



UNIVERSITI PUTRA MALAYSIA

***BIOGENIC AMINE AND MICROFLORAL PROFILING OF INDIAN
MACKEREL, RASTRELLIGER KANAGURTA CUVIER DURING STORAGE***

CHONG CHEONG YEW

FSTM 2012 21

**BIOGENIC AMINE AND MICROFLORAL PROFILING OF INDIAN
MACKEREL, *RASTRELLIGER KANAGURTA* CUVIER DURING STORAGE**



By

CHONG CHEONG YEW

**Thesis Submitted to the School of Graduate Studies, University Putra
Malaysia, In Fulfilment of the Requirements for the Degree of Doctor of
Philosophy**

April 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

BIOGENIC AMINE AND MICROFLORAL PROFILING OF INDIAN MACKEREL, *RASTRELLIGER KANAGURTA* CUVIER DURING STORAGE

By

CHONG CHEONG YEW

April 2012

Chairman : Professor Fatimah binti Abu Bakar, PhD

Faculty : Food Science and Technology

Indian mackerel is the most common marine fish in Malaysia and is potentially associated with histamine poisoning incidence. However, no literature is available on the profile of biogenic amines in different storage conditions. Biogenic amines, biochemical, microbiological changes and sensory evaluation were investigated in Indian mackerel stored at different temperature and modified atmosphere packaging. Dominant spoilage microflora was identified in fish stored in air, vacuum packaging and 100% CO₂.

Indian mackerel was subjected to storage at ambient temperature (25-29°C), chilled temperature (5 °C) and ice temperature (0 °C). Biogenic amines and various biochemical analysis such as pH, total volatile basic nitrogen and amino acids were carried out. All amines except for spermidine and spermine increased significantly during storage at ambient and chilled temperatures. The concentration of histamine reached the FDA acceptable limit after 16 hours at ambient temperature and 5 days at chilled temperature. Proper icing procedure retarded the formation of histamine effectively. This study confirmed the relationship between histidine and formation of histamine at ambient and chilled temperature. As storage time progressed, the amines forming bacteria grew significantly except for that stored in ice.

The Indian mackerel was stored in air, vacuum packaging, 30% CO₂/5% O₂/ 65% N₂, 60% CO₂/5% O₂/ 35% N₂, 80% CO₂/5% O₂/ 15% N₂ and 100% CO₂ at chilled temperature (5°C) for 12 days. Each biogenic amine responded differently to a different level of CO₂. High level of CO₂ (≥60% CO₂) was effective in retarding the formation of histamine in Indian mackerel. Fish stored in vacuum packaging and 30% CO₂/5% O₂/ 65% N₂ tended to have a high concentration of histamine and tyramine. Vacuum packaging and all modified atmosphere packaging treatments were effective in reducing the pH and total volatile basic nitrogen.

This study confirmed that the inhibitory effect of CO₂ increases as the level of CO₂ increases. The aerobic psychrotrophic bacteria were responsible for the formation of cadaverine and putrescine during the later stage of storage. The

application of modified atmosphere packaging improved the shelf life of Indian mackerel. According to sensory evaluation, the fish was still acceptable by the panelists when stored for 5 days in air, 7 to 8 days in vacuum packaging and 30% CO₂/5% O₂/ 65% N₂, 9 days in 60% CO₂/5% O₂/ 35% N₂, 10 to 11 days in 100% CO₂.

In general, *Aeromonas* sp. was found as the dominant spoilage microflora during storage in air, vacuum packaging and 100% CO₂. However, the diversity of microflora changed toward the end of the storage. The microbial diversity was directed to *Pseudomonas* sp. and enterobacteria in fish stored in air. In vacuum packaging, the prevalence of lactic acid bacteria and enterobacteria increased at the end of storage.

