Texture characteristics of chilled prepared Mandarin fish (*Siniperca chuatsi*) during storage

Yi Sun, Liang Ma, Mingsi Ma, Hong Zheng, Xiaojie Zhang, Luyun Cai, Jianrong Li, and Yuhao Zhang

College of Food Science, Southwest University, Chongqing, China; College of Food Science and Technology, Bohai University, Food Safety Key Lab of Liaoning Province, Jinzhou, China

**ABSTRACT**

Mandarin fish that have been prepared and chilled have a shelf life of 4 days at 4°C storage, according to the total volatile basic nitrogen values. During the storage process, we observed that the texture characteristics of these fish significantly deteriorated. Certain low-abundance proteins were degraded during the shelf life, which resulted in changes in protein conformation and protein–water interaction patterns. The water-holding capacity of the fish decreased during 4°C storage; nuclear magnetic resonance analysis showed that the binding of water in fish was weakened as shelf storage prolonged and a portion of the free water was also "squeezed" to the surface of the fish in the later stages of storage. A correlation analysis showed that a decrease in the immobilized water content and mobility was associated with quality deterioration of the fish, which may be a result of increase in hydrophobic interaction and disulfide bonds in the fish during storage.

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**Introduction**

With the acceleration of the pace of life and improvements in the quality of life during modern times, the market for chilled prepared foods is developing rapidly. In recent times, chilled prepared fish has become a popular product in supermarkets in China. However, the shelf life of chilled prepared fish during chilled storage is short due to its high water activity, neutral pH, high amino acid content, bacteria, and autolytic enzymes. The postmortem changes that occur in fish muscle result in a deterioration in quality of chilled prepared fish during their shelf life. The postmortem changes that occur in fish muscle result in a deterioration in quality of chilled prepared fish during their shelf life.

Generally, during the postmortem period, fish muscles are prone to becoming soft, which further affects their textural quality. Texture is one of the primary qualities that affect consumer acceptability. Furthermore, texture is frequently employed to examine and evaluate fish quality. During chilled storage, the texture index for fish muscle has typically shown a decreasing trend or increasing first and then decreasing during chilled or frozen storage at least 15 days. Previous research has hypothesized that the change in these indices is related to the proteolysis of myofibrillar proteins during storage. Sánchez-valencia et al. reported that the conformational transitions of proteins and the change in the interaction between myofibrillar proteins and water during storage influenced the textural quality of fish. Therefore, the mechanism of fish texture change should be studied primarily by examining protein degradation and the interaction between proteins and water.

In general, the shelf life of chilled prepared fish in the supermarket is up to 5 days. Li et al. reported that the hardness and elasticity of dark and white muscle in common carp (*Cyprinus carpio*)...
have a tendency to increase and then decrease within 72 h of chilled storage after slaughter; these findings indicate that the texture quality can deteriorate during chilled storage. Nevertheless, there has been no specific study of the change in texture quality and the mechanism of this change during the shelf life of chilled fish.

In China, the Mandarin fish (Siniperca chuatsi) is a type of freshwater fish that has a relatively high market value and is often sold chilled and prepared in supermarkets. The aim of this study is to investigate the texture quality change in Mandarin fish and the mechanism of this change during shelf life storage at 4°C.

Materials and methods

Fish samples

Live Mandarin fish (S. chuatsi) weighing 600.0 ± 50.0 g and 20.0 ± 5.0 cm in length were purchased from a local supermarket in Chongqing, China, and transported to the laboratory. The fish were killed by a blow to the head, scaled, eviscerated, and washed with running water. The treated fish were placed in a box covered by a plastic film, and the box was placed in a refrigerator at 4°C. The day of killing fish was set as first day.

Determination of total volatile basic nitrogen (TVBN)

The measurement method used to determine TVBN was similar to that described in Mousakhani-Ganjeh et al.,[8] with slight modification. The TVBN content of the fish samples was determined in boric acid solution (1%, w/v) after steam distillation. Approximately 10 g of a fish sample was mixed with 90 ml of 0.6 M perchloric acid solution, homogenized for 2 min (JYL-C012 Joyoung food processor, Shandong, China) at high speed and then filtered through a filter paper. Next, 5 ml of the filtrate was loaded into a Kjeldahl-type distillation tube, followed by the addition of 5 ml of 0.75 M aqueous NaOH solution. Steam distillation was performed using a vertical steam distillation unit; the distillate was received into a beaker containing 10 ml of 30 g/L aqueous boric acid solution and phenolphthalein indicator, and incubated for 5 min. The volatile base nitrogen contained in the distillate solution was determined by titration with 0.01 M HCl and calculated with the formula

\[
TVBN \text{ (mg/100g sample)} = (V_1 - V_0) \times \frac{14 \times 20 \times 100}{W}
\]

where \(V_0\) is the volume of hydrochloric acid used in the blank titration, \(V_1\) is the volume of hydrochloric acid used in the sample titration, and \(W\) is the weight of the fish sample in grams. All the analyses were performed in duplicate three times.

