

CONTENTS

	Page
Contents	(8)
List of tables	(13)
List of figures	(14)
Chapter	
1. Introduction	1
Literature review	2
1. Pepsin	2
1.1 Pepsin from pepsinogen	3
1.2 Catalytic mechanism of pepsin	4
1.3 Fish pepsin	5
1.4 Characteristics of fish pepsin	6
1.4.1. Optimal pH	6
1.4.2. Optimal temperature	7
1.4.3. Catalytic activity	8
2. Collagen	8
2.1 Marine fish collagen	10
2.2 Extraction and characterization of fish collagen	12
2.2.1 Acid-solubilized collagen	12
2.2.2 Pepsin-solubilized collagen	13
2.3 The factors affecting collagen properties	14
2.3.1 Imino acid content	14
2.3.2 Age and starvation of animals	15
2.3.3 pH and salt	16
2.3.4 Processing	16
3. Gelatin	17
3.1 Fish gelatin extraction	17
3.2 Uses of enzymes in collagen/gelatin manufacturing	20

CONTENTS (Continued)

	Page
3.3 Gelatin dehydration	21
3.4 Gelatin properties	21
3.5 Gelatin applications	22
Objectives	24
2. Materials and Methods	25
1. Chemical reagents	25
2. Fish stomach and skin preparation	25
3. Instruments	26
4. Preparation and characterization of pepsin from bigeye snapper stomach	26
4.1 Preparation of stomach extract	26
4.2 Enzyme assay	27
4.3 Enzyme fractionation	27
4.4 Characterization of fish pepsin	28
4.4.1 pH and temperature profile	28
4.4.2 pH and thermal stability	28
4.4.3 Inhibitory study	28
5. Extraction and characterization of pepsin solubilized collagen from bigeye snapper skin	28
5.1 Preparation of skin for collagen extraction	28
5.2 Effect of pepsin on collagen extraction and composition	29
5.2.1 Effect of pepsin levels and reaction time on extraction and composition of collagen	29
5.2.2 Effect of acid swelling process in combination with pepsin on extraction and composition of collagen	29
5.3 Comparative studies on different collagen extracting methods	30
5.3.1 Acid solubilization process	30
5.3.2 One-step acid/pepsin solubilization process	30

CONTENTS (Continued)

	Page
5.3.3 Two-step acid/pepsin solubilization process	30
5.4 Collagen precipitation	30
5.5 Characterization of collagen	30
5.5.1 Hydroxyproline content	30
5.5.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)	30
5.5.3 Differential scanning calorimetry (DSC)	31
5.5.4 Collagen solubility	31
5.5.4.1 Preparation of collagen solution	31
5.5.4.2 Effect of pH on collagen solubility	32
5.5.4.3 Effect of NaCl on collagen solubility	32
6. Extraction and characterization of gelatin from bigeye snapper skin using pepsin aided process	32
6.1 Non-collagenous protein removal process	32
6.2 Effect of bigeye snapper pepsin on gelatin extraction	32
6.3 Inactivation of skin endogenous proteases	33
6.3.1 Uses of heat treatment	33
6.3.2 Use of protease inhibitor	33
6.4 Preparation of fish skin gelatin	33
6.5 Characterization and functional properties of gelatin	34
6.5.1 Determination of chemical compositions	34
6.5.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)	34
6.5.3 Gelation of gelatin	34
6.5.3.1 Gelatin gel preparation	34
6.5.3.2 Determination of bloom strength	34
6.5.3.3 Color of gelatin gel	35
6.5.3.4 Turbidity of gelatin solution	35

CONTENTS (Continued)