The findings of the present study could contribute to the knowledge of food safety in controlling biogenic amine formation and microbial ecology in tropical fish. Aerobic plate count and total volatile basic nitrogen were correlated well with histamine at different storage temperature and atmosphere. Among the biogenic amines, cadaverine or cadaverine + putrescine can serve as good freshness indicator. A higher percentage of CO₂ and a lower percentage of O₂ in modified atmosphere packaging can be applied successfully in retarding the formation of histamine.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**AMIN BIOGENIK DAN PROFIL MIKROFLORA IKAN KEMBONG,
RASTRELLINGER KANAGURTA CUVIER SEMASA PENSTORAN**

By

CHONG CHEONG YEW

April 2012

Pengerusi: Profesor Fatimah binti Abu Bakar, PhD

Fakulti : Sains dan Teknologi Makanan

Ikan kembong merupakan ikan marin yang paling penting di Malaysia tetapi berpotensi terlibat dalam keracunan makanan disebabkan histamina. Walaubagaimanapun, tiada maklumat mengenai pembentukan pelbagai jenis amin biogenik di dalam spesies ikan ini semasa penstoran. Perubahan amin biogenik, biokimia, mikrobiologi and penilaian deria ikan kembong dikaji semasa penstoran dalam suhu dan atmosfera yang berbeza. Microflora yang dominan dalam ikan kembong ditentukan semasa penstoran dalam udara, vakum and 100% CO₂.

Eksperimen penstoran dijalankan pada suhu bilik (25-29°C), suhu dingin (5°C) dan suhu ais (0°C). Amin biogenik and analysis biokimia dijalankan

seperti pH, jumlah bes bernitrogen dan asid amino. Semua amin biogenik bertambah semasa penstoran pada suhu bilik dan suhu dingin melainkan spermidina and spermina. Kepekatan histamina mencapai tahap maximum FDA selepas 16 jam pada suhu bilik dan 5 hari pada suhu dingin. Penstoran ais dapat menghalang pembentukan histamina dengan berkesan. Histidina berhubung kait dengan histamina pada suhu bilik and suhu dingin. Semasa penstoran, bakteria penghasil amin bertambah melainkan ikan yang distor di dalam ais.

Ikan juga distor dalam udara, vakum, 30% CO₂/5% O₂/ 65% N₂, 60% CO₂/5% O₂/ 35% N₂, 80% CO₂/5% O₂/ 15% N₂ and 100% CO₂ pada suhu dingin (5°C) selama 12 hari. Respon amin biogenik adalah berlainan pada tahap CO₂ yang berlainan. Tahap CO₂ yang tinggi (≥60% CO₂) didapati berkesan untuk menyekati pembentukan histamina di dalam ikan kembong. Ikan dibungkus dalam vakum dan 30% CO₂/5% O₂/ 65% N₂ mengandungi histamina and tyramina yang tinggi. Pembungkusan vakum and campuran gas berlainan didapati berkesan untuk mengurangkan pH dan jumlah bes bernitrogen.

Peningkatan tahap CO₂ meninggikan halangan bagi pertumbuhan bakteria.

Bakteria aerobik and psikrotrofik didapati bertanggungjawab dalam pembentukan cadavarina and putrescina dalam kembong di peringkat akhir penstoran. Aplikasi pembungkusan atmosfera memperbaiki tempoh simpanan ikan kembong. Merujuk kepada penilaian deria, ikan dapat disimpan selama 5 hari dalam udara, 7 ke 8 hari dalam bungkusan vakum

and 30% CO₂/5% O₂/ 65% N₂, 9 hari dalam 60% CO₂/5% O₂/ 35% N₂ dan 10 ke 11 hari dalam 100% CO₂.

Aeromonas sp. ditentukan sebagai spesies dominan yang wujud semasa penstoran dalam udara, vakum dan 100% CO₂. Diversiti bakteria berubah semasa peringkat akhir dalam penstoran. *Pseudomonas* sp. and *Enterobakteria* dijumpai di dalam ikan yang distor dalam udara. Bakteria asid laktik dan enterobakteria bertambah dalam ikan kembong dibungkus dalam vakum semasa peringkat akhir penstoran.

Keputusan kajian ini menyumbangkan ilmu dalam pengawalan keselamatan makanan berhubung dengan pembentukan amin biogenik and ekologi bakteria dalam ikan di kawasan tropika. Jumlah bakteria aerobik dan jumlah bes bernitrogen berhubung kait rapat dengan histamina dalam penstoran pada suhu dan atmosfera berbeza. Cadavarina atau cadavarina + putrescina boleh berfungsi sebagai penunjuk kesegaran ikan yang bagus. Komposisi CO₂ yang tinggi dan O₂ yang rendah didapati mengawal pembentukan histamina dengan berkesan.

