Concentration-Dependent Effect of Shrimp Head Protein Hydrolysate on Freeze-Induced Denaturation of Lizardfish Myofibrillar Protein during Frozen Storage

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With the aim of finding an effective way of utilizing shrimp waste, the suppression of freeze-induced denaturation of lizardfish myofibril by proteolytic shrimp head protein hydrolysates (SHPH) during frozen storage at −25°C was investigated by determining the amount of unfrozen water and Ca-ATPase activity in myofibrillar samples containing SHPH from three different species of shrimp at various concentrations (2.5–10% dried matter). The amount of unfrozen water increased markedly after freezing and decreased gradually during frozen storage in the samples containing SHPH, regardless of shrimp species. Over 120 days of storage, the Ca-ATPase activity of myofibril containing SHPH slowly decreased, whereas that of the control fell drastically after the initial freezing. These results suggest that the interactions between myofibrillar protein and the active components of SHPH, such as hydrophilic amino acids and peptides, may retard freeze-induced denaturation in fish myofibril during frozen storage. SHPH exhibited optimum suppression of freeze-induced denaturation at concentrations of 5–7.5%, regardless of species differences.

Keywords: shrimp head protein hydrolysate, frozen storage, lizardfish, myofibril, Ca-ATPase activity, unfrozen water, freeze-induced denaturation

Introduction

Fish myofibrillar protein is recognized as being more unstable in frozen storage than muscle proteins from poultry and mammals (Ramirez et al., 2000). Several cryoprotectants have been used to reduce alterations in fish myofibrils due to denaturation and aggregation during freezing and storage (Nozaki et al., 1986; Noguchi and Matsumoto, 1975; Noguchi et al., 1975; Sych et al., 1990; MacDonald and Lanier, 1994; Sultanbawa and Li-Chan, 1998; Badii and Howell, 2002). However, the stability of frozen fish muscle protein varies among species (Jiang and Lee, 1985; Hastings et al., 1985). One particular species, the lizardfish, is commonly used to make fish jelly products in western Japan. The freshness of lizardfish is known to decrease rapidly, and its stability in frozen storage is very low (Morrissey and Tan, 2000). Changes in the functional properties of lizardfish during storage have been reported by several authors (Nozaki et al., 1975, 1978, 1986; Yasui and Lim, 1987; Benjakul et al., 2003).

In Thailand, shrimp production, particularly of black tiger shrimp, has undergone a dramatic increase in recent years. During processing, solid wastes, including heads and shells, are generated in amounts of up to 49–60% of the original raw material, depending on the species and the method of processing (INFOFISH, 1991). As regulations have become stricter, there is now a need to treat and utilize these wastes in the most efficient manner. The use of shrimp waste to obtain protein hydrolysates has recently been reviewed by several investigators (Ferrer et al., 1996; Synowiecki and Al-Khateeb, 2000; Gildberg and Stenberg, 2001). In this study, we attempted to utilize the head waste of three species of shrimp (northern pink shrimp, endeavour shrimp and black tiger shrimp), obtained from a shrimp processing factory in Thailand, to produce shrimp head protein hydrolysate (SHPH) using an enzymatic hydrolysis method. Previous studies have reported that protein hydrolysates from fishery byproducts such as shellfish meat, krill and fish scraps suppressed the denaturation of fish myofibrillar protein (Darnanto et al., 1997; Zhang et al., 2002; Khan et al., 2003). We therefore decided to investigate the possibility of utilizing the functional properties of SHPH in the seafood industry by using it as a cryoprotectant for frozen lizardfish. The purpose of this study was to investigate the cryoprotective effect of various concentrations of SHPH on lizardfish myofibrillar protein during frozen storage by measuring Ca-ATPase activity and amounts of unfrozen water.

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Materials and Methods

Materials  Frozen heads of three species of shrimp—northern pink shrimp (Pandalus eous), endeavour shrimp (Metapenaeus endeavouri) and black tiger shrimp (Penaeus monodon)—were supplied by Hoko Fishing Co. Ltd. (Samutsakorn, Thailand) and used as raw materials for preparation of protein hydrolysate. Fresh lizardfish (Saurida waniro) were purchased from Nagasaki Fish Market in Nagasaki, Japan, and used for preparation of fish myofibril samples.

