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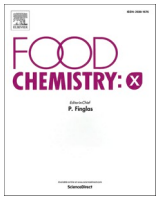

Factors affecting the quality of frozen large yellow croaker (*Pseudosciaena crocea*) in cold chain logistics: Retention time and temperature fluctuation

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ABSTRACT

The purpose of this study is to provide a reference for avoiding the quality loss of large yellow croaker in cold chain transportation. The effects of retention time before freezing and temperature fluctuation caused by transshipment in logistics were evaluated by TVB-N, K value, TMA value, BAs, FAAs content and protein-related characteristics. The results showed that the retention would lead to the rapid increase of TVB-N, K value, and TMA value. And the temperature fluctuation would further lead to deterioration of these indicators. We concluded that the influence of retention time was far greater than that of temperature fluctuation. In addition, the bitter free amino acids (FAAs) were highly correlated with the freshness-related indicators, which could reflect the freshness changes of samples, especially the quantity of histidine. Therefore, it is suggested to freeze samples immediately after catching and try to avoid temperature fluctuations during cold chain to maintain the quality.

Introduction

China is rich in fishery resources, including freshwater resources and seawater resources (Cai et al. 2020). With its high nutritional value and rich taste, large yellow croaker (*Pseudosciaena crocea*) has become one of the most popular marine fish in China (Lan et al. 2021). In 2021, the outputs of marine caught and breeding large yellow croaker were 38,167 and 254,224 tons (China Fishery Statistical Yearbook, 2022) respectively. Due to the working mode of offshore fishing, the large yellow croaker will be transported to the shore in a way of keeping alive. However, due to insufficient manpower and heavy load on equipment, some fish has to stay for a period of time before being processed. This will cause a rapid decline in the quality of the fish. Consequently, it is of great significance to study the effect of retention time before processing on quality changes of large yellow croaker after fishing.

After the death of sea fish, complex chemical reactions occur in the body, the protein, fat and carbohydrate polymer compounds in sea fish degrade into simple compounds (Yi and Xie 2022). At the same time, the changes of the body of sea fish after death can be divided into three stages: fish stiffness stage, fish autolysis stage and tissue corruption stage (Cheng et al. 2014). Different degrees of freshness appear in each stage, and the freshness decreases with the extension of time. Therefore, it is necessary to carry out some treatment after landing the catch to protect it from corruption and deterioration. Freezing is one of the most common preservation methods of sea fish (Tan et al. 2022).

In addition, temperature fluctuation is inevitable for frozen large yellow croaker during cold storage, transportation and sales. Many researchers found that the temperature fluctuation during the storage had an irreversible impact on the quality of fish (Göransson et al. 2018; Mu et al. 2017). Margeirsson et al. (2012) also believed that in the simulation of cold chain logistics, temperature fluctuations were the main reason for the shortened shelf life of cod. However, it has not been reported to systematically evaluate the effects of retention time and temperature fluctuation on the quality of large yellow croaker.

Therefore, in order to provide a reference for avoiding the quality loss of frozen large yellow croaker, this paper studies mainly from two parts. One part was to study the effect of retention time before freezing on large yellow croaker quality; the other part was the effect of

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temperature fluctuation caused by transshipment on the quality.

Materials and methods

Raw material treatment

A total of 60 live large yellow croaker with an average weight of 650 ± 100 g were purchased from the local aquatic product market (Shanghai Luchao Port seafood market), of which 6 were used for the test on 0 d to determine the relevant parameters of fresh fish. They were immediately transported to the laboratory and died suddenly by ice. Before freezing treatment, all samples were divided into 3 groups (20 fish per group). To simulate the effect of retention time, two groups were placed at 25 °C for 4 h (4 h group) and 8 h (8 h group), respectively. Two samples were randomly taken from each group for testing on 0 d. Subsequently, three groups of samples were frozen using a spiral freezer (Yantai Moon Co., Ltd., Shandong, China), and the machine parameters were set with reference to a previous research (Chu, Cheng, et al. 2021). Then, the frozen samples were stored at −18 °C and the quality was evaluated every 30 d. The simulated transport process was carried out for every group in the following ways: after being stored at −18 °C for 90 d, part of the large yellow croaker was transferred to −10 °C for 24 h which simulated the transport process of frozen fish, and then transferred to −18 °C again. The quality was evaluated on the 120 d, 150 d and 180 d respectively. The treatment process is shown in Fig. 1.

Determination of total volatile base nitrogen (TVB-N) and K value

The TVB-N of fish was performed using the method of Chu et al. (2021) by an automated Kjeldahl nitrogen tester.

The measurement of the content of ATP-related compounds was performed using a high performance liquid chromatography (HPLC, Waters 2695, Milford, CT) described by Chu et al. (2021), and the K value was calculated as follows:

$$kvalue = \frac{HxR + Hx}{ATP + ADP + AMP + IMP + HxR + Hx} \times 100\%$$

Where ATP, ADP, AMP, IMP, Hx, and HxR represent adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, inosine monophosphate, hypoxanthine, and inosine, respectively.

Determination of trimethylamine (TMA)

According to AOAC method 971.14 with slight modifications, 2 g sample was homogenized in 18 mL of 7.5% trichloroacetic acid solution (TCA, v/v) and centrifuged for 10 min (10,000 g, 4 °C), and 5 mL of supernatant was taken in a test tube and mixed with 1 mL of formaldehyde (10%, v/v), 10 mL of anhydrous toluene and 3 mL of KOH (25%, w/v) and allowed to stand for 5 min at 30 °C. The toluene layer was transferred to a test tube containing 0.5 g of anhydrous Na_2SO_4 and shaken to dehydrate it. The absorbance A_1 was measured at 410 nm using a spectrophotometer (Winooski, VT, USA), and the blank experiment was performed by 7.5% TCA according to the same procedure, and the absorbance was recorded as A_0. The TMA content was calculated according to the standard curve, and the results were expressed as mg/100 g.

