



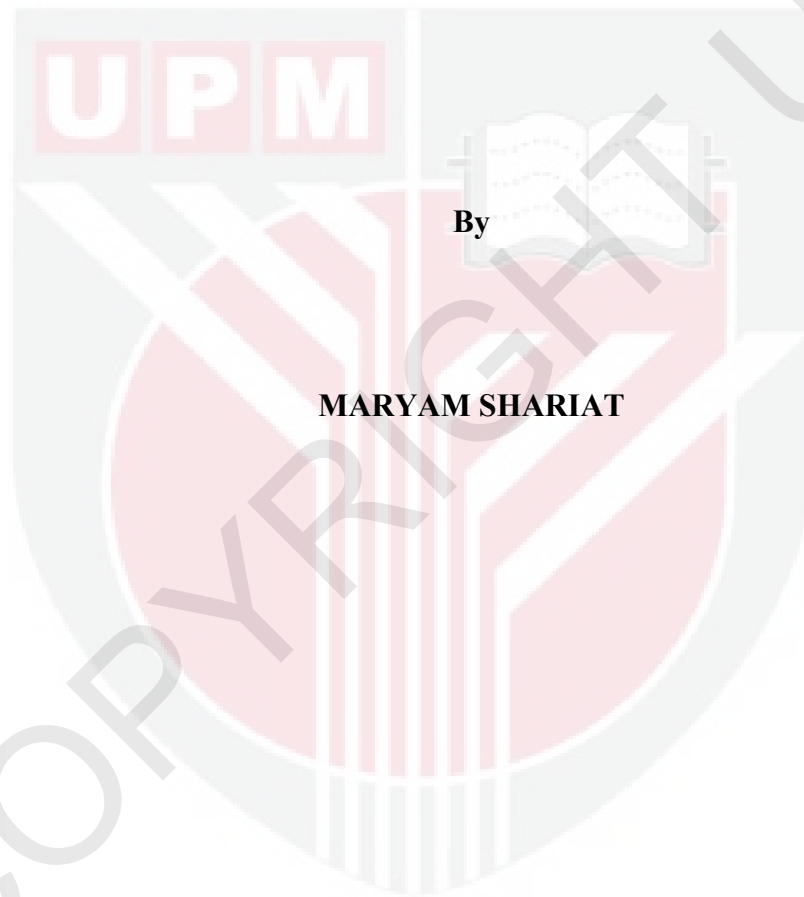
UNIVERSITI PUTRA MALAYSIA

MICROBIOLOGICAL AND BIOCHEMICAL QUALITY OF PATIN (PANGASIUS PANGASIUS) FILLETS UNDER MODIFIED ATMOSPHERE PACKAGING

MARYAN SHARIAT

FS 2012 33

MICROBIOLOGICAL AND BIOCHEMICAL QUALITY OF PATIN (*PANGASIUS PANGASIUS*) FILLETS UNDER MODIFIED ATMOSPHERE PACKAGING



By

MARYAM SHARIAT

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirement for the Degree of Master of Science**

May 2012

I would like to dedicate my thesis

to my lovely parents



© COPYRIGHT UPM

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

MICROBIOLOGICAL AND BIOCHEMICAL QUALITY OF PATIN (*PANGASIVS PANGASIVS*) FILLETS UNDER MODIFIED ATMOSPHERE PACKAGING

By

MARYAM SHARIAT

May 2012

Chairman: Prof. Fatimah BT Abu Bakar, PhD

Faculty : Food Science and Technology

Fresh fish are very susceptible to spoilage with a short shelf life due to their biological composition. The combined effects of microbiological and chemical changes gradually reduce the quality of fish during storage and are involved in spoilage process. Modified Atmosphere Packaging (MAP) is generally recognized as a useful preservation technique in extending the shelf life of fish and fish products. Consumers' tendency towards selection of fresh over processed or frozen fish has led MAP to become the most widely used technique in recent years. Thus, the present study was undertaken to determine the effects of vacuum packaging and MAP with different gas mixtures on the shelf life of patin fillets stored in refrigeration temperature. Patin (*Pangasius pangasius*) is a freshwater catfish which is widely aquacultured in Malaysia. Although patin fish is considered as a popular and high valued species, none has been reported about the modified atmosphere storage of this fish previously. In the present study, after

