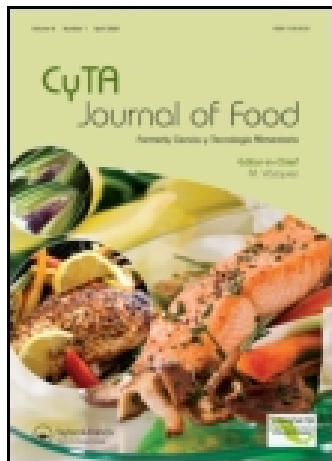


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CyTA - Journal of Food

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/tcyt20>

Cryoprotective effect of low-sweetness additives on protein denaturation of threadfin bream surimi (*Nemipterus* spp.) during frozen storage

R. Nopianti^{a,b}, N. Huda^a, I. Noryati^a, A. Fazilah^a & A.M. Easa^a

^a Fish and Meat Processing Laboratory, Food Technology Programme, School of Industrial Technology Universiti Sains Malaysia, 11800, Minden, Malaysia

^b Department of Fish Product Technology, Sriwijaya University, Inderalaya, 30662, South Sumatera, Indonesia

Published online: 23 Feb 2012.

To cite this article: R. Nopianti, N. Huda, I. Noryati, A. Fazilah & A.M. Easa (2012) Cryoprotective effect of low-sweetness additives on protein denaturation of threadfin bream surimi (*Nemipterus* spp.) during frozen storage, *CyTA - Journal of Food*, 10:3, 243-250, DOI: [10.1080/19476337.2011.639033](https://doi.org/10.1080/19476337.2011.639033)

To link to this article: <http://dx.doi.org/10.1080/19476337.2011.639033>

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Cryoprotective effect of low-sweetness additives on protein denaturation of threadfin bream surimi (*Nemipterus* spp.) during frozen storage

Efecto crioprotector de aditivos de bajo dulzor en la desnaturalización de proteínas de surimi de besugo (*Nemipterus* spp) durante almacenamiento en congelación

R. Nopianti^{a,b}, N. Huda^{a*}, I. Noryati^a, A. Fazilah^a and A.M. Easa^a

^aFish and Meat Processing Laboratory, Food Technology Programme, School of Industrial Technology Universiti Sains Malaysia, 11800, Minden, Malaysia; ^bDepartment of Fish Product Technology, Sriwijaya University, Inderalaya, 30662, South Sumatera, Indonesia

(Received 12 July 2011; final version received 2 November 2011)

The cryoprotective effects of lactitol, maltodextrin, palatinit, polydextrose, trehalose on the protein denaturation of threadfin bream surimi during six months of frozen storage were investigated. The characteristics analyzed were Ca²⁺-ATPase activity, protein solubility, sulfhydryl (SH) content, electrophoresis, surface morphology, and thermal stability. The effectiveness of all cryoprotectants generally decreased as the storage time increased. Polydextrose was able to maintain Ca²⁺-ATPase activity, protein solubility, and SH content of 33.5%, 71.3%, 71.8%, respectively. Sucrose was able to maintain Ca²⁺-ATPase activity, protein solubility, and SH content of 32.4%, 68.1%, 75.6%, respectively. Electrophoresis showed that the myosin heavy chain (MHC) and actin band intensity almost disappeared by the end of storage. Analysis by scanning electron microscopy (SEM) demonstrated that the number of pores formed increased after storage. Thermal stability analysis showed a decrease in myosin but no significant change in actin content. This study suggests that polydextrose as a cryoprotectant is able to maintain protein from denaturation as the same as sucrose.

Keywords: cryoprotectant; low-sweetness additives; myofibrillar protein; physicochemical properties; surimi

Se estudiaron los efectos crioprotectores de lactitol, maltodextrina, palatinita, polidextrosa, trehalosa en la desnaturalización de proteínas de surimi besugo durante seis meses de almacenamiento por congelado. Las características analizadas fueron actividad Ca²⁺-ATPasa, solubilidad de proteína, contenido de sulfhidrilos, electroforesis, morfología de superficie y estabilidad termal. La eficacia de todos los crioprotectores disminuyó en general al aumentar el tiempo de almacenamiento. La polidextrosa fue capaz de mantener la actividad Ca²⁺-ATPasa, solubilidad de proteína y contenido de sulfhidrilos de 33.5%, 71.3% y 71.8%, respectivamente. La sacarosa fue capaz de mantener la actividad Ca²⁺-ATPasa, solubilidad de proteína y contenido de sulfhidrilos en un 32.4%, 68.1% y 75.6%, respectivamente. La electroforesis mostró que MHC y la intensidad de bandas de actina casi habían desaparecido al final del periodo de almacenamiento. El análisis con SEM demostró que el número de poros formados aumentó tras el almacenamiento. El análisis de estabilidad termal mostró una disminución de miosina pero no un cambio significativo en el contenido de actina. Este estudio sugiere que la polidextrosa como crioprotector es capaz de mantener la proteína de desnaturalización como la sacarosa.