Determination of biogenic amines (BAs)

BAs were analyzed according to the method of Wang et al. (2014) with slight modifications. Took 5 g of sample and 20 mL of TCA (5%, v/v) to be homogenized, and centrifuged for 10 min (5000 r/min, 4 °C). This step was repeated twice, and the supernatant was combined and fixed to 50 mL by 5% TCA. The 10 mL of the extract was dissolved with 0.5 g NaCl by sonication, and then 10 mL of hexane was added to sonicate for 5 min. The lower layer of the resting liquid was degreased once more using 10 mL of hexane in the same way. A tube of 5 mL of degreased solution whose pH value was adjusted to 12 (TCA, NaOH) was mixed with 5 mL of *n*-butanol/chloroform mixture (1:1, v/v) for 5 min, and then centrifuged for 5 min (5000r/min, 4 °C). After standing, took the upper organic phase and fix the volume to 10 mL with *n*-butanol/trichloromethane mixture. 1 mL of the purified solution was mixed with 1 mL of saturated $NaHCO_3$, 100 μL of NaOH (1 M) and 1 mL of acetone solution of dansyl chloride (1%, w/v) and sonicated for 1 min, and the solution was derivatized by water bath at 60 °C for 20 min. The derivatized solution was mixed with 200 μL of ammonia and sonicated for 1 min. After standing for 30 min, 0.5 g of NaCl and 5 mL of ethyl ether were added and sonicated again for 2 min. The organic phase was

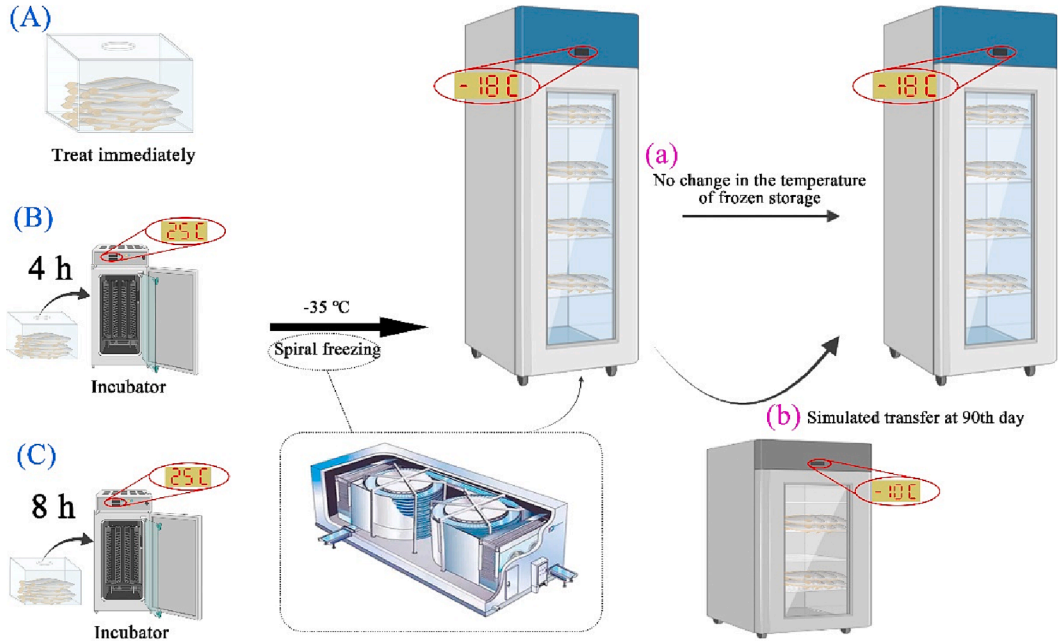

Fig.1. Schematic diagram of treatment process of large yellow croaker after fishing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

separated by standing, aspirated from the upper layer, blown dry in a 40 °C water bath and mixed into 1 mL of acetonitrile for sonication, and finally filtered using a 0.22 μm membrane and stored for testing.

A HPLC equipped with a PDA detector and a C_{18} column (T3, 250 mm × 4.6 mm, USA) was used for the analysis of BAs. The equipment parameters were set with reference to Wang et al. (2021).

Free amino acids (FAAs)

FAAs were measured with reference to Li et al. (2020) and the content was determined with an amino acid analyzer (Hitachi L-8800, Tokyo, Japan) and each measurement was repeated three times.

Taste activity value (TAV) was calculated as the ratio between the FAAs concentration measured in sample and its threshold value usually measured in water or in a simple substrate.

Secondary structure of myofibrillar protein (MP)

MP was extracted by reference to Li et al. (2020). The analysis of secondary structure of MP was accomplished by Fourier transform infrared spectroscopy (FT-IR) (Nicolet iS5, Thermo Scientific Inc.).

Tertiary structure of MP

The analysis of tertiary structures of was measured by a fluorescence spectrophotometer (F-7100, Hitachi, Japan) referring to the method of

