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Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage

Aimei Zhou [a,c,*], Soottawat Benjakul [b], Ke Pan [a], Jie Gong [a], Xin Liu [a]

[a] *Department of Food Science and Engineering, College of Food Science, South China Agricultural University, Guangzhou 510642, China*
[b] *Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai 90112, Thailand*
[c] *Department of Food Engineering, College of Food and Bioengineering, South China University of Technology, Guangzhou 510640, China*

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Abstract

The cryoprotective effects of trehalose and sodium lactate at level of 8% (w/w) in tilapia surimi were studied in comparison with a conventional cryoprotectant (sucrose/sorbitol, 1:1) during extended storage at −18 °C for up to 24 weeks. All present cryoprotectants retarded the protein changes as evidenced by the lowered decrease in salt extractable protein (SEP), Ca^{2+}-ATPase activity, total sulfhydryl content as well as the impeded increase in disulfide bond content and surface hydrophobicity. The gel-forming ability of frozen surimi was more retained with addition of cryoprotectants. Among all cryoprotectants used, trehalose exhibited the greatest protective effect on protein denaturation as shown by the effectiveness in maintaining Ca^{2+}-ATPase activity and protein solubility. Additionally, the greatest breaking force and deformation were obtained in surimi added with 8% trehalose throughout the frozen storage up to 24 weeks. Sodium lactate showed a similar cryoprotective effect to sucrose/sorbitol blend. Therefore, trehalose and sodium lactate appeared to be promising alternative cryoprotectants for surimi owing to their low sweetness and caloric value.

Keywords: Cryoprotectant; Protein denaturation; Gelation; Frozen storage; Functional properties

1. Introduction

Surimi is the wet concentrate of the myofibrillar proteins of fish muscle, that is mechanically deboned, water-washed and frozen (Okada, 1992). It possesses some important functional properties such as gel-forming ability and water-holding capacity. Therefore, it has become the intermediate material for surimi-based products. Surimi can be produced from both marine and fresh water fish. Tilapia has been used for surimi production due to its availability and good gel-forming ability. It is reported that the total output of tilapia in the world amounted to 1,266,000 tons in 2000 (Li, 2003). In China, tilapia farming is developing rapidly and its output in 2002 was 707,000 tons, accounting for 55% of the total output in the world (Li, 2003).

During frozen storage, surimi may lose its functional properties as a result of denaturation and/or aggregation of myofibrillar proteins (Shenouda, 1980). The addition of cryoprotectants is required in order to retain its functional properties (MacDonald & Lanier, 1994). Many compounds, including some low molecular weight sugars and polyols as well as many amino acids, carboxylic acids and polyphosphates, were found to be cryoprotective (Arakawa & Timasheff, 1982; Park & Lanier, 1987; Sych, Lacroix, & Carrier, 1991). The most commonly used cryoprotectant in the surimi industry is the 1:1 mixture of sucrose and sorbitol at a concentration of

* Corresponding author. Fax: +86 20 85280270.
E-mail address: zuojiahui@21cn.com (A. Zhou).

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8% (w/w). Though this commercial blend has been shown to have excellent cryoprotective effects on fish myofibrillar proteins (Lee, 1984; Yoon & Lee, 1990), it can impart an excessive sweet taste and high caloric value in final surimi products. As a consequence, other cryoprotectants with reduced sweetness and caloric value have been used in surimi. So far, the cryoprotective effects of lactitol, Palatinit®, polydextrose®, starch hydrolysate products such as maltodextrin, highly concentrated branched oligosaccharides and linear oligosaccharides have been extensively studied (Auh, Lee, Kim, Yoon, & Park, 1999; Carvajal, MacDonald, & Lanier, 1999; Miura, Takayanagi, & Nishimura, 1992; Park, Lanier, & Green, 1988; Sultanbawa & Li-Chan, 1998, 2001; Sych, Lacroix, Adambounou, & Castaigne, 1990; Sych et al., 1991).

Sodium lactate has no sweetness and contains low caloric value. It is currently GRAS (generally recognized as safe) for use as an emulsifier, flavour enhancer, flavouring agent, humectant, and pH control agent (MacDonald & Lanier, 1994). Sodium lactate has been demonstrated to be an effective stabilizer against both freeze–thaw- and heat-denaturation of tilapia (*Tilapia nilotica* × *Tilapia aurea*) actomyosin (MacDonald & Lanier, 1994; MacDonald, Lanier, & Carvajal, 2000).

