Variation of Histamine Development in Different Budy IIM cations of YeHowfin Tuna (*Thummus albacares*) Stored at 0⁶C

Gamage Rumeshi Chaturika Hewawasam

M.Sc.

2009

Variation of Histamine Development in Different Body Locations of Yellowfin Tuna (*Thunnus albacares*) Stored 0°C

By

Gamage Rumeshi Chaturika Hewawasam

Thesis submitted to the University of Sri Jayewardenapura in partial fulfilment of the requirements for the degree of Master of Science in Fisheries and Aquatic Resources Management

M.Sc.

2009

Memorandum

I hereby certify that the work described in this thesis was carried out by me under the supervision of Dr. Kamal Ranathunga, Department of Zoology, University of Sri Jayewardenapura and Dr. Ranjith Edirisinghe, Head /Post Harvest Technology Division, National Aquatic Resources Research and Development Agency (NARA), Sri Lanka, and a report on this has not been submitted to any university for another degree.

papasan

G.R.C.Hewawasam

Certification

We certify that the work of G.R.C.Hewawasam on 'Variation of Histamine Development in Different Body Locations of Yellowfin Tuna (*Thunnus albacares*) Stored at 0°C', for the Degree of Master of Fisheries and Aquatic Resource Management in the Faculty of Graduate Studies at University of Sri Jayawardenapura.

We feel that the candidate's work is complete and suitable for submitting to the University for the purpose of evaluation.

Dr. Kamal Ranatunge Supervisor Lecturer Department of Zoology University of Sri Jayewardenapura

Jabben

Dr. Ranjith Edirisinghe External Supervisor Head, Post Harvest Technology Division National Aquatic Resources Research and Development Agency (NARA)

Dr. Nissanka de Sílva

Coordinator, MSc in Fisheries and Aquatic Resources Mgt. Head, Department of Zoology University of Sri Jayewardenapura Nugegoda, Sri Lanka.

TABLE OF CONTENTS

Table of Contents	i
Lists of Tables	iii
List of Figures	iv
Abbreviations	
Acknowledgements	vi
ABSTRACT	vii

CHAPTER ONE: INTRODUCTION	
1.1 Background	1

CHAPTER TWO: LITRETURE REVIEW	3
2.1. Tuna Industry	3
2.2 Histamine Fish Poisoning	5
2.2.1 Histamine	5
2.2.2 Scombrotoxin Formation	6
2.2.3 Enzyme-forming Bacteria	8
2.2.4 Toxicity of Histamine and Toxic Dose	10
2.2.5 Histamine Poisoning Potentiation	11
2.2.6 Factors Affecting Histamine Fish Poisoning	12
2.3 Within-fish Variation of Histamine Concentration	14
2.4 Histamine Analysis Methods	15

CHAPTER THREE: MATERIALS AND METHODS	
3.1 Collection of Yellowfin tuna Fish Samples	
3.2 Temperature Measurement	
3.3 Histamine Analysis	
3.3.1 Sample Preparation	19
3.3.2 Extraction	
3.3.3 Column Chromatography Elution	21

3.3.4 Derivatization	
3.3.5 HPLC Analysis	
3.3.6 Preparation of Calibration Curves	
3.3.7 Calculations	
3.3.8 Reagents Preparation	
3.4 Microbial Enumeration by Aerobic Plate Count Method3.4.1 Sample Preparation	
3.4.2 Experimental Procedure	
3.4.3 Media and Diluent Preparation	
3.5 Statistical Analysis	

 CHAPTER FOUR: RESULTS & DISCUSSION
 4.1 Temperature Measurements
 4.2 Microbiological Examination
 4.3 Histamine Formation

CONCLUSION	41
References	42
APPENDICES	45
Appendix 1: Levels of Histamine Found in Tested Samples	45
Appendix 2: Statistical Analysis of Results	

LIST OF TABLES

Table 1: Export Figures of the Fishery Sector	4
Table 2: Length Measurements for Different Portions of Fish Sampled	18
Table 3: Histamine Standard Solutions and Absorption Peaks – Calibration Plot 1	23
Table 4: Histamine Standard Solutions and Absorption Peaks – Calibration Plot 2	24
Table 5: Histamine Content of Fresh Yellowfin Tuna	34
Table 6: Histamine Development in Different Sections of Fish – L	35
Table 7: Histamine Development in Different Sections of Fish – R	37