purchasing live patin fish from fish land pond, which was located in Serdang, they were transferred to the laboratory and filleted immediately. Patin fillets were packed under vacuum and four different MAP treatments namely MAP1 (5% O₂ + 40% CO₂ + 55% N₂), MAP2 (5% O₂ + 60% CO₂ + 35% N₂), MAP3 (5% O₂ + 80% CO₂ + 15% N₂) and MAP4 (100% CO₂) and subjected to microbial, chemical and sensory analyses at time points of 0, 3, 6, 9, 12, 15, 18 and 21 days during storage at 4°C. Air stored samples were used as control and the results obtained for all treatments were compared with the control. Microbiological analyses were performed for total mesophilic and psychrotrophic aerobic counts, total mesophilic and psychrotrophic anaerobic counts, proteolytic bacterial counts and *Enterobacteriaceae* counts. Biochemical analyses were carried out for pH, total volatile bases nitrogen (TVBN) and lipid oxidation (TBA test). Before packaging the fillets, proximate composition and fatty acids profile of the fillets were also determined in fresh fish at day 0. The dominant aerobic mesophilic microflora in the fillets under air storage and MAP4 (100% CO₂) were identified at each sampling day. The highest bacterial counts for all the bacterial groups were observed in air storage, followed by vacuum packaging (VP), MAP1, MAP2, MAP3 and MAP4, respectively. Aerobic mesophilic counts for the patin fillets under air storage exceeded the threshold value for microbial spoilage (10⁷cfu/g) after 9 days, whereas those kept under VP, MAP1, MAP2 and MAP3 reached the same value on day 14, 17, 18 and 21, respectively. Patin fillets packaged in MAP4 (100% CO₂) did not reach the spoilage value throughout 21 days of storage. Total aerobic mesophilic counts in air stored patin fillets increased approximately 5.5 log cycle throughout storage period, whereas those packaged under 100% CO₂ showed around 3 log cycle increase within 21 days. This clearly indicated the powerful effect of carbon dioxide against bacterial growth at the

highest concentration. The sensory quality of all patin fillets was acceptable during the first 13 ± 1 days of aerobic storage, 16 ± 1 days of storage in vacuum packaging and MAP1, 18 ± 1 days of storage in MAP2 and 19 ± 1 days of storage in MAP3. The overall sensory scores for the fillets packed in 100% CO₂ (MAP4) was higher than the acceptable limit at the end of storage period (21 days). A good correlation was found between the microbial (TVC) and sensory data (overall acceptability) for all storage conditions (correlation coefficient (r) between -0.943 to -0.987). Microbiological identification showed that *Aeromonas* species were the dominant bacteria during air storage while lactic acid bacteria (LAB) formed the majority of the isolates from fillets kept under 100% CO₂ (MAP4). The proximate and fatty acids composition of patin fillets showed they consisted of 5.71g lipid/100g which was susceptible to oxidation due to the high amount of unsaturated fatty acids (63.86%) versus saturated fatty acids (31.14%). The chemical parameters revealed 100% CO₂ (MAP4) and vacuum packed fillets had the lowest TBARS values whereas air-stored fillets showed the highest TBA values. TVBN increased negligibly during storage of patin fillets in all treatments and did not exceed the acceptability limit (35Nmg/100 g). The results of this study indicated that 100% CO₂ was found to be the most effective atmosphere for storage of patin fillets at 4°C since this condition was associated with superior microbiological, biochemical and sensory attributes.

