Investigation on Structural Changes of Myofibrillar Proteins from Silver Carp (*Hypophthalmichthys molitrix*) during Frozen Storage

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The effect of frozen storage at -20°C and -50°C on the structure of myofibrillar proteins (MP) from silver carp was determined using several analytical methods. Differential scanning calorimetry thermograms demonstrated that the thermal stability of MP decreased and some structural changes occurred particularly in the rod-like tail portion of myosin. The trend of surface hydrophobicity was first an increase and then a decrease, indicating that the unfolding of protein molecules and exposure of buried hydrophobic residues occurred firstly, followed by the aggregation of MP through hydrophobic interactions, and this was also supported by intrinsic fluorescence and Raman. Circular dichroism and Raman data indicated that there was a decrease in α -helix content with a concomitant increase in β -sheet during storage. All the experimental data confirmed that the MP stored at -20°C suffered more pronounced structural alteration than those stored at -50°C .

Keywords: silver carp (*Hypophthalmichthys molitrix*), structural change, myofibrillar protein (MP), frozen storage

Introduction

Due to its rapid growth, high yield and low price, silver carp (*Hypophthalmichthys molitrix*) is one of the most important economic freshwater fish in China, ranking the second place among China's total freshwater fish production in 2011 with the yield of over 3,607,500 tons (China Fishery Statistic Yearbook, 2011). Apart from being cooked freshly, a majority of silver carp is consumed in the forms of frozen muscles such as frozen fillets and raw material for surimi-based products.

Frozen storage, which can greatly prolong the shelf life while keeping maximum levels of flavor and nutritional value of aquatic products and expanding the market circulation radius, is widely used as a long-term preservation method for fish species such as silver carp. Despite the rates of microbiological, chemical and biochemical reactions reduced at low temperatures, quality losses are still inevitable, particularly in terms of texture, flavor, colour and functional properties (Benjakul and Sutthiphan, 2009; Leelapongwattana *et al.*, 2005). It has been found that changes in functional and physical attributes of frozen fish depend on fish species,

treatments after catch, freezing rate, storage temperature, temperature fluctuation, storage time and so on (Benjakul and Sutthiphan, 2009; Roy *et al.*, 2012; Mignino *et al.*, 2008; Benjakul *et al.*, 2003a).

Myofibrillar protein, contributing to 55 – 65% of total muscle protein, is the major protein responsible for many functional and textural properties of fish and fish products, such as water holding capacity, emulsification and gelation capacity (Xiong, 1994). In addition, myofibrillar protein is most prone to alteration in frozen storage (del Mazo *et al.*, 1999). Therefore, those quality changes during frozen storage are associated with structural modifications of proteins (Matsumoto, 1980). During freezing and frozen storage, the damage caused by the formation of ice crystal, partial dehydration by freezing of water, increased concentration of solutes in tissues and lipid oxidation, can cause structural changes in myofibrillar proteins, subsequently causing protein denaturation, degradation and aggregation, thereby contributing to quality deterioration (Roy *et al.*, 2012; Saeed and Howell, 2002). Some researchers have mentioned that changes in the secondary and tertiary structure and integrity of fish proteins after different treatments might affect fat and water binding affinities of protein molecules or result in an inferior gel network formation of fish products (He *et al.*,

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2010; Benjakul *et al.*, 2003b).

The denaturation and degradation of muscle proteins during frozen storage have been postulated to cause the loss of functional and physicochemical properties of frozen fish, and this phenomenon has seriously limited the production of better frozen fish products. There are a number of publications about the effects of frozen storage on various fish species, tropical fish in particular, such as catfish, cod, haddock, lizardfish, croaker, salmon and so on (Leelapongwattana *et al.*, 2005; Benjakul *et al.*, 2003a; del Mazo *et al.*, 1999; Korzeniowska *et al.*, 2012; Badii and Howell, 2002). However, to our knowledge, most of these studies focused on the effects of frozen storage on physicochemical and biochemical properties or quality losses of fish, little has gone deep down into the structural changes of fish proteins during frozen storage, especially the fresh-water-fish, but the understanding of structural changes is fundamental and crucial for giving us insight into the basic mechanism of frozen denaturation of fish proteins and supplying sufficient theoretical proof for improving the quality of frozen fresh-water fish products. Hence, the objective of this study is to investigate structural changes in the myofibrillar proteins from silver carp during frozen storage.