Preparation of SHPH  SHPH was prepared according to the method of Iwamoto et al. (1991) with slight modifications. Two volumes of distilled water were added to the shrimp heads and the mixture was heated at 90°C for 30 min to inactivate endogenous hydrolyzing enzymes. The mixture was then homogenized and adjusted to pH 8.0 with 1 N NaOH at 60°C to optimize protease activity. Endo-type protease derived from Bacillus subtilis was added to the mixture at 0.1% (w/w) and continuous hydrolysis was carried out with stirring for 2 h. The mixture was then heated at 90°C for 30 min to inactivate the protease, and the pH of the mixture was adjusted to 6.0 by adding 10% HCl at 60°C. Exo-type protease derived from Aspergillus oryzae was added at 0.1% (w/w) to the mixture using the approach described above. After removal of the resulting sludge, the solution was heated at 80°C for 10 min. To remove fat, the solution was centrifuged at 8,000 × g for 20 min. It was then desalted with a Micro Acilyzer G3 type (Asahi Kasei Inc., Kawasaki, Kanagawa, Japan) and filtered with a Pellicon-2 Mini Holder Ultrafiltration system (Millipore Corp., Bedford, MA, USA) in order to create a molecular weight cut-off of 30,000. Finally, the solution was made into a powder, to which we subsequently refer as shrimp head protein hydrolysate (SHPH), by spray drying with IGA 32 (Yamato Science Inc., Tokyo, Japan). Shrimp heads of all types were converted to SHPH by the same method.

Proximate composition, amino acid analysis and molecular weight distribution of SHPH  SHPH from each species was analyzed for moisture content, crude protein, crude lipid and crude ash by the standard method (AOAC, 1984), and sugar content was analyzed by a phenol-sulfuric acid colorimetric method. Amino acid composition was determined after digestion of SHPH in 6 N HCl (analytical grade) at 110°C for 20 h, using high performance liquid chromatography (HPLC; JLC-300; Nihon Electronic Industries Inc., Sagamihara, Kanagawa, Japan). Molecular weight distributions were estimated using gel filtration on a Sephadex G-25 column (2.2 × 60 cm). A 2-ml sample containing 5 mg protein/ml was eluted with 30 mM phosphate buffer (pH 7.0) containing 0.1 M NaCl at a flow rate of 30 ml/h, and the absorbance of each fraction was measured at 220 nm.

Preparation of fish myofibril and frozen samples  Fish myofibrillar protein was prepared according to the method of Katoh et al. (1977) with slight modifications by Nozaki et al. (1991). Fresh minced lizardfish meat was washed three times with five volumes of 0.1 M KCl-20 mM Tris-maleate buffer (pH 7.0) and compressed with a hydraulic pressing machine to remove excessive water. After dewatering, three volumes of the same buffer were added to the meat, and the mixture was homogenized in a foam-preventive blender at 10,000 rpm for 90 s, and then filtered through a nylon net (#16) to remove connective tissue. Next, 20% TritonX-100 was added to the minced meat to obtain a final concentration of 1%. After being left to sit for 30 min, the suspension was centrifuged at 1,800 × g for 10 min. The residue was washed several times with five volumes of the same buffer until the supernatant appeared clear, and the sediment was then washed with cold distilled water in order to remove buffer-derived KCl. The sediment was centrifuged at 5,000 × g for 10 min and 27,500 × g for 20 min to remove excess water, and then collected. This component is hereafter referred to as myofibril. All procedures were performed at 5°C.

SHPH from three species of shrimp was added to fish myofibril at 2.5, 5.0, 7.5 and 10.0% (dried weight/wet weight), and each sample was mixed well in a mortar at 5°C for 20 min. The pH of the mixture was adjusted to 7.0 by adding 0.1 M NaOH or 0.1 M HCl. About 1 g of each sample was packed into a micro-tube (inner diameter 9 mm, length 40 mm) and stored at −25°C. Myofibril without additive was used as a control.

Measurement of Ca-ATPase activity  In order to evaluate the freeze-induced denaturation of lizardfish myofibril, Ca-ATPase activity was measured, according to the method of Arai et al. (1970), in a reaction medium containing 100 mM KCl, 5 mM CaC2O4·25 mM Tris-maleate (pH 7.0), 1 mM ATP and 0.2–0.4 mg/ml myofibril at 25°C. The reaction was terminated after 5 min by adding 30% TCA until the final concentration was 5%, and free inorganic phosphate (Pi) was measured by colorimetric analysis (Katoh et al., 1977). Myofibrillar protein concentration was measured by the biuret method (Gornall et al., 1949). Relative myofibrillar Ca-ATPase activity was expressed as a ratio of the activities before and after frozen storage. The rate constant of freeze-denaturation of myofibrillar Ca-ATPase (Kd) was calculated according to the method of Matsumoto et al. (1985) using the equation Kd=ln(C0/Ct)/t, where C0 and Ct are the Ca-ATPase activity of myofibril before and after frozen storage for t days, respectively.