ACKNOWLEDGEMENTS

I am indebted to all the people at UPM for their invaluable assistance in the completion of this thesis. Special thanks to my supervisor Professor Dr Fatimah binti Abu Bakar for his invaluable support and guidance on this project. Thanks go to co-supervisor Professor Dr Russly Abdul Rahman and Professor Jamilah binti Bakar for their advice and assistance.

Appreciation goes to Mr Zukhruf Zaman for his advice in the analysis of biogenic amines in fish muscle sample. For help in modified atmosphere packaging study, my thanks go to Miss Selvi Vellu and Miss Maryam Shariat. Their co-operation are highly appreciated.

I would like to thank the Universiti Putra Malaysia for the research grant that was provided for this study. I also like to thank Faculty of Food Science and Technology for the use of the laboratory facilities. Special thanks to Miss Suraya from the faculty whose assistance was responsible for the operation of high performance liquid chromatography.

Finally, I want to thank my wife and family for their continued support in this project.

I certify that an Examination Committee has met on the **18 April 2012** to conduct the final examination of **Chong Cheong Yew** on his thesis entitled "**Biogenic amine and microfloral profiling of Indian mackerel (*Rastrelliger kanagurta*) during storage**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

Abdulkarim Sabo Mohammed, PhD

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

Son Radu, PhD

Y. Bhg. Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal examiner)

Farinazleen binti Mohamad Ghazali, PhD

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

I. Karunasagar, PhD

Y. Bhg. Professor
Animal and Fisheries Sciences University
College of Fisheries, India
(External examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 June 2012

This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory Committee were as follows:

Fatimah Abu Bakar, PhD

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

Russly Abdul Rahman, PhD

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

Jamilah Bakar, PhD

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

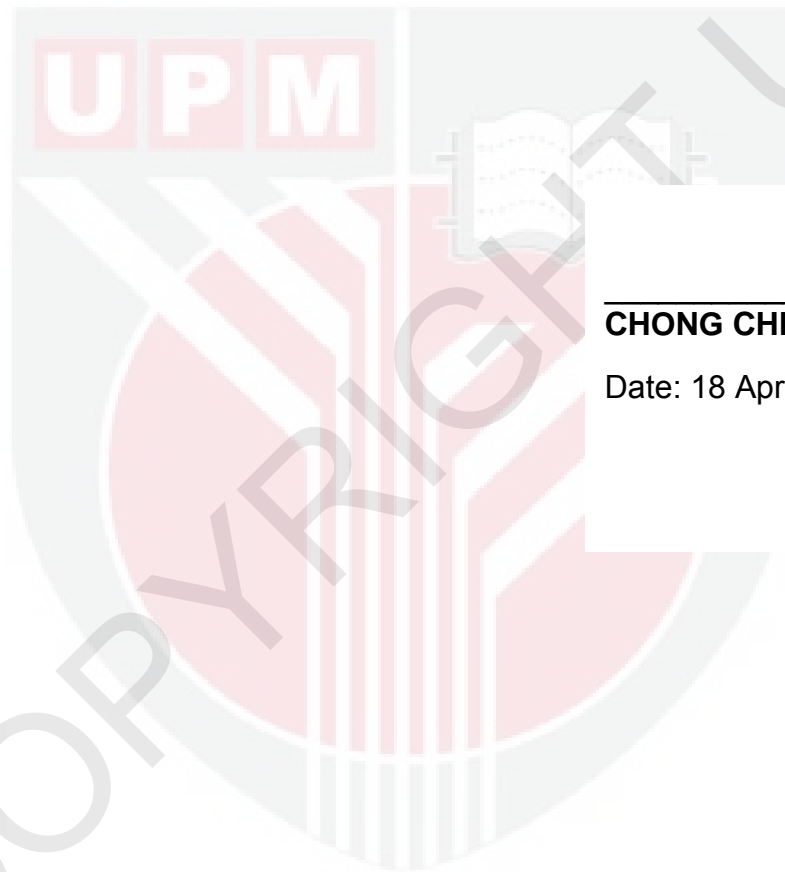
BUJANG BIN KIM HUAT, PHD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 June 2012

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 not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.



