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Cryoprotective Effects of Different Levels of Polydextrose in Threadfin Bream Surimi During Frozen Storage

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ABSTRACT

The aim of the study was to determine the influence of different levels of polydextrose (0, 3, 6, 9 and 12%) in maintaining the physicochemical properties of threadfin bream surimi during 16 weeks of frozen storage. The analysis of these effects included the studying the changes in gel strength, gel whiteness, juiciness, folding, water-holding capacity, protein solubility and the pH level of the surimi. The cryoprotective effectiveness generally increased with the increase in polydextrose content. The highest gel strength, water-holding capacity and protein solubility were maintained by 12% of polydextrose at 83, 94 and 83%, respectively; moreover, 32% of the moisture in juiciness was lost and an average gel whiteness of 76.33 was maintained over 16 weeks of storage. These values are measured against those of 0% polydextrose and surimi with 0.3% sodium trypolyphosphate. The addition of 6% polydextrose can maintain the gel strength, water-holding capacity and protein solubility at 82, 97 and 78%, respectively, lose 28% of moisture in juiciness and produce an average gel whiteness of 78.15. However, higher levels of polydextrose result in a larger extent of reduction in gel whiteness due to the Maillard reaction. Therefore, a 6% level of polydextrose was determined to be the optimal level for the cryoprotection of surimi.

Key words: Cryoprotectant, frozen storage, myofibrillar protein, physicochemical properties, polydextrose, surimi

INTRODUCTION

Surimi can be defined as a stabilized myofibrillar protein obtained from mechanically deboned fish flesh which is leached with cold water and mixed with cryoprotectants (Park and Lin, 2005). Fish myofibrillar proteins have excellent functional properties such as gel formation, water-holding activity and emulsion formation (Sato *et al*., 2003). The denaturation of myofibrillar proteins play a dominant role in the quality changes of frozen stored fish muscle. Fish muscle proteins are reported to be much less stable than those in beef, pig and poultry muscles (Hui, 2006).

Threadfin bream (*Nemipterus* sp.) is the main fish species used in Malaysia and Southeast Asia for surimi production. These fish are commonly found in the Indo-West Pacific region in tropical and subtropical waters (Guenneugues and Morrissey, 2005). Threadfin bream has been shown to produce high-quality surimi with good gel strength (Yongsawatdigul *et al*., 2002). Due to its white color, smooth texture, strong gel-forming ability and easy processing, threadfin bream surimi is widely used as a raw material for the production of Japanese kamaboko and surimi-based crabstick (Guenneugues and Morrissey, 2005).

The most widely used cryoprotectants for surimi are 4% sucrose, 4% sorbitol and 0.2-0.3% polyphosphates and they confer stability to surimi proteins during frozen storage (Sultanbawa and Li-Chan, 2001). However, 6% sucrose is typically used in surimi produced from warm-water species, perhaps due to higher thermal stability (Park and Lin, 2005). Sucrose and sorbitol have been shown to have excellent cryoprotective effects on fish myofibrillar proteins (Benjakul *et al*., 2005). However, a high level of sucrose may impart a sweet taste that may be undesirable to consumers. Besides, the sucrose added to the surimi has high tendency towards Maillard reaction, resulting in a brown color change in surimi during frozen storage (Pigott, 1986). Today's consumer is conscious of calorie content; thus, surimi containing low-calorie cryoprotectants may be preferred (Sultanbawa and Li-Chan, 1998). Low-sweetness sugar can be used as an alternative cryoprotectant to reduce the sweetness and calorie content of surimi.

Recently, many studies concerning the effect of low-sweetness sugars, such as polydextrose, lactitol, litesse, palatinit and trehalose on the physicochemical properties of surimi have been conducted. Herrera and Mackie (2004) reported that polydextrose performed excellent cryoprotective effect in the cryoprotection of actomyosin on rainbow trout (*Onchorhynchus mykiss*). Zhou *et al*. (2006) reported that 8% trehalose exhibited the greatest protective effect against protein denaturation, as shown by the effectiveness in maintaining Ca^{2+}-ATPase activity and protein solubility in tilapia (*Sarotherodon nilotica*) surimi during frozen storage. The mix of litesse, lactitol, sucrose and sorbitol in a 1:1:1:1 ratio offers the advantages of both sweetness and cost reduction (Sultanbawa and Li-Chan, 1998).