Measurement of unfrozen water  Using the method of Wakamatu and Sato (1979), the amount of unfrozen water was determined using a differential scanning calorimeter (DSC-100, Seiko Electronics Inc., Tokyo, Japan). Myofibrillar samples of about 20 mg (accuracy ±0.02 mg) were sealed tightly in aluminum pans. Al2O3 (20 mg) was sealed in reference pans and used for balancing the heat capacity of the sample. The heat of fusion was measured using a starting temperature of −40°C and a final temperature of 25°C, with an increase rate of 1°C/min. The amount of water in the sample was determined by the heat drying method at 105°C. The amount of unfrozen water was obtained by subtraction of the amount of free water from the amount of water in the sample. The amount of unfrozen water is expressed as mg H2O per unit of dried matter.
Statistical analysis The linear relationships between Ca-ATPase activity (logarithm values) and the amount of unfrozen water were obtained using Excel software (Office 2000 edition) for Windows.

Results

Properties of SHPH The proximate compositions of SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp are summarized in Table 1. The major component of SHPH was protein, which accounted for 89.79%, 91.47% and 91.00% of SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp, respectively. As shown in Table 2, the acidic amino acids, namely Glx and Asx, were abundant in all three types of SHPH, accounting for 9.77 and 6.36% of northern pink SHPH, 11.45 and 7.99% of endeavour SHPH, and 11.81 and 8.50% of black tiger SHPH. Moreover, their molecular weight distributions were below 12,000 and the average molecular weight of SHPH ranged from 300 to 1,400 (Fig. 1). These results indicate that SHPH is dominated by short-chain peptides.

Changes in Ca-ATPase activity Ca-ATPase activity is a good indicator of the integrity of fish myofibrillar protein. The logarithms of the Ca-ATPase activities of lizardfish myofibril containing 2.5–10% (dried matter) concentrations of SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp during frozen storage, plotted against the storage period (days), are shown in

### Table 1. Proximate composition of shrimp head protein hydrolysates (dried matter).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Northern pink SHPH</th>
<th>Endeavour SHPH</th>
<th>Black tiger SHPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein + SD (%)</td>
<td>89.79 ± 1.05</td>
<td>91.47 ± 0.51</td>
<td>91.00 ± 0.73</td>
</tr>
<tr>
<td>Crude lipid + SD (%)</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Crude ash + SD (%)</td>
<td>4.72 ± 0.18</td>
<td>5.20 ± 0.08</td>
<td>5.09 ± 0.08</td>
</tr>
<tr>
<td>Sugar + SD (%)</td>
<td>4.58 ± 0.05</td>
<td>2.99 ± 0.11</td>
<td>3.61 ± 0.31</td>
</tr>
</tbody>
</table>

* Standard deviation.

### Table 2. Amino acid composition of shrimp head protein hydrolysates (g/100 g dried matter).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Northern pink SHPH</th>
<th>Endeavour SHPH</th>
<th>Black tiger SHPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>6.36</td>
<td>7.99</td>
<td>8.50</td>
</tr>
<tr>
<td>Glx</td>
<td>9.77</td>
<td>11.45</td>
<td>11.81</td>
</tr>
<tr>
<td>Arg</td>
<td>4.35</td>
<td>6.27</td>
<td>5.17</td>
</tr>
<tr>
<td>Lys</td>
<td>5.07</td>
<td>5.84</td>
<td>6.02</td>
</tr>
<tr>
<td>His</td>
<td>2.82</td>
<td>2.74</td>
<td>2.62</td>
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<tr>
<td>Gly</td>
<td>5.54</td>
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<td>Ala</td>
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<td>Thr</td>
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<tr>
<td>Val</td>
<td>3.15</td>
<td>3.54</td>
<td>3.46</td>
</tr>
<tr>
<td>Leu</td>
<td>4.90</td>
<td>5.64</td>
<td>5.67</td>
</tr>
<tr>
<td>Ile</td>
<td>2.45</td>
<td>2.82</td>
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<tr>
<td>Phe</td>
<td>3.69</td>
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<td>Tyr</td>
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<tr>
<td>Pro</td>
<td>4.42</td>
<td>4.18</td>
<td>5.83</td>
</tr>
<tr>
<td>Cys</td>
<td>0.48</td>
<td>0.56</td>
<td>0.53</td>
</tr>
<tr>
<td>Met</td>
<td>2.42</td>
<td>2.10</td>
<td>2.12</td>
</tr>
</tbody>
</table>
Fig. 1. Gel chromatograms of SHPH on a Sephadex G-25 column. The numbers denote elution positions of the following standard compounds: (1) cytochrome C (M.W. 12,500); (2) aprotinin (M.W. 6,511); (3) bacitracin (M.W. 1,411); (4) glutathione (reduced) (M.W. 307); (5) glycyl-L-phenylalanine (M.W. 222); (6) L-phenylalanine (M.W. 165).

