

CONTENTS

| | Page |
|---|-------------|
| Contents..... | (8) |
| List of Tables..... | (14) |
| List of Figures..... | (16) |
| | |
| Chapter 1. Introduction and Literature Review | |
| Introduction..... | 1 |
| Literature review..... | 2 |
| Proteolytic enzymes..... | 2 |
| Acid proteases (Lysosomal cathepsins)..... | 3 |
| Neutral and Ca ²⁺ activated proteases..... | 3 |
| Alkaline proteases..... | 4 |
| Role of protease on fish muscle..... | 4 |
| Effect of protease on fish muscle..... | 5 |
| Effect of protease on textural properties of fish gels..... | 5 |
| Protease inhibitor..... | 6 |
| Natural protease inhibitor..... | 7 |
| Classification of protease inhibitors..... | 8 |
| Cysteine protease inhibitors..... | 8 |
| Serine protease inhibitors..... | 9 |
| Aspartic protease inhibitors..... | 10 |
| Metalloprotease inhibitors..... | 10 |
| Plasma protease inhibitor..... | 11 |
| Cysteine protease inhibitor in plasma..... | 12 |
| α_2 -macroglobulin..... | 12 |
| Kininogens..... | 13 |
| Albumin..... | 13 |
| Application of protease inhibitors in food processing..... | 13 |
| Objectives of study..... | 17 |

CONTENTS (Continued)

| | Page |
|---|-------------|
| Chapter 2. Chicken plasma protein affects gelation of surimi from bigeye snapper (<i>Priacanthus tayenus</i>) | |
| Abstract..... | 18 |
| Introduction..... | 18 |
| Materials and Methods..... | 20 |
| Results and Discussion | |
| Characterization of surimi and chicken plasma protein..... | 24 |
| Effect of CPP on textural properties of surimi..... | 28 |
| Effect of CPP on whiteness and expressible drip of surimi gels..... | 30 |
| Effect of CPP on protein solubility of surimi gels..... | 31 |
| Effect of CPP on TCA-soluble peptides of surimi gels..... | 33 |
| Effect of CPP on protein pattern of surimi gels..... | 34 |
| Effect of CPP on microstructure of surimi gels..... | 35 |
| Conclusion..... | 37 |
| Chapter 3. Rheological and textural properties of Pacific whiting surimi gels as influenced by chicken plasma protein | |
| Abstract..... | 38 |
| Introduction..... | 38 |
| Materials and Methods..... | 40 |
| Results and Discussion | |
| Effect of CPP on rheological property of Pacific whiting surimi..... | 43 |
| Effect of CPP on textural property and whiteness of Pacific whiting surimi gels..... | 47 |
| Effect of CPP on protein patterns of Pacific whiting gels..... | 50 |
| Conclusion..... | 53 |
| Chapter 4. Combination effect of chicken plasma protein and setting phenomenon on gel properties and cross-linking of bigeye snapper muscle proteins | |
| Abstract..... | 54 |

CONTENTS (Continued)

| | Page |
|--|-------------|
| Introduction..... | 54 |
| Materials and Methods..... | 56 |
| Results and Discussion | |
| Effects of CPP in combination with thrombin and/or CaCl_2 on textural properties of surimi gels..... | 61 |
| Effects of CPP in combination with thrombin and/or CaCl_2 on protein solubility and TCA-solubility peptides of surimi gels..... | 63 |
| Effect of CPP in combination with thrombin and/or CaCl_2 on pattern and microstructure of surimi gels..... | 64 |
| Effect of CPP in combination with setting on natural actomyosin cross-linking..... | 66 |
| Effect of TGase inhibitor on textural properties and electrophoretic patterns of surimi proteins..... | 69 |
| Effect of CPP in combination with setting on myosin cross-linking..... | 71 |
| Conclusion..... | 73 |
| Chapter 5. Chicken plasma protein: proteinase inhibitory activity and its effect on surimi gel properties | |
| Abstract..... | 74 |
| Introduction..... | 74 |
| Materials and Methods..... | 76 |
| Results and Discussion | |
| Inhibitory activity of CPP on sarcoplasmic proteinases..... | 81 |
| Inhibitory activity of CPP on the autolysis of mince and washed mince..... | 82 |
| Effect of CPP on textural properties of surimi gels..... | 86 |
| Effect of CPP on whiteness and expressible moisture of surimi gels..... | 86 |
| Effect of CPP on protein degradation in surimi gels..... | 89 |
| Microstructure of surimi gels..... | 91 |
| Conclusion..... | 92 |