However, there is a lack of published studies on the cryoprotective effects of different levels of polydextrose on tropical fish surimi (threadfin bream surimi) during frozen storage. Polydextrose is an odorless, white to light cream amorphous powder that has virtually no sweetness and a calorie content of only 1 kcal g^{-1}. Polydextrose has a higher average molecular weight and therefore, has a reduced and favorable effect on the freezing point depression compared with the effects of smaller molecules such as sucrose or sorbitol. Solutions of polydextrose have higher viscosities than sucrose or sorbitol solutions at equivalent concentrations and temperatures. This characteristic enables polydextrose to provide the desirable mouthfeel and textural qualities important in replacing sugars and fats (Roller and Jones, 1996).

The objectives of this study were to investigate the cryoprotective effects of different levels of polydextrose as well as to determine the level of polydextrose necessary for adequate stabilization of the functional properties of proteins in threadfin bream surimi during frozen storage.

MATERIALS AND METHODS

Sample preparation: The preparation of threadfin bream surimi samples was carried out at the surimi manufacturer plant QL Foods Sdn. Bhd. located in Hutan Melintang, Perak, Malaysia, according to commercial product procedure. This research project was conducted from July 2009 to July 2010.

The level of polydextrose used in this experiment is shown in Table 1. Frozen surimi blocks (10 kg) were sent to Universiti Sains Malaysia (USM) in a refrigerated truck. The blocks of surimi were cut into smaller a meat bone saw pieces using (Powerline USA) and divided into 10 portions for 16 weeks of frozen storage at -18°C.

Frozen storage analyses: Every three weeks for 4 months, samples were taken out from the freezer and thawed overnight in a chiller at 4°C prior to analysis. The analyses were carried out for gel strength, gel whiteness, juiciness, folding test, water holding capacity, protein solubility and pH.

Table 1: Levels of polydextrose used in experimental surimi preparation

Surimi blocks	Treatments
Control	0% (without any cryoprotectant)
A	0.3% sodium tripolyphosphate (STPP)
B	3% polydextrose+0.3% STPP
C	6% polydextrose+0.3% STPP
D	9% polydextrose+0.3% STPP
E	12% polydextrose+0.3% STPP

Moisture and total protein content: Moisture and protein content of surimi from each treatment was determined in triplicate by using direct oven method, No. AOAC: 950.46 and Micro-Kjeldahl Method, No. AOAC: 955.04, respectively (AOAC International, 1990).

Surimi gel preparation: Surimi was weighed to 400 g and mixed with 3.0% of salt using a high-speed cutter mixer (Blixer®3B, France) for 2 min to obtain a homogenous sol. The sol. was then placed into polyvinylidine casing with a diameter of 2.5 cm and both ends of the casing were sealed tightly with rubber bands to avoid the penetration of water. The surimi sol. was subjected to setting at 36°C for 30 min in a water bath (WB-22 DAIHAN, Korea), followed by heating at 90°C for 10 min in a water bath (WB-22 DAIHAN, Korea), based on the procedures of Park *et al.* (1988). The surimi gels formed were then cooled on ice for 30 min and stored for 24 h at 4°C. The surimi gels were equilibrated to room temperature prior to analysis.

Gel strength: Gel strength was measured using the standard puncture test according to Lanier (1992) and Nielsen and Pigott (1994). The polyvinylidine casing was removed and the gels were cut into 2.5 cm long sections. The gels were prepared in triplicate and two measurements were made for each gel, one at the top and another at the bottom. The breaking force (g) and deformation distance (cm) were measured by using a texture analyzer (TA.XT2 Texture Analyzer, Stable Micro Systems, UK) equipped with ¼" spherical plunger (p/0.25s) at a test speed of 1.00 mm sec^{-1}. Gel strength was expressed as the breaking force (g) multiplied by the deformation distance (cm).