CHONG CHEONG YEW

Date: 18 April 2012

Table of contents

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvii
LIST OF PLATES	xxi
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Fishing industry	5
2.1.1 Indian mackerel	6
2.1.2 Composition of Indian mackerel	7
2.2 Changes in fish during storage	8
2.2.1 Autolytic spoilage	9
2.2.2 Chemical spoilage	10
2.2.3 Microbiological spoilage	11
2.3 Biogenic amines	13
2.4 Formation of biogenic amines	14
2.5 Health aspects of biogenic amines	16
2.6 Outbreak and epidemiology	18
2.7 Histamine poisoning	19
2.8 Toxicology	21
2.9 Permissible limit for histamine in fish	23
3.0 Biogenic amine as freshness indices	24
3.1 Factors influencing biogenic amines formation	27
3.1.1 Temperature	27
3.1.2 Amino acid	28
3.1.3 pH	29
3.1.4 Salt content	30
3.2 Biogenic amines producing bacteria	32
3.3 Modified atmosphere packaging	41
3.4 Modified atmosphere packaging in controlling biogenic amine formation	44

3	METHODOLOGY	46
3.1	Proximate analysis	46
3.2	Fish Sampling for different temperature storage study	46
3.3	Fish Sampling for modified atmosphere packaging study	47
3.4	Determination of total volatile basic nitrogen	48
3.5	Determination of pH	49
3.6	Preparation of standard amines solution	50
3.7	Preparation of the standard curve	50
3.8	Extraction and derivatization of amines from a fish sample	51
3.9	Separation of biogenic amines by HPLC	52
3.10	Biogenic amines indicator	53
3.11	Determination of amino acids	53
3.12	Separation of amino acids by HPLC	54
3.13	Sensory evaluation	56
3.14	Shelf life evaluation	56
3.15	Headspace analysis	57
3.16	Microbiological analysis	57
3.17	Microbial identification	58
3.18	Bacteria identification using Biolog GEN III	59
3.19	Statistical analysis	59
4	RESULTS AND DISCUSSIONS	60
	Effect of different temperature storage on Indian mackerel	60
4.1	Proximate composition	60
4.2	Biochemical changes	61
4.2.1	pH	61
4.2.2	Total volatile basic nitrogen	63
4.2.3	Biogenic amines	66
4.2.4	Amino acids	75
4.3	Microflora changes	81
4.4	Sensory evaluation	87
4.5	Biogenic amine indicator	90
4.6	Relationship between freshness indices, biogenic amines and temperature in Indian mackerel	94
4.7	Shelf life of Indian mackerel	99
	Effect of modified atmosphere packaging on Indian mackerel	101
4.8	Headspace analysis	101
4.9	The biochemical changes	103
4.9.1	pH	103
4.9.2	Total volatile basic nitrogen	105
4.9.3	Histamine	109
4.9.4	Putrescine	113
4.9.5	Cadaverine	115
4.9.6	Tyramine	117
4.9.7	Spermidine	120
4.9.8	Spermine	122
4.9.9	Total biogenic amines	124

4.10	The microflora changes	126
4.10.1	Aerobic mesophilic bacteria	126
4.10.2	Psychrotrophic bacteria	131
4.10.3	Anaerobic bacteria	133
4.10.4	Proteolytic bacteria	135
4.10.5	Histidine decarboxylase bacteria	137
4.10.6	Lysine decarboxylase bacteria	139
4.10.7	Ornithine decarboxylase bacteria	141
4.11	Sensory evaluation	144
4.12	Relationship between freshness indices and biogenic amines in Indian mackerel stored under MAP conditions	147
4.13	Shelf life of Indian mackerel	153
4.14	The identification of spoilage microflora in Indian mackerel packed with different atmosphere packaging	156
5	SUMMARY, CONCLUSION AND RECOMMENDATION	168
	BIBLIOGRAPHY	172
	APPENDIX A	193
	APPENDIX B	197
	APPENDIX C	201
	APPENDIX D	205
	BIODATA OF STUDENT	206
	LIST OF PUBLICATION	207