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

**KUALITI MIKROBIOLOGI DAN BIODIVERSITI IKAN PATIN (PANGASIU
PANGASIU) DI BAWAH PENGUBAHSUAIAN ATMOSFERA
PEMBUNGKUSAN**

Oleh

MARYAM SHARIAT

Mei 2012

Pengerusi : Profesor Fatimah Abu Bakar, PhD

Fakulti : Sains Makanan dan Teknologi

Ikan segar sangat mudah untuk rosak dengan jangka masa yang pendek disebabkan komposisi biologinya. Gabungan kesan perubahan mikrobiologi dan kimia menurun secara beransur-ansur kualiti ikan semasa penyimpanan dan terlibat dalam proses kerosakan tersebut. Pembungkusan atmosfera diubahsuai (MAP) dikenali umum sebagai teknik mengawet untuk memanjangkan hayat simpanan ikan dan produk ikan. Kecenderungan pengguna dalam pemilihan ikan segar selepas proses dan sejuk beku telah menjadikan MAP sebagai teknik yang lebih meluas digunakan baru-baru ini. Oleh itu, pembelajaran ini adalah untuk menentukan kesan vakum dan MAP dengan campuran gas yang berbeza ke atas hayat simpanan daging ikan semasa penyimpanan pada suhu sejuk beku. Daging ikan patin dibungkus di bawah keadaan vakum dan rawatan MAP yang berbeza termasuk MAP1 (5% O₂ + 40% CO₂ + 55% N₂), MAP2 (5% O₂ + 60% CO₂ + 35% N₂), MAP3 (5% O₂ + 80% CO₂ + 15% N₂) and MAP4 (100% CO₂) dan tertakluk kepada analisis mikrob, kimia dan analisis deria pada masa 0, 3, 6, 9,

12, 15, 18 dan 21 hari semasa penyimpanan pada 4 °C. Sampel simpanan udara telah dijadikan sebagai “control” dan keputusan yang diperolehi dibandingkan dengan “control” tersebut. Analisis mikrob telah dijalankan ke atas kiraan jumlah mesofilik dan psychrotropik aerobik, jumlah kiraan mesophilik dan psychrotropik anaerobik, kiraan bacteria proteolitik bacterial dan kiraan *Enterobacteriaceae*. Analisis biokimia dilakukan untuk pH, “Total Volatile Bases Nitrogen” (TVBN) dan pengoksidaan lipid (ujian TBA). Sebelum membungkus daging ikan, anggaran komposisi dan profil asid lemak pada daging ikan tersebut telah ditentukan pada hari 0. Mikroflora mesophilik aerobik yang dominan di dalam daging ikan yang disimpan di dalam udara dan MAP4 (100% CO₂) telah dikenalpasti pada setiap hari pensemplan. Kiraan bakteria yang tertinggi untuk semua kumpulan bacteria yang diperhati di udara diikuti vacuum, MAP1, MAP2, MAP3 and MAP4. Kiraan mesofilik aerobik untuk daging ikan patin yang disimpan di udara melebihi nilai ambang kerosakan (10⁷cfu/g) selepas 9 hari, di mana sampel yang disimpan di vakum, MAP1, MAP2 dan MAP3 mencapai nilai yang sama pada hari ke 14, 17, 18 dan 21. Sampel yang disimpan pada MAP4 (100% CO₂) tidak mencapai nilai kerosakan sepanjang penyimpanan selama 21 hari. Jumlah kiraan mesophilik aerobik di dalam penyimpanan udara daging ikan patin meningkat kira-kira kitar 5.5 log sepanjang masa penyimpanan, di mana sampel 100% CO₂ menunjukkan peningkatan kitar 3 log selama 21 hari. Ini jelas menunjukkan kesan karbon dioksida yang hebat ke atas pertumbuhan microbial pada kepekatan yang tinggi. Kesan deria kualiti semua daging ikan patin diterima semasa 13 ±1 hari penyimpanan di udara, 16 ±1 hari penyimpanan di vacuum dan MAP1, 18 ±1 hari penyimpanan di MAP2 dan 19 ±1 hari penyimpanan di MAP3. Keseluruhan ujian deria untuk daging ikan yang dibungkus 100% CO₂ adalah lebih tinggi daripada kadar yang diterima pada hari terakhir penyimpanan (21