Materials and Methods

Materials Silver carp (*Hypophthalmichthys molitrix*) used in the present study was purchased from a local supplier in Wuxi, China, and transported to the laboratory alive in 20 min. Upon arrival, it was immediately headed, gutted and washed thoroughly with tap water. The carps used in this study were all male, 2–2.5 kg and obtained in the middle of January. The chemicals were of analytical grade.

Preparation of myofibrillar protein (MP) The extraction of myofibrillar protein from silver carp dorsal muscle was according to Xu *et al.* (2012) with a slight modification. Minced muscle was homogenized for 30 s with 5 volumes of 20 mM sodium phosphate buffer (pH 6.8) containing 50 mM NaCl in a Waring blender. The mixture was centrifuged at $10,000 \times g$ for 15 min (4°C) (Sigma Laborzentrifugen, Model 4K15, Osterode, Germany), and the precipitate was washed twice using the same buffer. The resulting precipitate was homogenized with 5 volumes of 20 mM sodium phosphate buffer (pH 6.8) containing 0.6 M NaCl, then centrifuged at $10,000 \times g$ for 15 min (4°C) after standing in a refrigerator at 4°C for 1 h. The supernatant was filtered through three layers of cheesecloth to remove connective tissue and diluted with 10 volumes of cold deionized water and let to stand at 4°C for 20 min until the protein precipitated. The top layer was siphoned off and the rest was centrifuged at $10,000 \times g$ for 15 min (4°C). Final precipitate was collected as myofibrillar

protein, and certain amounts of proteins were immediately packaged in polythelene bags as samples, frozen on board at SANYO ultra-low temperature freezer (Model MDF-U53V) until the core temperature reached -20°C or -50°C , and thereafter stored respectively at -20°C and -50°C . At the time designated, frozen samples were thawed by staying at 4°C .

Differential scanning calorimetry (DSC) Changes in the thermal stability of MP stored at -20°C and -50°C were examined using a DSC machine (TA Instruments, Inc., New Castle, DE, USA). The sample (7.0–9.0 mg) was heated from 35°C to 85°C at a constant heating rate of $10^{\circ}\text{C}/\text{min}$ with an empty pan as a reference. The transition temperature (T_m) was measured by the universal analysis 2000 Thermal advantagesTM software supplied by the DSC manufacture (Ramirez- Suarez and Xiong, 2002).

Measurement of surface hydrophobicity Myofibrillar protein was diluted to 0.05, 0.1, 0.2, 0.5 mg/mL using 0.6 M NaCl solution. After the temperature was stabilized at 20°C , 20 μL of 8 mM ANS (1-anilinonaphthalene-8-sulfonic acid) dissolved in 50 mM sodium phosphate buffer (pH 7.0) was added to 4.0 mL of diluted MP solution and mixed. 0.6 M NaCl solution was used as blank (Benjakul *et al.*, 2003a).

Fluorescence intensity was measured using a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) at the excitation and emission wavelength of 374 and 485 nm, respectively. Surface hydrophobicity was calculated from the initial slope (S_0) of the plot of fluorescence intensity versus protein concentration and referred to as ' $S_0\text{ANS}$ '.

Measurement of intrinsic fluorescence Intrinsic fluorescence was measured by scanning diluted MP (0.05 mg/mL) in 0.6 M NaCl solution with a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan). Intrinsic fluorescence spectroscopy was obtained between 300 and 400 nm with excitation wavelength of 295 nm at a scanning speed of 12000 nm/min. The excitation and emission slit widths were 5.0 nm and 0.6 M NaCl solution was used as blank for all samples (Xu *et al.*, 2012).

Circular dichroism (CD) analysis CD analysis was carried out with an MOS-450 spectropolarimeter (Biologic, Claix, France) in the far-UV region (200–250 nm) at 25°C as described by Zhao and Yang (2008). MP was diluted to 0.10 mg/mL with 0.6 M NaCl solution and measured in a 1.0 cm quartz cuvette (light path length of 10 mm). The values of spectral resolution, scanning rate, response, and bandwidth were 0.2 nm, 60 nm/min, 0.25 s and 1.0 nm, respectively. 0.6 M NaCl solution was used as blank for all samples.

The percentage of α -helicity (%) was estimated from ellipticities $[\theta]$ at 222 nm by the following equation (Ogama *et al.*, 1995): $\% \alpha\text{-helicity} = 100 \times \{[\theta]_{222}/-40,000\}$.