Fig. 2. The logarithm of Ca-ATPase activity decreased as the storage period continued, irrespective of the presence or absence of SHPH. The logarithm of Ca-ATPase activity in the control decreased rapidly within 15 days and then decreased gradually until 120 days of frozen storage, indicating that the denaturation pattern proceeded in two steps, with a turning point at 15 days of frozen storage. Myofibrillar samples containing various concentrations of each SHPH also exhibited two-step denaturation patterns, but the process was slower and the turning point later (at 20 days of frozen storage) than those of the control. At 120 days of frozen storage, myofibril with SHPH from black tiger shrimp showed the highest logarithm values of Ca-ATPase activity ($-0.481$ to $-0.781$ Pi $\mu$mol/mg/min), followed by those of northern pink shrimp SHPH ($-0.553$ to $-0.869$ Pi $\mu$mol/mg/min) and endeavour shrimp ($-0.592$ to $-0.949$ Pi $\mu$mol/mg/min), while the value of the control was the lowest ($-1.283$ Pi $\mu$mol/mg/min). Regardless of shrimp species, a significant effect was observed in myofibril containing 5-7.5% SHPH, whereas the myofibrillar samples containing 2.5% and 10% SHPH showed smaller effects, and the smallest effect was observed in the control.

The freeze-induced denaturation rate constant ($K_D$) can be used to detect the protective effect of cryoprotectants (Matsumoto et al., 1985; Matsumoto and Arai, 1986). The $K_D$ values ($\times 10^{-3}$/day) of myofibril with 2.5-10% SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp are summarized in Table 3. Both the $K_D$ of myofibril containing SHPH were lower than that of the control. The $K_D$ ranges of myofibril containing SHPH from all species of shrimp were 34.48-52.52, 41.47-65.74 and 28.36-50.00 ($\times 10^{-3}$/day), respectively. The $K_D$ ranges were 5.83-11.08, 4.94-9.05 and 5.40-7.98 ($\times 10^{-3}$/day), respectively. The $K_D$ and $K_{D2}$ of the control were 137.78 and 8.53 ($\times 10^{-3}$/day). These results indicated that the addition of SHPH caused the suppression of freeze-induced denaturation of myofibrillar protein during frozen storage. Significant effects on $K_D1$ and $K_D2$ were found for the myofibril containing 5% SHPH, while the effects on $K_D1$ and $K_D2$ were lower for the myofibril containing 2.5% SHPH, regardless of shrimp species.

Changes in amounts of unfrozen water Changes in the amount of unfrozen water in myofibril containing 2.5-10% (dried matter) of SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp during frozen storage are shown in Fig. 3. The amount of unfrozen water in the myofibrillar samples increased markedly after the addition of SHPH when compared with the control (0.833 mg H$_2$O/mg dried matter). Larger amounts of unfrozen water were observed for increasing concentrations of SHPH. During frozen storage, the amount of unfrozen water decreased as the storage period continued, regardless of the presence or absence of additives.

The amount of unfrozen water in the control rapidly decreased to 85% of the initial value at 5 days of storage and decreased gradually thereafter, reaching 74% of the initial value at 120 days of frozen storage. In contrast, the amount of unfrozen water in all myofibrillar samples containing SHPH decreased gradually, and the values of the SHPH-containing samples were higher than those of the control throughout the storage period. At the end of the storage period, the amount of unfrozen water in the control was 0.613 mg H$_2$O/mg dried matter, and the corresponding values for myofibril with SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp were 0.751-0.821, 0.745-0.835 and 0.743-0.891 mg H$_2$O/mg dried matter, respectively. Irrespective of species differences, the presence of 2.5% SHPH had a smaller effect on the amount of unfrozen water compared to other SHPH concentrations, while the differences in effect observed in myofibrillar samples containing 5-10% SHPH were slight.

