UNIVERSITI PUTRA MALAYSIA

BIOCHEMICAL AND MICROBIOLOGICAL CHANGES IN FRESHWATER CATFISH (Mystus nemurus V.) DURING STORAGE AT DIFFERENT TEMPERATURES

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FSTM 2010 13

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By

WILLY PRANATA WIDJAJA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2010

***Especially dedicated to* :**

My wife Lilis Rusmiati, my son Rafi K. Pranata Kusumah,

my daughter Navesa Norazlina Pranata Kusumah, my beloved mother

Hjh. Nani Kartawinata, and my late father alayarham Hj. Hadi Widjaja…

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

BIOCHEMICAL AND MICROBIOLOGICAL CHANGES IN FRESHWATER CATFISH (*Mystus nemurus* V.) DURING STORAGE AT DIFFERENT TEMPERATURES

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Chairman : Associate Professor Fatimah Abu Bakar, PhD

Faculty : Food Science and Technology

Mystus nemurus is a species of Malaysian freshwater catfish. The freshness of fish is important for quality measurement. Changes in the biochemical components of *M. nemurus* during storage were analyzed based on lipid changes, formation of amines (volatile and biogenic), pH, and K-Value. Analyses were carried out such as peroxidation by iodometric titration, thiobarbituric acid by spectrophotometry, anisidine value by colorimetry, polyene index by gas chromatography, free fatty acid by acidoalcalimetry titration, K-Value by high performance liquid chromatography (HPLC), volatile base nitrogen by distillation, pH by pH meter, free amino acids by HPLC, and production of biogenic amines by HPLC. The samples were stored at ambient temperature (28±2 ^{o}C) for 24 hours, chilled temperature (10±2 ^{o}C) for 10 days, and iced temperature (2±1 ^{o}C) for 20 days. Lipid oxidation and lipid hydrolysis were significantly increased ($p<0.05$) by increasing in storage time and temperature with the highest value was 28±2 ^{o}C during 24 h. However, polyene index was decrease. The levels of peroxide,

thiobarbituric acid, p-anisidine, polyene index and free fatty acid were 2.69 meq active oxygen kg^{-1} lipids, 17.99 mg malonaldehyde g^{-1} lipids, 1.28 absorbance g^{-1}, 3.67, and 7.40%, respectively. The K-value of all samples which represent nucleotide degradation reached the spoilage limit of 60% at the end of the storage periods, except for iced treated samples. The total volatile base (TVB) level showed slight differences throughout the storage period. TVB level increased from 17.16 to 45.76 mg $N100g^{-1}$ at ambient temperature for 24 hours. pH value increased from 6.85 to 7.18 for 24 hours at ambient storage, 6.85 to 7.00 for 10 days at chilled storage and 6.85 to 7.03 for 20 days at iced storage. In this study, 12 hours at ambient, 8 days at chilled, and 16 days at iced temperature showed an unacceptable quality of *M. nemurus*. The amino acids of histidine, arginine, and lysine decreased along storage time. However, the biogenic amines namely histamine, putrescine, and cadaverine significantly increased ($p<0.05$) in accordance with the decreased of free amino acids during storage. The kinetic study showed that cadaverine has the highest value of constant rate (k) at 5.380 with activation energy E_a of 3539.9 kcal mol^{-1} at ambient storage. Therefore, in biochemical study cadaverine could be considered as spoilage indicator for *M. nemurus* for all storage temperatures.

Studies on the bacteriological changes of pond water were also carried out to evaluate the relationship between water quality and microbiological changes during storage of *M. nemurus*. The analyses involved were total mesophilic and psychrophilic aerobic count, lipase-producing bacterial count by plate count agar, proteolytic bacterial count by skim milk agar, histamine-producing bacterial count

by modified Niven's agar, putrescine producing bacterial count by arginine decarboxylase agar, cadaverine-producing bacteria count by lysine decarboxylase agar and *Pseudomonas* count by cetrimide fusidin cepaloridine agar, and lipolytic activity by Rodhamine-β olive oil agar. For 20 days of iced storage, the total mesophilic aerobic count increased from log 5.12 CFU g^{-1} to log 7.30 CFU g^{-1}. Psychrophilic bacterial count also increased for both 10 days of chilled storage and 20 days of iced storage. However, this result was slightly lower in one log cycle than mesophilic count. The lipase-producing bacterial count showed increase for all storage temperatures. Total mesophilic proteolytic count increased significantly ($p<0.05$) and attained a maximum level of 6.61 CFU g^{-1} at the end of 20 days of iced storage. The same trend was observed for total psychrophilic proteolytic count. The count for cadaverine-producing bacteria was the highest as compared to the other amine-forming bacteria. The count of *Pseudomonas* spp. that contributed to the highest production of cadaverine reached the value of log 7.20 CFU g^{-1} for 20 days in iced storage could be a dominance indicator for the decomposition of *M. nemurus* during storage.