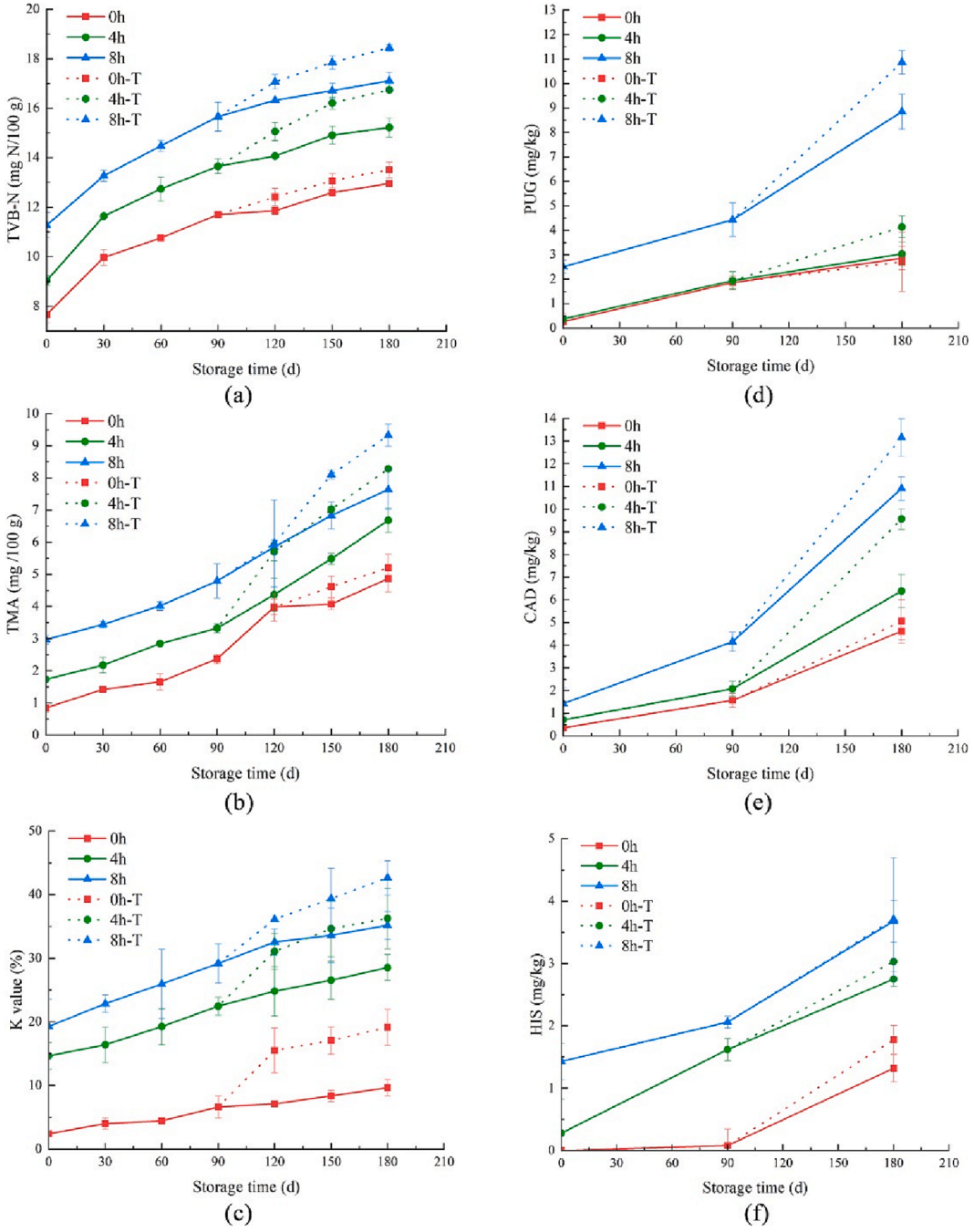

Fig.2. TVB-N (a), TMA value (b), K value (c) and BAs content (d-f) of large yellow croaker during frozen storage. Where T represents temperature fluctuation treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Shi et al. (2019).

Statistical analysis

Unless otherwise specified, all tests were repeated three times. One-way ANOVA was performed in SPSS 26.0 (SPSS Inc., Chicago, IL, USA), and the final results were expressed as mean ± SD. Significance was determined by Duncan's test, and different lowercase letters indicate significant differences ($P < 0.05$).

Results

TVB-n

TVB-N is a marker of protein and amine degradation, and higher TVB-N values indicate more destroyed amino acids (Jia et al. 2019), which has been widely used to evaluate the quality changes of aquatic products. According to the national industry standard (SC/T 3101–2010), the TVB-N value of the first grade large yellow croaker is limited to below 13 mg/100 g (Chu et al. 2022). The TVB-N value of samples are shown in Fig. 2(a). The fresh fish had a TVB-N value of 7.66 mg N/100 g, and gradually increased during 180 days frozen storage. Retention caused a rapid increase of TVB-N value, which reached 9.03 mg N/100 g (4 h) and 11.27 mg N/100 g (8 h) on 0 d. In particular, in the 8 h group, the large yellow croaker was no longer in the state of first grade after 30 d frozen storage, reaching 13.27 mg N/100 g. The temperature fluctuation treatment at 90 d also caused an increase in TVB-N values, the 0 h-T group showed little change in TVB-N values compared with the sample without temperature fluctuation, increasing by only 4.24% at 180 d. On the contrary, there was a significant increase in TVB-N values for samples that had been held for 4 h or 8 h before freezing, by 9.99% (4 h) and 7.77% (8 h), respectively. This may be due to the strong effect of microorganism and enzyme reaction during the retention at 25 °C, and with the extension of retention time, more proteins would be decomposed, resulting in the rapid increase of TVB-N value. Although the temperature fluctuation treatment was carried out on the 90th day, the sample was still in the frozen state, which had little impact on microorganisms and enzymes, so TVB-N value would not increase significantly. This shows that the quality of fish decreases quickly under ambient temperature before freezing, the temperature fluctuation during cold chain also causes a slight deterioration of the quality.

TMa

TMA is produced by decomposing trimethylamine oxide (TMAO) through bacterial and enzymatic activities. It is one of the compounds that cause the unique fishy taste of fish (Yu et al. 2021), and can be used to evaluate the freshness of fish (Zhu et al. 2016). TMAO is the main component of the non-protein nitrogen fraction in fish and has an osmolarity-regulating function, while promoting aerobic and anaerobic growth of microorganisms (Heising et al. 2014).When molecular oxygen is exhausted, TMAO acts as the terminal receptor of anaerobic respiration and is reduced to TMA (Zhu et al. 2016). TMA content of all groups is shown in Fig. 2 (b). During the first 90 d of storage, TMA increased slowly in all groups, and only increased by 1.53 (0 h), 1.59 (4 h) and 1.82 (8 h) mg / 100 g compared with that on 0 d. At the same time, the temperature fluctuation on 90 d also became an important reason for the further increase of TMA value in relative group. The upper acceptable limit for fish freshness is 10 mg/ 100 g (El-Obeid et al. 2018), the retention of large yellow croaker for 8 h was essentially close to the upper limit of unacceptability after 180 d of frozen storage, especially for samples affected by transit with temperature fluctuation (8 h-T), with TMA values reaching 9.33 mg/100 g. In addition to this, the TMA value of sample with 4 h retention was 6.68 mg/100 g after 180 d of frozen storage without temperature fluctuation, while the fish affected by transit reached 8.29 mg/100 g, which was also close to the upper limit of unacceptability. In some similar studies, the role of spoilage bacteria is the main reason for the significant increase of TMA level (Lou et al. 2021; Yi and Xie 2022). This explains why the retention time of 8 h leads to the highest TMA content at 0 d. In addition, the inhibition of freezing on microbial growth slowed down the accumulation of TMA, even though the temperature fluctuation treatment would increase the TMA content slightly. These results indicated that transit had a significant impact on the freshness of fish during frozen storage, while post-fishing retention also has an impact on the freshness. The TMA value of fish directly frozen (0 h) was only 4.87 mg/100 g at the end of frozen storage under 180 d constant temperature of −18 °C, indicating that freezing fish samples immediately and avoiding transshipment could maximally inhibit the oxidative transformation level of TMAO in fish, and also played a positive role in reducing fishy smell, which was consistent with the results of TVB-N analysis.