hari). Kesan toleransi yang baik telah dikenalpasti di antara mikroba (TVC) dan data ujian deria (keseluruhannya diterima) untuk semua keadaan penyimpanan (correlation coefficient (r) di antara -0.943 to -0.987). Pengalpastian mikrobiologi menunjukkan *Aeromonas* adalah spesies yang utama semasa penyimpanan udara sementara Bakteria Acid Laktik (LAB) membentuk majoriti yang dikenalpasti daripada daging ikan semasa 100% CO₂ (MAP4). Komposisi asid laktik di dalam daging ikan patin menunjukkan ia mengandungi 5.71g lipid/100g yang cenderung kepada pengoksidaan disebabkan jumlah asid laktik tidak tepu setinggi 63.86% berbanding asid laktik tepu setinggi 31.14%. Parameter kimia mendedahkan 100% CO₂ dan bungkusan vakum daging ikan mempunyai nilai TBARS yang tinggi, di mana penyimpanan udara daging ikan menunjukkan nilai TBA yang paling tinggi. TVBN yang meningkat semasa penyimpanan daging ikan patin boleh diabaikan pada semua rawatan dan tidak melebihi had yang diterima (35Nmg/100 g). Keputusan kajian ini menunjukkan 100% CO₂ adalah atmosfera yang paling berkesan untuk penyimpanan sejukbeku daging ikan patin pada 4°C dari aspek kualiti yang berkaitan dengan mikroba, biokimia dan ujian deria.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my supervisor, Prof. Dr. Fatimah Abu Bakar as the chairman of my supervisory committee, who gave me confidence and continues support during the course of this study. I would also like to show my gratitude to my co supervisors Prof. Dr. Jamilah Abu Bakar and Prof. Dr. Russly Abdul Rahman for providing me with valuable guidance and giving me the encouragement I needed to complete this research work.

I owe my deepest gratitude to my beloved family for their love, their supports and their confidence throughout my life.

Last but not least, I also would like to thank all of my friends and lab mates, particularly, Chong and Selvi, for their helpful assistance in completing my lab work. My particular gratitude to Shadi Samaram for her friendly help and guidance during this project. Also special thanks to Mohammad Raftari for his guidance and supportive advices during all parts of my work.



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:

Fatimah Binti Abu Bakar, PhD

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

Jamilah Binti Bakar, PhD

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

Russly Bin Abdul Rahman, PhD

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

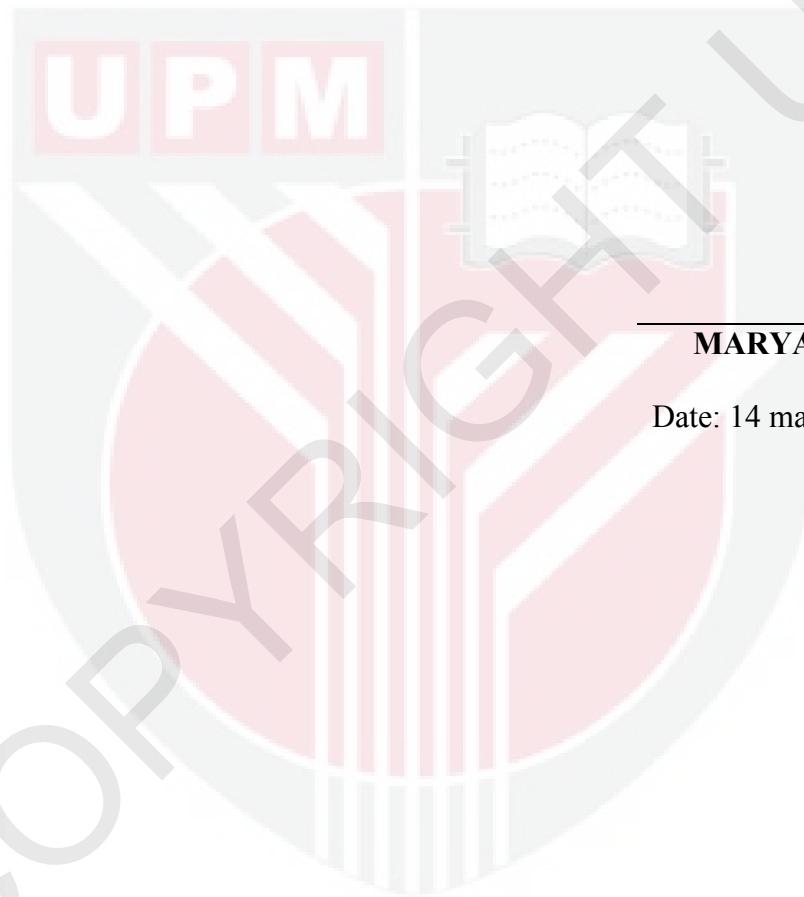
BUJANG BIN KIM HUAT, PhD

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 citation 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 other institutions.