CONTENTS (Continued)

| | Page |
|---|-------------|
| Chapter 6. Comparative study of chicken plasma protein and some protein additives on proteolysis and gel-forming ability of sardine (<i>Sardinella gibbosa</i>) surimi | |
| Abstract..... | 93 |
| Introduction..... | 93 |
| Materials and Methods..... | 95 |
| Results and Discussion | |
| Autolysis study of sardine surimi..... | 99 |
| Effect of CPP on autolysis of sardine surimi..... | 100 |
| Effect of CPP on textural properties of sardine surimi gels..... | 103 |
| Effect of CPP on whiteness and expressible moisture of sardine surimi gels... | 107 |
| Effect of CPP on degradation of sardine surimi gels..... | 109 |
| Effect of CPP on protein pattern of sardine surimi gels..... | 110 |
| Conclusion..... | 114 |
| Chapter 7. Cysteine proteinase inhibitor from chicken plasma: fractionation, characterization and autolysis inhibition of fish myofibrillar proteins | |
| Abstract..... | 115 |
| Introduction..... | 115 |
| Materials and Methods..... | 117 |
| Results and Discussion | |
| Polyethylene glycol fractionation..... | 122 |
| Ammonium sulfate fractionation..... | 124 |
| Protein pattern and inhibitory activity staining of CPI fraction..... | 126 |
| Thermal stability of CPI fraction..... | 127 |
| pH stability of CPI fraction..... | 129 |
| Salt stability of CPI fraction..... | 130 |
| Effect of CPI fraction on inhibition of fish mince autolysis..... | 131 |
| Effect of CPI fraction on inhibition of washed mince autolysis..... | 133 |
| Conclusion..... | 135 |

CONTENTS (Continued)

| | Page |
|---|------|
| Chapter 8. Effect of cysteine proteinase inhibitor containing fraction from chicken plasma on autolysis and gelation of Pacific whiting surimi | |
| Abstract..... | 136 |
| Introduction..... | 136 |
| Materials and Methods..... | 138 |
| Results and Discussion | |
| Autolysis profile of Pacific whiting surimi..... | 142 |
| Autolysis inhibition of Pacific whiting surimi by CPI fraction..... | 144 |
| Effect of CPI fraction on textural properties..... | 145 |
| Effect of CPI fraction on dynamic viscoelastic properties..... | 148 |
| Effect of CPI fraction on whiteness of surimi gels..... | 150 |
| Effect of CPI fraction on TCA-soluble peptides of surimi gels..... | 151 |
| Effect of CPI fraction on protein pattern of surimi gels..... | 152 |
| Conclusion..... | 153 |
| Chapter 9. Partial purification and characterization of cysteine proteinase inhibitor from chicken plasma | |
| Abstract..... | 154 |
| Introduction..... | 154 |
| Materials and Methods..... | 155 |
| Results and Discussion | |
| Purification of CPI from chicken plasma..... | 160 |
| Protein pattern and inhibitory activity staining of purified CPI..... | 161 |
| Thermal stability of purified CPI..... | 163 |
| Salt stability of purified CPI..... | 165 |
| Autolysis of arrowtooth flounder and Pacific whiting NAM..... | 166 |
| Autolysis of purified CPI on inhibition of NAM autolysis..... | 168 |
| Conclusion..... | 169 |

CONTENTS (Continued)

| | Page |
|--|-------------|
| Chapter 10. Summary and future works..... | 170 |
| References | 172 |
| Vitae | 194 |

LIST OF TABLES

| Table | Page |
|--|------|
| 1. Properties of some lysosomal proteases found in muscle..... | 4 |
| 2. Selected sources of protease inhibitors..... | 8 |
| 3. Mode of action of selected protease inhibitors..... | 11 |
| 4. Selected plasma protease inhibitors..... | 12 |
| 5. Commonly food grade inhibitors used in surimi processing..... | 15 |
| 6. Potential uses of inhibitors in food industry..... | 16 |
| 7. Proximate composition of surimi and dried chicken plasma protein (CPP)..... | 25 |
| 8. Solubility of surimi gels from bigeye snapper added with various concentrations of chicken plasma protein..... | 32 |
| 9. TCA-soluble peptide of surimi gel from bigeye snapper with various concentrations of chicken plasma protein..... | 33 |
| 10. TCA-soluble peptide content of surimi gels from Pacific whiting added with different levels of chicken plasma..... | 51 |
| 11. Protein solubility and TCA-soluble peptides of surimi gels added with chicken plasma protein in combination with thrombin and/or CaCl_2 | 64 |
| 12. Inhibitory activity of chicken plasma protein at different concentrations on sarcoplasmic proteinases from bigeye snapper and lizardfish muscle..... | 82 |
| 13. Whiteness and expressible moisture of surimi gels added with different concentrations of chicken plasma protein..... | 88 |
| 14. TCA-soluble peptides of surimi gels added with different concentrations of chicken plasma protein..... | 89 |
| 15. Effect of CPP on whiteness and expressible moisture of modori and kamaboko gel of sardine surimi..... | 108 |
| 16. Fractionation of cysteine proteinase inhibitor from chicken plasma by polyethylene glycol (PEG-4000)..... | 123 |
| 17. Fractionation of cysteine proteinase inhibitor from chicken plasma by ammonium sulfate (AS)..... | 125 |
| 18. Purification of cysteine proteinase inhibitor from chicken plasma protein..... | 160 |