LIST OF FIGURES

Figure 1: Yellowfin Tuna Production	3
Figure 2: YellowfinTuna Exports	4
Figure 3: Histamine Molecular Structure	6
Figure 4: Formation of Histamine	7
Figure 5: Within -fish Variation in Histamine Concentrations (Frank et al., 1981)	.14
Figure 6: Numbering Scheme for Identification of Fish Section	.17
Figure 7: Overall Procedure for Histamine Detection in Fish Samples	.20
Figure 8: Calibration Curve No.1	.23
Figure 9: Calibration Curve No.2	.24
Figure 10: Change in Backbone Temperature with Time for Fish L	.31
Figure 11: Change in Backbone Temperature with Time for Fish R	.31
Figure 12: Microbial Counts in Different Sections of Fish L	. 32
Figure 13: Microbial Counts in Different Sections of Fish R	.33
Figure 14: Variation of Initial Histamine Contents in Different Sections	. 34
Figure 15: Histamine Development in Fish - L during Storage	. 36
Figure 16: Histamine Development in Fish – R during Storage	. 37
Figure 17: Histamine Development in Four Sections up to 18 Days	. 38

ABBREVIATIONS

NARA	National Aquatic Resources Research and Development Agency
НАССР	Hazard Analysis Critical Control Point
EU	European Union
FDA	US Food and Drug Administration
HFP	Histamine Fish Poisoning
IUPAC	International Union of Pure and Applied Chemistry
GMP	Good Manufacturing Practices
DAO	Di-amine Oxidase
HMT	Histamine Methyl Transferase
AOAC	Association of Official Analytical Chemists
OPT	o-phthaldehyde
TCA	Trichloroacetic Acid
APC	Aerobic Plate Count
ANOVA	Analysis of Variance

V

ACKNOWLEDGEMENTS

I would like to thank my external supervisor Dr. Ranjith Edirisinghe, Head of the Post Harvest Technology Division of NARA for the supervision, guidance and the assistance given throughout the study. Secondly, I thank to Dr. Kamal Ranatunga of Department of Zoology, my supervisor, for the fruitful discussions in statistical methods and analysis of this study.

This study would be impossible to accomplish without the kind assistance in finding a research supervisor and the noble opportunity given by Mr. G. Piyasena, Secretary of the Ministry of Fisheries and Aquatic Resources.

I wish to thank the National Aquatic Resources Research and Development Agency (NARA) that provided the research opportunity, technical support and other facilities for this study. My thanks are due to Mrs. K.T.R.Prathapasinghe, Director General of NARA for giving the opportunity to use NARA laboratory facilities for this study. I want to thank all the researchers and staff of chemistry and microbiological laboratories and other staff of NARA who have helped and provided a nice working environment. I would like to thank especially Ms. Shakeela Nasir Ahamad and Mr. B.K. Kolitha, research officers of the chemistry laboratory for their assistance and guidance during the study.

A wonderful time has been created during my two years study here at the University of Sri Jayewardenapura. I would like to thank the Department of Zoology, all the lecturers, my MSc colleagues and all other parties for the help and support during my study.

G.R.C.Hewawasam

Sri Jayewardenapura, Sri Lanka May, 2009

Variation of Histamine Development in Different Body Locations of Yellowfin Tuna (*Thunnus albacares*) Stored at 0°C

G.R.C. Hewawasam

ABSTRACT

The biogenic histamine is an essential quality parameter in fish products and strict upper limits have been introduced in US and EU markets. The present study was undertaken to assess the within-fish variation of histamine concentrations in Yellowfin tuna (*Thunnus albacares*) by studying the histamine levels of different portions of the fish during storage at 0°C. In this study the histamine contents of fish samples taken from 4 different portions were determined by reversed phase high-performance liquid chromatography (HPLC) at regular intervals. In addition to the histamine determinations, total aerobic plate counts were also performed as a safety indicator.

Fresh fish contained negligible amounts of histamine as well as microbial flora. The section near the gut cavity and tail showed higher histamine values and the initial average histamine content was 2.00 ± 0.86 ppm. The mean histamine level remained at a low value of 2.32 ± 1.01 ppm up to 18 days for different fish portions studied. Upon storage, significant increases in the concentrations of histamine were noticed especially between 17 and 24 days. The initial variation was changed and the highest content was found in the section adjacent to the gills.