Gel whiteness: The whiteness of the surimi gels was measured using a colorimeter (Minolta Spectrophotometer, Model CM-3500d, Osaka, Japan). The measurement of L*, a* and b* was performed in triplicate. Whiteness was calculated using the following equation (Park, 2005):

$$\text{Whiteness} = 100-[(100-L^*)^2+a^{*2}+b^{*2}]^{1/2}$$

Juiciness: Juiciness was measured in triplicate according to the method of Gujral *et al.* (2002). The cylindrical gel samples were cut to a thickness of 5 mm. Gel samples with a thickness of 5 mm were weighed (X) and placed between two pieces of Whatman filter paper No. 1. A standard weight (5 kg) was placed on the top of the sample for 2 min. The sample was then removed and weighed again (Y). Juiciness was calculated using the following equation:

$$\text{Juiciness} = [(X-Y)/X]\times 100\%$$

Folding test: The folding test was determined according to Lanier (1992). Cooked samples were cut into 3 mm thick portions. The slices were held between the thumb and the forefinger and folded

to observe the way that they broke. The scale used was as: (1) breaks by finger pressure, (2) cracks immediately when folded in half, (3) cracks gradually when folded in half, (4) no cracks showing after folding in half and (5) no cracks showing after folding twice.

Water-holding capacity: The water-holding capacity of surimi was measured in triplicate according to the method of Porteous and Wood (1983). Ten grams of surimi was homogenized with 40 mL of distilled water using a homogenizer (IKA®T25 digital, Model T25D, Germany) and A 45 mL aliquot of the homogenate was placed in a 50 mL centrifuge tube. The homogenate was centrifuged at 2000 rpm for 5 min using a centrifuge (Kubota, Model 4000, Japan). After centrifugation, the volume of supernatant was measured. The water holding capacity was determined using the following formula:

$$\text{Water holding capacity} = \frac{(a-b)}{b} \times 100\%$$

where, a is volume of homogenate placed in centrifuge tube (mL) and b is volume of supernatant (mL)

Protein solubility: The protein solubility of surimi was determined in triplicate according to the method of Hashimoto *et al.* (1979). Five grams of sample was homogenized in 50 mL of phosphate buffer (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4) at pH 7.5 using a homogenizer (IKA®T25 digital, Model T25D, Germany). The homogenate was placed in a 50 mL centrifuge tube and centrifuged at 5000x g for 25 min at 4°C using the KUBOTA high-speed refrigerated centrifuge (KUBOTA, Model 6500, Japan). After centrifugation, the supernatant was decanted and the precipitate was added to 50 mL of the same phosphate buffer solution, homogenized and centrifuged again. The supernatant was decanted. For the precipitate, 10% of phosphate buffer (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4) containing 0.45 M KCl, pH 7.5 was added. The mixture was homogenized and centrifuged at 5000×g for 25 min at 4°C using the same centrifuge. After centrifugation, the supernatant was collected. The precipitate obtained was added to 10 vol. % of the same phosphate buffer solution containing KCl, homogenized and centrifuged again. After centrifugation, the supernatant was collected. The two supernatants were combined and the protein contents of the supernatants were determined using the Biuret method (Gornall *et al.*, 1949). The protein solubility was calculated by using the following formula:

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

Statistical analysis: All of the data were analyzed statistically by using one-way ANOVA (for comparing more than 2 means) testing at a 5% significance level. Duncan's test was also conducted to compare between treatments at a 5% significance level. All statistical analyses were conducted using SPSS, version 14.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Moisture and total protein content: The moisture content of the surimi samples was in the range 74.29-81.31%. The moisture content for industrial surimi should be less than 85%

Table 2: Average moisture and total protein contents of surimi

Treatments	Moisture (%)	Total protein (%)
0%	81.31±0.38^{a}	16.04±2.98^{abc}
0.3% STPP	81.12±0.74^{a}	16.49±1.12^{ab}
3% PD+0.3% STPP	77.79±0.53^{b}	17.19±1.47^{a}
6% PD+0.3% STPP	76.92±0.70^{c}	16.98±1.07^{a}
9% PD+0.3% STPP	76.12±0.45^{d}	14.55±1.39^{bc}
12% PD+0.3% STPP	74.29±0.20^{e}	13.91±1.20^{c}

Values shown are averages of triplicate analyses on duplicate surimi blocks. Values in the same column with different superscripts are significantly different ($p<0.05$)

(Sych *et al*., 1990). According to Lee (1986) the moisture content of industrial surimi should be less than 85% and for top-grade surimi, the content should be less than 82%. The range of moisture content surimi sample indicated that surimi sample met the criterion of moisture content for industrial surimi. The sample without polydextrose had higher average moisture than the other samples (Table 2). Over 16 weeks of frozen storage, the moisture content of surimi decreased significantly with increasing levels of added polydextrose ($p<0.05$). This result is in agreement with the study conducted by Sych *et al*. (1991) which showed a decreasing trend in the moisture content of surimi with an increase in the level of lactitol added during frozen storage.