Palabras clave: crioprotector; aditivos de bajo dulzor; proteína miofibrilar; propiedades físico-químicas; surimi

Introduction

Surimi is composed of stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with a cryoprotectant. Myofibrillar protein still undergoes deterioration, especially protein denaturation, which results in poor functionality due to freezing (Park & Lin, 2005). The addition of a cryoprotectant to raw surimi can overcome this problem and retain the functional properties of surimi (Okada, 1992).

A commonly cryoprotectant used is a mixture of sucrose and sorbitol in a 1:1 ratio with sodium tripolyphosphate added as synergist (MacDonald & Lanier, 1991; Yoon & Lee, 1990). Surimi manufactured from warm-water species typically used 6% of sucrose due to higher thermal stability (Park & Lin, 2005). However, the incorporation of sucrose imparts a sweet taste and high calorie value in surimi (Carvajal,

MacDonald, & Lanier, 1999). Low-sweetness additives can be used as an alternative to overcome this problem. Many studies have been conducted regarding the use of low-sweetness additives. Pan, Shen, and Luo (2010) and Zhou, Benjakul, Pan, Gong, and Liu (2006) reported that trehalose exerts excellent cryoprotective effects on grass carp and tilapia surimi. Herrera and Mackie (2004) reported that polydextrose and lactitol are highly effective in preventing Ca²⁺-ATPase activity in rainbow trout actomyosin during the first 24 h of storage. Sych, Lacroix, Adambounou, and Castaigne (1990) suggested that palatinit stabilizes cod surimi proteins as well as a sucrose/sorbitol mixture does. Carvajal et al. (1999) have reported the effective cryoprotection of Alaska pollock surimi by maltodextrin.

Threadfin bream (*Nemipterus* spp.) is the main fish species used in Southeast Asia for surimi production. Guenneugues and Morrisey (2005) describe threadfin bream

*Corresponding author. Email: nrlhd@usm.my

as being white in color and possessing a smooth texture, a strong gel-forming ability, and easy process ability; thus, it is widely used as a raw material for the production of Japanese “kamaboko” and surimi-based crabstick.

However, studies concerning the cryoprotective effect of low-sweetness additives on marine tropical fish are lacking. The objectives of this work were to investigate the effect of different types of low-sweetness additives (lactitol, maltodextrin, palatinit, polydextrose, trehalose) on the protein denaturation of threadfin bream surimi by measuring Ca^{2+} -ATPase activity, protein solubility, sulfhydryl (SH) content, electrophoresis, surface morphology, thermal stability and to compare their effectiveness with that of sucrose.

Materials and methods

Surimi preparation

Threadfin bream surimi was prepared at QL Food Sdn. Bhd., Hutan Melintang, Perak, Malaysia. Different types of low-sweetness additives at the same concentration (6%) with 0.3% sodium tripolyphosphate were added to the surimi. The samples are identified in Table 1. After mixing, the surimi was packed in polyethylene bags; each package weighed 10 kg and was frozen using a contact plate freezer. Frozen surimi blocks were transported to Universiti Sains Malaysia by a refrigerated truck.

Storage study

Surimi samples were stored in a freezer at -18°C . Surimi samples were evaluated every month over a six-month period of frozen storage. The following analyses were conducted: Ca^{2+} -ATPase activity, protein solubility, SH content, electrophoresis, scanning electron microscopy, and thermal stability.

Ca^{2+} -ATPase activity

Actomyosin was prepared according to the method of Benjakul, Seymour, and Morrisey (1997). Surimi (3 g) was homogenized in 30 ml of chilled 0.6 M KCl (pH 7.0) for 4 min with an IKA[®]T25 digital ultra-turrax[®], Germany homogenizer, and the sample container was placed on 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 in a Kubota 6500 centrifuge. Three volumes of chilled distilled water were added to precipitated actomyosin. Actomyosin pellets were collected by centrifugation at 8370 g for 20 min

at 4°C and then 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 . Actomyosin was kept on ice during all analyses. The following analyses and techniques required actomyosin: Ca^{2+} -ATPase activity, SH content, protein solubility, and SDS-PAGE.

The Ca^{2+} -ATPase activity measurement was performed as described by Wang, Xiong, and Srinivasan (1997), in which 0.2 mL actomyosin was diluted in a reaction solution (7.6 mM ATP; 15 mM CaCl_2 ; 150 mM KCl; and 180 mM Tris-HCl, pH 7.4). The reaction was performed at 25°C for 10 min, and 1.0 mL of 10% trichloroacetic acid was added to stop the reaction. The mixture was subsequently centrifuged at 3500 g for 5 min, and 1.0 mL of the supernatant was reacted with 3.0 mL of 0.66% ammonium molybdate in 0.75 N sulfuric acid. A 0.5-mL aliquot of freshly prepared 10% FeSO_4 in 0.15 N sulfuric acid was then added, and the mixture was allowed to react for 2 min for color development. The absorbance was read at 700 nm to determine the ATPase activity. NaH_2PO_4 was used to prepare the standard curve for phosphate calculation.