MARYAM SHARIAT

Date: 14 may 2012

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xix
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Fish as Food	5
2.2 Freshwater Fish Industry	7
2.3 Patin (<i>Pangasius pangasius</i>)	8
2.4 Fish Composition	9
2.5 Fish Lipid Composition	11
2.6 Changes Occurring in Fish During Storage	12
2.6.1 Microbiological Changes	13
2.6.1.1 Fish Microflora	13
2.6.1.2 Specific Spoilage Organisms (SSO)	15
2.6.2 Chemical (biochemical) Changes	16
2.6.2.1 Trimethylamine (TMA), Total Volatile Bases Nitrogen (TVBN)	17
2.6.2.2 Lipid Oxidation	19
2.6.3 Sensory Changes	23
2.7 Vacuum and Modified Atmosphere Packaging (MAP)	24
2.8 MAP Gases	25
2.8.1 Carbon Dioxide (CO ₂)	25
2.8.2 Oxygen (O ₂)	27
2.8.3 Nitrogen (N ₂)	28
2.8.4 Gas Mixture	28
2.9 Packaging Material for MAP	30
2.10 The Effects of MAP on the Microbiological and Chemical Properties of Fish	31
3 METHODOLOGY	39
3.1 Materials	39
3.1.1 Raw Materials (Fish Sample)	39
3.1.2 Reagents	40
3.2 Preparation of Fish Sample and Packaging	42

3.3	Sampling	44
3.4	Microbiological Analyses	45
	3.4.1 Total Aerobic Mesophilic Bacteria	45
	3.4.2 Total Anaerobic Mesophilic Bacteria	46
	3.4.3 Total Aerobic Psychrotrophic Bacteria	46
	3.4.4 Total Anaerobic Psychrotrophic Bacteria	46
	3.4.5 Total Aerobic Proteolytic Bacteria	46
	3.4.6 Determination of <i>Enterobacteriaceae</i>	47
3.5	Bacterial Identification	47
3.6	Biochemical Analysis	49
	3.6.1 Determination of pH	49
	3.6.2 Determination of Total Volatile Bases Nitrogen (TVBN)	49
	3.6.3 Determination of Lipid Oxidation	51
	3.6.3.1 Preparation of TBA Solution	51
	3.6.3.2 Determination of TBA	51
	3.6.4 Determination of Fish Composition	52
	3.6.4.1 Moisture Content Determination	52
	3.6.4.2 Protein Content Determination	53
	3.6.4.3 Ash Content Determination	54
	3.6.4.4 Lipid Extraction	55
	3.6.5 Fatty Acid Determination by Gas Chromatography (GC)	56
3.7	Gas Analysis	57
3.8	Sensory Analysis	57
3.9	Statistical Analysis	68
4	RESULTS AND DISCUSSION	60
	4.1 Microbial Changes of Patin Fillets during Storage	60
	4.1.1 Aerobic Mesophilic Bacteria	60
	4.1.2 Anaerobic Bacterial (mesophilic and psychrotrophic) Counts	65
	4.1.3 <i>Enterobacteriaceae</i>	69
	4.1.4 Aerobic Psychrotrophic Bacteria	71
	4.1.5 Aerobic Proteolytic Bacteria	73
4.2	Bacterial Composition of Patin	77
4.3	Chemical Changes in Patin Fillets during Storage	83
	4.3.1 pH	83
	4.3.2 Total Volatile Bases Nitrogen (TVBN)	86
	4.3.3 Lipid Oxidation (TBA Test)	90
	4.3.4 Proximate Analysis	93
	4.3.5 Fatty Acid Composition	94
4.4	Gas Analysis	99
4.5	Sensory Evaluation of Patin fillets	100

5	CONCLUSION AND RECOMMENDATION	105
	REFERENCES	108
	APPENDICES	120
	BIODATA OF STUDENT	126