Textural parameters

The measurement method used to determine textural quality was similar to that described in Gao et al.,[9] with slight modification. Texture measurements were determined using a TC3 Texture Analyzer (Brookfield, Massachusetts, USA). Mandarin fish (dorsal muscle) fillets were cut into small cubes (3.0 × 2.0 × 1.0 cm\(^3\)) and kept on ice prior to texture analysis. Each sample was compressed using a flat-ended aluminum cylindrical plunger (50 mm diameter, type TA25) at a constant test speed of 1 mm/s until it reached 30% deformation and a trigger point of 5 g. The compressive force was perpendicular to the muscle fiber orientation. This was repeated 15 times in parallel to the muscle fiber orientation for each sample. Hardness was defined as the maximum force detected during first compression, expressed in grams. Cohesiveness was measured as the ratio of the positive force during the second compression to the positive force during the first compression.\[10\] Gumminess was defined as the product of hardness × cohesiveness. Springiness was defined as the ratio of the time or distance from the start of the second area to the second probe reversal over the
distance or the time between the start of the first area and the first probe reversal. Chewiness was defined as the product of hardness × cohesiveness × springiness.\[^{11}\] Cohesiveness and springiness are dimensionless.

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE)**

SDS–PAGE was performed according to Shi et al.\[^{12}\] with some modifications. Three grams of fish muscle was blended with 27 ml of dissolving solution (2\% SDS, 8 mol/L urea, pH 8.8) using a JYL-C012 food processor (Joyoung, Shandong, China). The homogenates were incubated at 80°C for 1 h to allow maximal protein solubilization and extraction, and subsequently centrifuged at 3000 g for 15 min, as the supernatant was kept diluted to a 0.2\% protein concentration with water.\[^{13}\]

Treated protein samples (0.2\% protein concentration) were mixed (at a 1:1 v/v ratio) with SDS–PAGE sample buffer (4\% SDS, 20\% glycerol, 10\% b-mercaptoethanol, 0.125 M Tris, pH 6.8) and dissolved by heating in boiling water for 5 min. For SDS–PAGE, 10 μL of the sample per well was loaded onto a polyacrylamide gel made of 4\% stacking gel and 10\% separating gel, then subjected to electrophoresis at 15 mA until the bromophenol blue band reached the separation gel of the adhesive interface, at which point, the current was switched to 25 mA. After electrophoresis, the gels were stained using 0.05\% (w/v) Coomassie Brilliant Blue G-250 in 50\% (v/v) methanol and 9.2\% (v/v) glacial acetic acid, then destained using 7.5\% (v/v) glacial acetic acid and 5\% (v/v) methanol.

**Moisture content, centrifugation loss, and cooking loss (CL)**

The moisture content was measured according to Marimuthu et al.\[^{14}\] with slight modification. Approximately 5 g of minced muscle was mixed with sand in a ceramic bowl for 4 h at 103 ± 2°C. The moisture content was recorded based on the weight differences before and after the drying of four replicates for each sample.

Centrifugation loss was measured according to the method in Etemadian et al.,\[^{15}\] with slight modification. Approximately 5 g of each fish sample was centrifuged at 4000 g for 15 min at 4°C onto a filter paper pad placed at the bottom of the centrifugal tube. Then, the water was poured out of the centrifuge tubes and the remaining fish samples were weighed. The centrifugation loss was expressed as a percentage of weight loss of the initial sample weight.

In accordance with the method of Bouton et al.,\[^{11}\] 10 g of each sample was heated by water vapor for 5 min, then cooled and stored at 4°C until analysis. CL was determined with the following equation:

\[
\text{CL} = \frac{(m_1 - m_2)}{m_1} \times 100\% ,
\]

where \(m_1\) is the weight of the fish muscle before cooking, and \(m_2\) is the weight of the fish muscle after cooking.