K Value

In the frozen storage of fish, the degradation of ATP is attributed to the autolysis and decomposition of fish. Its derivative index K value mainly represents the accumulation of ATP degradation products Hx and HxR (Xiong et al. 2020). At present, it has been widely used to evaluate the freshness of fish. Freshness decreased with increasing K value, and 60% is the maximum acceptable limit (Liu et al. 2020). The K values of different groups are shown in Fig. 2(c). The K value of fresh sample was 2.42% and the low temperature made the K value of large yellow croaker increase to only 9.65% during 180 d of storage, which was always in a very fresh state, because the low temperature inhibited most of the vital activities. During the retention at 25 °C, the K value increased sharply, reaching 14.66% (4 h) and 19.28% (8 h) respectively. With the frozen storage, the K value of 4 h group and 8 h group finally increased to 28.54% and 35.18%, which could only reach the state of moderate freshness. The temperature fluctuation also had an effect on the K values, which all increased significantly relative to the group without temperature fluctuation, but it is of interest that the K value of the 0 h-T group was still within 20% of the very fresh state at the end of frozen storage (19.15%) despite the temperature fluctuation, while the 8 h-T group entered a stale state. It may be that microorganisms cause ATP to decompose rapidly due to the retention on 0 d at 25 °C, which eventually lead to the increase of K value. Almost all microbial growth was inhibited during frozen storage, which explains why the K value did not increase significantly. The treatment of temperature fluctuation may cause the destruction of muscle tissue, leading to the release of enzymes in cells and accelerating the increase of K value. As shown above, according to the analysis of TVB-N, TMA and K values, as far as freshness is concerned, large yellow croaker should be frozen immediately after fishing on board, and retention will lead to a decrease in freshness, and the degree of freshness decreases more drastically as the extension of retention time.

BAs

BAs are important quality indicators of fish because they have the potential risk of food poisoning and bad smell (Costa et al. 2018). Free amino acids are decarboxylated by microorganisms to produce low molecular weight organic bases called biogenic amines. BAs are diverse and include cadaveric amines (CAD), putrescine (PUT) and histamine (HIS), which are formed from lysine, ornithine and histidine, respectively (Zhao et al. 2022). PUT and CAD can induce histamine allergy by inhibiting histamine methylase (Bulushi et al. 2009). Therefore, they are usually used as indicators of fish quality and safety, reflecting the freshness of fish. As seen in Fig. 2(d-f), PUT, CAD and HIS were barely detectable in fresh large yellow croaker, which was consistent with the study of Dai et al. (2021) and Zhao et al. (2022). It could be detected after 90 d, and increased with the extension of frozen storage. Since the low temperature inhibited the growth of almost all microorganisms, the

contents of PUT, CAD and HIS showed small levels of 2.86, 4.62 and 1.32 mg/kg, respectively, after 180 d of frozen storage. After detention, the contents of PUT, CAD and HIS increased rapidly, and with the increase of retention time, the content of BAs increased rapidly. Some microorganisms with decarboxylation ability can be active in low temperature environment, so such microorganisms, which caused proliferation in fish due to retention, continued to be active during the frozen storage and may have been the main reason for the greater increase in BAs content in the 4 h and 8 h groups. In addition, the temperature fluctuation had an effect on the increase of BAs, which was consistent with the results of the studies on TVB-N, K values and TMA.

FAAs

A variety of amino acids, are closely related to bitterness, sweetness and umami of fish (Yu et al. 2021). A total of 17 common FAAs represented by serine (Ser), glutamate (Glu), glycine (Gly), alanine (Ala) and lysine (Lys) were analyzed, as shown in Table 1. Among the five representative FAAs, Ser, Glu, ALA and Aly can produce pleasant taste, accounting for 62.55% in fresh samples. At the same time, it could be found that their content decreased significantly with the extension of residence time, and decreased to 55.99% (4 h) and 47.22% (8 h) on 0 d. This may be due to the gradual transformation of FAAs in the sample to BAs during retention, which could also be seen from the analysis of BAs. In order to more intuitively compare the changes in fish flavor during frozen storage, this study analyzed the changes of FAAs content in frozen samples using principal component analysis (PCA) (Fig. 3(a)), and further analyzed the relative abundance of FAAs in samples by using heat map visualization and clustering results (Fig. 3(b)). The TAV was also calculated for FAAs exhibiting positive effects (Umami/Sweet) and those exhibiting negative effects (Bitter) as shown in Fig. 3(c-d). PCA is usually used to analyze the relationship between variables (Zhang et al., 2020). The cumulative variance contribution of the PC1 (52.9%) and PC2 (26.2%) was 79.1%. And the samples were well distinguished in the distribution map, showing obvious differences. It can be seen from the figure that there was a significant difference between the immediately frozen groups (0 h) and the retained frozen groups (4 h and 8 h), and there was a large overlap between the confidence intervals of 4 h and 8 h groups, indicating that there is no significant difference between 4 h and 8 h groups. The results showed that FAAs changed significantly in the retention process, but the change was not obvious with the extension of retention time. Based on FAA abundance, clustering trees for 17 FAAs and 15 samples were constructed on the top and left of the graph, respectively (Fig. 3(b)). It can be seen from the cluster tree at the top that FAAs (Asp, Arg, Thr, Pro, Glu, Ala, Ser, Gly and Leu) with positive effects and FAAs (Cys, Val, Met, Ile, Lys, Tyr, Phe and Hys) with negative effects were obviously divided into two groups, indicating that there were obvious differences between the two groups.

The clustering tree on the left reflected the relationship between storage time, retention time and the temperature fluctuation. There was no doubt that there were differences between groups at the beginning and end of frozen storage. In addition, it could be found that there was a significant difference between immediately frozen groups (0 h) and the retained frozen groups (4 h and 8 h), which was consistent with the analysis of PCA. For the treatment of temperature fluctuation, significant differences could also be found compared with the group without temperature fluctuation, but the differences were smaller than those of retained samples. This indicated that the temperature fluctuation during cold chain would have an impact on FAAs, but the impact of retention was more obvious.

According to the calculation of TAV, it was found that TAV-umami/sweet and TAV-bitter produced significant differences at 0 d. The difference was that FAAs with negative effects were accumulated gradually with the retention time. Due to the transformation of FAAs to BAs, TAV

Table 1
FAAs content (mg/100 g) of large yellow croaker during frozen storage under different treatments.