The protein content of surimi samples was in the range of 13-17% wet bases as shown in Table 2. The protein content (in dry bases) of surimi without polydextrose was higher than that in the other samples. According to Table 2, it was observed that an increase in the level of polydextrose incorporated into the surimi was associated with a decrease in the total protein content (in dry bases). A similar trend was also reported by Sych *et al*. (1991). The higher amount of cryoprotectant added would result in proportionally lower contents of other components, including the percentage of protein content (Huda *et al*., 2001).

Gel strength and folding test: Gel strength is influenced by the proportion of myofibrillar protein in surimi (Ramadhan *et al*., 2010). The gel strength of surimi in all treatments decreased significantly over the 16 weeks of frozen storage ($p<0.05$) (Fig. 1). The range of values of gel strength was 150-312 g.cm. During the first week of frozen storage, the surimi treated with different levels of polydextrose and 0.3% STPP exhibited higher gel strength compared to the sample without addition of cryoprotectant (0%). The gel strength of the surimi without a cryoprotectant (0%) was the lowest throughout the 16 weeks. The surimi treated with sodium tripolyphosphate and different levels of polydextrose always showed higher gel strength than the 0% polydextrose throughout the frozen storage. This indicated that the addition of polydextrose and sodium tripolyphosphate to the surimi was able to protect the myofibrillar protein and retard protein denaturation and aggregation during frozen storage. Therefore, the loss of the gel-forming ability of cryoprotected surimi was retarded which contributed to higher gel strength.

A decrease in gel strength was observed by Hui (2006) as the gel-forming ability of fish protein was affected by the frozen storage treatment. Poor frozen storage can also encourage enzymatic lipid oxidation cause protein denaturation and negatively impact gel formation. Freeze denaturation of surimi actomyosin is thought to proceed by the aggregation of protein chains via disulfide bonds and hydrophobic interactions, rendering the chains unavailable for subsequent gel formation during the heat processing of surimi. The freeze denaturation of surimi actomyosin occurs at varying rates depending on the treatment and level of cryoprotectant (Sych *et al*., 1991).

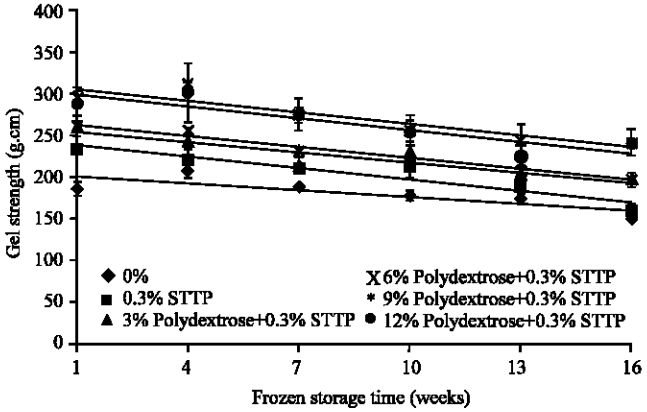

Fig. 1: Changes in gel strength of threadfin bream surimi as a function of storage time at -18°C for each treatment. Values shown are averages of triplicate analysis on duplicate surimi blocks. Bars indicate standard deviation

A previous study by Yoon and Lee (1990) reported higher levels of cryoprotectants produced more cohesive gels due to the better-protected proteins in frozen surimi. In comparison to surimi with 3% polydextrose, surimi containing 6 and 12% of polydextrose exhibited outstandingly better gel strength during frozen storage. However, the gel strength of surimi with 9% added polydextrose was lowered than that of surimi treated with 6% polydextrose although it was expected to be higher. It was presumed that the myofibrillar protein in surimi treated with 9% polydextrose had undergone a certain extent of denaturation before the freezing process and frozen storage.