	Page
6.5.4 Gelatin solubility	35
6.5.5 Emulsifying properties	35
6.5.6 Foaming properties	36
6.5.7 Scanning electron microscopy	36
7. Statistical analysis	37
3. Results and discussion	38
1. Extraction and characterization of pepsin from bigeye snapper stomach	38
1.1 Extraction and fractionation of pepsin from bigeye snapper stomach	38
1.2 pH and temperature profile of bigeye snapper pepsin (BSP)	41
1.3 pH and thermal stability of BSP	43
1.4 Effect of inhibitors on BSP	45
2. Preparation and characterization of pepsin solubilized collagen from bigeye snapper skin	47
2.1 Effect of pepsin on collagen extraction and composition	47
2.1.1 Effect of pepsin levels and reaction time	47
2.1.2 Effect of prior acid swelling process in combination with pepsin	49
2.2 Composition of collagen extracted from bigeye snapper skin with different conditions	51
2.3 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of collagens from bigeye snapper skin	52
2.4 Thermal stability of collagens from bigeye snapper skin	54
2.5 Solubility of skin collagens from bigeye snapper skin	56
3. Effect of pepsin-aided process on extraction and characteristics of gelatin from bigeye snapper skin	58
3.1 Effect of pepsin levels on extraction and characteristics of gelatin	58
3.2 Inactivation of endogenous proteases in BSP pretreated bigeye snapper skin	61
3.3 Proximate compositions of bigeye snapper skin and gelatins	63

CONTENTS (Continued)

	Page
3.4 Protein patterns of different gelatins	65
3.5 Gel characteristics of different gelatins	66
3.6 Solubility of gelatins	69
3.7 Emulsifying properties	70
3.8 Foaming properties	71
3.9 Microstructure of gelatin gels	72
4. Conclusions	74
Future works	75
References	76
Appendix	89
Vitae	100

LIST OF TABLES

Table	Page
1. Collagens and their distribution	10
2. Amino acid composition of collagen from fish and mammalian	11
3. Effect of extracting media on pepsin extraction	39
4. Fractionation of pepsin from bigeye snapper stomach	40
5. Effect of various inhibitors on the activity of proteinases from bigeye snapper stomach	46
6. Total hydroxyproline (Hyp) and yield of collagen extracted from bigeye snapper skin using bigeye snapper pepsin (BSP) or porcine pepsin (PP) for 24 h and 48 h	48
7. Total hydroxyproline (Hyp) and the yield of collagen extracted from bigeye snapper skin with prior acid swelling, followed by the treatment of bigeye snapper pepsin (BSP) or porcine pepsin (PP) for 24 h and 48 h	50
8. Yield, hydroxyproline and collagen contents of different collagens from bigeye snapper skin	52
9. The maximum transition temperature (T_{max}) and total denaturation enthalpy (ΔH) of different collagens from bigeye snapper skin rehydrated in 0.05M acetic acid	55
10. Hydroxyproline (Hyp) yield of gelatin extracted from bigeye snapper skin treated with bigeye snapper pepsin (BSP) at different levels	60
11. Proximate compositions of bigeye snapper skin and different gelatins	64
12. Color and bloom strength of gel and turbidity of gelatin solution (6.67% protein) from different gelatins	67
13. Emulsifying properties and foaming properties of gelatin from bigeye snapper skin and gelatin from bovine bone	71

LIST OF FIGURES

Figure	Page
1. Schematic representation of the structure of pepsinogen and its conversion to pepsin. The major points of hydrolysis are marked with P and result in release of several peptides (A), pepsin inhibitor (B), and pepsin (C). Hydrolysis of the bond <u>P</u> is essential for activation.	4
2. Catalytic mechanism of pepsin	5
3. Arrangement of tropocollagen	9
4. Collagen conversions into gelatin	17
5. pH profiles of proteinases from bigeye snapper stomach. Proteinase activity was determined using hemoglobin as a substrate at 45°C at various pHs for 20 min. TCA-soluble peptides released were determined by the Lowry assay. The activity was expressed as units/ml. Bars represent the standard deviation from triplicate determinations.	42
6. Temperature profiles of proteinases from bigeye snapper stomach. Proteinase activity was determined using hemoglobin as a substrate at pH 2.5 at various temperatures for 20 min. TCA-soluble peptides released were determined by the Lowry assay and the activity was expressed as units/ml. Bars represent the standard deviation from triplicate determination.	43
7. pH stability of proteinases from bigeye snapper stomach. Enzyme at a level of 184.84 units/ml was subjected to different pHs for 30, 60 and 120 min. Residual activity was determined at 45°C and pH 2.5 for 20 min using hemoglobin as a substrate. Bars represent the standard deviation from triplicate determinations.	44
8. Thermal stability of proteinases from bigeye snapper stomach. Enzyme at a level of 181.15 units/ml was subjected to incubation at different temperatures for 30, 60 and 120 min, followed by cooling in iced water. Residual activity was determined at 45°C and pH 2.5 for 20 min using hemoglobin as a substrate. Bars represent the standard deviation from triplicate determinations.	45