Trehalose (D-glucopyranosyl-α (1 → 1)-D-glucopyranoside) is a non-reducing disaccharide with low caloric value and low sweetness, only 45% of that of sucrose (Hu, Xia, Chen, & Cai, 2004). It is very stable in properties and can protect biological cells under adverse circumstances (Ren, Zhuang, Liao, & Mu, 2001). Recently, trehalose has been found to have protective effect against thermal inactivation of enzymes and its effectiveness was correlated with its large hydration volume (MacDonald et al., 2000). So far, no information regarding the cryoprotective effect of both sodium lactate and trehalose, especially in the surimi produced from freshwater fishes, including tilapia, has been reported. The objectives of this work were to investigate the ability of sodium lactate and trehalose to stabilize tilapia surimi during 6 months frozen storage and to compare their effectiveness with that of the commercial blend (4% sucrose and 4% sorbitol).

2. Materials and methods

2.1. Chemicals and cryoprotectants

Adenosine 5′-triphosphate (disodium salt) was obtained from Duchefa Biochemie (Haarlem, The Netherlands). Bovine serum albumin, tris(hydroxymethyl) amino-methane, urea and glycine were purchased from Shanghai Baiao Biotechnology Co., Ltd (Shanghai, China). 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), 8-anilino-1-naphthalene-sulfonic acid (ANS) and sodium dodecyl sulfate (SDS) were obtained from Sigma–Aldrich Inc. (Saint Louis, MO, USA). Guanidinethiocyanate was purchased from Amresco (Solon, OH, USA). Trehalose (food grade) was provided by Nanning Zhongruo Bioengineering Co., Ltd. (Guangxi, China). Sodium lactate (analytical grade) was obtained from Chengdu United Chemical & Pharmaceutical Co., Ltd. (Chengdu, China). Sucrose (food grade) was purchased in the local department store. Liquid sorbitol (food grade) was obtained from Guangzhou Honsea Chemical Co., Ltd. (Guangzhou, China).

2.2. Surimi preparation

Fresh Tilapia (*Sarotherodon nilotica*) surimi (about 80% moisture content) was purchased from Nanhai Food Import & Export Co., Ltd. (Guangzhou, China) and was transported in ice to the College of Food Science, South China Agricultural University, Guangzhou, China within 1 h. Surimi was kept in ice during preparation.

Fresh surimi was added with 8% trehalose, 8% sodium lactate or 8% commercial blend (sucrose/sorbitol, 1:1). The mixture was mixed thoroughly using a CH-10 mixer (Taixing Medical Machine Manufacturer, Jiangsu, China) for 90 s in a walk-in cold room. Surimi without cryoprotectant was used as the control. The samples (400 g) were packaged in a polyethylene bag and frozen by air-blast freezer at −18 °C. The frozen samples were kept at −18 °C and taken for analyses at week 0, 1, 3, 5, 9, 20 and 24. Each experiment was run in triplicate.

2.2.1. Preparation of natural actomyosin (NAM)

NAM was prepared according to the method of Benjakul, Seymour, and Morrissey (1997) with a slight modification. Surimi (3 g) was homogenized in 30 ml of chilled 0.6 M KCl, pH 7.0 for 4 min using a FJ-200 homogenizer (Shanghai Specimen Model Co., Shanghai, China). The container with sample was placed in ice. Each 20 s of homogenization was followed by a 20 s rest interval to avoid overheating during extraction. The homogenate was centrifuged at 8370*g* for 30 min at 4 °C using a TGL-16G centrifuge (Shanghai Anting Scientific Instrument Co., Ltd., Shanghai, China). Three volumes of chilled distilled water were added to precipitate NAM. NAM was collected by centrifuging at 8370*g* for 20 min at 4 °C, and the pellet was dissolved by stirring in an equal volume of chilled 0.6 M KCl, pH 7.0. Undissolved debris was removed by centrifugation at 8370*g* for 30 min at 4 °C. NAM was kept in ice during all analyses.

2.2.2. Determination of Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity was assayed according to the method of Benjakul et al. (1997) with a slight

modification. NAM was diluted to 2.5 mg/ml with 0.6 M KCl, pH 7.0. A 0.5 ml volume of diluted solution was added into 0.3 ml of 0.5 M Tris–maleate, pH 7.0. To the mixture, 0.5 ml of $CaCl_2$ and 3.45 ml of distilled water were added, followed by the addition of 0.25 ml of 20 mM ATP to initiate the reaction. The reaction was conducted at 25 °C for 10 min and terminated by adding 2.5 ml of chilled 15% TCA (w/v). The reaction mixture was centrifuged at 6045*g* for 5 min. The inorganic phosphate liberated in the supernatant was measured by ammonium molybdate (Zhang & Qu, 2003). Specific activity was expressed as μmoles inorganic phosphate released per mg protein per minute. A blank measurement was conducted by adding the chilled TCA prior to the addition of ATP.

