Contents

	Page
บทคัดย่อ	(3)
Abstract	(5)
Acknowledgment	(8)
Contents	(9)
List of Tables	(14)
List of Figures	(16)
Chapter	
1. Introduction	1
Literature Review	3
Collagen	3
Collagen in marine animals	9
Collagen in Invertebrates	12
Collagen in fish	14

	The factors affecting collagen properties	17
	Gelatin	21
	Conversion of collagen to gelatin	21
	Production of gelatin	21
	Composition of gelatin	24
	Gelatin structure	25
	Isoelectric point of gelatin	28
	The Mechanism of gelation	28
	Factors affecting the properties of gelatin gels	30
	Production and characterization of fish gelatins	35
	Use of collagen and gelatin	39
Ob	ojectives	41
2. Ma	aterial and methods	42
1.	Chemical reagents	42
2	Fish skin and hone preparation	42

3.	Instruments	44
4.	Preparation and characterization of acid soluble collagens of collagen from	45
	skin and bone	
	4.1 Extraction of collagen	45
	4.2 Characterization of collagen	46
5.	Extraction and characterization of gelatin from skin and bone of bigeye	50
	snapper	
	5.1 Deproteinization process for gelatin extraction from skin and bone	50
	5.2 Demineralization process for gelatin extraction from bone	
	5.3 Swelling process for gelatin extraction from skin and bone	51
	5.4 Extraction of gelatin from pretreated skin and bone	51
5.	Characterization and functional properties of gelatin of skin from bigeye	52
	snapper	53

Contents (continued)

	Page
6.1 Determination of chemical compositions	53
6.2 SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	53
6.3 Gelation	53
6.4 Solubility	54
6.5 Emulsifying Properties	55
6.6 Foaming Properties	56
7. Improvement of gel properties of gelatin from the skin of bigeye snapper	56
7.1 Use of $MgSO_4$	
7.2 Use of microbial transglutaminase (MTGase)	56
8. Statistical analysis	57
3. Results and Discussion	59
Characterization of acid soluble collagen from skin and bone from bigeye	59

1.1	Compositions of skin, bone and their collagens	
1.2	Amino acid composition of skin and bone collagens	59
1.3	SDS-polyacrylamide gel electrophoresis patterns of skin and bone	60
colla	gens	63
1.4	Peptide mapping of skin and bone collagens	65
1.5	Thermal stability of skin and bone collagens	68
1.6	Viscosity of skin and bone collagen solutions	70
1.7	Solubility of skin and bone collagens	71
Extraction	n of gelatin from skin and bone from bigeye snapper	74
2.1	Deproteinization for gelatin extraction	74
2.2	Demineralization for gelatin extraction from bone	78
2.3	Swelling for gelatin extraction from skin and bone	79
Character	rization and functionality of gelatin from skin of bigeye snapper	84
3.1	Proximate compositions of bigeve snapper skin and its gelatin	

2.

3.

	3.2	SDS-polyacrylamide gel electrophoretic (SDS-PAGE) patterns of	84
	skin	gelatin	85
	3.3	Gelation of gelatin from bigeye snapper skin	
	3.4	Solubility of gelatin	86
	3.5	Emulsifying properties	90
	3.6	Foaming properties	91
4.	Improver	nent of gel properties of gelatin from skin of bigeye snapper	93
	4.1	Turbidity of gelatin solution and color of gelatin gels	95
	4.2	Bloom strength of skin gelatin gels	
	4.3	Color of gelatin gel	95
			96
			99

Contents (continued)

		Page
4.4	SDS- polyacrylamide gel electrophoretic (SDS-PAGE) patterns of	100
gelat	in gels	
4.5	Scanning Electron Microscopy of Gelatin gels	102
4. Conclusion		110
Suggestion		111
References		112
Appendix		129
Vitae		142

List of Tables

Table		Page
1.	Collagen and their distribution	5
2.	Amino acid composition of muscle collagens of marine fish and	11
	invertebrates	
3.	Main neutral sugars in collagens of marine fish and invertebrates	12
4.	Collagen content in white muscle of fishes	15
5.	Chemical compositions of skin, bone and their collagen from bigeye	60
	snapper	
6.	Amino acid composition of skin and bone collagens from bigeye snapper	62
	(residue/1,000 residues)	
7.	Ash content in residues of deproteinized bone from bigeye snapper after	79
	demineralization process under different conditions	
8.	Effect of swelling on the yield of gelatin from bigeye snapper skin and bone	80
	with different demineralization conditions	

9.	Proximate compositions of skin and skin gelatin from bigeye snapper	84
10.	Color of gelatin gel from bigeye snapper skin at different pHs	89
11.	Emulsifying properties of gelatin from bigeye snapper skin and egg white at	92
	different concentrations	
12.	Foaming properties of bigeye snapper skin gelatin and egg white at	94
	different concentrations	
13.	Color of gelatin gel from bigeye snapper skin with add with MgSO ₄ or	99
	MTGase at different concentrations	