Folding is one of parameters that reflect the gel strength (Venugopal, 2006). In addition to testing for gel strength, the folding test can be carried out to qualitatively determine gel quality (Lanier, 1992). The scores of the folding test for gels prepared from surimi under all treatments during the frozen storage are shown in Table 4. The range of scores was 2-5. The scores of the folding test for surimi gels under all treatments did not change significantly during the 16 weeks of frozen storage ($p<0.05$). This indicates polydextrose can maintain its folding properties throughout frozen storage.

Gel whiteness: The whiteness of surimi gels prepared from surimi under all treatments decreased significantly ($p<0.05$) as the storage time increased, as shown in Table 3. At the same time increasing level of polydextrose will decrease the whiteness value of surimi gels. Benjakul *et al.* (2005) suggested that the decrease in whiteness during frozen storage may be caused by the addition of pigment proteins, especially oxidized pigments to surimi proteins. The cross-linking of pigment proteins and muscle proteins occurs via the free radical process as a result of lipid oxidation in muscle during frozen storage (Saeed *et al.*, 1999).

Table 3 also shows that surimi gels prepared from surimi treated with 0.3% STPP display higher whiteness than surimi gels prepared from the sample containing 0% polydextrose. It is proposed that STPP had a certain whitening effect on the surimi gels. Furthermore, the gel whiteness decreased with an increase in the level of polydextrose incorporated into the surimi. This result is postulated to be due to the Maillard reaction during the preparation of surimi gel. Chung and Min (2004) also support that polydextrose has reducing carbonyl groups which participate in the Maillard reaction. Surimi gel formation involved a setting and heating process. During the heating

Table 3: Whiteness of surimi gels prepared from threadfin bream surimi for each treatment during frozen storage at -18°C

Storage time (weeks)	0%	0.3% STPP	3% PD +0.3% STPP	6% PD +0.3% STPP	9% PD +0.3% STPP	12% PD +0.3% STPP
1	80.44±0.29^{a}	81.52±0.18^{a}	79.50±0.39^{a}	78.48±0.33^{a}	77.49±0.22^{a}	76.55±0.18^{a}
4	80.20±0.16^{a}	81.48±0.14^{a}	78.99±0.25^{b}	78.22±0.28^{ab}	77.28±0.10^{abc}	76.29±0.23^{bc}
7	79.93±0.08^{b}	81.41±0.05^{a}	78.57±0.21^{c}	78.10±0.23^{b}	77.39±0.16^{ab}	76.05±0.14^{d}
10	79.69±0.29^{bc}	80.73±0.12^{b}	78.67±0.24^{bc}	78.03±0.19^{b}	77.08±0.24^{bc}	76.19±0.11^{cd}
13	79.45±0.20^{c}	80.56±0.68^{b}	78.97±0.26^{b}	78.12±0.21^{b}	76.99±0.06^{c}	76.41±0.09^{ab}
16	79.20±0.15^{d}	80.61±0.25^{b}	78.76±0.27^{bc}	77.95±0.25^{b}	77.09±0.18^{bc}	76.49±0.15^{a}

Values shown are averages of triplicate analyses on duplicate surimi blocks. Values in the same column with different superscripts are significantly different ($p<0.05$)

Table 4: Scores of folding tests for surimi gels prepared from threadfin bream surimi for each treatment during frozen storage at -18°C

Storage time (weeks)	0%	0.3% STPP	3% PD +0.3% STPP	6% PD +0.3% STPP	9% PD +0.3% STPP	12% PD +0.3% STPP
1	3±0	3±0	4±0	4±0	4±0	4±0
4	3±0	3±0	4±0	5±0	4±0	4±0
7	2±0	3±0	4±0	4±0	4±0	4±0
10	2±0	3±0	4±0	4±0	4±0	4±0
13	2±0	2±0	3±0	4±0	3±0	4±0
16	2±0	2±0	3±0	4±0	3±0	4±0

Values shown are averages of triplicate analyses on duplicate surimi blocks

process, the Maillard reaction between reducing carbonyl groups of polydextrose and the amino groups of surimi protein is suggested to have been induced, resulting in a browning effect on the surimi gels formed later. The results obtained suggest that the higher the level of polydextrose added, the higher the tendency towards undergoing the Maillard reaction is resulting in lower gel whiteness.