Protein solubility

Protein solubility was determined using the Kjeldahl method according to Association of Official Analytical Chemist [AOAC] (2000). Briefly, a sample (actomyosin) was digested, distilled and titrated using 0.02 N HCl. Protein levels were calculated using a conversion factor of 6.25. The protein solubility was calculated using the following formula:

$$\text{Protein solubility (\%)} = \frac{\text{Protein content in the supernatant}}{\text{Total protein content}} \times 100$$

Sulfhydryl (SH) content

The procedure used to measure the SH content was previously described by Wang et al. (1997). A 0.5-mL aliquot of actomyosin was mixed with 1.0 mL of a urea and sodium dodecyl sulfate (SDS) solution (8.0 M urea, 3% SDS and 100 mM phosphate buffer with a final pH of 7.4). The titration of SH was initiated by the addition of 0.5 mL of 5,5'-dithio-bis (2-nitrobenzoic-acid) (DTNB) reagent (10 mM DTNB in 0.1 M phosphate buffer with a final pH of 7.4). A sample blank was run with 0.5 mL of phosphate buffer without DTNB. A reagent blank was run with only water. The absorbance was read at 420 nm after 15 min at room temperature. The concentration of SH was calculated using a molar extinction coefficient of $11400 \text{ M}^{-1}\text{cm}^{-1}$.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE procedure was followed according to the instructions provided by Wealtec for their V-Ges vertical gel electrophoresis version 1.0 instrument and the method previously described by Laemmli (1970). A 12% resolving gel and a 5% stacking gel were used. The sample buffer consisted of 1.5 M Tris-HCl pH 6.8, 2 M Dithiothreitol (DTT), SDS, bromophenol blue, and glycerol in deionized water. Sample preparation was conducted by mixing protein samples with loading buffer in a ratio 1:1 and heating the

Table 1. Low-sweetness additives used in experimental surimi.

Tabla 1. Aditivos de bajo dulzor usados en surimi experimental.

Surimi blocks	Treatments
A	Raw surimi (without any cryoprotectant)
B	6% sucrose + 0.3% sodium tripolyphosphate
C	6% lactitol + 0.3% sodium tripolyphosphate
D	6% maltodextrin + 0.3% sodium tripolyphosphate
E	6% palatinit + 0.3% sodium tripolyphosphate
F	6% polydextrose + 0.3% sodium tripolyphosphate
G	6% sorbitol + 0.3% sodium tripolyphosphate
H	6% trehalose + 0.3% sodium tripolyphosphate

mixture to 100°C for 5 min to denature the protein samples. An SDS-PAGE broad-range molecular weight standard (6.5 to 200 kDa) (Bio-Rad, Alfred Nobel Drive, Hercules, CA) was used. The running buffer consisted of 12 mM Tris base, 192 mM glycine and 0.1% SDS (w/v) in deionized water. The voltage that was used to pre-run the gel was 80 V for 20 min and 120 V for 80 min to run the gel. Gels were then immersed in deionized water for 5 min and stained with a coomassie blue stain buffer for 20 min destained with a destaining buffer for 1 min. The gels were then immersed in 1% acetic acid and incubated for 1 h on a shaker. Acetic acid was replenished every hour until the gel was completely destained, and the gels were visualized using an UV Tec gel imaging system (Cambridge, UK) that used UVI band version 12.11 software (serial number 08.630445).

Scanning electron microscopy (SEM)

The microstructure of the surimi gels was determined via SEM. The SEM procedure followed was previously described by Rawdkuen and Benjakul (2008). Briefly, the surimi gels were prepared by cutting them into slices of 2–3 mm thickness. The slices of surimi gel were freeze-dried for three days. The dried samples were cut (0.5 cm × 0.5 cm) and placed on an aluminum stub using carbon tape; the samples were then coated with gold for approximately 60 s. The specimens were observed with a scanning electron microscope (LEO supra 50VP field emission SEM Carl-Zeiss SMT; Oberkochen, Germany).

Changes in thermal stability

The changes in the thermal stability of actin and myosin in threadfin bream surimi were monitored via Differential Scanning Calorimetry (DSC) as previously described (Nasab, Alli, Ismail, & Ngadi, 2005). Briefly, freeze-dried samples were subjected to thermal analysis using a differential scanning calorimeter Q100 equipped with a DSC refrigerated cooling system and monitored by Universal Analysis 2000 software (ver.3.7A). Nitrogen was used as the purge gas (50 mL/min) for all scans. The heat flow and melting points were calibrated from the melting endotherm of indium. The heating rate used for calibration was the same as that used to analyze the scanned samples. Samples were weighed (2–3 mg) into aluminum pans with the addition of phosphate buffer (pH 7.4) until a total weight of 13–15 mg was reached. The pans were sealed hermetically and placed on the calorimetric cell of the Q100. The cell was allowed to equilibrate to 25°C and was then heated to 100°C at a rate of 5°C/min.

Statistical analysis

The experiments were run in two replications; each replication was performed in triplicate. Data were subjected to analysis of variance (ANOVA) at a 5% significance level. A comparison of means was performed by Duncan's test to determine the significant difference between treatments at $P < 0.05$.

