CONTENTS

	Page
Contents	X
List of Tables	xv
List of Figures	xvi
List of Abbreviations and Symbols	XX
Chapter 1. Introduction and Literature Review	
Introduction	1
Literature review	2
Protein of fish muscle	2
Freeze-induced protein denaturation.	4
Mechanical damage	6
Dehydration	7
Concentration of salts	8
Lipid oxidation	9
Cellular metabolites	11
Hydrolysis of lipid	11
Formaldehyde formation	12
Physicochemical changes of muscle proteins during frozen storage	16
Solubility	16
Ca ²⁺ -ATPase activity	18
Sulfhydryl group/disulfide bond	18
Surface hydrophobicity/hydrophobic interaction	19
Thermal stability	20
Rheological behavior	20
Structural changes	21
Trimethylamine-N-oxide demethylase (TMAOase; EC 4.1.2.32)	21
Distribution of TMAOase	21
Characteristics and properties of TMAOase	24
Inhibitors and activators	24
pH and temperature profile and stability	24

		Page
	Molecular characteristics	25
	Retardation of demethylation of TMAO in fish muscle	27
	Physical methods	27
	Chemical inhibition	28
(Objectives of study	29
Chapt	er 2. Physicochemical and biochemical changes during frozen storage of	
	minced flesh of lizardfish (Saurida tumbil)	
	Abstract	30
]	Introduction	30
]	Materials and Methods	32
]	Results and Discussion	37
	Changes in TMAO, FA and DMA contents of muscle of lizardfish	37
	Changes in TMAOase activity in lizardfish muscle	40
	Changes in Ca2+-ATPase activity of NAM extracted from lizardfish	
	mince	41
	Changes in SH and disulfide bond contents of NAM extracted from	
	lizardfish mince	43
	Changes in surface hydrophobicity of NAM extracted from lizardfish	
	mince	46
	Changes in TNBS-reactive amino groups of NAM extracted from	
	lizardfish mince	47
	Changes in protein solubility of lizardfish muscle	48
(Conclusion	51
Chapt	er 3. Physicochemical and biochemical changes in whole lizardfish (Saurida	
	tumbil) muscles and fillets during frozen storage	
	Abstract	52
]	Introduction	53
,	Materials and Methods	54

	Page
Results and Discussion	59
Changes in TMAO, FA and DMA contents of whole lizardfish and its	
fillets	59
Changes in TMAOase activity in whole lizardfish and its fillets	62
Changes in protein solubility of whole lizardfish and its fillets	63
Changes in surface hydrophobicity of NAM extracted from whole	
lizardfish and its fillets	66
Changes in SH and disulfide bond contents of NAM extracted from	
whole lizardfish and its fillets	67
Changes in Ca2+-ATPase activity of NAM extracted from whole	
lizardfish and its fillets	70
Changes in TNBS-reactive amino groups of NAM extracted from whole	
lizardfish and its fillets	71
Conclusion	72
Chapter 4. Effect of some additives on the inhibition of lizardfish trimethylamine-	
N-oxide demethylase and frozen storage stability of minced flesh	
Abstract	73
Introduction	73
Materials and Methods	74
Results and Discussion.	78
Effect of some additives on lizardfish muscle TMAOase activity	78
Changes in TMAOase activity in lizardfish muscle	82
Changes in TMAO, FA and DMA contents of lizardfish mince	83
Changes in protein solubility of lizardfish mince	86
Conclusion	87
Chapter 5. Effect of trimethylamine–N-oxide demethylase from lizardfish kidney	
on biochemical changes of haddock natural actomyosin stored at 4 and	
-10°C	

	Page
Abstract	88
Introduction	89
Materials and Methods	90
Results and Discussion.	95
Partially purification of TMAOase from lizardfish kidney	95
Effect of TMAOase on FA, DMA, and TMAO contents of	
simulated system	99
Effect of TMAOase on protein solubility of simulated system	103
Effect of TMAOase on hexanal content of simulated system	105
Effect of TMAOase on DSC thermogram of simulated system	107
Conclusion	110
Chapter 6. Structural and rheological changes in haddock natural actomyosin stored	
at 4 and -10° C as affected by trimethylamine-N-oxide demethylase	
from lizardfish kidney	
Abstract	109
Introduction	112
Materials and Methods	113
Results and Discussion	117
Rheological behavior of haddock NAM	117
Effect of TMAOase on rheological behavior of haddock NAM	119
Raman spectra of haddock NAM	124
Effect of TMAOase on Raman spectra of NAM	128
Conclusion	132
Chapter 7. Effect of inhibitors and antioxidants on physicochemical and	
biochemical changes of haddock muscle induced by lizardfish	
trimethylamine- N -oxide demethylase during frozen storage	
Abstract	133
Introduction	133