2.2.3. Determination of total sulfhydryl content

Total sulfhydryl content was determined using DTNB according to the method of Benjakul et al. (1997) with a modification. To 0.5 ml of NAM solution (4 mg/ml), 4.5 ml of 0.2 M Tris–HCl buffer, pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA, were added. To 4 ml of the mixture, 0.4 ml of 0.1% DTNB in distilled water (pH 7.2) was added and incubated at 40 °C for 25 min. The absorbance at 412 nm was measured using a 752-UV/Grating Spectrophotometer (Shanghai Accurate Scientific Instrument Co., Ltd, Shanghai, China). A blank was conducted by replacing the sample with 0.6 M KCl. Sulfhydryl content was calculated using the extinction coefficient of 13,600 M^{-1} cm^{-1}.

2.2.4. Determination of disulfide bond content

Disulfide bond in NAM was assayed using 2-nitro-5-thiosulfobenzoate (NTSB) according to the method of Benjakul, Visessanguan, Thongkaew, and Tanaka (2003) with a slight modification. To 0.1 ml of NAM solution (4 mg/ml), 3 ml of freshly prepared NTSB assay solution, pH 9.5, were added. The mixture was incubated in dark at room temperature (25–27 °C) for 25 min. Absorbance at 412 nm was measured. A blank was conducted by replacing the sample with distilled water. Disulfide bond was calculated using the extinction coefficient of 13,900 M^{-1} cm^{-1}.

2.2.5. Determination of surface hydrophobicity

Surface hydrophobicity was determined as described by Benjakul et al. (1997) using 1-anilinonaphthalene-8-sulphonic acid (ANS) as a probe. NAM dissolved in 10 mM phosphate buffer, pH 6.0 containing 0.6 M NaCl was diluted to 0.125, 0.25, 0.5 and 1 mg/ml using the same buffer. The diluted protein solution (2 ml) was added with 10 μl of 8 mM ANS in 0.1 M phosphate buffer, pH 7.0. The fluorescence intensity of ANS-conjugates was measured using a 960 MC Spectrofluorophotometer (Shanghai China Analytical Instrument General Factory, Shanghai, China) at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. The initial slope of the plot of fluorescence intensity versus NAM concentration was referred to as SoANS.

2.2.6. Determination of salt extractable protein (SEP) content

Determination of SEP content of surimi samples was carried out according to the method of Wu, Wang, and Han (1999). The sample (1 g) was mixed with 100 ml of either high ionic strength phosphate buffer (0.5 M KCl–0.01 M NaH_2PO_4–0.03 M Na_2HPO_4) or low ionic strength phosphate buffer (0.025 M NaH_2PO_4–0.025 M Na_2HPO_4). The mixtures were homogenized for 4 min using a FJ-180 homogenizer. Both homogenates were kept at room temperature for 3 and 1 h, respectively, to extract proteins before centrifuging at 7440*g* for 10 min using a TGL-16G centrifuge. The 10 ml of the supernatant of each mixture were added with 10 ml 15% TCA to precipitate proteins followed by centrifuging at 7440*g* for 10 min. In each precipitate, 5 ml of 1 M NaOH was added to dissolve the proteins followed by protein determination. SEP content was expressed as the protein content extracted by the high ionic strength phosphate butter subtracted by the protein content extracted by the low ionic strength phosphate buffer.

2.2.7. Protein determination

Protein concentration was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as a standard.

2.2.8. Surimi gel preparation

Surimi (200 g) was mixed with 2.5% salt in a DS-1 High Speed Mashing Machine (Shanghai Specimen Model Co., Shanghai, China) and the moisture content was adjusted to 85% with iced water. The mixture was chopped below 10 °C for 5 min to obtain the homogeneous sol. The sol was stuffed into polyvinylidine casing with a diameter of 3 cm and both ends of casing were sealed tightly. Surimi sol was then subjected to setting at 40 °C for 30 min before heating at 90 °C for 20 min. The gels were cooled in iced water and stored for 24 h at 4 °C prior to analyses.