Results and discussion

Changes in Ca^{2+} -ATPase activity

Ca^{2+} -ATPase activity can reflect the integrity of myosin molecules (Benjakul et al., 1997). The Ca^{2+} -ATPase activity

of all the samples is shown in Figure 1. The results show that each cryoprotectant produced a different effect on surimi during freezing. The Ca^{2+} -ATPase activity of each treatment was in the range 49.44–380.97 $\mu\text{g Pi/mg protein/10 min}$. Benjakul, Visessanguan, Thongkaew, and Tanaka (2005) reported that the Ca^{2+} -ATPase activity of surimi made from fish commonly used for surimi production in Thailand (croaker, lizardfish, threadfin bream, bigeye snapper) mixed with sorbitol and sucrose was in the range 23.25–100.75 $\mu\text{g Pi/mg protein/10 min}$ over 25 weeks of frozen storage, which is lower than the activity observed for the surimi samples in this study. The highest Ca^{2+} -ATPase activity was observed for surimi treated with polydextrose as the cryoprotectant after six months of frozen storage; the recorded activity was significantly different ($P < 0.05$) from that of the other samples. Herrera and Mackie (2004) also reported that polydextrose was highly effective in preserving Ca^{2+} -ATPase activity, decreasing sharply after eight weeks of storage compared with glucose syrup and sucrose/sorbitol in the actomyosin of rainbow trout. The lowest Ca^{2+} -ATPase activity was observed in raw surimi. Surimi treated with sucrose also showed lower Ca^{2+} -ATPase activity than surimi treated with polydextrose.

The decrease in Ca^{2+} -ATPase activity for all the treatments over six months of frozen storage varied significantly ($P < 0.05$) from month to month. The initial Ca^{2+} -ATPase activities of raw surimi, sucrose, lactitol, maltodextrin, palatinit, polydextrose, sorbitol, and trehalose were 267.2, 320.4, 375.2, 365.4, 379.9, 381.0, 341.6, and 322.9 $\mu\text{g Pi/mg protein/10 min}$, respectively. The final values were 49.4, 103.9, 99.2, 75.0, 117.9, 127.8, 103.2, and 119.2 $\mu\text{g Pi/mg protein/10 min}$, respectively. The same trends were reported by Benjakul et al. (2005) and Pan et al. (2010); the Ca^{2+} -ATPase activity of threadfin bream surimi and grass carp surimi mixed with sorbitol/sucrose and trehalose also decreased over six months and 25 weeks of frozen storage, respectively.

The denaturation of muscle protein was indicated by the reduced Ca^{2+} -ATPase activity and the change in surface hydrophobicity. This might result in poor gel-forming ability

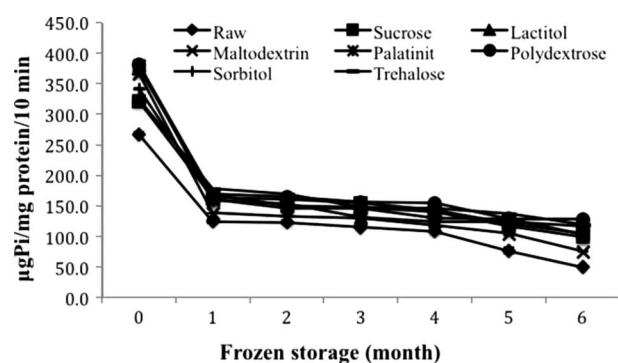


Figure 1. Changes in Ca^{2+} -ATPase activity of surimi gel with different cryoprotectants during six months of frozen storage. Values shown are averages of triplicate analysis of duplicate surimi blocks. Data are shown as the mean \pm SD.

Figura 1. Cambios en la actividad Ca^{2+} -ATPasa de gel de surimi con diferentes crioprotectores durante seis meses de almacenamiento por congelado. Los valores que se muestran son el promedio de análisis triplicados de bloques de surimi duplicados. Los datos se muestran como media \pm desviación estándar.

(Chaijan, Benjakul, Visessanguan, & Faustman, 2006). Lanier, Carvajal, and Yongsawatdigul (2005) have also suggested that a decrease in the Ca^{2+} -ATPase activity of actomyosin during the frozen storage of fish is accompanied by the reduced gel strength of surimi made from the fish.

In this study, the sharp decrease in the Ca^{2+} -ATPase activity of raw surimi indicates the large degree of denaturation of myosin. The added cryoprotectants, however, were able to provide protection against drastically reduced Ca^{2+} -ATPase activity. According to this study, surimi treated with a cryoprotectant could maintain its Ca^{2+} -ATPase activity during frozen storage. This study also proved that polydextrose is slightly better at maintaining Ca^{2+} -ATPase activity than sucrose during six months of frozen storage.

Changes in protein solubility

Myofibrillar protein is a protein that is soluble in salt. Salt solubility is one of the main characteristics of myofibrillar protein. The protein solubility of all the samples is shown in Figure 2. This figure shows that each cryoprotectant produced a different effect in the surimi during the freezing process. The protein solubility values were in the range 9.63–60.10%. Benjakul et al. (2005) reported that the protein solubility of surimi made from some tropical fish in Thailand mixed with sorbitol and sucrose was in the range 17.50–90.00% during 25 weeks of frozen storage, which is higher than the protein solubility values observed in surimi samples in this study. Among all of the samples, raw surimi showed the lowest protein solubility from the beginning until the end of frozen storage ($P < 0.05$). For samples treated with cryoprotectant, the lowest protein solubility was exhibited by the maltodextrin-treated surimi. Polydextrose showed the highest value for protein solubility among the other samples from zero to six months of frozen storage. Zhou et al. (2010) reported that polydextrose could retard the decrease in protein solubility of darce (*Cyprinus* sp) surimi until 26 weeks of frozen storage.