**Low-field nuclear magnetic resonance (LF-NMR)**

Relaxation time was analyzed using low-field 1H NMR. Over 40 samples (approximately 3-cm long and 2 × 1 cm in cross-sectional area, weighing approximately 10 g) were cut along the fibers using a scalpel. Four pieces of a cooked sample were randomly selected and heated for 5 min at 100°C. Once the temperature returned to 32°C, each sample was placed in a cylindrical glass tube with the fiber direction perpendicular to the tube wall. Transverse relaxation times (\(T_2\)) were measured on a Niumag Benchtop Pulsed NMR analyzer (MesoQMR23-060H; Niumag Electric Corporation, Shanghai, China) operating at 21.7673 MHz. The \(T_2\) was measured using the Carr–Purcell–Meiboom–Gill sequence with the test parameters: SFO1 (MHz) = 21.7673, P1 (us) = 10.4, P2 (us) = 21.4, SW (KHz) = 100, TW (ms) = 3000, TE (ms) = 0.3, RG1 = 20, DRG1 = 3, PRG = 1, NECH = 8000, and NS = 8.\[^{16,17}\] The LF-NMR relaxation curve was fitted to a multi-exponential
curve using Multi Exp Inv Analysis software (Niumag Electric Corporation, Shanghai, China), which uses the inverse Laplace transformation algorithm. The following parameters were presented: $T_{21}$, $T_{22}$, and $T_{23}$ as the relaxation components; and $A_{21}$, $A_{22}$, and $A_{23}$ as the corresponding area fractions. The relaxation time acts as an indicator for water mobility, while the area under the curve can indicate the amount of water within each component.\[18\]

**Determination of the presence of intermolecular bonds**

Selective solubility of the proteins was determined as described by Dan et al.\[19\] Chopped fish muscle (2 g) was homogenized in 10 ml of various solvents including SA (0.05 M NaCl), SB (0.6 M NaCl), SC (0.6 M NaCl + 1.5 M urea), SD (0.6 M NaCl + 8 M urea), and SE (0.6 M NaCl + 8 M urea + 2-β-mercaptoethanol), and kept at 4°C for 1 h, followed by centrifugation at 10,000g for 15 min using an Eppendorf centrifuge (5810R, Hamburg, Germany). Protein contents of the supernatants were measured by the Lowry method to determine the existence of ionic bonds (difference between protein dissolved in SB and SA), hydrogen bonds (difference between protein dissolved in SC and SB), hydrophobic interactions (difference between protein dissolved in SD and in SC), and disulfide bonds (difference between protein dissolved in SD and SE).\[20–22\] The results were expressed as grams of soluble protein per liter of homogenate.

**Results and discussion**

**Total volatile basic nitrogen**

The putrefaction of fish represents severe proteolysis in which fish are degraded by both microorganisms and autolytic enzymes.\[23–26\] TVBN represents the quantity of nonprotein nitrogen, such as nucleotides, sulfur containing amino acids, and trimethylamine oxide, converted to volatile basic nitrogenous substances such as trimethylamine, methylmercaptan, and ammonia. Therefore, the accumulation of TVBN is usually used as a reliable freshness index for fishery products.\[27\] As expected, the TVBN of Mandarin fish increased gradually as storage time was prolonged. In Fig. 1, the TVBN value (mg/100 g muscle) increased from 10.47 ± 0.88 to 20.49 ± 0.55 at 4°C within 5 days of storage. According to the official standards of China (GB 2733–2015), the TVBN value of freshwater fish and shrimp during the storage should be less than or equal to 20 mg/100 g. Some

![Figure 1. Total volatile basic nitrogen (TVBN) (mg/100 g fish muscle) of fish during cold storage at 4°C.](image-url)
articles in the literature have also reported the limit level of TVBN. Alparslan et al. [28] and Arfat et al. [29] found that TVBN content of 30–35 mg of N per 100 g is usually regarded as the limit of acceptability for fish. Smichi et al. [30] found that the limit authorized of TVBN for human consumption is set at 30 mg N/100 g. Huang et al. [31] reported that the limit of acceptability for TVBN in fresh fish is 20 mg/100 g fish muscle. In general, the TVBN limit of freshwater fish should be less than that of marine fish. Therefore, 20 mg/100 g was chosen as the limit, and within these parameters, the shelf life of Mandarin fish was 4 days at 4°C.