Groups	Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met
0h-0d	0.52 ± 0.03a	4.39 ± 0.51a	15.14 ± 2.00a	12.63 ± 1.46a	32.68 ± 3.45a	26.44 ± 3.07a	0.57 ± 0.06b	4.33 ± 0.38c	2.35 ± 0.24b
4h-0d	0.46 ± 0.03a	3.97 ± 0.17ab	12.2 ± 0.46ab	11.64 ± 1.88a	29.48 ± 5.75a	23.00 ± 2.53a	1.12 ± 0.13a	6.07 ± 0.23b	4.64 ± 0.46a
8h-0d	0.37 ± 0.03b	3.24 ± 0.10b	9.89 ± 0.60b	8.81 ± 1.13a	23.72 ± 3.60a	18.56 ± 2.53a	1.33 ± 0.14a	7.28 ± 0.44a	5.44 ± 0.35a
0h-30d	0.49 ± 0.03a	4.31 ± 0.13a	13.27 ± 0.60a	11.93 ± 1.29a	30.60 ± 1.82a	24.58 ± 3.2a	0.62 ± 0.07b	2.58 ± 0.23c	1.88 ± 0.19b
4h-30d	0.42 ± 0.02ab	3.58 ± 0.16b	11.19 ± 0.59b	9.89 ± 0.93ab	23.77 ± 2.13a	21.52 ± 2.38a	1.21 ± 0.14a	3.61 ± 0.13b	3.67 ± 0.38a
8h-30d	0.33 ± 0.03b	2.93 ± 0.07c	9.08 ± 0.58c	8.01 ± 0.94b	23.86 ± 4.31a	16.79 ± 2.12a	1.45 ± 0.15a	4.33 ± 0.28a	4.3 ± 0.27a
0h-90d	0.42 ± 0.02a	3.32 ± 0.27a	6.12 ± 0.43c	13.63 ± 1.14a	14.54 ± 2.51b	24.29 ± 2.10a	0.68 ± 0.08b	2.04 ± 0.18c	1.49 ± 0.14b
4h-90d	0.39 ± 0.02ab	3.33 ± 0.17a	10.25 ± 0.49a	9.16 ± 0.96b	23.82 ± 1.55a	20.08 ± 2.18ab	1.32 ± 0.15a	2.84 ± 0.09b	2.92 ± 0.29a
8h-90d	0.32 ± 0.03b	2.85 ± 0.10a	8.56 ± 0.61b	7.64 ± 0.89b	19.73 ± 0.89ab	16.19 ± 2.58b	1.59 ± 0.17a	3.43 ± 0.21a	3.39 ± 0.22a
0h-180d	0.37 ± 0.01a	2.92 ± 0.23a	5.29 ± 0.33cd	11.86 ± 0.84a	15.43 ± 2.18ab	21.10 ± 1.81a	0.73 ± 0.08b	1.62 ± 0.13b	1.18 ± 0.12b
0h-T-180d	0.32 ± 0.01abc	2.56 ± 0.21ab	4.6 ± 0.27d	10.36 ± 0.62a	11.10 ± 1.89b	18.45 ± 1.52ab	0.8 ± 0.10b	1.92 ± 0.16b	1.28 ± 0.13b
4h-180d	0.32 ± 0.02ab	2.86 ± 0.13ab	8.88 ± 0.44ab	7.78 ± 0.88b	20.02 ± 4.88a	16.97 ± 1.85abc	1.74 ± 0.18a	2.7 ± 0.16a	2.68 ± 0.17a
4h-T-180d	0.26 ± 0.01cd	2.33 ± 0.09bc	7.36 ± 0.43bc	6.51 ± 0.70b	16.58 ± 1.20ab	14.05 ± 1.37bc	1.89 ± 0.20a	3.19 ± 0.19a	2.91 ± 0.19a
8h-180d	0.28 ± 0.02bcd	2.51 ± 0.10abc	7.49 ± 0.59ab	6.69 ± 0.82b	17.53 ± 0.92ab	14.05 ± 2.11bc	0.87 ± 0.1b	1.52 ± 0.13b	1.02 ± 0.10b
8h-T-180d	0.23 ± 0.02d	2.04 ± 0.08c	6.17 ± 0.43bc	5.53 ± 0.71b	14.44 ± 0.45ab	11.63 ± 1.72c	0.95 ± 0.11b	1.81 ± 0.17b	1.11 ± 0.11b