RT-Raman spectroscopic analysis Raman experiments were conducted using a portable Raman spectrometer Ram-Tracer-200-HS (OptoTrace Technologies Co., Ltd., Silicon Valley, USA) with the following parameters: an excitation light source of 785 nm, a sweep range of 500 – 2000 cm^{-1} , and a resolution of 6 cm^{-1} . The scanning power of Raman spectrometer was 300 mW and the duration was 5 s.

Statistical analysis All the specific assays were performed at least in triplicate.

Results and Discussion

DSC analysis DSC is a widely used analytical technique for studying thermal stability of proteins. On the thermogram, lower transition temperatures and decreased peak areas or disappearance of specific peaks implying decreases in protein structural stability (Korzeniowska *et al.*, 2012).

Thermal denaturation profiles of myofibrillar proteins stored at -20°C and -50°C are shown in Fig. 1. When subjected to thermal scan, fresh MP of silver carp exhibited three major peaks with maximum transition temperatures (T_m) at 40.6, 50.8 and 75.2°C , respectively. The first two peaks were attributed to denaturation of different parts of myosin, the two globular heads and a rod-like tail portion, respectively (“myosin 1” and “myosin 2”), and the third peak was associated with denaturation of actin (Korzeniowska *et al.*, 2012). Tironi *et al.* (2002) reported that the DSC thermograms of fresh myofibrillar proteins from sea salmon showed 3 endothermic transition peaks with T_m at 43.4°C , 52.6°C and 68.7°C .

During the storage, T_m of myosin 1 decreased from 40.6°C to 38.2°C (-20°C) and 39.0°C (-50°C) and T_m of myosin 2 shifted from 50.8°C to 48.0°C (-20°C) and 50.2°C ($\leq 50^{\circ}\text{C}$), while T_m of actin remained relatively similar to the fresh. The peaks of myosin both became lower, flatter and broader, while the shape and size of actin peak basically remained the same (Fig. 1). The results implied that the thermal stability of myosin had decreased compared to the fresh sample and some changes had occurred in myosin structure, and actin was more resistant to frozen denaturation than myosin. Tironi *et al.* (2010) reported that sea salmon stored at -11°C for 4 months did not present significant change in denaturation temperature or denaturation enthalpy of actin peak. Badii and Howell (2002) also pointed out that myosin was the main protein denatured during frozen storage assessed by electrophoresis and amino acid analysis. It had been suggested that there was a connection between seasons and the thermal stability of myosin, winter-type myosin (myosin in winter season carp including December, January, February and March) of silver carp like our samples tended to be more unstable during storage on ice than summer-type

myosin (myosin in summer season carp including June, July, September and October) due to the water temperature adaptation of fish (Yuan *et al.*, 2006).

In addition, the peak area of myosin 2 decreased much more remarkably than that of myosin 1 after storage at both temperatures, which indicates that myosin 2 is more sensitive to freezing as the rod portion made a large contribution to the stability of myosin (Nagai *et al.*, 1999). Deng *et al.* (2002) reported that the denaturation of the rod-like tail of myosin in food thermal processing was the most crucial factor affecting the water-holding capacity in meat and meat products. Furthermore, it can be seen from Fig. 1 that the peak of myosin 2 almost disappeared at -20°C while it still existed at -50°C , which might be due to greater protein denaturation at -20°C (Badii and Howell, 2002). Careche *et al.* (2002) once pointed that different temperatures of frozen storage affected myosin molecules in different ways.

Hydrophobicity In native myofibrillar proteins, hydrophobic residues especially nonpolar aromatic amino acids buried inside the natural folded structure, while the surface

Fig. 1. DSC thermograms of myofibrillar proteins from silver carp stored at -20°C (a) and -50°C (b), respectively.

of proteins has a high density of charged groups and accordingly lacks in hydrophobic groups (Riebroy *et al.*, 2009). Therefore, the changes in the surface hydrophobicity can be a sensitive tool for evaluating subtle structural changes in proteins during frozen storage.

In this study, surface hydrophobicity was determined using ANS as a fluorescent probe and the changes in surface hydrophobicity (S_0 ANS) of MP during frozen storage are shown in Fig. 2. The surface hydrophobicity of samples stored at -20°C and -50°C both increased during the first 4 weeks of frozen storage, and then gradually decreased until the end of storage. Similarly, Leelapongwattana *et al.* (2005) also reported an increase in surface hydrophobicity of natural actomyosin from lizardfish after 4 weeks of frozen storage, followed by a decrease upon further storage to 16 weeks.