Juiciness: Figure 2 shows that the juiciness of surimi gels for each treatment increased significantly as the storage time increased ($p<0.05$). This indicates that less and less water was imbibed in the gel matrix as the storage time increased. According to Benjakul *et al.* (2005), protein denaturation is induced by extended frozen storage and denatured protein has a low affinity for water. Moreover, denatured protein causes a loss of gel-forming ability and the formed gel matrix possesses a lower water-holding capacity.

During the first week of frozen storage, it was observed that the juiciness of gels prepared from surimi treated with different levels of polydextrose was lower than that of the sample with 0% polydextrose. This was postulated to be due to the incorporation of polydextrose into the surimi prior to freezing. When cryoprotectants are added prior to freezing, they are dissolved and remain bound to protein molecules. This results in an increased hydration of the protein molecules, slower ice crystal growth due to increased resistance to the displacement of water from protein surfaces and an incomplete freezing of water due to increased amounts of bound water. As a result, the degree of protein unfolding or denaturation is lessened (Matsumoto and Noguchi, 1992). In other words, surimi that was treated with different level of polydextrose maintained levels of juiciness lower than those in surimi without polydextrose because of the increase in the amount of bound water. Polyphosphate, when added to surimi, reduces juiciness. According to Paredi *et al.* (1996) polyphosphate reduced the amount of juiciness in frozen *Aulacomya ater ater* (Molina) muscles.

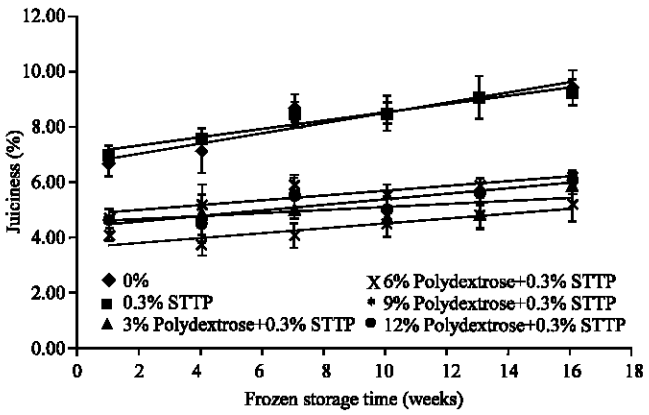

Fig. 2: Changes in gel juiceness of threadfin bream surimi as a function of storage time at -18°C for each treatment. Values shown are averages of triplicate analysis on duplicate surimi blocks. Bars indicate standard deviation

The gels prepared from samples without polydextrose (0%), showed much higher juiciness in comparison with those of surimi cryoprotected with polydextrose. It is proposed that the myofibrillar protein in the sample without polydextrose (0%) underwent denaturation to a larger extent without cryoprotection. As a result, the sample without a cryoprotectant formed a poor gel matrix with lower water-holding capacity, resulting in higher juiciness. Among the gels with different levels of added polydextrose, gels prepared from surimi with 6% added polydextrose showed the lowest juiciness throughout the frozen storage.

Water-holding capacity: The water-holding capacity of surimi under all treatments slightly decreased during frozen storage, except for the sample without a cryoprotectant (0%) and surimi treated with 3% and 6% polydextrose (Fig. 3). According to Fennema *et al.* (1973), the water-holding capacity of fish muscle is related myofibrillar proteins. The denaturation of myofibrillar proteins is indicated by a decrease in water-holding capacity. During the first week of frozen storage, the water-holding capacities of surimi with different levels of added polydextrose were higher compared to the sample with 0% polydextrose and the surimi treated with 0.3% STPP. During the freezing process, polydextrose and sodium tripolyphosphate exert their cryoprotective effects by minimizing the denaturation and aggregation of myofibrillar proteins in surimi.