LIST OF FIGURES (Continued)

Figure	Page
9. SDS-PAGE patterns of collagens from bigeye snapper skin extracted using BSP or PP for 24 h and 48 h. Numbers denote pepsin activity in kUnits/g defatted skin.	49
10. SDS-PAGE patterns of collagen extracted from bigeye snapper skin with prior acid swelling, followed by the treatment of bigeye snapper pepsin (BSP) or porcine pepsin (PP) for 24 h and 48 h.	51
11. SDS-PAGE patterns of collagens from bigeye snapper skin. A48: collagen extracted with acid for 48 h; BSP48: collagen extracted with acid containing BSP (20 kUnits/g defatted skin) for 48 h; A24/BSP48: collagen extracted with acid for 24 h, followed by extracting using BSP (20 kUnits/g defatted skin) for 48 h; A24/PP48: collagen extracted with acid for 24 h, followed by extracting using PP (20 kUnits/g defatted skin) for 48 h. HM and CSC denote high MW protein markers and collagen type I, respectively.	54
12. Relative solubility (%) of collagens extracted from bigeye snapper skin with different methods at different pHs.	57
13. Relative solubility (%) of collagens extracted from bigeye snapper skin with different methods in the presence of NaCl at different concentrations.	58
14. SDS-PAGE patterns of BSP pretreated skin and gelatin extracted from bigeye snapper treated with BSP at different levels. Skin was pretreated in acid solution at 4°C for 48 h in the presence of BSP at different levels. Gelatin was extracted after pretreatment at 45°C for 12 h. Number denote activity levels (kUnits/g alkaline-treated skin).	60
15. SDS-PAGE patterns of gelatin extracted from bigeye snapper skin, treated with BSP at 15 kUnits/g skin, at 45 °C for different times. Numbers denote time of gelatin extraction (h).	61

LIST OF FIGURES (Continued)

Figure	Page
16. SDS-PAGE patterns of BSP pretreated skin heated at different temperatures for 5 min and gelatins extracted from BSP pretreated skin heated at different temperatures. Gelatin was extracted at 45 ^o C for 12 h. C denote swollen skin treated with bigeye snapper pepsin before heat-treatment.	62
17. SDS-PAGE patterns of gelatin extracted from bigeye snapper skin in the absence and the presence of proteinase inhibitors at different concentrations. I and C denote collagen type I and gelatin extracted without proteinase inhibitor.	63
18. SDS-PAGE patterns of gelatin from bigeye snapper skin extracted by typical process (GT); gelatin extracted from skin treated with bigeye snapper pepsin (GA) and porcine pepsin (GP) at levels of 15 kUnits/g skin and gelatin from bovine bone (GB) under reducing and non-reducing conditions. HM and I denote high MW protein markers and collagen type I, respectively.	66
19. SDS-PAGE patterns of gelatin from bigeye snapper skin extracted by typical process (GT); gelatin extracted from skin treated with bigeye snapper pepsin (GA) and porcine pepsin (GP) and gelatin from bovine bone (GB) before and after gel setting.	69
20. Relative solubility of different gelatins from bigeye snapper skin. GT, GA and GP denote gelatin extracted by typical process, gelatin extracted from skin treated with bigeye snapper pepsin and porcine pepsin, respectively. GB denote gelatin from bovine bone.	70
21. Microstructure of gelatin gels (magnification: 10,000 X). GT: gelatin gel of bigeye snapper skin extracted by typical process. GA and GP: gelatin gels of bigeye snapper skin extracted by pepsin-aided process using with bigeye snapper pepsin and porcine pepsin, respectively. GB: gelatin gel of bovine bone.	73