LIST OF FIGURES

| Figure | Page |
|--|------|
| 1. Protein pattern of surimi from bigeye snapper and chicken plasma protein..... | 27 |
| 2. Breaking force and deformation of surimi gels added with various concentrations of chicken plasma protein..... | 29 |
| 3. Whiteness of surimi gels from bigeye snapper added with various concentrations of chicken plasma protein..... | 30 |
| 4. Expressible drip of surimi gels from bigeye snapper added with various concentrations of chicken plasma protein..... | 31 |
| 5. SDS-PAGE protein pattern of surimi gels from bigeye snapper added with various concentrations of chicken plasma protein..... | 35 |
| 6. Electron microscopic image of surimi gels from bigeye snapper..... | 36 |
| 7. Effect of chicken plasma on changes in storage modulus (G') and loss modulus (G'') of Pacific whiting surimi paste heated from 20 to 90°C at a rate of 1°C/min..... | 44 |
| 8. Effect of chicken plasma on changes in phase angle of Pacific whiting surimi paste heated from 20 to 90°C at a rate of 1°C/min..... | 46 |
| 9. SDS-PAGE pattern of Pacific whiting surimi paste added with chicken plasma at different levels induced by linear heating from 20 to 90°C at a rate of 1°C/min..... | 47 |
| 10. Effect of chicken plasma on the textural properties of Pacific whiting surimi gels..... | 49 |
| 11. Whiteness of surimi gels from Pacific whiting added with various levels of chicken plasma..... | 50 |
| 12. SDS-PAGE pattern of modori gels from Pacific whiting surimi added with various levels of chicken plasma..... | 52 |
| 13. SDS-PAGE pattern of kamaboko gels from Pacific whiting surimi added with various levels of chicken plasma..... | 53 |
| 14. Breaking force (A) and deformation (B) of surimi gel added with chicken plasma protein in combination with thrombin and/or CaCl_2 | 62 |

LIST OF FIGURES (Continued)

| Figure | Page |
|--|------|
| 15. SDS-PAGE patterns of surimi gels added with chicken plasma protein in combination with thrombin and/or CaCl_2 | 65 |
| 16. Electron microscopic image of surimi gels from bigeye snapper (magnification: 10,000X)..... | 66 |
| 17. SDS-PAGE patterns of natural actomyosin (NAM) paste added with chicken plasma protein in combination with thrombin and/or CaCl_2 | 67 |
| 18. SDS-PAGE patterns of natural actomyosin (NAM) paste added with chicken plasma protein in combination with thrombin and/or CaCl_2 as affected by incubation time at 40°C | 68 |
| 19. Breaking force (A) and deformation (B) of surimi gels added with/without CPP in the presence of TGase inhibitor..... | 69 |
| 20. SDS-PAGE patterns of surimi gels without chicken plasma protein (A) and with chicken plasma protein (B) in the absence and presence TGase inhibitors..... | 70 |
| 21. SDS-PAGE pattern of myosin added with chicken plasma protein in combination with thrombin and/or CaCl_2 | 71 |
| 22. SDS-PAGE patterns of myosin added with chicken plasma protein in combination with thrombin and/or CaCl_2 as affected by incubation time at 40°C | 72 |
| 23. Inhibitory activity of chicken plasma protein at different concentrations on the autolysis of mince and washed mince from bigeye snapper (A) and lizardfish (B)..... | 83 |
| 24. Protein patterns of mince (A) and washed mince (B) added with different concentrations of chicken plasma protein..... | 85 |
| 25. Breaking force and deformation of surimi gels added with different concentrations of chicken plasma protein..... | 87 |
| 26. Protein patterns of bigeye snapper (A) and lizardfish (B) gels prepared under different conditions..... | 90 |
| 27. Scanning electron microscopic image of modori gel without (A) and with 2 % (w/w) chicken plasma protein (B)..... | 92 |