**Texture profile analysis (TPA)**

A TPA was used to measure factors such as hardness, cohesiveness, springiness, gumminess, and chewiness. These factors were the primary variables used to describe the tissue properties. [15,32] Table 1 shows that the changing trends of all factors in raw fish fillet resembled those of cooked fish, which indicates that changes to texture characteristics in raw fish are similar in effect to those of cooked fillets.

During shelf life, some indices of raw and cooked fish fillet showed decreasing trends, such as in hardness, springiness, gumminess, and chewiness. The cohesiveness of raw fish fillet did not show a significant change throughout the storage period. Conversely, the cohesiveness of cooked fish fillet increased in the first 2 days and then decreased gradually during shelf life. The results are in accordance with previous studies. Liu et al. [33] found that the hardness of grass carp fillets stored at −3°C and 0°C both decreased sharply within the first 3 days. Hassoun and Karoui [34] reported that the cohesiveness of whiting fillets showed no significant change at 4°C over 15 days. Gao et al. [9] discovered that the average values for hardness, gumminess, chewiness, and springiness of pompano fillets displayed significant decreases throughout 15 days of storage at 4 ± 1°C. Zhao et al. [35] found that the springiness of large Yellow Croaker fillets under vacuum conditions at 0°C showed a significant decreasing trend over a period of 20 days. Compared with other references in the literature, textural changes determined by mouthfeel decreased, and quality deteriorated with extended storage times.

The decrease in texture quality of fish stored at 4°C may be due to protein changes during the storage process. Ayala et al. [36] found deterioration of sea bream quality during 22 days of storage at 4°C, which may have been due to the degradation of collagen and cytoskeletal proteins by the action of endogenous enzymes during storage. In addition, Ishiwatari et al. [37] discovered that protein denaturation further affected the change in the state of water. In summary, the texture quality decrease may be due to the degradation of proteins by the action of endogenous enzymes and the interaction between proteins and water during storage. As a result, the acceptable storage time exceeded shelf life in the previous studies. There has not been a systematic study of the deterioration of fish quality during the early stages of storage.

| Time (day) | 1 | 2 | 3 | 4 | 5 |
|-----------|---|---|---|---|---|
| **Raw**   |   |   |   |   |   |
| Hardness  (g) | 3145 ± 301<sup>a</sup> | 2739 ± 318<sup>b</sup> | 2493 ± 302<sup>c</sup> | 1498 ± 293<sup>d</sup> | 958 ± 186<sup>e</sup> |
| Springiness | 0.7 ± 0.1<sup>a</sup> | 0.7 ± 0.1<sup>ab</sup> | 0.64 ± 0.09<sup>bc</sup> | 0.6 ± 0.1<sup>cd</sup> | 0.5 ± 0.1<sup>de</sup> |
| Cohesiveness | 0.50 ± 0.05<sup>a</sup> | 0.49 ± 0.07<sup>a</sup> | 0.49 ± 0.06<sup>a</sup> | 0.47 ± 0.05<sup>b</sup> | 0.48 ± 0.06<sup>a</sup> |
| Gumminess (g) | 1574 ± 168<sup>a</sup> | 1351 ± 208<sup>b</sup> | 1214 ± 198<sup>b</sup> | 710 ± 151<sup>c</sup> | 464 ± 105<sup>d</sup> |
| Chewiness (g) | 1163 ± 193<sup>a</sup> | 982 ± 227<sup>b</sup> | 784 ± 168<sup>c</sup> | 417 ± 105<sup>d</sup> | 255 ± 80<sup>e</sup> |
| **Cooked** |   |   |   |   |   |
| Hardness (g) | 572 ± 190<sup>a</sup> | 349 ± 60<sup>b</sup> | 341 ± 106<sup>b</sup> | 340 ± 116<sup>c</sup> | 285 ± 116<sup>d</sup> |
| Springiness | 1.0 ± 0.4<sup>a</sup> | 0.9 ± 0.2<sup>ab</sup> | 0.9 ± 0.5<sup>bc</sup> | 0.9 ± 0.3<sup>ab</sup> | 0.77 ± 0.08<sup>b</sup> |
| Cohesiveness | 0.4 ± 0.1<sup>a</sup> | 0.63 ± 0.09<sup>a</sup> | 0.6 ± 0.1<sup>a</sup> | 0.6 ± 0.1<sup>ab</sup> | 0.5 ± 0.1<sup>b</sup> |
| Gumminess (g) | 261 ± 124<sup>a</sup> | 220 ± 53<sup>b</sup> | 210 ± 73<sup>a</sup> | 219 ± 116<sup>a</sup> | 157 ± 83<sup>b</sup> |
| Chewiness (g) | 245 ± 118<sup>a</sup> | 207 ± 88<sup>ab</sup> | 193 ± 136<sup>ab</sup> | 197 ± 133<sup>ab</sup> | 124 ± 70<sup>b</sup> |