2.2.9. Texture analysis

Texture analysis of surimi gels was conducted using a texture analyzer TA-XT2I/25 (Stable Micro System, UK). Gels were equilibrated and evaluated at room temperature. Six cylinder-shaped samples with a length of 2.5 cm, were prepared and subjected to determination. Breaking force (gel strength) and deformation (elasticity/deformability) were measured using the texture analyzer equipped with a spherical plunger (diameter 5 mm; depression speed 60 mm/min).

2.2.10. Statistical analysis

One-way ANOVA analysis of variance and least significant difference (LSD) were performed for comparisons among means using the Statistical Analysis System for 1997.

3. Results and discussion

3.1. Effect of different cryoprotectants on the salt extractable protein (SEP) content of tilapia surimi during frozen storage

The changes in SEP of surimi added with different cryoprotectants during the frozen storage at −18 °C are shown in Fig. 1. Generally, SEP in all samples decreased sharply during the first week of storage. However, the highest rate of decrease was found in the control. SEP of the control at week 24 was reduced by 44.8% compared with that obtained in fresh surimi (week 0). For surimi added with trehalose, sodium lactate and sucrose/sorbitol, SEP of 56.8, 56.3 and 53.0 mg/g were observed, respectively, and were decreased by 24.4%, 29.6% and 24.7%, compared to their corresponding initial SEPs, respectively. From the result, it was noted that the addition of cryoprotectants at a level of 8% resulted in the lowered SEP, compared with the control at week 0. This was probably due to the dilution effect of cryoprotectants on the protein content in the surimi. In general, the control showed the lowest SEP throughout the storage up to 24 weeks, compared with other samples ($p < 0.05$), suggesting the preventive effect of the cryoprotectants on protein denaturation. From the result, no difference in SEP between surimi added with trehalose, sodium lactate and sucrose/sorbitol was found at week 20–24 ($p > 0.05$). However, the greater SEP was found in samples added with trehalose and sodium lactate during the first 9 weeks of storage, compared with that of sample added with sucrose/sorbitol.

The decrease in protein solubility is a primary indicator of protein denaturation during frozen storage resulting from the formation of hydrogen or hydrophobic bonds, as well as disulfide bonds and ionic interactions (Auh et al., 1999; Benjakul & Bauer, 2000; Jiang, Hwang, & Chen, 1988; Lim & Haard, 1984). The sharp decrease in SEP of tilapia surimi without any cryoprotectant showed that proteins underwent denaturation induced by frozen storage. The rapid decrease in protein solubility during frozen storage has been noted for other fish muscle systems when cryoprotectants are not incorporated (Sych et al., 1990, 1991; Sultanbawa & Li-Chan, 1998; Auh et al., 1999). Slower decrease in SEP of cryoprotectant-added surimi suggested that trehalose, sodium lactate and the commercial cryoprotectant had good cryoprotective effect on tilapia surimi proteins, and the effects of trehalose and sodium lactate were comparable to those of the commercial cryoprotectant, especially when the storage time was extended.

3.2. Effect of different cryoprotectants on the Ca^{2+}-ATPase activity of tilapia surimi during frozen storage

During storage of 24 weeks at −18 °C, Ca^{2+}-ATPase activity of the NAM from surimi without cryoprotectants decreased markedly and no activity was observed after 9 weeks (Fig. 2). Ca^{2+}-ATPase activity of NAM from the surimi added with trehalose, sodium lactate and sucrose/sorbitol decreased at a slower rate than that of the control. No marked changes in Ca^{2+}-ATPase activity of NAM were found in cryoprotectant-added samples after 9 weeks of storage ($p > 0.05$). When comparing the activity of NAM from different samples, that added with trehalose showed the greatest activity within the first 9 weeks ($p < 0.05$). During week 20 and 24, Ca^{2+}-ATPase activity of NAM extracted from surimi added with sodium lactate tended to show a slightly

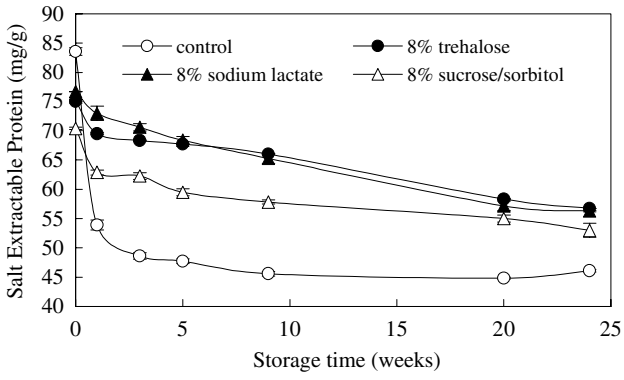

Fig. 1. Effect of different cryoprotectants on the salt extractable protein content (mg/g) of tilapia surimi as a function of storage time at −18 °C. Bars indicate the standard deviation from triplicate determinations.

