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Physicochemical and Rheological Changes of Myofibrillar Proteins from Big-Eye Tuna (Thunnus Obesus) During Frozen Storage

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Abstract: Physicochemical changes of muscle from tuna were monitored during 60 days of storage at -18 and -30 ℃. Ca^{2+}-ATPase activity of myofibrillar protein (MP) storage at both temperature decreased continuously during storage ($P<0.05$). A decrease in sulfhydryl group content was observed during the storage ($P<0.05$). Rheology indicated that MP at -18 ℃ showed higher storage modulus (G') than -30 ℃. The loss of free sulphydryls associated with the decrease in Ca^{2+}-ATPase activity could result in an ascent in the storage modulus (G').

Key words: Frozen storage; myofibrillar protein; rheology; tuna

1. Introduction

Tuna processing is an industry of well known economic importance due to their high value and their frequent use for canning and sashimi production in many countries. Deterioration of marine products after catch occurs very quickly compared to other food stuffs[1]. Structural and physicochemical changes accompanied by deterioration of muscles still could happen during frozen storage[2].The understanding of the protein interaction is very important in developing novel food with tailored textural properties. Sea foods are highly perishable and usually spoil faster than other muscle foods. They are more vulnerable to texture deterioration than other meats.

Rheological techniques can be used to determine subtle changes in muscular tissue [3]. The rheological characteristics of fish muscle are thought to be governed by both the myofibrillar and connective tissue proteins, while the sarcoplasmic proteins contribute very little to texture [4]. Small amplitude oscillatory shear (SAOS) testing is extremely sensitive to the physical structure and chemical composition of the sample; therefore, it is useful for evaluating the linear behavior in rheology in complex macromolecular food system [5]. Understanding physicochemical characteristics is of utmost importance as it is directly related to the final quality of products like surimi, sausages and battered products. However, up till now, information concerning the effect of storage temperature on the changes of big-eye tuna myofibrillar proteins is still limited. The purpose of the present study was to investigate the physicochemical changes of muscle protein as well as the rheological properties of tuna during frozen storage at -18 and -30 ℃.

2. Materials and methods

2.1. Materials

Frozen big-eye tuna (*T. obesus*), which had been skinned and finely chopped, were supplied by Zhongshui Fisheries General Corporation (Shanghai, China). Tuna were cut into blocks of approximately 50 g. All samples were wrapped with polyethylene film, packed individually in zip-lock packets, and stored at -60 ℃ until use. All chemicals used in this study were of analytical grade.

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2.2. Myofibril isolation

Myofibrils were prepared according to the method of Katoh [6] with some modifications. All steps were performed at 4℃ to minimize proteolysis and protein denaturation. The washed myofibril pellet was stored at 4℃ until required for use. Samples were suspended and adjusted to 3% (w/v) protein using 0.6 M NaCl, 50 mM sodium phosphate buffer.

2.3. Determination of total sulfhydryl content

Total sulfhydryl content was measured using 5,5'-dithiobis(2-nitro benzoic)(DTNB) according to the method of Ellman[7] as modified by Benjakul[8]. The absorbance at 412nm was measured using an UV-spectrophotometer (Persee，China). A blank was conducted by replacing the sample with 0.6 M NaCl, 50 mM sodium phosphate buffer

2.4. Determination of Ca2+-ATPase activity

Ca^{2+}-ATPase activity was determined according to the method of Benjakul [9]. Specific activity was expressed as μmol inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP.

2.5. Dynamic rheological measurement

The oscillatory rheological experiments were conducted in a controlled-stress/strain rheometer (Physica MCR 301; Anton Paar, Ostfildern, Germany) using a parallel measuring system (50mm diameter and 1mm gap) to avoid wall slip phenomena. Temperature sweep analysis, to measure the changes in dynamic rheological parameters, including storage moduli (G') and loss moduli (G''), during heating, were performed at a constant frequency of 1 rad/s and an amplitude stress of 1 Pa, which was within the linear viscoelastic region, from 20 to 60℃, at an increasing rate of 1℃/min.

