

Contents

	Pages
Abstract	(2)
Acknowledgment	(7)
Contents	(8)
List of Tables	(12)
List of Figures	(14)
Chapter	
1. Introduction	1
Literature Review	3
1. Myofibrillar proteins composition	3
2. Functional properties of fish proteins	7
2.1 Water holding capacity	8
2.2 Solubility	9
2.3 Gel-forming ability	9
3. Denaturation of surimi proteins during freezing	11
3.1 Effect of freezing and frozen storage on muscle proteins	11
3.2 Effect of freezing and frozen storage on gel-forming ability	13
3.3 Effect of freeze-thawing on muscle proteins	13
4. Cryoprotectants for surimi	14
4.1 Sugar and polyhydric alcohol	14
4.2 Mechanisms of protein cryoprotectant	17
4.3 Application of cryoprotectants	21
Objective	30

Contents (continued)

	Pages
2. Materials and methods	31
1. Fish preparation	31
2. Chemicals	31
3. Instrument	32
4. Natural actomyosin and surimi preparation	32
5. Cryoprotective study in NAM	35
6. Effect of cryoprotectant on the aggregation of NAM	35
7. Cryoprotective study in surimi	36
8. Statistical analysis	39
3. Result and Discussion	40
1. Cryoprotective effect of trehalose, sucrose and sorbitol alone at different concentration in NAM with 1 and 2 freeze-thaw cycles	40
1.1 Changes in Ca ²⁺ -ATPase activity	40
1.2 Changes in sulfhydryl content	42
1.3 Changes in disulfide bonds content	44
1.4 Changes in surface hydrophobicity	47
1.5 Changes in solubility in 0.6 M KCl	48
2. Cryoprotective effect of different formula of cryoprotectants in NAM with 1 and 2 freeze-thaw cycles.	51
2.1 Changes in Ca ²⁺ -ATPase activity	51
2.2 Changes in sulfhydryl content and disulfide bonds content	52
2.3 Changes in surface hydrophobicity	55

Contents (continued)

	Pages
2.4 Changes in solubility in 0.6 M KCl	57
3. Characterization of NAM aggregate with and without cryoprotectants after multiple freeze-thaw cycles	60
1. Solubility of NAM aggregate in various denaturing solutions	60
2. SDS-PAGE of NAM aggregate in various denaturing solutions	63
4. Changes in physicochemical and gelling properties surimi with and without cryoprotectants during frozen storage	66
1. Changes in pH during frozen storage	66
2. Changes in ATPase activity during frozen storage	67
3. Changes in Ca ²⁺ -sensitivity during frozen storage	70
4. Changes in total sulfhydryl content during frozen storage	71
5. Changes in disulfide bonds content during frozen storage	73
6. Changes in surface hydrophobicity during frozen storage	74
7. Changes in solubility in 0.6 M KCl during frozen storage	76
8. Changes in protein patterns during frozen storage	77
9. Changes in breaking force and deformation during frozen storage	80
10. Change in expressible moisture during frozen storage	82
11. Changes in microstructure of surimi gel during frozen storage	84
12. Changes in whiteness during frozen storage	87

Contents (continued)

	Pages
5. Change in physicochemical and gelling properties of surimi with and without cryoprotectants subjected to multiple freeze-thaw cycles	89
1. Changes in pH of surimi added with different cryoprotectants subjected to multiple freeze-thaw cycles	89
2. Changes in ATPase activity	89
3. Changes in Ca ²⁺ -sensitivity	91
4. Changes in total sulfhydryl contents and disulfide bonds content	93
5. Changes in surface hydrophobicity	96
6. Changes in solubility in 0.6 M KCl	97
7. SDS-PAGE of surimi	98
8. Changes in breaking force and deformation	101
9. Changes in expressible moisture of surimi gel	103
10. Changes in whiteness of surimi	105
4. Conclusion	107
References	108
Appendices	128
Vitae	142

List of Tables

Tables	Pages
1. Characteristics of sugars and polyalcohols	15
2. Relative sweetness of sweeteners	23
3. Cryoprotectant formulae used in bigeye snapper NAM	35
4. Effect of individual cryoprotectant and cryoprotectant blends on Ca^{2+} ATPase activity in NAM subjected to 1 and 2 freeze-thaw cycles.	52
5. Effect of individual cryoprotectant and cryoprotectant blends on total sulfhydryl content in NAM subjected to 1 and 2 freeze-thaw cycles.	54
6. Effect of individual cryoprotectant and cryoprotectant blends on disulfide bonds content in NAM subjected to 1 and 2 freeze-thaw cycles.	55
7. Effect of individual cryoprotectant and cryoprotectant blends on surface hydrophobicity in NAM subjected to 1 and 2 freeze-thaw cycles	57
8. Effect of individual cryoprotectant and cryoprotectant blends on solubility in 0.6 M KCl in NAM subjected to 1 and 2 freeze-thaw cycles	58
9. Changes in pH of surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	66
10. Changes in whiteness of surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	88