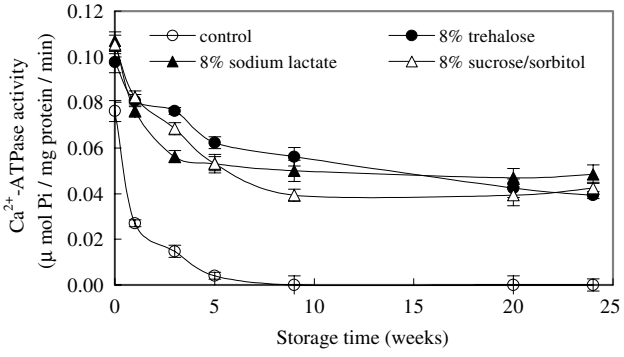

Fig. 2. Effect of different cryoprotectants on the Ca^{2+}-ATPase activity of natural actomyosin extracted from tilapia surimi as a function of storage time at −18 °C. Bars indicate the standard deviation from triplicate determinations.

higher activity, while no differences were found between samples added with trehalose and sucrose/sorbitol ($p > 0.05$).

Ca^{2+}-ATPase activity is a good indicator of the integrity of the myosin molecule (Benjakul et al., 1997, 2003; Benjakul, Visessanguan, & Tueksuban, 2003; Roura & Crupkin, 1995). The globular heads of myosin are responsible for Ca^{2+}-ATPase activity. A sharp decrease in activity for the tilapia surimi without cryoprotectants during extended frozen storage indicated the denaturation of myosin, especially in the head region. Similar results are found in other fish muscle systems without cryoprotectants (Auh et al., 1999; Benjakul et al, 2003; Damodaran & Gopakumar, 1992; Jiang et al., 1988; Ramirez, Martin-Polo, & Bandman, 2000). Benjakul et al. (2003) reported the marked decrease in Ca^{2+}-ATPase activity in lizardfish, croaker, threadfin bream and bigeye snapper muscle after 24 weeks of frozen storage at −18 °C and the decrease in ATP activity was postulated to be due to the conformational changes of myosin globular head as well as the aggregation of this portion. Myosin head possesses ATPase activity and its conformational changes were caused by ice crystals and the increase in ionic strength of the system (Benjakul et al., 1997). The rearrangement of protein via protein–protein interaction also contribute to the loss in activity (Benjakul & Bauer, 2000). In addition, the oxidation of sulfhydryl groups on the active site of actomyosin might also induce the decrease in Ca^{2+}-ATPase activity (Benjakul & Bauer, 2000).

The results showed that the incorporation of trehalose, sodium lactate or sucrose/sorbitol could prevent the decrease in Ca^{2+}-ATPase activity, indicating that these compounds had cryoprotective effects on tilapia muscle proteins during extended frozen storage. The good cryoprotective effect of sodium lactate was also confirmed by MacDonald and Lanier (1994). Sodium lactate at the level of 6% could recover the Ca^{2+}-ATPase activity of the tilapia (*Tilapia nilotica* × *Tilapia aurea*) actomyosin similarly to sucrose at the level of 25%.

3.3. Effect of different cryoprotectants on the total sulfhydryl and disulfide bond content of tilapia surimi during frozen storage

During frozen storage for up to 24 weeks, sulfhydryl content of NAM from surimi without any cryoprotectants decreased sharply, while that of NAM from the cryoprotectant-added surimi decreased to a lower extent (Fig. 3(a)). Among all samples, trehalose-added surimi showed the greatest sulfhydryl content, compared with other samples, throughout the storage of 24 weeks. After 24 weeks of storage, the sulfhydryl contents of the control, trehalose-, sodium lactate- and sucrose/sorbitol-added surimi were 5.37, 6.14, 5.92 and 5.90 mol/10^5 g protein, respectively, and were reduced by 14.4%, 1.5%, 5.2% and 6.4% in comparison with their initial values, respectively. Within the first 9 weeks, sulfhydryl content of NAM from sucrose/sorbitol-added surimi was much higher than that from sodium lactate-added surimi ($p < 0.05$). However, no differences were observed between those two samples at week 20 and 24 ($p > 0.05$). From the result, the surimi without any cryoprotectant addition had the lowest sulfhydryl content.