The water-holding capacity of the sample without a cryoprotectant (0%) was much lower than those of surimi with different levels of added polydextrose. It is suggested that the myofibrillar proteins in the sample without polydextrose were more susceptible to denaturation and aggregation during the freezing process and frozen storage which resulted in the loss of water-holding ability. The surimi treated with 0.3% STPP had slightly higher water-holding capacity than the sample without a cryoprotectant. Phosphates increase moisture retention and increase the ability of a protein to reabsorb liquid. Phosphates will increase the pH slightly, thereby causing the water-holding capacity to increase which will also lead to improved gel-forming ability and gel strength (Hui, 2006).

Figure 3 shows that the higher the level of polydextrose incorporated into the surimi, the greater the water-holding capacity of the surimi. This result is in agreement with that of Yoon and Lee (1990) who showed that cryoprotective effectiveness increased significantly with an increase in levels of sorbitol in red hake surimi.

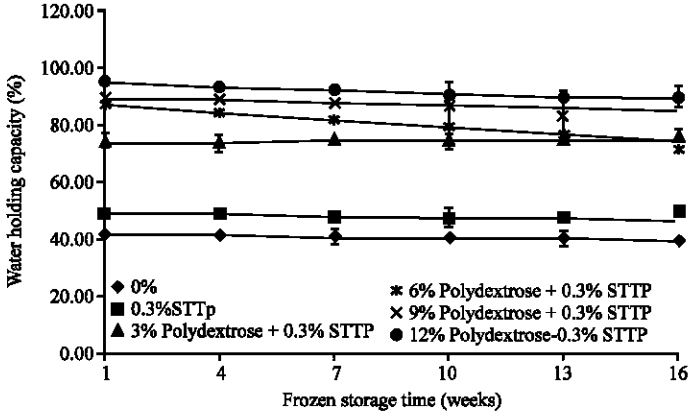

Fig. 3: Changes in water holding capacity of threadfin bream surimi as a function of storage time at -18°C for each treatment. Values shown are averages of triplicate analysis on duplicate surimi blocks. Bars indicate standard deviation

Protein solubility: The solubility of myofibrillar proteins depends on ionic strength as well as pH. Most myofibrillar proteins can be solubilized in 0.5-0.6 KCl at alkaline pH (Yamamoto *et al*., 2002). Therefore, changes in protein solubility in salt solutions can be used to evaluate the extent of protein denaturation and aggregation of surimi during frozen storage (Lian *et al*., 2000). As shown in Fig. 4, the protein solubility of surimi under all treatments decreased significantly as the storage time increased ($p<0.05$). The protein solubility of the sample ranged from 20 to 46%. During the first week of frozen storage, the protein solubility of the 0% polydextrose sample was lower compared to the surimi treated with different levels of polydextrose. This suggests that the myofibrillar proteins in the surimi without polydextrose had undergone a larger degree of denaturation and aggregation during the freezing process. Herrera and Mackie (2004) also reported that polydextrose showed a higher effectiveness in maintaining solubility levels than glucose and a mixture of sucrose and sorbitol in rainbow trout (*Oncorhynchus mykiss*) muscle. It is clear that these cryoprotectants prevented the drastic changes in proteins associated with freezing and thawing, causing aggregation to occur more slowly.

Protein denaturation and aggregation are promoted because water molecules in cooler locations start to crystallize during the freezing process. The addition of cryoprotectants to the surimi prior to freezing can retard protein denaturation because these additives protect against protein freezing and chilling injury (Best, 2008). Therefore, the myofibrillar proteins in surimi treated with different levels of polydextrose underwent lesser degrees of denaturation and aggregation after the freezing process. Moreover, there was a smaller loss of protein solubility compared to the surimi without polydextrose during the first week of frozen storage.

The cryoprotective role of polydextrose was expressed as higher protein solubility in surimi treated with different levels of polydextrose at the end of frozen storage compared to the surimi without polydextrose (0%). This indicates that the extent of protein denaturation and aggregation in surimi cryoprotected with polydextrose was reduced. This is in agreement with the studies by Yoon and Lee (1990), who again showed that cryoprotective effectiveness increases with an increase in levels of cryoprotectant in surimi.

pH: The pH level can reflect the elasticity of a product. The pH value of minced fish should be 6.5-7.0 because it allows for maximum water-holding capacity (Gomez-Guillen *et al*., 1997).