Groups	Ile	Leu	Tyr	Phe	Lys	His	Arg	Pro	Total
0h-0d	3.05 ± 0.38b	5.02 ± 0.12a	2.33 ± 0.12b	2.07 ± 0.15a	18.39 ± 2.41c	2.76 ± 0.27b	0.17 ± 0.01a	6.08 ± 0.27a	138.91 ± 13.13a
4h-0d	5.00 ± 0.60a	4.02 ± 0.09b	4.07 ± 0.21a	1.44 ± 0.10b	20.50 ± 2.63b	3.31 ± 0.41ab	0.14 ± 0.01ab	5.23 ± 0.22b	136.31 ± 11.49a
8h-0d	5.79 ± 0.65a	3.63 ± 0.27b	3.56 ± 0.22a	1.27 ± 0.09b	27.49 ± 1.71a	4.33 ± 0.37a	0.11 ± 0.01b	4.30 ± 0.23c	129.12 ± 4.26b
0h-30d	2.41 ± 0.29b	3.48 ± 0.06a	4.12 ± 0.29b	0.93 ± 0.08b	16.24 ± 2.03a	2.88 ± 0.34a	0.15 ± 0.02a	5.76 ± 0.29a	126.21 ± 9.82a
4h-30d	3.95 ± 0.45a	2.80 ± 0.09b	3.16 ± 0.21c	1.08 ± 0.08ab	20.03 ± 2.57a	3.64 ± 0.46a	0.13 ± 0.01ab	4.88 ± 0.22b	118.52 ± 5.68b
8h-30d	4.56 ± 0.52a	2.53 ± 0.18b	5.26 ± 0.28a	1.35 ± 0.11a	21.75 ± 4.45a	4.27 ± 0.55a	0.1 ± 0.01b	4.00 ± 0.21c	114.92 ± 8.41b
0h-90d	1.92 ± 0.24b	2.41 ± 0.06a	4.59 ± 0.32a	1.58 ± 0.11a	14.98 ± 2.99a	3.17 ± 0.36a	0.12 ± 0.01a	4.12 ± 0.3ab	99.42 ± 9.25b
4h-90d	3.14 ± 0.34a	1.94 ± 0.05b	4.63 ± 0.34a	1.18 ± 0.08b	18.09 ± 3.48a	3.97 ± 0.52a	0.12 ± 0.01a	4.48 ± 0.13a	111.66 ± 8.76a
8h-90d	3.59 ± 0.40a	1.75 ± 0.13b	4.22 ± 0.24a	1.47 ± 0.11ab	20.06 ± 2.38a	4.53 ± 0.53a	0.1 ± 0.01a	3.71 ± 0.19b	103.13 ± 8.15b
0h-180d	1.52 ± 0.18b	1.67 ± 0.04b	6.58 ± 0.4b	1.7 ± 0.12a	9.92 ± 0.44b	2.72 ± 0.25c	0.11 ± 0.01a	3.56 ± 0.29ab	88.29 ± 4.31ab
0h-T-180d	1.65 ± 0.20b	2.48 ± 0.05a	8.10 ± 0.48a	1.85 ± 0.13a	12.83 ± 0.68ab	3.28 ± 0.29bc	0.10 ± 0.01ab	3.14 ± 0.28bc	84.81 ± 4.95b
4h-180d	2.87 ± 0.32a	1.21 ± 0.09c	3.22 ± 0.15c	0.81 ± 0.05b	12.94 ± 0.95ab	3.01 ± 0.40c	0.10 ± 0.01ab	3.79 ± 0.10ab	91.89 ± 7.15a
4h-T-180d	3.12 ± 0.34a	1.79 ± 0.12b	3.94 ± 0.19bc	0.88 ± 0.06b	11.95 ± 1.79ab	3.69 ± 0.45bc	0.08 ± 0.01ab	3.08 ± 0.09bc	83.61 ± 3.23b
8h-180d	1.31 ± 0.16b	1.72 ± 0.04b	3.74 ± 0.25bc	0.97 ± 0.08b	13.75 ± 0.68a	4.51 ± 0.34ab	0.09 ± 0.01ab	3.25 ± 0.13abc	81.3 ± 5.66a
8h-T-180d	1.43 ± 0.18b	2.55 ± 0.08a	4.59 ± 0.32b	1.05 ± 0.09b	13.96 ± 1.34a	5.13 ± 0.47a	0.07 ± 0.01b	2.69 ± 0.10c	75.37 ± 2.91c

The results are expressed as mean ± SD (n = 3). The means between different treatments on the same days with different lowercase letters (a-d) differ significantly ($P < 0.05$).

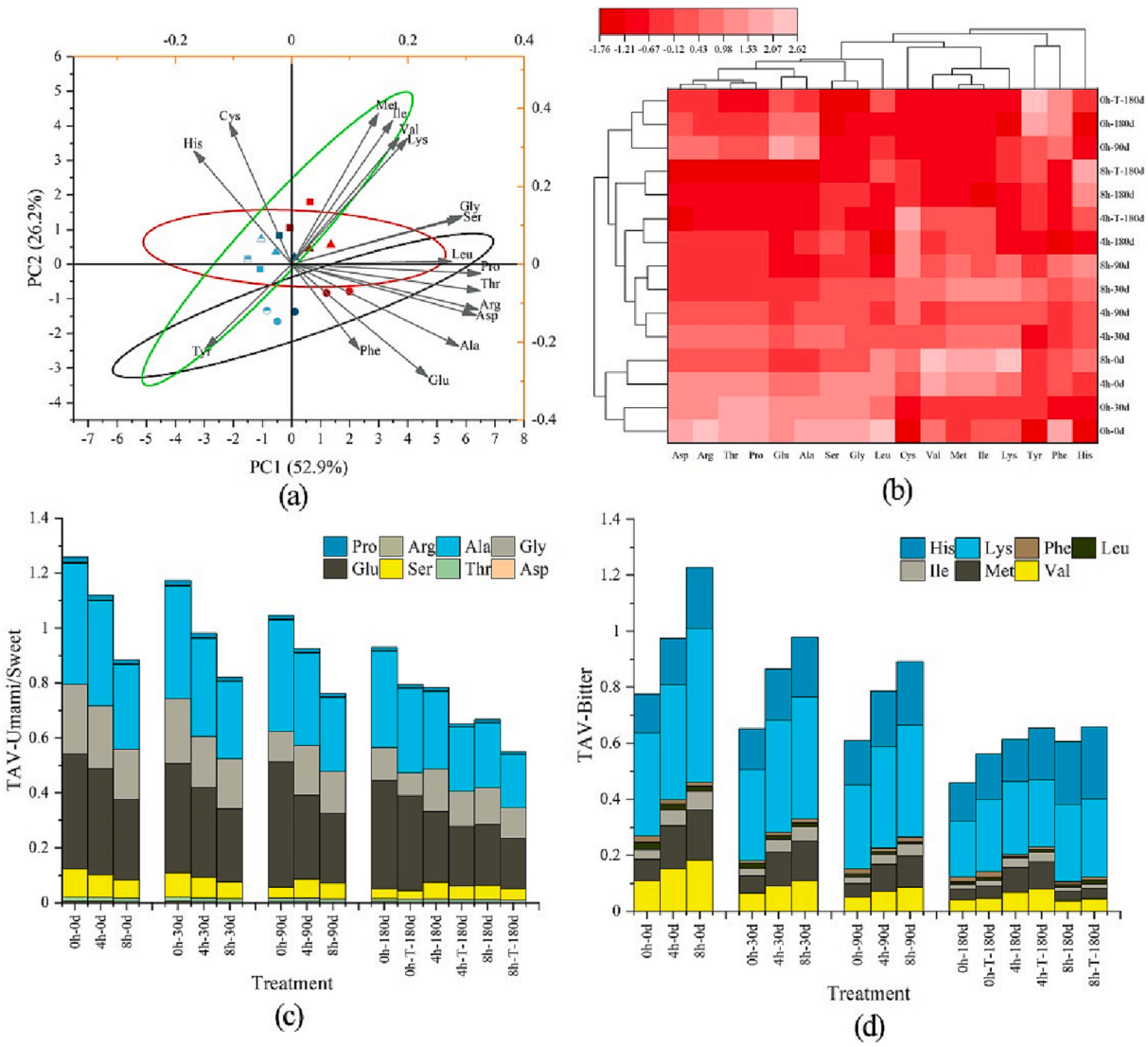

Fig.3. PCA (a), Heat map visualization and clustering results (b) and changes in TAV-Umami/Sweet (c) and TAV-Bitter (d) of FAAs with different treatments. (In (a), ●: 0 h; ▲: 4 h; ■: 8 h. ■: 0 d; ■: 30 d; ■: 90 d; ■: 180 d. The semi hollow legend indicates temperature fluctuation).

also showed a tendency to decrease with storage time. At the end of frozen storage, the TAV-bitter did not change much between groups, which might be caused by the loss of a large amount of water during the fish deterioration, resulting in the loss of some FAAs with water.