List of Tables (continued)

Tables	Pages
11. Changes in pH of surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles	89
12. Changes in whiteness of surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles	106

List of Figures

Figures	Pages
1. Structure of myosin	4
2. Structure of actin, troponin and tropomyosin	6
3. Conversion of protein native state (N) to the denatured state (D) in water or sucrose solution	18
4. Hydrophobic interaction in an aqueous environment	20
5. Effect on the mobility of water, and consequently on the entropy of the system, when two hydrophobic groups are separated in solution (top) as opposed to being in association (bottom)	21
6. Structure of sucrose	22
7. Structure of sobitol	24
8. Structure of trehalose	26
9. Structure of trehalose-water complex	28
10. Scheme for surimi preparation	33
11. Scheme for surimi gel preparation	37
12. Effect of different cryoprotectants at various concentrations on changes of Ca^{2+} -ATPase activity in NAM (2.5 mg/ml, pH 7.0, 0.6 M KCl) subjected to 1(a) and 2(b) freeze-thaw cycles.	42
13. Effect of different cryoprotectants at various concentrations on changes of total sulfhydryl content in NAM (2.5 mg/ml, pH 7.0, 0.6 M KCl) subjected to 1(a) and 2(b) freeze-thaw cycles.	45

List of Figures

Figures	Pages
14. Effect of different cryoprotectants at various concentrations on changes of disulfide bonds content in NAM (2.5 mg/ml, pH 7.0, 0.6 M KCl) subjected to 1(a) and 2(b) freeze-thaw cycles.	46
15. Effect of different cryoprotectants at various concentrations on changes of surface hydrophobicity in NAM (2.5 mg/ml, pH 7.0, 0.6 M KCl) subjected to 1(a) and 2(b) freeze-thaw cycles.	48
16. Effect of different cryoprotectants at various concentrations on changes of %solubility in 0.6 M KCl in NAM (2.5 mg/ml, pH 7.0, 0.6 M KCl) subjected to 1(a) and 2(b) freeze-thaw cycles.	50
17. Extractability of protein aggregate from NAM with and without different cryoprotectants in free solutions after freeze-thawing for 2 (a) and 4(b) cycles.	62
18. SDS-PAGE pattern of NAM aggregate with and without cryoprotectants after 2 (a) and 4 (b) freeze-thaw cycles, dissolved in free denaturing solution, N: non-reducing; R: reducing; S ₁ : 1%SDS; S ₂ : 1%SDS+8Murea; S ₃ : 1%SDS+8Murea+2%βME	64

List of Figures

Figures	Pages
19. Changes in Ca^{2+} -ATPase (a), Mg^{2+} -ATPase (b), Mg^{2+} - Ca^{2+} -ATPase (c), Mg^{2+} -EGTA-ATPase (d) activity of surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	69
20. Changes in Ca^{2+} -sensitivity in surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	71
21. Changes in total sulfhydryl content in surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	72
22. Changes in disulfide bonds content in surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	73
23. Changes in surface hydrophobicity in surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	75
24. Changes in solubility in 0.6 M KCl in surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	77
25. SDS-PAGE of surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks, 1: control; 2: 8%trehalose; 3: the blend including 5.34%trehalose+ 1.33%sucrose+1.33%sorbitol; and 4: commercial cryoprotectants; R: reducing condition; N: non-reducing condition.	79

List of Figures

Figures	Pages
26. Changes in breaking force (a) and deformation (b) in surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	82
27. Changes in expressible moisture in surimi added with different cryoprotectants during frozen storage at -18°C for 12 weeks.	84
28. Scanning electron micrographs of bigeye snapper surimi gels with and without cryoprotectants after frozen storage for week 0 and 12, at -18°C	86
29. Changes in Ca^{2+} -ATPase (a), Mg^{2+} -ATPase (b), Mg^{2+} - Ca^{2+} -ATPase (c), Mg^{2+} -EGTA-ATPase (d) activity of surimi added with and without different cryoprotectants and subjected to multiple freeze-thaw cycles.	92
30. Changes in Ca^{2+} -sensitivity in surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles.	93
31. Changes in sulfhydryl content in surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles.	95
32. Changes in disulfide bonds content in surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles.	95
33. Changes in surface hydrophobicity in surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles.	97

List of Figures

Figures	Pages
34. Changes in solubility in 0.6 M KCl in surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles.	98
35. SDS-PAGE of surimi added with and without different cryoprotectants and subjected multiple freeze-thaw cycles, 1: control; 2: 8%trehalose;3: the blends including 5.34%trehalose+1.33%sucrose+1.33%sorbitol; and 4: commercial cryoprotectants; R: reducing condition; N: non-reducing condition.	100
36. Changes in breaking force (a) and deformationin (b) surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles.	103
37. Changes in expressible moisture in surimi added with different cryoprotectants and subjected to multiple freeze-thaw cycles.	105