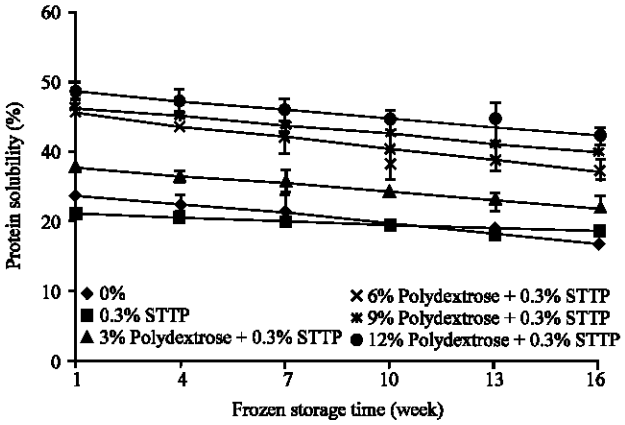

Fig. 4: Changes in protein solubility of threadfin bream surimi as a function of storage time at -18°C for each treatment. Values shown are averages of triplicate analysis on duplicate surimi blocks. Bars indicate standard deviation

Table 5: pH value of threadfin bream surimi for each treatment during frozen storage at -18°C

Storage time (weeks)	0%	0.3% STPP	3% PD +0.3% STPP	6% PD +0.3% STPP	9% PD +0.3% STPP	12% PD +0.3% STPP
1	7.02±0.01^{ab}	7.04±0.01^{a}	7.04±0.01^{a}	7.05±0.01^{a}	7.03±0.02^{a}	7.04±0.01^{a}
4	7.02±0.01^{b}	7.04±0.02^{a}	7.01±0.02^{b}	7.03±0.01^{a}	7.02±0.02^{ab}	7.02±0.01^{b}
7	7.03±0.01^{a}	7.03±0.01^{a}	6.99±0.03^{bc}	7.00±0.02^{b}	7.00±0.02^{b}	7.00±0.02^{c}
10	7.01±0.01^{b}	7.03±0.01^{a}	7.01±0.03^{bc}	6.99±0.01^{b}	7.01±0.01^{ab}	7.03±0.01^{ab}
13	6.97±0.01^{c}	7.00±0.01^{b}	7.00±0.01^{bc}	7.00±0.01^{b}	7.00±0.01^{ab}	7.01±0.02^{bc}
16	6.95±0.02^{c}	6.98±0.01^{c}	6.98±0.01^{c}	6.99±0.01^{b}	6.99±0.01^{b}	7.00±0.01^{c}

Values shown are averages of triplicate analyses on duplicate surimi blocks. Values in the same column with different superscripts are significantly different ($p<0.05$)

According to Gomez-Guillen *et al.* (1997) the result showed the pH of all the samples in this study was still in the range that recommended. Another study conducted by Liu *et al.* (2010) reported that a compact and uniform gel of silver carp myosin was obtained at pH 7.0. The range of pH of the surimi samples in this study was 6.95-7.05. The pH of surimi under all treatments decreased significantly after 16 weeks of frozen storage ($p<0.05$) (Table 5). This result is in agreement with the studies by Suvanich *et al.* (2000) that the decrease in pH during frozen storage was probably due to an increased amount of organic acids from glycogen, particularly lactic acid, as a result of glycolysis. The addition of a cryoprotectant raised the initial pH slightly because of the alkaline polyphosphate components in the cryoprotectant.

CONCLUSION

In general, there was a significant decrease in the quality of surimi without a cryoprotectant during frozen storage ($p<0.05$). The myofibrillar proteins in surimi cryoprotected by different levels of polydextrose and 0.3% STPP still maintained better gel-forming ability and water-holding capacity after 16 weeks of frozen storage than the 0% polydextrose sample and surimi treated with 0.3% STPP alone. Moreover, higher levels of polydextrose in surimi exerted better cryoprotective effects in surimi. In addition to higher cost, another disadvantage of using large amounts of

polydextrose is its browning effect on surimi gel whiteness as a result of the Maillard reaction during gel formation. Therefore, 6% polydextrose was considered to be the optimal level for providing good cryoprotective effects and reducing the tendency toward the Maillard browning reaction.

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