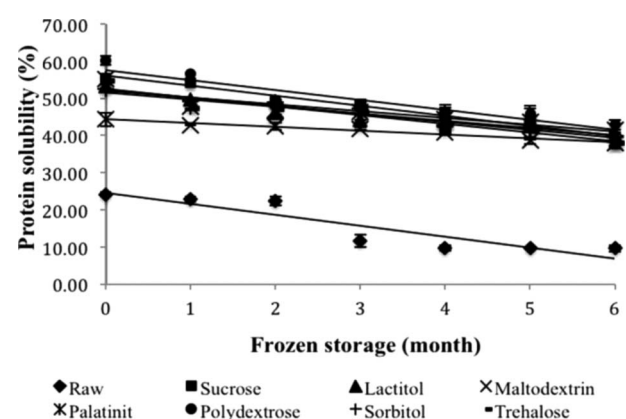


Figure 2. Changes in protein solubility of surimi gel with different cryoprotectants during six months of frozen storage. Values shown are averages of triplicate analysis of duplicate surimi blocks. Data are shown as the mean \pm SD.

Figura 2. Cambios en solubilidad de proteínas de gel de surimi con diferentes crioprotectores durante seis meses de almacenamiento por congelado. Los valores que se muestran son el promedio de análisis triplicados de bloques de surimi duplicados. Los datos se muestran como media \pm desviación estándar.

Generally, the protein solubilities of all the samples decreased as the storage time increased and were significantly different ($P < 0.05$). The initial protein solubility values of raw surimi, sucrose, lactitol, maltodextrin, palatinit, polydextrose, sorbitol, and trehalose were 24.15%, 55.37%, 53.22%, 44.28%, 55.07%, 60.10%, 52.41%, and 54.61%, respectively. The values at the end of frozen storage were 9.63%, 37.71%, 39.94%, 37.81%, 41.48%, 42.84%, 39.64%, and 37.82%, respectively. The decreasing trend in protein solubility was also reported by Pan et al. (2010) with respect to the protein solubility of grass carp surimi treated with trehalose over 25 weeks of frozen storage.

The decrease in protein solubility is the main indicator of protein denaturation during frozen storage (Benjakul & Bauer, 2000). The sharp decrease in the protein solubility of threadfin bream surimi without a cryoprotectant means that proteins underwent denaturation induced by frozen storage. The rapid decrease in protein solubility during frozen storage has been noted for tilapia surimi treated with trehalose when a cryoprotectant is not incorporated (Zhou et al., 2006). The slower decrease in the protein solubility of surimi with the addition of a cryoprotectant suggests that cryoprotectants prevent proteins from denaturation. According to this study, polydextrose exhibits a greater effect in maintaining the protein solubility of threadfin bream surimi as the same as sucrose.

Changes in SH content

SH groups are the most reactive functional groups in proteins (Sultanbawa & Li-Chan, 2001). The SH groups of proteins can be oxidized to disulfide groups and cause a decrease in the SH content during frozen storage (Pan et al., 2010). The SH content of each treatment is shown in Figure 3. The SH content of all the samples was in the range 7.39–15.71 mol SH/10⁵g protein. Pan et al. (2010) reported that the SH content of grass carp surimi was in the range 4.75–7.38 mol SH/10⁵g protein during 25 weeks of frozen storage, which is lower than that observed for surimi samples in this study.

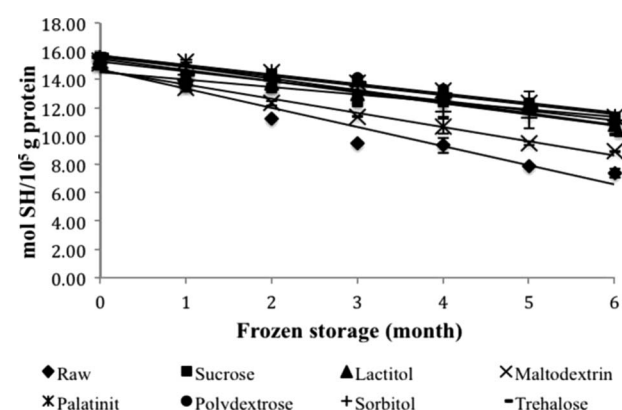


Figure 3. Changes in SH content of surimi gel with different cryoprotectants during six months of frozen storage. Values shown are averages of triplicate analysis of duplicate surimi blocks. Data are shown as the mean \pm SD.

Figura 3. Cambios en contenido de SH de gel de surimi con diferentes crioprotectores durante seis meses de almacenamiento por congelado. Los valores que se muestran son el promedio de análisis triplicados de bloques de surimi duplicados. Los datos se muestran como media \pm desviación estándar.