* a-e: Values with unlike superscript letters in the same line are significantly different (P < 0.05).
The value of TCA soluble peptides during storage is illustrated in Fig. 2; TCA soluble peptides gradually increased with storage time. This could be due to protein degradation by endogenous enzymes or microorganisms in the fish that produce exogenous enzymes during the storage process.\textsuperscript{38}

The protein changes in Mandarin fish during cold storage (4°C) are shown in Fig. 3. The characteristic bands for myosin heavy chain (MHC), actin, tropomyosin, and α-actinin were present in the gels. Myosin and actin are the major proteins that contribute to most of the functional

![Figure 2](image1.png)

**Figure 2.** Changes in TCA-soluble peptide contents of fish during cold storage at 4°C.

**Trichloroacetic acid (TCA) soluble peptide content and SDS-PAGE**

![Figure 3](image2.png)

**Figure 3.** SDS-PAGE patterns of fish during cold storage at 4°C. Numbers designate the storage time (days). Marker: molecular weight marker.
properties of myofibrillar proteins.\cite{39} As shown in Fig. 3, there were no obvious changes in MHC and actin over the shelf life period. This result is inconsistent with previous research. Ramirez-Suarez et al.\cite{13} found that MHC decreased over the 15 days of ice storage with a concomitant appearance of a protein band at 153 kDa in jumbo squid. Yang et al.\cite{40} reported a slight decrease in MHC and actin content in salmon during 12 days of storage at 4°C. Because of the shorter storage time in our study, differences existed between these previous studies and this paper. A decrease in the intensity of the 50–80 kDa bands was discovered in the early stages of storage, which may include some effects of protein degradation. A possible reason for the above situation is a weakening of Z-disks and release of sarcomeres via the action of calpains.\cite{41} Previous studies showed that degradation of myosin and actin was primarily associated with cathepsin, but cathepsin was not activated during early storage.\cite{42} Through the weakening of Z-disks, release of costameres, breakdown of desmin and dystrophin, loss of α-actinin, and proteolysis of nebulin, titin and troponin-T, calpains play a leading role in early storage.\cite{41,43} Therefore, the quality deterioration of Mandarin fish during their shelf life at 4°C is mainly due to the degradation of low-abundance proteins, including desmin, dystrophin, and α-actinin.

**Moisture content, CL and centrifugal loss**

The interaction of water and protein was measured (Table 2). Fish muscle was shown to be a high-moisture-content food. The moisture content was volatile between 82.20 ± 0.44% and 83.48 ± 0.22%, which did not change regularly. CL appeared to slightly increase from 9.58 ± 2.35% to 11.83 ± 0.66%. Centrifugation loss showed a trend of significant increase from 11.41 ± 1.50% to 16.53 ± 1.11%. The trends of these three indicators illustrate that the water-holding capacity of the fish decreased. Association with changes in protein structure could explain the above trend.\cite{44,45} During storage, the degradation of some low-abundance proteins can expose hydrophobic groups and alter protein conformation, which can change the pattern of protein–water interaction.\cite{46,47} For validation, the effect of protein and water was further analyzed by LF-NMR.

**Low-field nuclear magnetic resonance**

A typical NMR \( T_2 \) curve showing the \( T_2 \) transverse relaxation time distribution of Mandarin fish fillets stored at 4°C is presented in Fig. 4. Based on previous studies,\cite{48} the first peak (\( P_1 \)), with a relaxation time (\( T_{21} \)) ranging from 0.1 to 10 ms, is considered to be bound water. The second peak (\( P_2 \)), with a relaxation time (\( T_{22} \)) of approximately 50 ms, is considered immobilized water. The last peak (\( P_3 \)), with a relaxation time (\( T_{23} \)) ranging from 100 to 1000 ms, is free water. The relaxation time (\( T_2 \)) represents the mobility of water, the drop in \( T_2 \) represents the decrease in the mobility of water, the peak area (\( A_2 \)) represents the amount of water, and the drop in \( A_2 \) represents the decrease in the amount of water.\cite{49}