Correlation between Ca-ATPase activity and amounts of unfrozen water Ca-ATPase activities (logarithms) were plotted against the amounts of unfrozen water in lizardfish myofibril during frozen storage at $-25^\circ C$ to calculate
their relationship; the results are shown in Fig. 4. The slope of the control was different from those of myofibrillar samples containing SHPH. Although the correlation between the logarithm of Ca-ATPase activity and the amount of unfrozen water was low for myofibril containing SHPH (R²=0.382, n=132), a close correlation was observed in the control (R²=0.867, n=11), indicating that the denaturation of myofibril is correlated with the amount of unfrozen water present.

Discussion
In this study, we attempted to elucidate the potential use of SHPH as natural cryoprotectant to suppress freeze-induced denaturation in lizardfish myofibril during frozen storage by evaluating the inactivation of Ca-ATPase and the amounts of unfrozen water present in the samples. As shown in Fig. 2, marked denaturation of myofibril was observed in the control after freezing and during frozen storage, as indicated by the residual Ca-ATPase activity, while the denaturation of myofibril containing SHPH was found to be slower. Lizardfish myofibril is particularly susceptible to freeze denaturation; the addition of SHPH may help to suppress denaturation during freezing and frozen storage. These results were in agreement with
the findings of Nozaki et al. (1975), who reported that Ca-ATPase activity in additive-free lizardfish actomyosin rapidly decreased during freezing, and that the addition of various additives such as sorbitol, sugar and polyphosphate resulted in retention of quality for a longer period (Nozaki et al., 1986). In a process known as freeze denaturation, frozen storage leads to extensive alterations in fish meat protein, which results in undesirable changes in textural and functional properties (Sikorski and Kolakowska, 1990; Pomeranz, 1991). Ice crystal formation, an increase in tissue salt concentration and lipid oxidation products are thought to be the major factors involved in protein denaturation and aggregation during freezing and frozen storage (Brown, 1986; Badii and Howell, 2002). A number of studies have reported suppression of freeze-denaturation by cryoprotectants such as sugars, polyols and inorganic salts in fish and minced-fish products, including amino acids and peptides (Nozaki et al., 1986; Sultanbawa and Li-Chan, 1998; MacDonald and Lanier, 1994; Noguchi and Matsumoto, 1975; Noguchi et al., 1975). Many amino acids have cryoprotective effects on fish muscle protein, while some amino acids such as Leu and Phe, which contain large hydrophobic side chains, trigger denaturation (Noguchi and Matsumoto, 1984; Jiang and Lee, 1985; Pan, 1990; Nozaki et al., 1991). Among the amino acids, glutamic acid and aspartic acid are the most effective, as the -COOH groups contained in their side chains are replaceable by -NH₂, -SH, -OH or -SO₃H without loss of freeze-denaturation preventive capacity (Noguchi and Matsumoto, 1971). As described in the Results section, SHPH is rich in peptides and hydrophilic amino acids; Glx and Asx are particularly abundant. The mechanism of suppression of freeze-induced denaturation by SHPH may be based on the ability of the hydrophilic amino acids and peptides in SHPH to prevent ice-crystal formation and structural alteration of fish myofibril during freezing and frozen storage.

The amount of unfrozen water in myofibril containing SHPH was 1.21–1.45 times higher than the control at 120 days of frozen storage (Fig. 3), indicating that SHPH stabilizes water molecules in lizardfish myofibril during frozen storage. It is likely that SHPH inhibits the freezing water by bonding with water molecules surrounding the myofibril. The amount of unfrozen water in myofibril containing less than 5% SHPH was lower than the amount contained in myofibril with SHPH concentrations of 5–10%, which indicates that SHPH has a concentration-dependent stabilization effect on hydration water. It may