Generally, the SH content of all the samples decreased as the storage time increased and was significantly different ($P < 0.05$) from month to month. The initial SH contents of raw surimi, sucrose, lactitol, maltodextrin, palatinit, polydextrose, sorbitol, and trehalose were 15.54, 15.02, 15.52, 15.18, 15.32, 15.54, 15.54, 15.71 mol SH/10⁵g, respectively. The final values after six months of frozen storage were 7.39, 11.35, 10.78, 8.93, 11.34, 11.16, 11.14, and 10.13 mol SH/10⁵g, respectively. Raw surimi lost about 52.48% of its initial SH content after six months of frozen storage; this decrease was the highest among all of the samples. For surimi featuring a cryoprotectant, the maltodextrin-treated sample showed the greatest decrease of 41.16%. Sucrose showed the lowest decrease among the other samples, and followed by palatinit 25.99%, polydextrose 28.15%, sorbitol 28.30%, lactitol 30.53%, and trehalose 35.55%. A similar decreasing trend was also reported by Zhou et al. (2006) and Pan et al. (2010) with respect to the SH content of tilapia and grass carp surimi treated with trehalose during 25 weeks of frozen storage.

According to Pan et al. (2010), the sharp decrease in the SH content of the raw surimi in this study indicates the denaturation of threadfin bream protein, and the lower rates of decrease of the other seven samples suggests a cryoprotective effect in alleviating the denaturation of threadfin bream protein. The decrease is considered to be due to the formation of disulfide bonds via the oxidation of SH groups or disulfide interchange (Benjakul & Bauer, 2000). Lanier et al. (2005) have suggested that the oxidation of SH groups to disulfide linkages leads to protein aggregation typically during the storage of fish on ice. This oxidation also inactivates the Ca²⁺-ATPase activity of actomyosin because SH groups located in the head portion of myosin have been found to play an important role in ATPase activity. Hence, the decrease in Ca²⁺-ATPase activity is in agreement with the decrease in the total SH content.

Changes in sodium electrophoresis

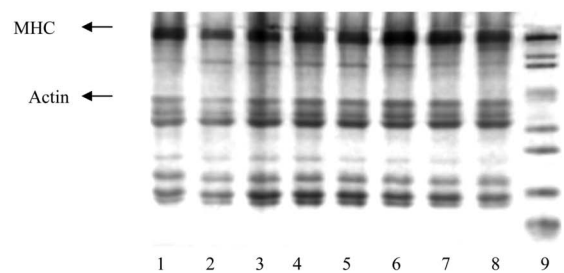
Myosin heavy chain (MHC) was found to be the major protein band in the studied samples, followed by actin. The MHC and actin band intensity of all the samples, which are shown to have decreased with storage time, is presented in Figure 4. The intensity of the MHC and actin bands at zero and three months did not show much change. At six months of frozen storage, the MHC and actin band showed a decrease in intensity and almost disappeared, especially in raw surimi. The disappearance of the MHC band in surimi without a cryoprotectant was also reported by Sultanbawa and Li-Chan (1998) with respect to the SDS-PAGE profile of ling cod surimi containing no added cryoprotectant at four months of frozen storage.

The decrease in the intensity of the MHC and actin bands was due to protein denaturation especially actomyosin during frozen storage. Surimi actomyosin treated with a cryoprotectant still exist, although at a smaller concentration, after six months of frozen storage. This indicates that the cryoprotectants added to surimi are effective in preventing the aggregation and subsequent insolubility of myosin and actin during frozen storage (Sultanbawa & Li-Chan, 1998).

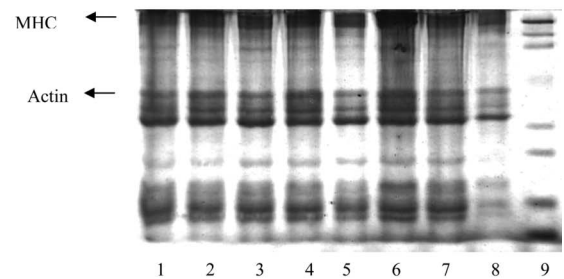
Changes in thermal stability

The differential scanning calorimetry thermograms of myosin and actin for each treatment during six months of frozen

a. SDS-PAGE 0 month



b. SDS-PAGE 3 month



c. SDS PAGE 6 month

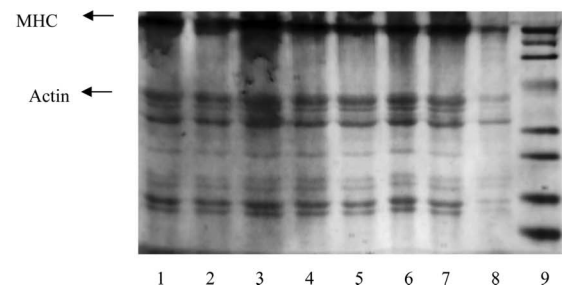


Figure 4. SDS-PAGE during six months of storage and a one-dimensional image of threadfin bream surimi at zero month (a), three months (b), and six months (c). The numbers represent the conditions as follows: L1 = Lactitol, L2 = Maltodextrin, L3 = Palatinit, L4 = Polydextrose, L5 = Sorbitol, L6 = Sucrose, L7 = Trehalose, L8 = Raw, L9 = Standard; MHC, myosin heavy chain.