The changes in the peak area (\( A_2 \)) and relaxation time (\( T_2 \)) of raw fish during storage is shown in Fig. 5. In the first 3 days of storage, the \( A_{21} \) decreased, indicating that the amount of bound water decreased. The \( A_{22} \) increased slightly during the first 2 days and significantly decreased on the third day, which directly indicated that the content of immobilized water also increased slightly and then decreased significantly. The \( A_{23} \) showed a trend of increase in the first 3 days, which indicated that

| Time (day) | 1 | 2 | 3 | 4 | 5 |
|-----------|---|---|---|---|---|
| Moisture content (%) | 82.5 ± 0.1\(^b\) | 83.5 ± 0.2\(^a\) | 83.4 ± 0.4\(^a\) | 83.3 ± 0.3\(^a\) | 82.2 ± 0.4\(^b\) |
| Cooking loss (%) | 10 ± 2\(^b\) | 10.3 ± 0.3\(^ab\) | 11 ± 1\(^b\) | 9 ± 1\(^a\) | 11.8 ± 0.7\(^b\) |
| Centrifugation loss (%) | 11.5 ± 0.2\(^e\) | 14.0 ± 0.2\(^d\) | 15.5 ± 0.3\(^c\) | 16.00 ± 0.08\(^b\) | 16.6 ± 0.2\(^a\) |

\(^a\)-\(^e\): Values with unlike superscript letters in the same line are significantly different (\( P < 0.05 \)).
the volume of free water increased in the first 3 days. Combined with the above observations, a conclusion could be drawn that the state of water changed in the first 3 days of shelf life. A portion of the bound water was transformed into immobilized water, and some of the immobilized water was transformed into free water, which brought about the increase in the amount of free water. In the fourth day, the content of bound water had almost no change. Conversely, both the immobilized water and the free water decreased significantly. According to the results from the total peak area, the total amount of water decreased as the shelf life was extended. These conclusions are not consistent with previous results (Table 2). As protein conformation changes during storage, a portion of the free water undetected by NMR can be “squeezed” to the surface of fish. However, this would have been measurable via the drying method. Therefore, the content of free water decreased even though some immobilized water was converted to free water in the fourth day. In the fifth day, the three types of water did not change significantly ($p < 0.05$), which indicates that the state of water was unchanged.
The changing tendencies in the mobility of water are shown in Fig. 5b. $T_{21}$ had no significant change over the course of storage. The $T_{22}$ values indicate that the mobility of immobilized water increased in the first day and then decreased. As usual, the higher the water content, the stronger the water mobility.$^{[50,51]}$ The trend in the immobilized water mobility and content was consistent with previous reports. However, the mobility and content of free water showed a different trend; $T_{23}$ decreased significantly during the first 3 days and then had no change during additional days of storage. Because part of the immobilized water transferred to free water and a portion of the free water with higher mobility was “squeezed” out to the surface of the fish, the mobility of free water was decreased, even though its content increased over the first 3 days; additionally, free water mobility did not decrease along with its content over the last 2 days.

The Pearson product-moment correlation coefficient (PPMCC) has been widely used to measure the degree of correlation between two variables.$^{[52,53]}$ The PPMCC of NMR and texture (hardness, springiness, cohesiveness, gumminess, and chewiness) is shown in Table 3. The immobilized water was strongly related to the texture index. This meant that the decrease in the immobilized water volume and mobility was associated with the quality deterioration of fish during shelf life. The immobilized water was also the most abundant part of the water content in fish muscle. Thus, the change in the immobilized water was dependent on the change in the pattern of protein–water interaction. According to earlier studies, the pattern of protein–water interaction was related to intermolecular bonds in protein.$^{[54,55]}$ As a result, the diversification of intermolecular bonds was further analyzed to clarify the reasons for the change in the immobilized water.

| PPMCC  | $A_{21}$ | $A_{22}$ | $A_{23}$ | $T_{21}$ | $T_{22}$ | $T_{23}$ |
|--------|---------|---------|---------|---------|---------|---------|
| Hardness | 0.723   | 0.957   | 0.342   | 0.302   | 0.933   | 0.664   |
| Springiness | 0.809   | 0.965   | 0.221   | 0.501   | 0.966   | 0.720   |
| Cohesiveness | 0.650   | 0.877   | 0.127   | 0.010   | 0.799   | 0.730   |
| Gumminess | 0.736   | 0.960   | 0.319   | 0.306   | 0.935   | 0.683   |
| Chewiness | 0.780   | 0.970   | 0.253   | 0.364   | 0.948   | 0.727   |

$A_{21}$, $A_{22}$, $A_{23}$ represent the water content of bound water, immobilized water, and free water, respectively.