	Page
Materials and Methods	135
Results and Discussion.	140
Effect of TMAOase, TMAOase inhibitors and antioxidants on FA,	
DMA and TMAO contents of haddock mince	140
Effect of TMAOase, TMAOase inhibitors and antioxidants on lipid	
oxidation of haddock mince	143
Effect of TMAOase, TMAOase inhibitors and antioxidants on	
protein extractability of haddock mince	145
Effect of TMAOase, TMAOase inhibitors and antioxidants on	
rheological behavior of haddock mince	148
Conclusion	150
Chapter 8. Summary and future works	
Summary	151
Future works	152
References	153
Vitae	174

LIST OF TABLES

Table		Page
1.	Causes of freeze-induced protein denaturation and possible prevention	
	methods	5
2.	Typical reactions of formaldehyde with functional groups of amino acids in	
	aqueous solutions	14
3.	TMAOase activity distribution in several marine organisms	22
4.	Effect of various additives on the inhibition of TMAOase activity from	
	lizardfish muscle	81
5.	Purification of TMAOase from lizardfish kidney	97
6.	Hexanal content in simulated system containing different levels of	
	TMAOase from lizardfish kidney after storage at 4 and -10°C	106
7.	Transition temperature and enthalpy of myosin and actin in simulated system	
	containing different levels of TMAOase from lizardfish kidney after storage	
	at 4°C for 15 days and -10°C for 8 weeks	109
8.	Storage modulus and loss modulus after heating to 90°C and cooling from	
	90°C to 20°C of simulated NAM system containing different levels of	
	TMAOase from lizardfish kidney after storage at 4 and 10°C	123
9.	Changes in the tyrosyl doublet of simulated NAM system containing 15	
	units of TMAOase from lizardfish kidney after storage at 4 and -10°C	126
10.	Storage modulus and loss modulus before heating to 90°C and after	
	heating/cooling from 90°C to 20°C of haddock mince with and without	
	TMAOase (15 units/g mince) in the presence of some additives after	
	storage at -10°C for 6 weeks	149

LIST OF FIGURES

Figure		Page
1.	Factors affecting muscle protein denaturation and consequent loss of protein	
	functionality during frozen storage	4
2.	Effect of freezing on the myofibrillar lattice	7
3.	Potential reactions of proteins with lipid radicals and their oxidation	
	products	10
4.	Decomposition of TMAO into DMA and FA by the action of TMAOase	12
5.	Possible interactions of FA with muscle proteins in frozen fish	15
6.	The myosin molecule	17
7.	Changes in FA, DMA and TMAO contents in lizardfish mince kept in air	
	and under vacuum during frozen storage at −20°C for 24 weeks	39
8.	Changes in TMAOase acitivity in lizardfish mince kept in air and under	
	vacuum during frozen storage at −20°C for 24 weeks	41
9.	Changes in Ca ²⁺ -ATPase activity of NAM extracted from lizardfish mince	
	kept in air and under vacuum during storage at -20°C for 24 weeks	42
10.	Changes in total sulfhydryl and disulfide bond contents of NAM extracted	
	from lizardfish mince kept in air and under vacuum during storage at -	
	20°C for 24 weeks	45
11.	Changes in surface hydrophobicity of NAM extracted from lizardfish mince	
	kept in air and under vacuum during storage at -20°C for 24 weeks	47
12.	Changes in TNBS-reactive amino groups of NAM extracted from lizardfish	
12.		
1.0	mince kept in air and under vacuum during storage at -20°C for 24 weeks.	48
13.	Changes in protein solubility of lizardfish mince kept in air and under	
	vacuum during storage at -20°C for 24 weeks	50
14.	Changes in FA, DMA and TMAO contents in whole lizardfish and its fillets	
	kept in air and under vacuum during frozen storage at -20°C for 24 weeks.	61
15.	Changes in TMAOase acitivity in whole lizardfish and its fillets kept in air	
	and under vacuum during frozen storage at −20 °C for 24 weeks	63
16.	Changes in protein solubility of whole lizardfish and its fillets kept in air	
	and under vacuum during storage at -20°C for 24 weeks	65

LIST OF FIGURES (Continued)