3. Results and Discussion

3.1. Ca^{2+}-ATPase activity of myofibrillar protein from tuna during frozen storage

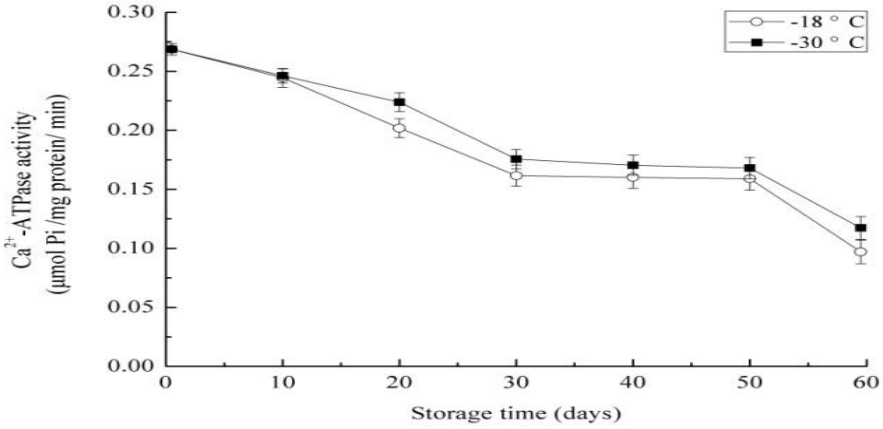

Fig. 1: Changes in Ca^{2+}-ATPase activity of myofibrillar protein extracted during frozen storage at -18℃ and -30℃ for 60 days. Error bars indicate the standard deviations from the mean of triplicate determinations.

Ca^{2+}-ATPase activity of myofibrillar protein (MP) from all sample decreased thought 60 days of frozen storage as shown in Fig.1. Decrease in Ca^{2+}-ATPase activity were noticeable during 30 days storage ($P<0.05$). Thereafter, no marked changes were found with increasing storage time up to 50 days ($P>0.05$).It was found that the activities in tuna storage at -18 and -30 ℃ were 0.09 and 0.12μmol (pi) mg^{-1} (pro) min^{-1} and decreased by 63.88% and 56.37%, respectively, compared to its initial value after 60 days of frozen storage. The decrease in Ca^{2+}-ATPase activity was possible due to the conformational changes of the myosin globular head as well as the aggregation of this portion[10]. Myosin consists of two heavy chains and two to four light chains. Each of the two subfragment-1s contains a heavy chain and two light chains. They comprise the head

of myosin that has ATPase activity and actin-binding ability. The globular heads of myosin are responsible for Ca^{2+}-ATPase activity activity. Moreover, denaturation of myosin was possibly associated with the oxidation of sulfhydryl groups on the myosin globular head.Ca^{2+}-ATPase activity is used as an indicator for the completeness of myosin molecules and it has been widely used as an indicator of fish or surimi protein denaturation[11]. Based on the decrease in Ca^{2+}-ATPase activity myosin from tuna storage at -18℃ underwent denaturation to the higher extent, compared to those of tuna at -30℃. Benjakul, Visessanguan Thongkaew and Tanaka[8] reported the continuous decrease in Ca^{2+}-ATPase activity of some tropical fish during frozen storage and the degree of decrease depended on species. Difference in the decrease of Ca^{2+}-ATPase activity among tuna and other species were probably due to different susceptibility to frozen denaturation of muscle protein.

3.2. Total sulphydryl contents of myofibrillar protein from tuna during frozen storage

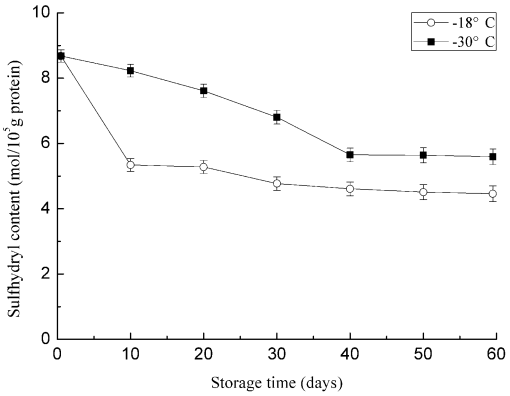

Fig. 2: Changes in sulfhydryl content of myofibrillar protein extracted during frozen storage at -18℃ and -30℃ for 60 days. Error bars indicate the standard deviations from the mean of triplicate determinations.