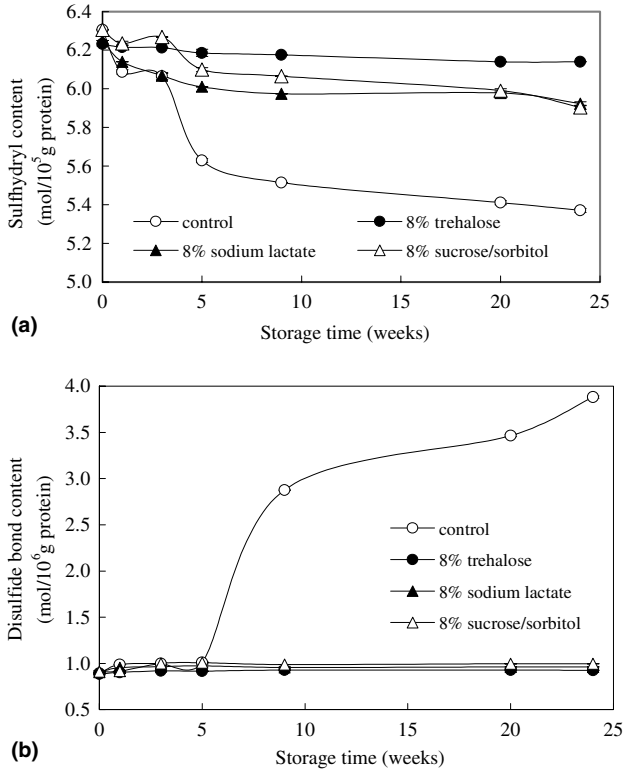

Fig. 3. Effect of different cryoprotectants on the sulfhydryl content (a) and disulfide bond content (b) of natural actomyosin extracted from tilapia surimi as a function of storage time at −18 °C. Bars indicate the standard deviation from triplicate determinations.

Disulfide bond content of NAM from surimi without cryoprotectants increased sharply throughout the frozen storage especially after 5 weeks (Fig. 3(b)) with the concomitant decrease in sulfhydryl content ($p < 0.05$) (Fig. 3(a)). Nevertheless, disulfide bond content in surimi added with cryoprotectants increased much more slowly during the first 5 weeks and did not change thereafter ($p > 0.05$) (Fig. 3(b)). After 24 weeks of frozen storage, disulfide bond contents of NAM extracted from the control, trehalose-, sodium lactate-, and sucrose/sorbitol-added surimi were 3.88, 0.93, 0.962 and 0.99 mol/10^6 g protein, which were increased by 334.6%, 4.7%, 6.3% and 9.5%, respectively. From the result, disulfide bond content of NAM from surimi added with sucrose/sorbitol was higher than that of surimi added with trehalose throughout the frozen storage ($p < 0.05$), and was higher than that of surimi added with sodium lactate after 3 weeks of storage ($p < 0.05$).

Sulfhydryl groups are considered to be the most reactive functional groups in proteins (Sultanbawa & Li-Chan, 2001). Sulfhydryl groups in fish muscle proteins were easily oxidized to disulfide groups during iced or frozen storage, or freeze–thawing process, hence resulting in a decrease in surface or total sulfhydryl contents (Benjakul et al., 1997, 2003; LeBlanc & LeBlanc, 1992; Ramirez et al., 2000; Sultanbawa & Li-Chan, 2001). Formaldehyde formation could favor the oxidation of sulfhydryl groups and the masking of sulfhydryl groups by protein aggregate might also cause the decrease in free sulfhydryl groups available for determination (Benjakul et al., 2003). The conformational changes of myosin molecules might cause the reactive sulfhydryl groups to be exposed, resulting in the increase of disulfide bond through oxidation. Our study showed trehalose exhibited the most effectiveness on retardation of oxidation of sulfhydryl groups to disulfide bonds among all cryoprotectants during frozen storage. Benjakul et al. (2003) found that the decrease in sulfhydyl groups of NAM extracted from lizardfish, croaker, threadfin bream and bigeye snapper with a concomitant disulfide bond formation was coincidental with the decrease in Ca^{2+}-ATPase activity during frozen storage at −18 °C. A similar result was shown in this study (Figs. 2 and 3). Myosin contains 42 sulfhydryl groups (Chan, Gill, Thompson, & Singer, 1995) located in the head portion. Myosin (SH_1 and SH_2) plays an important role in ATPase activity and the oxidation of these groups caused the decrease in Ca^{2+}-ATPase activity (Benjakul et al., 2003). Furthermore, sulfhydryl groups located in other portions of myosin also contributed to oxidation (Sompongse, Itoh, & Obatake, 1996), resulting in the loss of Ca^{2+}-ATPase activity (Benjakul et al., 2003). Our results suggested that the incorporation of trehalose, sodium lactate and sucrose/sorbitol could prevent the oxidation of sulfhydryl groups of NAM from tilapia surimi with the coincidental remaining Ca^{2+}-ATPase activity during frozen storage.