The initial increase of surface hydrophobicity is known due to the unfolding of protein molecules and exposure of some buried hydrophobic residues during freezing and frozen storage, which results in conformational changes in the peptide chain and a different arrangement from those in the native structure (Riebroy *et al.*, 2009). In some cases, the increase of surface hydrophobicity would mean an irreversible denaturation of proteins (Nakai *et al.*, 1986). The subsequent decrease in surface hydrophobicity might be attributed to the hydrophobic interactions of the previously exposed hydrophobic domains, leading to increased aggregation of partly unfolded protein molecules, thus causing the loss of protein solubility which may be associated with the degradation of function properties such as water-holding capacity and gel-forming ability of proteins (Benjakul *et al.*, 2003a). So there are lots of studies which aimed at studying the relationships between the changes of structure and function in food proteins that will emphasize the importance of protein hydro-

phobicity on functional properties (Mignino *et al.*, 2008; Nakai *et al.*, 1986).

The hydrophobicity was greater for MP stored at -20°C compared to the samples stored at -50°C throughout the frozen storage period. This result was in agreement with Badii and Howell (2002) who found the same situation in cod and haddock stored at -10°C and -30°C , and they attributed this to increased protein denaturation and unfolding at lower temperatures.

Intrinsic fluorescence Intrinsic fluorescence is sensitive to the polarity of microenvironment along the transition in tertiary structures of proteins (Zhao and Yang, 2008). The intrinsic fluorescence with the excitation wavelength at 295 nm of many proteins is almost ascribed to tryptophan (Trp) as the illumination with 295 nm light exclusively excites Trp, and the location of Trp in proteins affects the fluorescence energy (Zhao *et al.*, 2007). Since myosin has Trp residues both in its rod and head regions, Trp is important for moni-

Fig. 2. Changes in the surface hydrophobicity (S_0 ANS) of myofibrillar proteins from silver carp stored at -20°C and -50°C , respectively. Each point represents the mean of three determinations.

Fig. 3. Changes in the intrinsic fluorescence intensity (FI) of myofibrillar proteins from silver carp stored at -20°C (a) and -50°C (b), respectively. Each point represents the mean of three determinations. Each point represents the mean of three determinations.

toring conformational changes including alteration and aggregation of myofibrillar proteins (Kato and Konno, 1993).

The fluorescence spectra of MP stored at -20°C and -50°C are shown in Fig. 3. The fluorescence spectrum of fresh MP had a broad band with a maximum at 336 nm. As frozen storage time increased, emission intensity of MP from silver carp stored at both temperatures gradually reduced. Lefevre *et al.* (2007) pointed out that the decrease in intrinsic fluorescence could be an indication of denaturation and exposure of indole side chain of Trp to the polar or aqueous environment. The exposure of Trp residues may play an important role in the protein-protein interactions accompanying the detrimental changes emerged from frozen storage (Careche *et al.*, 1999). It could be deduced that the intermolecular hydrophobic interactions between exposed hydrophobic areas and the formation of aggregates would be promoted when the unfolding of native MP and exposure of buried Trp residues occurred firstly during frozen storage. The unfolding of fish MP would promote the inter/intra-molecular interactions leading to protein aggregation (Liu *et al.*, 2011).

The emission intensity of MP stored at -20°C remained almost constant during the further storage, while that of the samples stored at -50°C still decreased, however, the emission intensity of samples stored at -50°C were constantly higher than that of samples stored at -20°C (Fig. 3). The subsequent plateau of the emission intensity of samples stored -20°C may depend on the balance between unfolding and aggregation of the proteins. Moreover, a little blue shift (-20°C : from 336 nm to 333 nm; -50°C : from 336 nm to 334 nm) in the emission maximum with the extended storage time was observed.

CD analysis CD spectroscopy has been successfully proven to be sufficiently simple and reliable for rapid determination of structural changes in proteins in diluted solutions.

Secondary structure elements are of great importance to the stability of a protein (Zhao and Yang, 2008). Spectra in the far-UV wavelength region (200 – 250 nm), which are related to the polypeptide backbone structure and the amide region, were used to characterize secondary structures of samples (Zhao *et al.*, 2007). Fig. 4 showed the far-UV CD spectra of myofibrillar proteins from silver carp stored at -20°C and -50°C , respectively. The CD spectrum of fresh sample exhibits two main minima at around 208 nm and 222 nm. The two bands are rationalized by the $n\text{-}\pi^*$ transition in the peptide bond of α -helix, therefore, can be seen as indicators of α -helical structure (Neumann and Katchalsky, 1972).