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

PERUBAHAN BIOKIMIA DAN MIKROBIOLOGI IKAN "BAUNG" AIR TAWAR (*Mystus nemurus* V.) SEMASA PEYIMPANAN PADA SUHU BERBEZA

Oleh

WILLY PRANATA WIDJAJA

September 2010

Pengerusi : Profesor Madya Fatimah Abu Bakar, PhD

Fakulti : Sains Makanan dan Teknologi

Mystus nemurus ialah spesis ikan air tawar Malaysia. Kesegaran ikan adalah penting untuk penentuan kualiti. Perubahan komponen biokimia *M. nemurus* semasa penyimpanan telah dianalisa berdasarkan kepada perubahan lipid, pembentukan pelbagai jenis amina (meruap dan biogenik), pH dan Nilai-K. Analisis yang telah dijalankan adalah seperti penentuan peroksida menggunakan kaedah penititratan iodometrik, asid tiobarbitiurik menggunakan spektrofotometri, nilai anisidin menggunakan kolorimetri, indeks poliene menggunakan kromatografi gas, asid lemak bebas menggunakan penitratan asidoalkalimetri, Nilai-K menggunakan kromatografi cecair prestasi tinggi (HPLC), bes nitrogen menggunakan kaedah penyulingan, pH menggunakan pH meter, asid amino bebas dan biogenik amin menggunakan HPLC.

Sampel telah disimpan pada suhu bilik (28±2 ^{o}C) selama 24 jam, suhu sejuk (10±2 ^{o}C) selama 10 hari dan suhu ais (2±1 ^{o}C) selama 20 hari. Pengoksidaan

lipid dan hidrolisis lipid telah meningkat secara bererti ($p<0.05$) mengikut peningkatan tempoh dan suhu penyimpanan di mana nilai tertinggi ialah setelah simpanan 24 jam (28±2 ^{o}C). Walaubagaimanapun, indeks poliene telah menurun. Nilai masing-masing bagi tahap peroksida, asid tiobarbitiurik, p-anisidin, indeks poliene dan asid lemak bebas adalah 2.69 meq oksigen aktif kg^{-1} lipid, 17.99 mg malonaldehid g^{-1} lipid, 1.28 penyerapan g^{-1}, 3.67 dan 7.40%. Untuk semua sampel, nilai-K (mewakili pemecahan nukleotida), menunjukkan had kerosakan pada nilai 60% pada akhir tempoh penyimpanan, kecuali bagi sampel perlakuan ais. Tahap jumlah bes-meruap (TVB) telah menunjukkan perubahan kecil sepanjang tempoh penyimpanan. Tahap TVB meningkat daripada 17.16 kepada 45.76 $mgN100g^{-1}$ pada suhu bilik sepanjang 24 jam. Nilai pH meningkat daripada 6.85 kepada 7.18 selama 24 jam semasa penyimpanan pada suhu bilik, daripada 6.85 kepada 7.00 selama 10 hari pada suhu sejuk dan daripada 6.85 kepada 7.03 selama 20 hari pad suhu ais. Dalam kajian ini, penyimpanan selama 12 jam pada suhu bilik, 8 hari pada suhu sejuk dan 16 hari pada suhu ais menunjukkan penolakan kualiti bagi *M. nemurus*. Asid amino seperti histidin, arginin dan lisin berkurang sepanjang tempoh penyimpanan. Walaubagaimanapun, biogenik amin seperti histamin, putresin dan kadaverin meningkat secara bererti ($p<0.05$) seiring dengan penurunan asid amino bebas. Kajian kinetik menunjukkan kadaverin mempunyai nilai tertinggi bagi kadar tetap (k) iaitu 5.380 dengan tenaga pengaktifan Ea= 3539.9 kcal mol^{-1}, semasa penyimpanan suhu bilik. Jadi dalam kajian biokimia, kadaverin boleh dinilai sebagai penunjuk kerosakan bagi M. *nemurus* untuk semua suhu penyimpanan.