ANOVA revealed a significant variation (P = 0.00) of histamine level in various portions of the fish in relation to storage time and fish section. The mesophilic microbial counts showed an insignificant difference (p > 0.05) in relation to the storage time and fish section. The fish remained in sensorial acceptable condition until 24 days. However, histamine level exceeded the maximum limit of acceptability of 50 ppm between 24 and 32 days of storage.

It can be concluded from the present study that histamine development in Yellowfin tuna is very low for first 18 days under carefully controlled conditions at 0°C.

CHAPTER ONE: INTRODUCTION

1.1 Background

Histamine has been used as an important quality and safety indicator of tuna products. It is a product of bacterial spoilage due to time and temperature abuse. Histamine fish poisoning is considered a mild-to-moderate form of 'food poisoning' but it occurs throughout the world and is perhaps the most common form of toxicity caused by the ingestion of fish.

The tuna processing industry has quality and safety control measures once the raw material is received and also prior to purchasing. Because of the recurrence of histamine poisoning in many parts of the world and the importance of international trade of the concerned fish species, many countries have enacted maximal limits or guidelines on histamine levels in traded fish. In European Union (EU) regulations, the threshold toxic dose is 10mg/100g. The European Union Directive No.91/493 stipulates that nine samples must be taken from each batch of fish species of the following families: Scombridae, Clupeidae, Engraulidae and Coryphaenidae. These samples must fulfil the following requirements.

- the mean value must not exceed 10 mg/100g (100 ppm)
- two samples may have a value of more than 10 mg/100g (100 ppm) but less than 20 mg/100g (200 ppm)
- no sample may have a value exceeding 20 mg/100g (200 ppm).

The US Food and Drug Administration (FDA) has ruled that histamine must be addressed in Hazard Analysis Critical Control Point programmes for scombroid or scombroid-like fish. The FDA Compliance Policy Guidelines specify 50 mg/100 g (500 ppm) as the toxicity level, and 5 mg/100g (50 ppm) as the defect action level because histamine is not uniformly distributed in a decomposed fish. Therefore, if 5 mg/100g found in one section, there is a possibility that other units may exceed 50 mg/100g (FDA, 2001a).

Histamine level of fresh tuna fish is checked by exporting companies at the time of dispatching but there are some records investigating histamine levels increase when tuna catches reach their destinations. This is a major risk in the tuna export industry which results in the rejection of tuna consignments due to detection of histamine levels above the maximum acceptable level.

The objective of this study is to find out whether a significant variation in histamine development exists among different portions of a fish when similar storage conditions are given. Many studies have been conducted on development of histamine in different tuna varieties under varying storage conditions, but less attention has been paid on the histamine formation variations within different parts of a fish. This project deals with the study of histamine development in different portions of Yellowfin tuna during storage at 0°C. If a significant variation exists, it is necessary to set separate limits for different parts of the fish. Exporting Yellowfin tuna is an important industry in Sri Lanka and the findings will directly benefit the industry.

PCHAPTER TWO: LITRETURE REVIEW

2.1. Tuna Industry

The Sri Lankan fish and fishery products export industry is mainly composed of tunas and shrimps. The Indian Ocean which surrounds Sri Lanka is rich with tuna varieties. Sri Lanka produces around 85,000Mt of tuna annually with the 9th place among 49 countries those who harvest tuna in the Indian Ocean (Globefish, 2002). In 2007, Skipjack tuna and Yellowfin tuna contributed 52,540Mt and 39,260Mt respectively to the Sri Lankan marine fish production. (Fisheries Statistics, 2007) However, Skipjack tuna which contributed 21% of the marine fish production is mainly consumed domestically and contributes an insignificant quantity to fish exports. The Yellowfin tuna production in 2007 was the highest value reported during the past decade and it contributed to 16 % of the Sri Lankan marine fish production. The Figure 1 shows the increasing trend of Yellowfin tuna catch in the recent years except in the year 2005 due to Tsunami disaster in 2004.

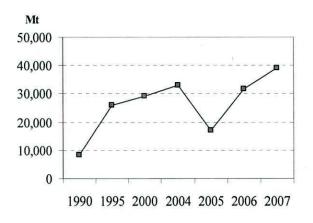


Figure 1: Yellowfin Tuna Production