<table>
<thead>
<tr>
<th>Sample</th>
<th>K_{D1}</th>
<th>K_{D2}</th>
<th>2.5%</th>
<th>5%</th>
<th>7.5%</th>
<th>10%</th>
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<tr>
<td>Northern pink SHPH</td>
<td>137.78(15)</td>
<td>52.52(20)</td>
<td>34.48(20)</td>
<td>39.74(20)</td>
<td>39.98(20)</td>
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</tr>
<tr>
<td></td>
<td>8.53</td>
<td>9.51</td>
<td>5.83</td>
<td>7.47</td>
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<tr>
<td>Endeavour SHPH</td>
<td>137.78(15)</td>
<td>65.74(20)</td>
<td>41.47(20)</td>
<td>44.47(20)</td>
<td>63.94(20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.53</td>
<td>7.44</td>
<td>5.34</td>
<td>4.94</td>
<td>9.05</td>
<td></td>
</tr>
<tr>
<td>Black tiger SHPH</td>
<td>137.78(15)</td>
<td>50.00(20)</td>
<td>28.36(20)</td>
<td>30.09(20)</td>
<td>44.53(20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.53</td>
<td>7.98</td>
<td>5.40</td>
<td>5.80</td>
<td>7.65</td>
<td></td>
</tr>
</tbody>
</table>

*a* × 10⁻⁷/day.

*b* Numerals in parentheses indicate the turning point (days) between K_{D1} and K_{D2}.

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**Table 3.** Rate constant (K_{D}) for inactivation of Ca-ATPase in lizardfish myofibril at various concentrations of SHPH from three shrimp species during frozen storage at −25°C.

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![Graph](image-url)

Fig. 4. Correlation between logarithm of Ca-ATPase activity and amounts of unfrozen water in lizardfish myofibril containing various concentrations of SHPH from three species of shrimp during frozen storage at −25°C. Symbols: (○) control; (●) myofibril containing SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp.
be considered that the amount of unfrozen water was unaffected by concentrations of SHPH higher than 5%. This result was in agreement with other studies that have reported concentration-dependent effects of additives such as protein hydrolysates and chitin hydrolysate on the amount of unfrozen water contained in fish myofibril during frozen storage (Zhang et al., 2002; Hossain et al., 2004; Yamashita et al., 2003). There was a close correlation between the logarithm of Ca-ATPase activity and the amount of unfrozen water in the control, but a lower correlation was observed for myofibril containing SHPH (Fig. 4). This is probably due to the lower structural stability of the control during freezing and frozen storage. These results corresponded with the findings of Yamashita et al. (2003), who observed a slight correlation between the amount of unfrozen water and the inactivation of Ca-ATPase in myofibril containing chitin hydrolysate, while a high correlation was found in myofibril without additive. Moreover, the same phenomenon was reported by Hossain et al. (2004), who studied the correlation between specific Ca-ATPase activity and the amount of unfrozen water in myofibril in the presence of squid protein hydrolysate.

At the same concentrations, the myofibrillar samples containing SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp exhibited slight differences in Ca-ATPase activity and amounts of unfrozen water. This was probably due to small differences in their compositions. In contrast, different concentrations of SHPH exhibited some differences in their effects, which is reasonable and may be due to differences in peptide formation and the positions of active groups. This is in agreement with the findings of Noguchi and Matsumoto (1971), who reported that the number and types of functional groups, molecular spatial structure and other physico-chemical properties are important factors in the cryoprotective effect of additives. Although SHPH showed considerable potential as a natural cryoprotectant, it must be used at optimum concentrations. As shown in the results, concentrations of 5–7.5% SHPH from northern pink shrimp, endeavour shrimp and black tiger shrimp resulted in superior inactivation of Ca-ATPase, but lower or higher concentrations were less effective. It is possible that at a SHPH concentration of 2.5%, there are insufficient active groups to enhance the suppressive effect on freeze-induced denaturation, while at a concentration of 10%, the large numbers of inactive groups such as hydrophobic amino acids interfere with the suppressive effect. Consequently, 5–7.5% is the optimum SHPH concentration for suppressing freeze-induced denaturation of lizardfish myofibril during frozen storage at −25°C, regardless of shrimp species.

Although SHPH was no more effective in suppressing freeze-induced denaturation than other protein hydrolysates such as pearl oyster meat (Darmanto et al., 1997), krill (Zhang et al., 2002), squid meat (Hossain et al., 2003) and fish scraps (Khan et al., 2003), the use of SHPH is an interesting way to take advantage of shrimp industry waste. Studies on the effects of SHPH on gel-forming ability and the denaturation of lizardfish surimi during frozen storage are now in progress, and the results will be published soon.

References