Kajian perubahan bakteriologi air kolam juga dijalankan untuk menilai hubungkait di antara kualiti air dengan perubahan mikrobiologi semasa penyimpanan *M. nemurus.* Analisis yang terlibat adalah jumlah mesofilik, hitungan aerobik-psikrofilik, hitungan bakteria penghasil-lipase menggunakan agar plat, hitungan bakteria-proteolitik menggunakan agar susu skim, hitungan bakteria penghasil-histamina menggunakan agar Niven's terubahsuai, hitungan bakteria penghasil-putresina menggunakan agar dikarboksilasi arginin, hitungan bakteria penghasil-kadaverina menggunakan agar dikarboksilasi lisin, hitungan *Pseudomonas* menggunakan agar sefaloridin-fusidin-setrimid dan aktiviti lipolitik menggunakan agar minyak zaitun Rodhamine-β. Selama 20 hari penyimpanan ais, jumlah hitungan aerobik-mesofilik meningkat daripada log 5.12 CFU g^{-1} kepada log 7.30 CFU g^{-1}.Hitungan bakteria-psikrofilik juga meningkat untuk kedua-dua cara penyimpanan iaitu simpanan sejuk (10 hari) dan ais (20 hari). Walaubagaimanapun, keputusan ini (psikrofilik) adalah lebih rendah sedikit dalam satu kitaran log berbanding hitungan mesofilik. Hitungan bakteria penghasil-lipase menunjukkan peningkatan untuk semua jenis suhu penyimpanan. Jumlah hitungan mesofilik-proteolitik meningkat secara bererti ($p<0.05$) dan mencapai tahap maksimum 6.61 CFU g^{-1} pada hari terakhir (20 hari) penyimpanan ais. Corak yang sama telah dicerap untuk jumlah hitungan psikrofilik-proteolitik. Hitungan untuk bakteria penghasil-kadaverina (amin) menunjukkan nilai tertinggi berbanding bakteria penghasil-amin lain. Hitungan *Pseudomonas* spp. mencapai log 7.20 CFU g^{-1} selama 20 hari penyimpanan ais dan merupakan penunjuk utama penguraian dengan menyumbang kepada penghasilan paling tinggi kadaverina semasa penyimpanan *M. nemurus.*

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and thanks to my supervisor, Associate Professor Dr. Fatimah Abu Bakar of the Department of Food Science, Faculty of Food Science and Technology for her guidance, confidence and encouragement during the study and all along I have known her since. I wish to thank also my co-supervisor Professor Dr. Nazamid Sa'ari for his guideance and giving me the encouragement I needed to complete this study. To my other co-supervisor, Dr. H. Zamri Ishak, I wish to thank you for the support and encouragement. I also wish to extend my sincere thanks to all the staff of the Department of Food Science and laboratory assistants for whatever help they have rendered me one way or the other during the course of this study.

To both of my beloved parents who have always blessed and bestowed upon me unending support during the study, I will forever be in your debt. To my wife, Lilis Rusmiati, I am utmost grateful and I highly appreciate you for your confidence, understanding and love for all these years. My appreciation would never be complete unless I extend it to both my wonderful children, Rafi and Navesa for their patience, inspiration and love. To Dr. Kuswata Kartawinata and Mrs.Yenny K., your help and support during my study is a huge driving force to keep me going.

My sincere gratitude is also extended to the financial support provided by the Intensification of Research in Priority Area (IRPA) fund for this research which was awarded to Assoc. Prof. Dr. Fatimah Abu Bakar. I am also indebted to all the staffs of the Department of Food Science for their generous cooperation.

Special thanks also to all her graduate friends, especially food safety and cocoa group members: Asep Edi Kusnadi, Helmi Wasoh, Muh. Zuhkrufuz Zaman, Nga Kea Soon, Tan Teng Ju, Yusep Ikrawan, Misnawi, Rashidah Sukor, Noorazimah Ali, Elham Moazami, Ismail Fitri, and Mohd. Safzan for sharing the literature and invaluable assistance. The time spent and memorable memories will always be cherished.

Finally, my thanks go to the Indonesian Government especially the Ministry of Higher Education of Indonesia, University Pasundan Bandung, my late Dean Professor Dr. Ir. H. Adang Kadarusman, MSc. and my current Dean Professor Dr. Ir. H. Sutarman, MSc. for giving me the opportunity to initiate, undergo, and complete this study. To all the staff of the Department of Food Technology, University Pasundan Bandung, I thank you for always being there for me.

I certify that a Thesis Examination Committee has met on 3 September 2010 to conduct the final examination of Willy Pranata Widjaja on his thesis entitled "Biochemical and Microbiological Changes in Freshwater Catfish (*Mystus nemurus* V.) During Storage At Different Temperatures" in accordance with the Universities and University College 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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

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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 at any other institutions.

WILLY PRANATA WIDJAJA

Date: 3 September 2010

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