3.4. Effect of different cryoprotectants on the surface hydrophobicity of tilapia surimi during frozen storage

The increases in surface hydrophobicity were observed throughout the storage of 24 weeks (Fig. 4). Drastic increase in surface hydrophobicity of NAM from the control surimi was found during storage. After 24 weeks, surface hydrophobicity of NAM from the control increased by 200.7%, compared with that from the fresh sample. When cryoprotectants were added, the increase in surface hydrophobicity of NAM from tilapia surimi could be retarded to different extents, depending on the cryoprotectants used. After 24 weeks, surface hydrophobicity of NAM from trehalose-, sodium lactate- and sucrose/sorbitol-added surimi was increased by 110.1%, 113.1% and 133.5%, compared with

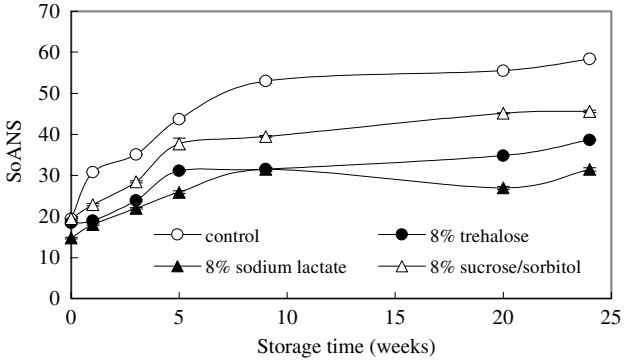

Fig. 4. Effect of different cryoprotectants on the surface hydrophobicity of natural actomyosin extracted from tilapia surimi as a function of storage time at −18 °C. Bars indicate the standard deviation from duplicate determinations.

the initial values. The difference in surface hydrophobicity found between samples suggested the varying efficacy of different cryoprotectants on stabilizing the protein structures. Surimi added with sucrose/sorbitol had greater surface hydrophobicity than surimi added with trehalose and sodium lactate throughout the storage ($p < 0.05$). The marked increase in surface hydrophobicity in surimi without cryoprotectants was presumably induced by protein denaturation caused by frozen storage. During denaturation, the hydrophobic portions buried in the interior of the protein molecule become exposed, leading to an increase in surface hydrophobicity (Benjakul & Bauer, 2000; Benjakul et al, 2003). When appropriate cryoprotectants were added, protein denaturation could be prevented to some extent. Thus the exposure of hydrophobic portions could be retarded.

The increase in surface hydrophobicity in surimi was coincidental with the decrease in protein solubility (Fig. 1). During extended frozen storage, the exposure of hydrophobic portions induced by conformational changes of proteins might cause hydrophobic interaction, leading to aggregation and loss in solubility. Benjakul et al. (2003) reported that the decrease in protein solubility was coincidental with the increase in surface hydrophobicity of croaker, threadfin bream and bigeye snapper muscle during frozen storage at −18 °C for up to 24 weeks. When cryoprotectants were added, the slower increase in surface hydrophobicity of tilapia surimi was also found in accordance with slower decrease in protein solubility. It was suggested that these cryoprotectants could prevent the exposure of hydrophobic portions of proteins to much extent. Therefore, the hydrophobic interaction might be inhibited, resulting in the remaining protein solubility.

3.5. Effect of different cryoprotectants on the gel-forming ability of tilapia surimi during frozen storage

The breaking force and deformation of gels made from tilapia surimi added with different cryoprotectants