Correlation of freshness-related indicators and FAAs

Redundancy analysis (RDA) is helpful to observe different data in low dimensional space to further understand their relationship. In this study, the changes of response variables (TVB-N, K value, TMA value and BAs) were summarized by using the measured free amino acids presenting bitter taste in eight species as explanatory variables. The degree of correlation between two variables was determined by the angle between vectors. Acute angle indicated positive correlation, and the lower the angle, the stronger the positive correlation. Obtuse angle indicated a negative correlation. Fig. 4 shows the sample, explanatory variables and response variables to describe the correlation between bitter FAAs and freshness. The high correlation presented by all freshness-related indicators indicated an important association between these indicators during frozen storage of large yellow croaker. For FAAs, the strongest positive correlation was found between histidine and freshness-related indicators, which was consistent with Dai et al. (2021). Secondly, Tyr and Cys also showed a positive correlation. At the same time, for Lys with high content, it had a negative correlation with freshness-related indicators. Based on the above findings, samples with high histidine-rich content were more likely to cause a decrease in freshness, followed by Tyr and Cys. The content of Lys did not have a significant effect on freshness. At the same time, from the distribution of sample points, the correlation between the groups with retention time of 4 h and 8 h was more obvious than that of the groups with retention time of 0 h, which showed that the retention has a greater impact on the quality of samples during frozen storage.

Secondary structure of MP

FT-IR spectroscopy is a widely used technique to determine changes in secondary structure of protein. (Wang et al., 2018). Fig. 5 (a) shows the evolution of FT-IR spectra of MP during frozen storage, and the relative percentage contents of α-helices, β-sheets, β-turns, and random coils are shown in Fig. 5 (b). The α-helices content of fresh large yellow croaker was 40.02% and decreased to 23.23% after 180 d of storage, which was caused by the degradation of secondary structure of protein during frozen storage. Retention at 25 °C caused a sharp decrease in the content of α-helices, which was 29.67% (4 h) and 21.88% (8 h) at

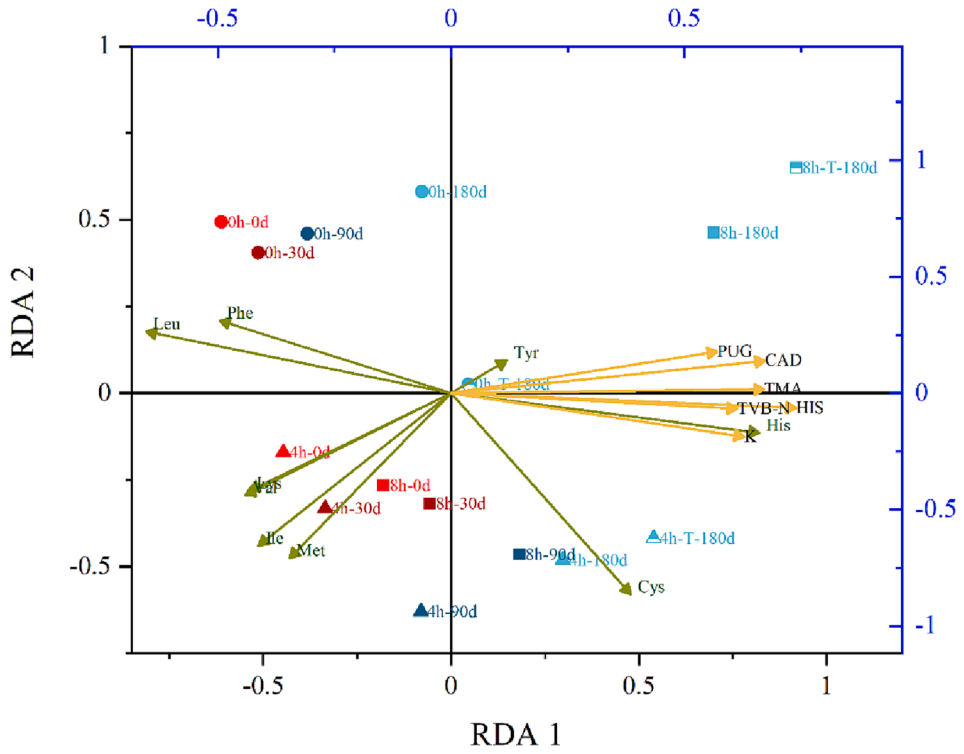

Fig.4. Triplot for the correlation assessment with FAAs and freshness-related indicators in large yellow croaker during frozen storage. (●: 0 h; ▲: 4 h; ■: 8 h. ■: 0 d; ■: 30 d; ■: 90 d; ■: 180 d. The semi hollow legend indicates temperature fluctuation). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0 d and reached 18.79% (4 h) and 16.38 (8 h) after 180 d of storage, respectively, suggesting that 4 h or 8 h of retention was more likely to cause degradation of secondary structure compared to 180 d of storage. In addition, temperature fluctuation also caused different degrees of damage to the secondary structure, with a decrease of 1.15% (0 h), 2.45% (4 h) and 1.06% (8 h), respectively, compared to those without temperature fluctuation at 180 d.

Tertiary structure of MP

Tryptophan residues are commonly used as indicators to monitor tertiary conformational changes in proteins (Royer 2006), including aggregation and unfolding of myogenic fibronectin. The fluorescence intensity of different treatments of large yellow croaker is shown in Fig. 5(c). There was a slight increase in λ_{max} with the extension of retention time, which indicated that the buried tryptophan was exposed to a polar environment was changed. A slight increase in λ_{max} was also observed for the 8 h group, while the 0 h and 4 h groups showed no further change in λ_{max} during frozen storage, which indicated that the large yellow croaker with long retention time is more likely to degrade the tertiary structure of MP during frozen storage. At the same time, FI_{max} can also reflect the degradation of tertiary structure of MP. It was found that the FI_{max} value of the 0 h group was always in the highest state during the 180 d of storage, which indicated that freezing immediately after fishing could reduce the degradation of MP during the frozen storage. In addition to this, it was of interest that the temperature fluctuation also had a negative effect on the tertiary structure of MP, but it can be seen from Fig. 5(c) that the retention time had a greater effect than the temperature fluctuation.