Earlier studies have shown that the α -helix structure was the dominant pattern of secondary structure in the myosin rod with the content above 95% (King and Lehrer, 1989). In

addition, the rod portion of myosin represents a majority of secondary structures of myosin. Therefore, changes in the α -helix content are of great importance to monitor secondary structural changes in myofibrillar proteins. As shown in Fig. 4, the ellipticity values of the negative peaks of 208 nm and 222 nm both decreased in prolonged frozen storage of MP at -20°C and -50°C , indicating a gradual loss of some amount of α -helical structures at both temperatures. The gradual reduction in the content of α -helix in this study suggests that myosin underwent significant structural changes including the unfolding of the α -helix structure of the myosin rod portion during frozen storage, and this is consistent with our DSC results that the rod-like tail portion might be more sensitive to freezing. Benjakul *et al.* (2003a) found the rod portion of myosin molecules underwent structural changes during frozen storage at -18°C for up to 6 months and resulted in a lower gel-forming ability. The decrease of gel-forming abilities of fish muscle during frozen storage may

Fig. 4. Changes in the far-UV CD spectra and the α -helix structure of myofibrillar proteins from silver carp stored at -20°C (a) and -50°C (b), respectively. Each point represents the mean of three determinations.

be ascribed to the large contribution made by the rod portion of myosin to the development of gel networks and structural changes in myosin rod during frozen storage.

Estimates of α -helix contents of MP under frozen storage were obtained from the molecular ellipticity values at the wavelength of 222 nm, which functioned as a standard measurement of α -helical content of the protein. As seen from Fig. 4, α -helix content reduced from 26.27% to 18.70% (-20°C) and 21.67% (-50°C), respectively. Liu *et al.* (2007) agreed that structural changes in myosin were highly sensitive to difference in temperatures.

Raman spectroscopic analysis Raman spectroscopy can be a useful tool to probe protein structure in solid samples and aqueous solutions as it is a fast, direct and non-destructive technique which needs little or no pre-treatment of samples, and only small portions of samples are necessary for the analysis. It is capable of providing abundant information, such as the peptide backbone structure, the environment of some aromatic side chains and the local conformation of disulfide bonds and methionine residues (Herrero, 2008a).

Fig. 5 revealed a typical Raman spectrum of fresh sample in the range of $500 - 2000\text{ cm}^{-1}$ wavenumber region. The most prominent band corresponding to C-H (CH_2) bending vibrations, centered in the range of 1440 to 1460 cm^{-1} . There was a decrease in the intensity of this band during frozen storage (Table 1). It is acknowledged that there is a relation between the changes in the C-H bending vibrations and the changes in tertiary structure of proteins. Some researchers believe that a decrease in the intensity of this band indicates exposure of aliphatic residues to a more polar environment (Herrero, 2008b, Howell and Li-Chan, 1996). This decrease in our study may result from the exposure of aliphatic residues and thereafter hydrophobic interactions between aliphatic residues. Hydrophobic interactions are considered to play an important role in protein folding and aggregation. It was suggested that one of the most important factors determining juiciness might be hydrophobic interactions of aliphatic residues (Herrero, 2008b). The changes in the C-H bending vibrations in myofibrillar proteins stored at -20°C were greater than those kept at -50°C , with a decrease of the

intensity from 1886 to 1029 at -20°C and a decrease from 1886 to 1214 at -50°C . Greater changes in C-H bending near 1450 cm^{-1} were also observed in hake fillets stored at -10°C than in those kept at -30°C (Careche *et al.*, 1999).