PPMCC: Pearson product-moment correlation coefficient; NMR: nuclear magnetic resonance.

The changing tendencies in the mobility of water are shown in Fig. 5b. $T_{21}$ had no significant change over the course of storage. The $T_{22}$ values indicate that the mobility of immobilized water increased in the first day and then decreased. As usual, the higher the water content, the stronger the water mobility.$^{[50,51]}$ The trend in the immobilized water mobility and content was consistent with previous reports. However, the mobility and content of free water showed a different trend; $T_{23}$ decreased significantly during the first 3 days and then had no change during additional days of storage. Because part of the immobilized water transferred to free water and a portion of the free water with higher mobility was “squeezed” out to the surface of the fish, the mobility of free water was decreased, even though its content increased over the first 3 days; additionally, free water mobility did not decrease along with its content over the last 2 days.

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Figure 6. Intermolecular bonds changes of fish during cold storage at 4°C.
Intermolecular bonds

The changes in intermolecular bonds as storage proceeds are shown in Fig. 6. Ionic bonds did not obviously change, while the primary changes were in the hydrogen bonds, hydrophobic interactions, and disulfide bonds. Hydrophobic interaction increased rapidly, and the strength of hydrogen bonds was decreased. The reason for this phenomenon may be due to protein conformation changes during storage, leading to the exposure of hydrophobic groups.\textsuperscript{[21,56]} Disulfide bonds in the storage process showed an upward trend, which was likely due to oxidation of sulfhydryl groups to disulfide bonds.\textsuperscript{[37,58]}

Combined with the LF-NMR data, the PPMCCs of the immobilized water and intermolecular bonds (hydrophobic interactions and disulfide bonds) are illustrated in Table 4. The change in the immobilized water could primarily be related to hydrophobic interaction and disulfide bonds. Hydrophobic interaction and disulfide bonds both revealed increasing trends, which suggest that the protein network was growing larger and closer. On the second day, new protein networks had formed, leading to a peak in the water volume and the relaxation time of immobilized water. With the enhancement of hydrophobic interactions and disulfide bonds, the protein network became tighter. A portion of the immobilized water was “squeezed,” causing the water volume and the relaxation time of the immobilized water to drop during the later stage.

Conclusions

According to the TVBN value of chilled prepared Mandarin fish during 4°C storage, the shelf life was determined to be 4 days. The texture quality of raw and cooked fish fillet showed deterioration tendency over prolonged shelf storage times. The TCA soluble peptide content analysis showed the protein in fish degrades gradually as the storage time increased. SDS–PAGE showed that there were no obvious changes in MHC and actin, and some low-abundance proteins were degraded during storage. Loss of fish during cooking and centrifugation showed an increasing trend, which indicated that the water-holding capacity of the fish decreased as the storage time increased. NMR analysis showed that the binding of water in fish was weakened as shelf storage prolonged and some of the free water was also “squeezed” to the surface of the fish in the later stages of storage, which also indicated that the water-holding capacity of the fish had decreased. The mobility of the immobilized water largely showed a decreasing trend, during storage due to its transformation into free water. Correlation analysis showed that the immobilized water was strongly related to the texture index, which implied that the decrease in the immobilized water content and mobility is associated with the quality deterioration of fish during shelf life. Analysis of intermolecular bonds within the fish showed that the increase in hydrophobic interactions and disulfide bonds resulted in the decrease in the immobilized water content and mobility during storage.

Considering these results, we conclude that the degradation of low-abundance proteins can alter protein conformation in fish during storage throughout its shelf life and that the increase in hydrophobic interactions and disulfide bonds can decrease both the immobilized water content and mobility, which results in the deterioration of fish texture quality.

| Table 4. PPMCCs of immobilized water and intermolecular bonds (hydrophobic interactions and disulfide bonds). |
|----------------|----------------|----------------|
| PPMCC          | Hydrophobic interactions | Disulfide bonds |
| $A_{22}$       | -0.903          | -0.870         |
| $T_{22}$       | -0.910          | -0.866         |

PPMCC: Pearson product-moment correlation coefficient.
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