Figure		Page
17.	Changes in surface hydrophobicity of NAM extracted from whole lizardfish	
	and its fillets kept in air and under vacuum during storage at -20°C for 24	
	weeks	67
18.	Changes in total sulfhydryl and disulfide bond contents of NAM extracted	
	from whole lizardfish and its fillets kept in air and under vacuum during	
	storage at -20°C for 24 weeks	69
19.	Changes in Ca ²⁺ -ATPase activity of NAM extracted from whole lizardfish	
	and its fillets kept in air and under vacuum during storage at −20 °C for 24	
	weeks	71
20.	Changes in TNBS-reactive amino groups of NAM extracted from whole	
	lizardfish and its fillets kept in air and under vacuum during storage at -	
	20°C for 24 weeks	72
21.	Changes in TMAOase acitivity in lizardfish mince without and with various	
	additives during frozen storage at -20°C for 24 weeks	83
22.	Changes in DMA, FA and TMAO contents in lizardfish mince without and	
	with various additives during frozen storage at -20°C for 24 weeks	85
23.	Changes in protein solubility of lizardfish mince without and with various	
	additives during storage at -20°C for 24 weeks	87
24.	Elution profile of TMAOase on Sephacryl S-300 column	98
25.	Elution profile of TMAOase on DEAE-cellulose column	99
26.	Effect of TMAOase from lizardfish kidney at different levels on FA content	
	of simulated system during storage at 4°C for 15 days and -10°C for 8	
	weeks	101
27.	Effect of TMAOase from lizardfish kidney at different levels on proton ¹ H	
	NMR spectra (400 MHz) of simulated system after storage at 4 °C for 15	
	days	102
28.	Effect of TMAOase from lizardfish kidney at different levels on proton ¹ H	
	NMR spectra (400 MHz) of simulated system after storage at -10°C for 8	
	weeks	102

LIST OF FIGURES (Continued)

Figure		Page
29.	Effect of TMAOase from lizardfish kidney at different levels on protein	
	solubility of simulated system during storage at $4^{\circ}C$ for 15 days and -	
	10°C for 8 weeks	104
30.	Differential scanning calorimetry thermogram of fresh haddock NAM	107
31.	Rheogram of fresh haddock NAM heated linearly from 20 to 90°C at the	
	rate of 2°C/min, followed by cooling to 20°C	119
32.	Changes in storage modulus and loss modulus of simulated haddock NAM	
	system containing different levels of TMAOase from lizardfish kidney after	
	storage at 4°C for 15 days	121
33.	Changes in storage modulus and loss modulus of simulated haddock NAM	
	system containing different levels of TMAOase from lizardfish kidney after	
	storage at -10°C for 8 weeks	122
34.	Raman spectra in the 800-1800 cm ⁻¹ wavenumber regions of haddock	
	NAM, after storage at $4^{\circ}C$ for 15 days or at $-10^{\circ}C$ for 8 weeks	125
35.	Raman spectra in the and 2500-3400 cm ⁻¹ wavenumber regions of	
	haddock NAM, after storage at 4°C for 15 days or at -10°C for 8 weeks	128
36.	Raman spectra in the 800-1800 cm ⁻¹ wavenumber region of haddock	
	NAM before and after storage at 4°C for 15 days or at -10°C for 8	
	weeks in the presence or the absence of 15 units TMAOase/g	130
37.	Raman spectra in the 2500-3400 cm ⁻¹ wavenumber region of haddock	
	NAM before and after storage at 4°C for 15 days or at -10°C for 8 weeks	
	in the presence or the absence of 15 units TMAOase/g	131
38.	Changes in FA content in haddock mince with and without TMAOase (15	
	units/g mince) in the presence of some additives during frozen storage at -	
	10°C for 6 weeks	142
39.	Proton ¹ H NMR spectra (400 MHz) of haddock mince with and without	
	TMAOase (15 units/g mince) in the presence of some additives after	140
	storage at -10°C for 6 weeks	143

LIST OF FIGURES (Continued)

Figure		Page
40.	Changes in TBARS value in haddock mince with and without TMAOase	
	(15 units/g mince) in the presence of some additives during frozen storage	
	at -10°C for 6 weeks	145
41.	Changes in water-soluble proteins and salt-soluble proteins in haddock	
	mince with and without TMAOase (15 units/g mince) in the presence of	
	some additives during frozen storage at -10°C for 6 weeks	147

LIST OF ABBREVIATIONS AND SYMBOLS

ANS 1-anilinonaphthalene-8-sulfonic acid

Ca²⁺-ATPase Ca²⁺-adenosine 5'-triphosphatase

°C degree Celsius

DEAE diethylaminoethyl
DMA dimethylamine

DSC differential scanning calorimetry

DTNB 5,5'-dithio-bis-(2-nitrobenzoic acid)

EDTA ethylenediaminetetraacetic acid

FFA free fatty acid FA formaldehyde

X g gravitational force

G' storage modulus

G'' loss modulus

 $K_{\rm m}$ Michaelis-Menton constant

 $\begin{array}{cc} \mu mol & micromole \\ mmol & millimole \end{array}$

NAM natural actomyosin

NMR nuclear magnetic resonance

NTSB 2-nitro-5-thiosulfobenzoate

SH sulfhydryl

SS disulfide bond

TBARS thiobarbituric acid-reactive substances

TMA trimethylamine

TMAO trimethylamine oxide

TMAOase trimethylamine-N-oxide demethylase

TNBS trinitobenzenesulfonic acid

 T_{m} maximum transition temperature