In summary, based on the analysis of the secondary structure and tertiary structure of MP, it was not difficult to find that the same conclusions as the previous studies on FAAs and freshness-related indicators were obtained, and reflected the effects of retention time and the temperature fluctuation on the quality of large yellow croaker during frozen storage from the perspective of the degradation of MP.

Conclusion

Extending shelf life and product freshness have always been the focus of aquatic product researchers. Therefore, in this study, the effects of retention time before freezing and temperature fluctuation caused by transshipment during frozen storage of large yellow croaker on its freshness and protein were compared by simulating the operation mode of ocean fishing. The differences in TVB-N, K value, TMA value, BAs content, FAAs content and protein-related characteristics were used to evaluate the effects of retention and temperature fluctuation. The results showed that retention caused a rapid increase in TVB-N and K values, which increased by 17.89–47.13% and 12.24–16.86% at 0 d compared to the group with retention time of 0 h, respectively, and the extent increased with storage time. At the same time, amines accumulated rapidly, even to an unacceptable level. The temperature fluctuation also had a further negative impact on the contents. The results of RDA showed that there was a high correlation between bitter FAAs and freshness-related indicators, which could fully reflect the freshness changes of large yellow croaker during frozen storage. In addition, there was a difference between 4 h and 8 h, but the difference was not obvious. The secondary structure and tertiary structure of MP also confirm the above conclusions. In conclusion, the correlation between FAAs and freshness-related indicators can be used to evaluate the quality of large yellow croaker during frozen storage, and attention should be focused on its handling before freezing to avoid unnecessary quality degradation.

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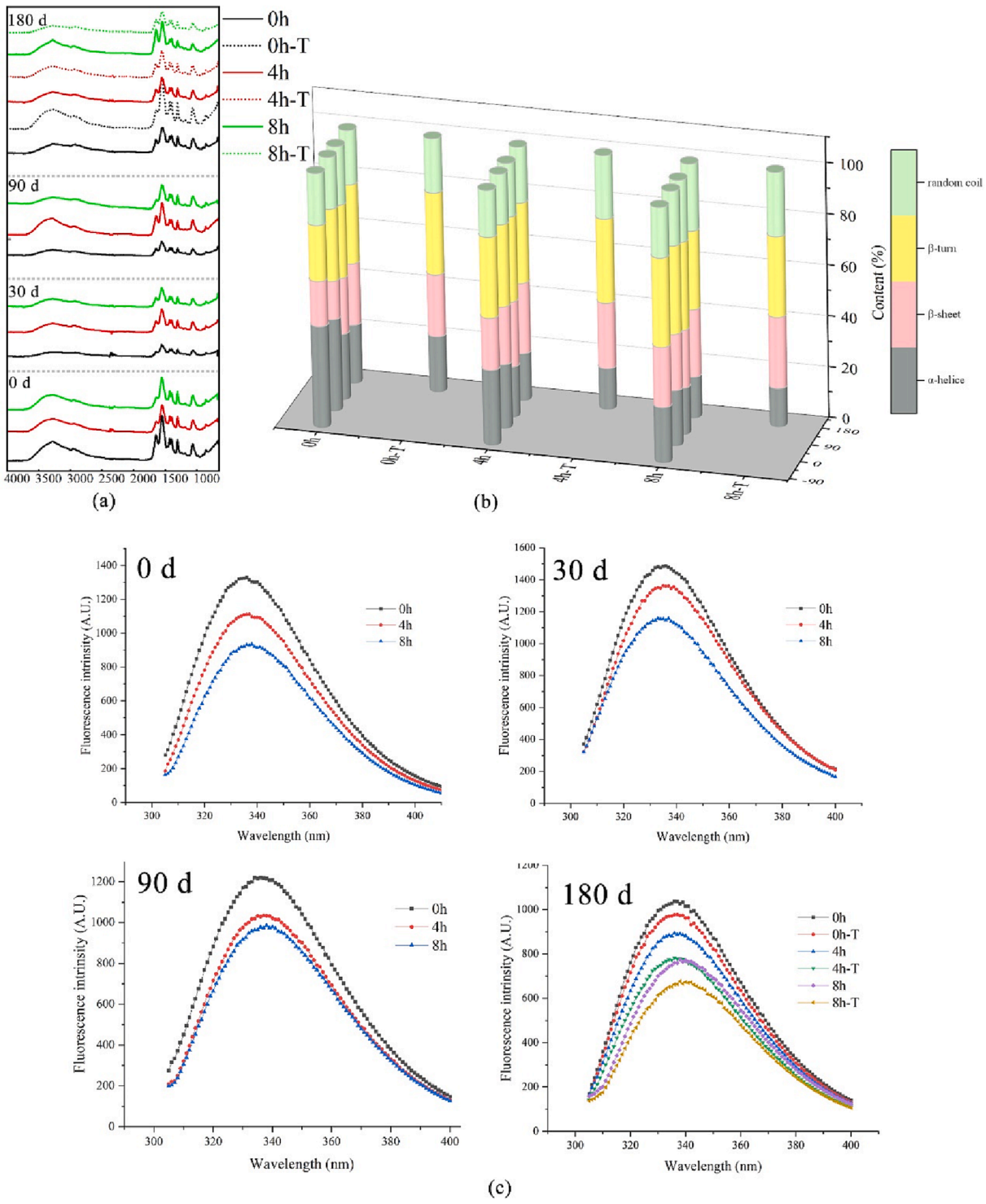

Fig.5. FT-IR spectral curves (a), the contents of α-helices, β-sheets, β-turns and random coils (b) and intrinsic fluorescence intensity (IFI) (c) of MP during frozen storage.

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Institutional Review Board Statement

In the present study, all procedures were performed in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China), and were approved by the Institutional Animal Care and Use Committee of Shanghai Ocean University (SHOU-DW-2020-150).

CRediT authorship contribution statement

Yuanming Chu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Zhaoyang Ding:** Validation, Conceptualization, Formal analysis. **Jinfeng Wang:** Validation, Conceptualization, Formal analysis.

Jing Xie: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Yuting Ding:** Validation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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