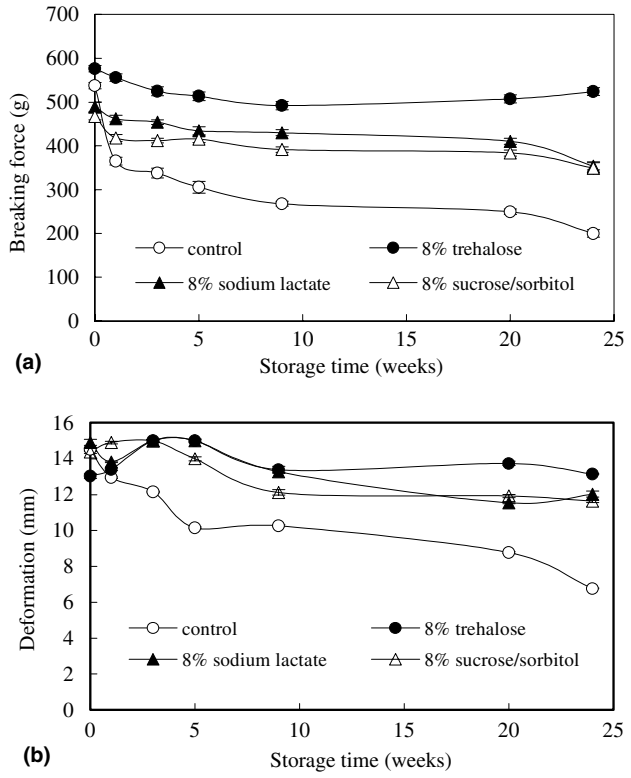

Fig. 5. Breaking force (a) and deformation (b) of gels made from tilapia surimi treated with different cryoprotectants during 24 weeks of storage at −18 °C. Bars indicate the standard deviation from determinations of six times repetition.

during frozen storage are depicted in Fig. 5. When the storage time increased, breaking force of all surimi gels decreased at varying rates, depending on cryoprotectant used (Fig. 5(a)). Among all samples, the control had the lowest breaking force ($p < 0.05$). After 24 weeks of storage, breaking force of the control surimi decreased by 62.8%, while that of surimi added with trehalose, sodium lactate or sucrose /sorbitol was reduced by 9.0%, 27.8% and 25.2%, respectively, compared to the initial values. The breaking force of surimi gels containing trehalose was markedly higher than that of surimi gels containing sodium lactate or sucrose/sorbitol ($p < 0.05$). On the other hand, no much difference in breaking force was found between surimi added with sodium lactate and with the commercial blend ($p > 0.05$).

Deformation of surimi gels without cryoprotectants decreased continuously with increasing frozen storage time. After 24 weeks, deformation decreased by 53.4% (Fig. 5(b)), while that of surimi gels containing cryoprotectants decreased to a lower extent. After 24 weeks, deformation of surimi gels added with trehalose was 13.13 mm, which was similar to that of week 0. Deformation of surimi gels added with sodium lactate and sucrose/sorbitol was 12.03 and 11.62 mm and was reduced by 19.3% and 19.1%, respectively, compared with their initial values. At week 20 and 24, no differences in deformation were found between surimi containing sodium lactate and that with the commercial blend ($p > 0.05$), while surimi with trehalose showed the highest deformation ($p < 0.05$).

Rapid decrease in gel-forming ability during frozen storage has been noted for other fish muscle systems when cryoprotectants were not incorporated (Auh et al., 1999; Benjakul et al., 2003; Sultanbawa & Li-Chan, 1998; Sych et al., 1991.).

Decrease in gel-forming ability of fish muscles during frozen storage was associated with the freeze denaturation of surimi actomyosin via the aggregation of protein chains, leaving them unavailable for subsequent gel formation during heating processing of surimi (Sych et al., 1991). An, Peters, and Seymour (1996) showed that myosin integrity was of paramount importance for surimi gelation. Denaturation or degradation of myosin during iced or frozen storage resulted in an inferior gel network formation, causing a lower elasticity with poor water-holding capacity in the gel matrix (Benjakul & Bauer, 2000; Benjakul et al, 2003). In this study, the decrease in gel-forming ability of tilapia surimi without cryoprotectants was concomitant with the decrease in salt extractable protein and with the increase in disulfide bond and surface hydrophobicity. Therefore, protein aggregation via disulfide bond or hydrophobic interaction might be an important factor causing the decrease in gel-forming ability. When trehalose, sodium lactate or sucrose/sorbitol was added, the gel-forming ability was maintained to some extent. The cryoprotective effects of trehalose were significantly greater than those of sucrose/sorbitol as evidenced by the greater gel-forming ability of surimi obtained.

4. Conclusion

Trehalose and sodium lactate at level of 8% (w/w) effectively prevented the protein denaturation of tilapia surimi during frozen storage at −18 °C for 24 weeks. Trehalose (8% w/w) appeared to achieve better cryoprotection than the commercial blend (sucrose/sorbitol, 1:1), while sodium lactate showed the comparable effect with the conventional blend. Therefore, trehalose and sodium lactate can be used as alternative cryoprotectants in surimi due to their low sweetness and caloric value. Further work should be conducted to investigate the optimal levels of these two compounds, and the sensory properties of the resulting products should be evaluated.

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