Sulfhydryl group content of myofibrillar protein from tuna decreased during 60 days of frozen storage as shown in Fig.2. For -18℃ , the marked decrease in sulfhydryl content was observed in the first 10 days of storage and only additional slight decreases were found thereafter ($P < 0.05$). This decrease of sulfhydryl group content was rapid and lower in tuna fillets storage -18℃ compared with those stored at -30℃. From Fig.2 during the storage of 60 days, the total sulphydryl content of the initial sample was 8.67×10-5 mol g^{-1}(pro). The sharp decrease in sulfhydryl content was observed in the first 10 days of storage at -18℃. At day 10, sulfhydryl content in tuna MP storaged at -18℃ and -30℃ decreased by 38.46% and 5.14%, respectively. Thereafter, gradual decrease was found. However, the marked decrease was observed at -30 ℃ from 10 to 40 days. After 60 days of storage, sulfhydryl content at -18 and -30℃ decreased by 48.60 and 35.59%, respectively, compared to that of initial values. The decrease in sulfhydryl content was due to either the oxidation of sulfhydryl disulfide interchanges or the formation of hydrogen and hydrophobic bonds, which masked the reactive sulfhydryl structure of actomyosin molecules[12]. Sulphydryl groups (the most reactive functional groups) in myofibrillar protein were readily oxidised to disulfide groups during frozen storage, therefore resulting in an obvious decrease in total sulphydryl content and active sulphydryl content[13]. Sulfhydryl groups on the head portion,named SH_1,SH_2, were reported to be involved in the ATPase activity of myosin[14]. Similar trends have also been reported in fish myofibrillar proteins and myosin. However, the degree of sulfhydryl oxidation and the decrease in Ca^{2+}-ATPase for different species vary greatly. Benjakul, Visessanguan, Thongkaew [8] reported decrease in sulfhydryl groups in the muscle of croaker, lizardfish, threadfin bream and bigeye snapper during storage at −18 °C up to 24 weeks was 29.6, 67.3, 43.2 and 47.8%, respectively. The differences in sulfhydryl group content between tuna and other species during frozen storage were probably caused by the difference in susceptibility to oxidation of sulfhydryl groups of myofibrillar proteins.

3.3. Dynamic rheological properties of tuna myofibrillar proteins

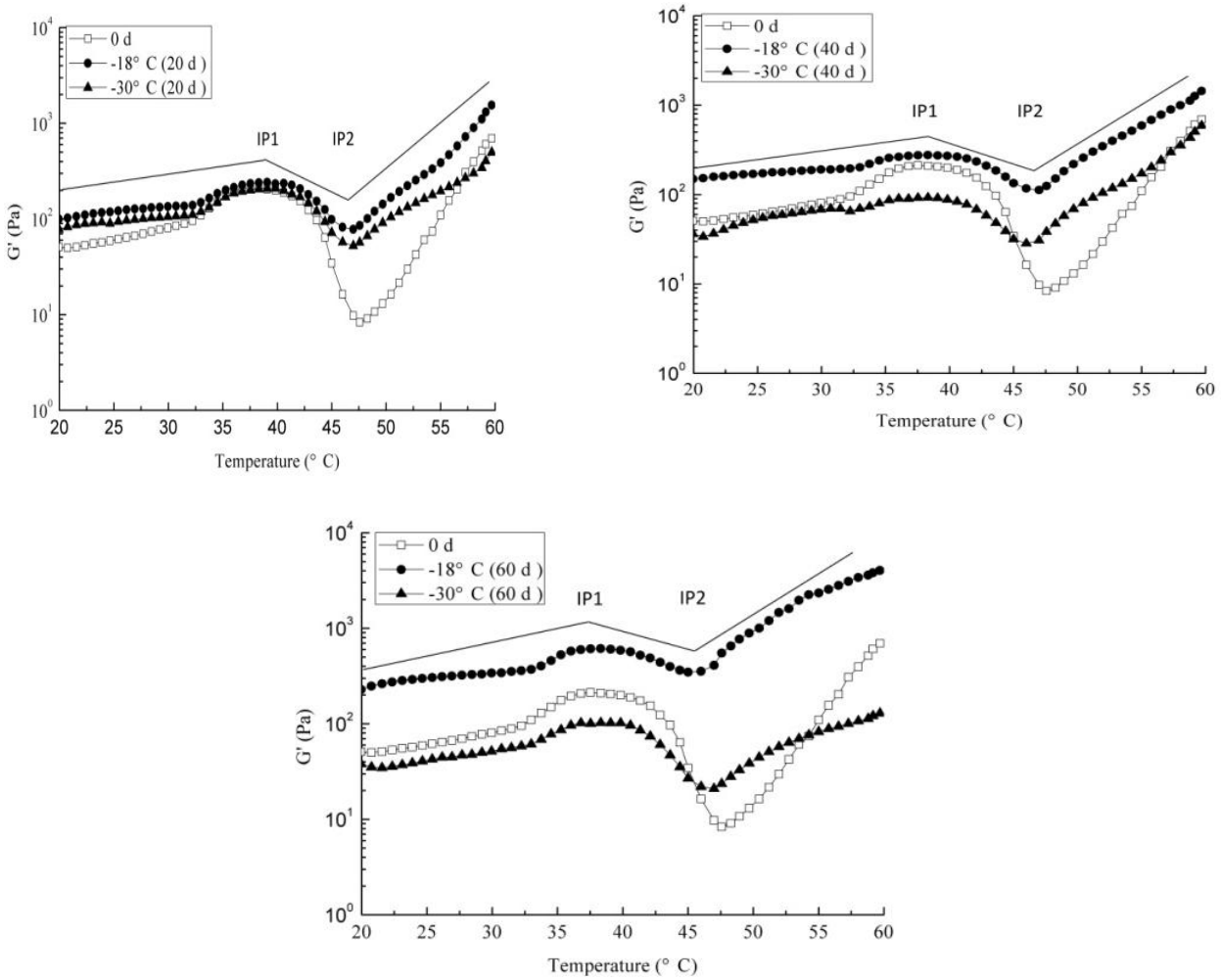

Fig.3 Storage modulus (G', Pa) of myofibrillar proteins (3.0%, w/v) during heating from 20 to 60 ℃ at a rate of 1 ℃ /min. Curves result from mean values of three samples for each storage temperature.