Figura 4. SDS-PAGE durante seis meses de almacenamiento e imagen unidimensional de surimi de besugo a 0 meses (a), tres meses (b) y seis meses (c). Los números representan las condiciones como se indica: L1 = lactitol, L2 = maltodextrina, L3 = palatinita, L4 = polidextrosa, L5 = sorbitol, L6 = sacarosa, L7 = trehalosa, L8 = crudo, L9 = estándar, MHC, cadena pesada de miosina.

storage are shown in Tables 2 and 3. The peak maximum temperatures (T_{max}) were used to describe the denaturation temperatures of the proteins. Thermograms normally consist of two endothermic transitions: the first peak for the myosin transition and the second peak for the actin transition (Sych et al., 1990). The myosin transition temperatures of all the samples were in the range 42.10–47.83°C. Sych et al. (1990) reported that the overall mean myosin transition temperatures for cod surimi treated with sucrose/sorbitol, polydextrose, palatinit, and lactitol were 48.7°C, 49.0°C, 49.1°C, and 49.1°C, respectively, during 12 weeks of frozen storage, which are higher than those observed for surimi samples in this study. The difference in temperature may be due to the different materials that were used. The myosin transition

Table 2. Changes in T_{\max} (°C) of myosin threadfin bream surimi with different cryoprotectants during six months of frozen storage.

Month	Raw	Sucrose	Lactitol	Maltodextrin	Palatinit	Polydextrose	Sorbitol	Trehalose
0	44.44 ± 2.07 ^{ab}	46.27 ± 0.37 ^{abAB}	46.79 ± 0.86 ^{abAB}	47.83 ± 1.85 ^{aA}	47.26 ± 0.41 ^{aA}	46.35 ± 0.11 ^{abAB}	46.77 ± 0.29 ^{abAB}	46.29 ± 0.25 ^{abAB}
3	41.80 ± 1.24 ^{ab}	45.44 ± 0.78 ^{abA}	46.22 ± 0.23 ^{aA}	46.02 ± 0.08 ^{abA}	46.29 ± 0.09 ^{abA}	45.30 ± 0.57 ^{abA}	46.65 ± 0.94 ^{aA}	46.03 ± 0.17 ^{abA}
6	42.10 ± 0.32 ^{ad}	44.27 ± 0.54 ^{bbC}	45.19 ± 0.17 ^{abABC}	43.79 ± 1.06 ^{bc}	45.80 ± 0.47 ^{bA}	45.19 ± 0.63 ^{abABC}	45.55 ± 0.62 ^{abAB}	44.91 ± 0.64 ^{abABC}

Note: Different superscript letters in the same column indicate a significant difference. Different superscript capitalized letters in the same row indicate a significant difference ($P < 0.05$). Values shown are averages of triplicate analysis of duplicate surimi blocks.

Table 3. Changes in T_{\max} (°C) of actin threadfin bream surimi with different cryoprotectants during six months of frozen storage.

Month	Raw	Sucrose	Lactitol	Maltodextrin	Palatinit	Polydextrose	Sorbitol	Trehalose
0	68.35 ± 0.90 ^{abAB}	67.69 ± 1.08 ^{abAB}	69.28 ± 1.00 ^{abAB}	67.35 ± 0.18 ^{ab}	69.38 ± 0.88 ^{abAB}	67.87 ± 0.18 ^{abAB}	69.62 ± 1.12 ^{abA}	68.28 ± 0.58 ^{abAB}
3	69.04 ± 0.60 ^{abA}	66.79 ± 0.19 ^{ab}	68.87 ± 0.29 ^{abA}	69.25 ± 0.73 ^{abA}	69.12 ± 0.52 ^{abA}	68.15 ± 0.11 ^{abAB}	69.52 ± 1.24 ^{abA}	69.03 ± 0.05 ^{abA}
6	69.50 ± 0.47 ^{abA}	66.49 ± 0.59 ^{bc}	68.75 ± 0.28 ^{abAB}	68.28 ± 1.24 ^{abABC}	68.92 ± 0.07 ^{abAB}	67.19 ± 0.32 ^{bbC}	68.61 ± 1.19 ^{abAB}	68.20 ± 1.02 ^{abABC}

Note: Different superscript small letters in the same column indicate a significant difference. Different superscript capitalized letters in the same row indicate a significant difference ($P < 0.05$). Values shown are averages of triplicate analysis of duplicate surimi block.

temperature showed that from zero to six months of frozen storage, the samples showed considerable stability and there were no significance differences ($P < 0.05$) among the samples treated with a cryoprotectant, except for maltodextrin, which showed a decrease and significantly different ($P < 0.05$) temperature from the other samples treated with a cryoprotectant. The decrease in the myosin transition temperature of maltodextrin, palatinit, sucrose and trehalose began at the end of frozen storage, and the samples' final temperatures were significantly different ($P < 0.05$) from their initial temperature. From zero to six months, the myosin transition temperature of raw surimi was lower than that of surimi treated with a cryoprotectant. This indicates that cryoprotectants can prevent myosin from rapid denaturation.