List of Figures

Figure		Page
1.	Triple-helical structure of collagen	4
2.	Illustration of the overlap structure of the collagen fiber responsible for	6
	the banding pattern of a negatively stained collagen fiber	
3.	Cross-link formation in collagen by side chain groups	8
4.	Relationship between the total imino acid content (proline +	19
	hydroxyproline) and the molecular melting temperatures (T_m) of various	
	collagens from different species	
5	Factors determining optimum gelatin extraction conditions	23
6	Molecular weight distribution showing the major structural components	27
	of gelatin	
7.	Scheme for the concentration and temperature-dependent pathways for	29
	helix formation in α chains derived from collagen	
8.	Gel strength of seven different commercial fish and pork gelatins as a	31

function of concentration

9.	Effect of the solution pH on the bloom strength of gelatin Variation in	32
	bloom strength (at 6.67% (w/v) concentration) with temperature of	
	maturation for 275-bloom (curve A) and 175-bloom (curve B) gelatins	
10.	Variation in bloom strength (at 6.67% (w/v) concentration) with	33
	temperature of maturation for 275-bloom (curve A) and 175-bloom	
	(curve B) gelatins	
11.	Effect of pH on the thermal degradation of gelatin at 70 $^{\rm o}{\rm C}$	34
12.	Effect of pH on the degradation of gelatin	35
13.	Protein patterns of the gelatin from sole, megrim, cod, hake, and squid	38
14.	The $\alpha 1/\alpha 2$ ratio and relative amount of molecular weight components	38
	of gelatin from fish skins	
15.	Bigeye snapper, Priacanthus tayenus	43
16.	Scheme for extraction of gelatin from skin and bone	50
17.	SDS-PAGE patterns of collagen from bigeye snapper skin and bone	65

- under reducing and non-reducing conditions
- 18. Peptide mapping of skin and bone collagens from bigeye snapper 67 digested by V8 protease and lysyl endopeptidase
- 19. Thermograms of bigeye snapper skin and bone collagens rehydrated in 690.05 M acetic acid and deionized distilled water
- 20. Relative viscosity of bigeye snapper skin and bone collagens solution at 71 different temperatures
- 21. Relative collagen solubility (%) of bigeye snapper skin and bone 72 collagens at different pHs
- 22. Relative collagen solubility (%) in 0.5M acetic acid of bigeye snapper 73 skin and bone collagens in presence of NaCl at different concentrations
- 23. Accumulative protein content and hydroxyproline content in 76 deproteinizing solution of bigeye snapper skin
- 24. Accumulative protein content and hydroxyproline content in 77 deproteinizing solution of bigeye snapper bone

25.	Microstructure of skin, deproteinized skin and skin swollen with acetic	81
	acid and citric acid at different concentrations	
26.	Bloom strength of gelatin gel from skins swollen with acetic acid or citric	83
	acid at different concentrations	
27.	SDS-PAGE pattern of gelaitn from bigeye snapper skin under reducing	86
	condition	
28.	Turbidity of gelatin solution from bigeye snapper skin at different pHs	87
29.	Bloom strength of bigeye snapper skin gelatin at different pHs	88
30.	Relative solubility of gelatin from bigeye snapper skin and egg white at	90
	different pHs	
31.	Turbidity of gelatin solution from bigeye snapper skin added with ${\rm MgSO_4}$	96
	or MTGase at different concentrations	
32.	Bloom strength of gelatin gel from bigeye snapper skin added with	98
	MgSO ₄ and MTGase at different concentrations	
33.	SDS-PAGE pattern of gelatin gel from bigeye snapper skin added with	101

MgSO ₄ at different concentrations

- 34. SDS-PAGE pattern of gelatin gel from bigeye snapper skin with MTGase 102 at different concentrations
- 35. Microstructure of gelatin gel from bigeye snapper skin with and without 103 addition of $MgSO_4$ at different concentrations
- 36. Microstructure of gelatin gel from bigeye snapper skin with and without 104 addition of MTGase at different concentrations
- 37. Microstructure of gelatin gel from bigeye snapper skin with and without 104 adding MTGase at the concentration of $0.01 \frac{9}{0} (\text{w/v})$
- 38. Bloom strength of gelatin gel from bigeye snapper skin containing 0.01 106

 % (w/v) MTGase incubated for different times
- 39. SDS-PAGE pattern of gelatin gel from bigeye snapper skin containing 107 $0.01 \, \%$ (w/v) MTGase incubated for different times
- 40. SDS-PAGE pattern of gelatin gel from bigeye snapper skin added with 109 0.1 or 1.0% (w/v) MTGase and incubated for different times