LIST OF FIGURES (Continued)

| Figure | Page |
|---|------|
| 28. TCA-soluble peptide content in sardine surimi incubated at different temperatures and times..... | 99 |
| 29. Effect of CPP or other protein additives at different concentrations on inhibition of autolysis in sardine surimi at 70°C for 60 min..... | 101 |
| 30. Autolysis pattern of sardine surimi added with CPP or other protein additives at different concentrations. Samples were incubated at 70°C for 60 min..... | 102 |
| 31. Breaking force (A) and deformation (B) of sardine modori gels added with CPP or other protein additives at different concentrations..... | 104 |
| 32. Breaking force (A) and deformation (B) of sardine kamaboko gels added with CPP or other protein additives at different concentrations..... | 106 |
| 33. TCA-soluble peptide content in sardine modori and kamaboko gels added with CPP or other protein additives at different concentrations..... | 110 |
| 34. Protein pattern of modori gels added with CPP or other protein additives at different concentrations..... | 111 |
| 35. Protein pattern of kamaboko gels added with CPP or other protein additives at different concentrations..... | 113 |
| 36. Protein pattern of chicken plasma and different fractions from polyethylene glycol (PEG-4000) fractionation..... | 124 |
| 37. Protein pattern of chicken plasma and different fraction from ammonium sulfate (AS) fractionation..... | 126 |
| 38. Protein pattern (A) and inhibitory activity staining for papain (B) of cysteine proteinase inhibitor fraction from chicken plasma..... | 127 |
| 39. Effect of heating temperature (A) and time (B) on stability of cysteine proteinase inhibitor fraction from chicken plasma..... | 128 |
| 40. Effect of pH on stability of cysteine proteinase inhibitor fraction from chicken plasma..... | 129 |
| 41. Effect of salt content on stability of cysteine proteinase inhibitor fraction from chicken plasma..... | 130 |
| 42. Effect of cysteine proteinase inhibitor fraction from chicken plasma on autolysis inhibition of arrowtooth flounder and Pacific whiting mince..... | 132 |

LIST OF FIGURES (Continued)

| Figure | Page |
|--|------|
| 43. Effect of cysteine proteinase inhibitor fraction from chicken plasma on Autolysis inhibition of arrowtooth flounder and Pacific whiting washed mince... | 134 |
| 44. Electrophoresis pattern of Pacific whiting surimi without (A) and with 1% CPI fraction from chicken plasma (B) incubated at 55°C for 0–180 min..... | 143 |
| 45. Inhibitory activity of CPI fraction from chicken plasma at different concentrations on the autolysis of Pacific whiting surimi at 55°C for 60 min.... | 145 |
| 46. Autolytic pattern of Pacific whiting surimi added with CPI fraction from chicken plasma at different concentrations..... | 146 |
| 47. Breaking force and deformation of surimi gels added with CPI fraction from chicken plasma at different concentrations..... | 147 |
| 48. Effect of CPI fraction from chicken plasma on storage modulus (G') of Pacific whiting surimi paste heated from 20 to 90°C at a rate of 1°C/min..... | 149 |
| 49. Whiteness of Pacific whiting surimi gels added with different concentrations of CPI fraction from chicken plasma..... | 150 |
| 50. TCA-soluble peptide content of Pacific whiting modori gels added with CPI fraction from chicken plasma at different concentrations..... | 151 |
| 51. SDS-PAGE pattern of Pacific whiting surimi gels added with different concentrations of CPI fraction from chicken plasma..... | 152 |
| 52. Elution profiles of cysteine proteinase inhibitor from chicken plasma on CM-papain-Sepharose-4B..... | 161 |
| 53. Protein pattern (A) and inhibitory activity staining for papain (B) of purified CPI from chicken plasma under non-reducing (a) and reducing (b) condition... | 162 |
| 54. Effect of heating temperature (A) and time (B) on stability of purified CPI from chicken plasma..... | 164 |
| 55. Effect of salt content on stability of purified CPI from chicken plasma..... | 166 |
| 56. SDS-PAGE of natural actomyosin without (A) and with 150 units/ml NAM (B) incubated at 60 and 55°C for arrowtooth flounder and Pacific whiting, respectively..... | 167 |

LIST OF FIGURES (Continued)

| Figure | | Page |
|---|--|-------------|
| 57. SDS-PAGE pattern of natural actomyosin added with purified CPI from chicken plasma at different levels..... | | 169 |