The actin transition temperature was in the range 66.49–69.62°C. Sych et al. (1990) reported that the actin transition temperatures of cod surimi treated with sucrose/sorbitol, polydextrose, palatinit, and lactitol were 72.8°C, 73.0°C, 73.2°C, and 72.9°C, respectively, during 12 weeks of frozen storage, which are higher than those of the surimi samples in this study. Overall, the decrease in the actin transition temperature of all the samples was not significantly different ($P < 0.05$) until after six months of frozen storage, except for polydextrose-treated sample, whose transition temperature decrease was significantly different from its initial value after six months of frozen storage. The actin transition temperatures of all the samples were also significantly different, except for the sucrose-treated sample after six months of frozen storage. Sych et al. (1990) reported that actin was more stable than myosin in cod surimi during 16 weeks of frozen storage. Hence, the actin transition temperature of all the samples in this study showed no significant changes.

Sych et al. (1990) suggested that DSC studies of T_{max} provide information regarding the protein stability of surimi during frozen storage. This study proves that cryoprotectants prevent extensive protein denaturation in threadfin bream surimi during six months of frozen storage.

Changes in scanning electron microscopy

Scanning electron microscopy images of surimi gel prepared from different cryoprotected samples are shown in Figure 5. Jafarpour and Gorczyca (2008) revealed that retention of more myofibrillar proteins resulted in a more compact gel matrix. Generally, at zero month of frozen storage, the gel microstructures of all the samples were compact and contained few of pores, but at three months of frozen storage, the number of pores increased. The number of pores became even greater at six months of frozen storage, and the gel microstructure became less compact compared with initial condition, especially in raw surimi. Surimi with a cryoprotectant generally showed that the cryoprotectant was able to prevent drastic protein aggregation and produce less pores for up to three months of frozen storage, but after six months of frozen storage, the number of pores in almost all of the samples increased, except for polydextrose. Hence, raw surimi shows a large number of pores and loose structure starting at three months of frozen storage.

There is correlation between the increasing numbers of pores in surimi gel and water-holding capacity. The increase in the number of pores indicates a poor gel matrix with a

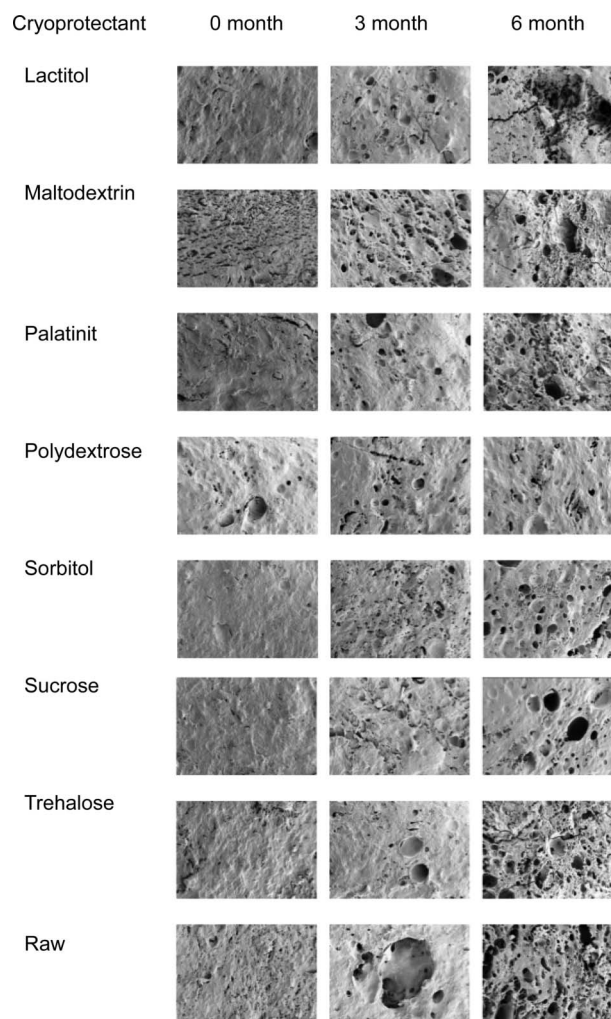


Figure 5. SEM images of threadfin bream surimi with different cryoprotectants during six months of frozen storage (magnification 50×).

Figura 5. Imágenes SEM de surimi de besugo con diferentes crioprotectores durante seis meses de almacenamiento por congelación (magnificación 50×).

lower water-holding capacity. Niwa (1992) suggested that the degradation of MHC would naturally lead to the breakdown of the formed network structure of surimi gel. Benjakul et al. (2005) also reported that large voids in frozen fish are caused by protein aggregation and are accompanied by a decrease in water-holding capacity. As a result, the inferior structure of surimi gel prepared from frozen fish confirms the poorer gel-forming ability of muscle proteins in frozen fish.

Conclusion

Surimi blended with a cryoprotectant is generally able to withstand protein denaturation during six months of frozen storage, whereas raw surimi is not. Polydextrose exerted better cryoprotection than other cryoprotectants due to higher Ca^{2+} -ATPase, protein solubility, and SH content and can be an alternative as a new kind of nonsweet cryoprotectant for application in surimi where sweetness is

undesirable and provides a sugar substitute with diminished caloric value.

Acknowledgments

The authors acknowledge with gratitude the support given by Universiti Sains Malaysia for their research in this area and USM Fellowship and also the aid of the research grant from Malayan Sugar Manufacturing Company Berhad.

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