The most useful Raman bands to provide secondary structure information about protein backbone are the amide I bands (centered $1645 - 1685\text{ cm}^{-1}$) composed of overlapping band components falling in the $1650 - 1658$, $1665 - 1680$, and $1660 - 1665\text{ cm}^{-1}$ ranges, which are attributable to α -helices, β -sheets, and random coil structures, respectively (Herrero, 2008a). As shown in Fig. 6, the peak maximum of the $1650 - 1658\text{ cm}^{-1}$ region (α -helices) of myofibrillar proteins shifted slightly to higher frequencies with a more remarkable changes after storage at -20°C (-20°C : from 1650 cm^{-1} to 1656 cm^{-1} ; -50°C : from 1650 cm^{-1} to 1654 cm^{-1}), indicating a decrease in α -helix structure and a slight increase in β -sheet and/or random coil contents. It can be deduced from our DSC and CD results that the decrease in α -helical structure were mainly due to the unfolding of the α -helix structure of myosin rod-like tail portion, which is consistent with Leelapongwattana *et al.* (2008) that the decrease in α -helical structure were mainly in the tail part of the myosin. Careche *et al.* (1999) found a decrease in the proportion of α -helix with a concomitant increase in β -sheet content of hake muscle during frozen storage at -18°C . As hydrophobic interactions between aliphatic residues play an important role in the protein aggregations during frozen storage (supported by our surface hydrophobicity, intrinsic fluorescence and C-H bending vibration results) and β -sheet was an important conformational component in aggregated protein, we may draw the conclusion that hydrophobic interactions make a large contribution to the increase of β -sheet formation during frozen storage (Choi and Ma, 2007). Bouraoui *et al.* (1997) once hypothesized that these changes could be due to an unfolding of α -helix structures, followed by the formation of β -sheet structures possibly through intermolecular interactions between exposed hydrophobic residues.

The tyrosine doublet bands, which are located near 855 and 828 cm^{-1} , are very useful for observing the microenvironment around tyrosine residues (Howell and Li-Chan,

Table 1. Tyrosine doublet ratio I_{850}/I_{830} and C-H bending intensities of Raman spectroscopic analysis of myofibrillar proteins obtained from silver carp stored at -20°C and -50°C , respectively.

Storage time	1850/1830		C-H bending	
	-20°C	-50°C	-20°C	-50°C
Fresh	1.05	1.05	1886	1886
2 weeks	1.08	1.06	1437	1467
4 weeks	0.96	0.98	1245	1406
8 weeks	0.94	0.95	1067	1369
12 weeks	0.89	0.94	1029	1214

1996). The doublet bands are assigned to vibrations of the para-substituted benzene ring of Tyr residues which are affected by the environment and the involvement of the pheno-

Fig. 5. Raman spectrum in the 500 – 2000 cm^{-1} wavenumber region of myofibrillar protein obtained from fresh silver carp.

Fig. 6. Raman spectra in the 1635 – 1685 cm^{-1} wavenumber region of myofibrillar proteins obtained from silver carp, after storage at -20°C (a) and -50°C (b), respectively. Fig. 1.

lic hydroxyl group in hydrogen bonding (Leelapongwattana *et al.*, 2008). The $I_{850/830}$ intensity ratio of fresh sample was 1.05, suggesting that the Tyr residues of fresh sample were exposed to a polar environment and able to participate in moderate or weak hydrogen bonding. The $I_{850/830}$ ratios for myofibrillar proteins increased to 1.08 (-20°C) and 1.06 (-50°C) in the first two weeks and then gradually decreased to 0.89 (-20°C) and 0.94 (-50°C), respectively (Table 1). Similar to the study of Leelapongwattanan *et al.* (2008) that the intensity ratio $I_{850/830}$ of natural actomyosin from had-dock decreased from 1.08 to 0.64 after storage at -10°C for 8 weeks. The increase of $I_{850/830}$ ratios indicated that the unfolding of MP molecules occurred, resulting in more Tyr residues to the surface of the protein, then the decrease suggesting that enhanced hydrophobic interactions might cause aggregation of proteins, and some exposed Tyr residues become reburied in a more hydrophobic environment and be involved into hydrogen bonding and cross-linking hydrogen bond donors. This is similar to the trend of Trp as indicated by our intrinsic fluorescence result, and is also in agreement with the low surface hydrophobicity values in the later experimental stage of this study.

Conclusion

Freezing and frozen storage caused significant secondary and tertiary structural changes in myofibrillar proteins of silver carp, including the unfolding of native protein molecules, exposure of some buried aromatic amino acids like Tyr and Trp, an increase of β -sheets at the expense of α -helices and protein aggregation via hydrophobic interactions. Hydrophobic interactions are considered to play an important role in the secondary structural change and aggregation of myofibrillar proteins during frozen storage. In addition, myosin is more susceptible to frozen denaturation than actin, and the rod-like tail portion of myosin is most sensitive to freezing. Throughout the frozen storage, structural changes were much smaller in myofibrillar proteins stored at -50°C than in those stored at -20°C . Therefore, ultra-low temperature storage may become a promising preservation method for better quality and longer shelf life of frozen fish and fish products in the coming future.

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