Rheological properties are mainly governed by molecular mass, molecular conformation. Storage modulus (G') is a measure of the energy stored and subsequently released and is the property that relates to molecular events of an elastic nature. In contrast, loss modulus (G'') is a measure of the energy dissipated. G'' is the property that relates to molecular events of a viscous nature [15]. Hence, changes in the storage modulus have been used to monitor gelation of food proteins [16]. Gelation of myofibrillar protein was monitored by dynamic rheological measurements (Fig. 3). Changes in G' of myofibrillar protein during heating from 20 to 60 ℃ can be divided into three distinct series by two inflection points (IP), where the inflection point is characteristic of the specific treatment curve. Rheological testing shows three temperature ranges in the storage modulus of tuna MP during heating phase.

G' for proteins extracted from tuna showed a typical initial increase, followed by a decrease, and then a steady increase. G' of myofibrillar protein initially increased with temperature up to IP1 (37.5–39.0 ℃). Upon further heating, the G' values for myofibrillar protein decreased significantly and reached their minimum values at IP2 (46.0–48.0 ℃). A similar curve for heating was observed by Westphalen[17], who studied the the impact of pH on heat-induced gelation properties of myofibrillar proteins from porcine semimembranosus muscle. Overall, the rheological characteristics of MP storage at different temperature were similar to those reported in the literature, reflecting unfolding and association of heavy meromyosin (HMM)(the initial increase in G′ up to IP1, which were followed by a decline thereafter due to unfolding of light meromyosin (LMM) and an eventual rise due to irreversible aggregation and gel network formation of MP[18]. The second increase in G' has been attributed to the formation of permanent irreversible cross-linked myosin filaments and a stable three-dimensional structure, due to the disulfide covalent bonding and hydrophobic interactions [19]. The gelling behavior of MP from different frozen temperature was

different. For -18°C, the average G' of MP increased with in frozen storage time. Nevertheless, for -30°C, G' decreased with in frozen storage time. The curves of MP at -18℃ that changed more and more gently with increasing time, indicating the stronger denaturation of myosin had taken place. The change of sulphydryl content seemed to be an important factor in regulating the rheological properties of MP, including the temperature of IP and the degree of HMM denaturation. Mangang [20] suggested that disulphide cross-linking between oil droplets and the gel matrix contributed to the elastic characteristics, the increase of G' can be explained in terms of disulphide reactions that involved myosin and other MP components. Another likely contributing factor to the rheological transition of MP of -18℃ was the loosening of internal bonds in myosin, so that myosin unfolded more to gain a greater hydrodynamic radius giving rise to a stronger elastic response.

Table 1: Inflection temperatures (℃) of rheological data of storage modulus (G', Pa) during heating phase

Storage Time	-18 ℃		-30 ℃	
	IP1(℃)	IP2(℃)	IP1(℃)	IP2(℃)
0 d	37.0	48.0	37.0	48.0
10 d	39.1	47.4	37.5	47.4
20 d	39.1	46.7	39.1	46.7
30 d	39.1	46.7	39.0	46.7
40 d	38.3	46.7	38.3	46.7
50 d	38.3	46.7	38.3	46.7
60 d	38.3	45.0	38.3	46.0

IP was determined for each transition during the heating stage. IP1 values of both temperature differed significantly ($P < 0.05$) as shown in Table 1. The same trend of IP2 of both frozen temperature was shown, indicating that the denaturation of MP did not change the occasion of the permanent irreversible cross-linked formation and the stable three-dimensional structure establishment. The initial increasing with temperature up to IP1 was due to unfolding and association of heavy meromyosin (HMM). The decrease in sulfhydryl groups with a concomitant disulfide bond formation may cause the change of IP1.

4. Conclusions

Frozen storage of tuna at -18 and -30℃ caused the transition in gel-forming behavior, which was associated with protein denaturation. Degree of changes was dependent upon species, storage time and storage temperature. The degree of changes was more pronounced with increasing storage temperature. Tuna was very susceptible to denaturation and loss its functional properties. Moreover, further research is warranted to elucidate the precise role of disulphide bonds in rheological properties of MP gels.

5. Acknowledgements

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