.5		outbreak detection and investigation		43					
	2.5	Frozen burger patties			44					
		2.5.1	Interests on vegetarian food products		45					
			Burger patties making process		46					
		2.5.3	Possible contamination sources for frozen bu patties	_	47					
			patties		4/					
3	PREVALENCE AND NUMBERS OF LISTERIA									
	MO	MONOCYTOGENES IN FROZEN BURGER PATTIES								
		Introduction								
	3.2	Materials and methods			50					
		3.2.1	Sample collection		50					
		3.2.2	Sample preparation and pre-enrichment		52					
		3.2.3	Detection and enumeration of Listeria							
			monocytogenes		52					
		3.2.4	Genomic DNA preparation		<b>5</b> 3					
		3.2.5	PCR assay		54					
		3.2.6	Isolation on culture media		55					
		3.2.7	Data analysis		55					
	3.3	Results Discussion								
	3.4									
	3.5	Concl	usion		67					
1	CIL	A D A CT	FEDITATION OF LICTEDIA MONOCYTOCI	CNIEC						
4		CHARACTERIZATION OF LISTERIA MONOCYTOGENES								
		ISOLATED FROM BURGER PATTIES BASED ON ANTIBIOTIC RESISTANT PROFILE AND RAPD BANDING								
		TERN		IDIN	G					
	4.1									
			rials and methods		69 71					
	7.2	4.2.1	Antimicrobial susceptibility testing (AST)		71					
			RAPD-PCR analysis		75 75					
			Data analysis		77					
	4.3	Resul	3		78					
	4.4	Discu			87					
		Concl			96					
	4.5	Conci	usion		90					
5	CIM	 	TION OF DECONTAMINATION OF LISTER	ΤΔ						
J		MONOCYTOGENES DURING COOKING OF								
		CONTAMINATED CHICKEN BURGER PATTIES IN								
		DOMESTIC KITCHENS								
		Introduction 97								
	5.2		rials and methods		99					

		5.2.1	Preparation of Listeria monocytogenes inocul	um 99	
		5.2.2	Sample preparation and inoculation	99	
		5.2.3	Cooking of burger patties	100	
		5.2.4	Enumeration of Listeria monocytogenes	101	
		5.2.5	Data analysis	101	
	5.3	5.3 Results		102	
	5.4	Discu	ssion	104	
	5.5	Conc	lusion	109	
6	GEN	NERAI	. DISCUSSION AND CONCLUSION	111	
REFERENCES					
APPENDICES					
BIODATA OF STUDENT					
LIS	ST OF	PUBLI	CATIONS	147	