Quality attributes of horse mackerel (Trachurus japonicus) during frozen storage as affected by double-glazing combined with theaflavins

XueSong Wang¹ and Jing Xie¹,a,b,c,d

¹College of Food Science & Technology, Shanghai Ocean University, Shanghai, China; ²Shanghai Engineering Research Center of Aquatic Product Processing & Preservation, Shanghai, China; ³National Experimental Teaching Demonstration, Center for Food Science and Engineering (Shanghai Ocean University), Shanghai, China; ⁴Shanghai Professional Technology, Service Platform on Cold Chain Equipment Performance and Energy Saving Evaluation, Shanghai, China

ABSTRACT
This study aims to investigate a new method of glazing for improving the storage quality of frozen horse mackerel. Specifically, this study explored the effect of double-glazing with theaflavins on the quality of horse mackerel stored at −20°C for 28 weeks by comparing with unglazed and those single glazed with theaflavins. Chemical indicators were used to assess deterioration during storage, the water-holding capacity (WHC), weight loss, drip loss, and low-field nuclear magnetic resonance (LF-NMR) were used to analyze the water retention of fish and water migration in tissues. The water retention analysis results showed that double-glazing can maintain higher WHC, T22 values and lower weight loss, drip loss, T23 values at the end of storage. Chemical analysis results showed that double-glazing combined with theaflavins was highly effective in maintaining lower TVB-N, FAAs, MDA values, and higher SH, IFI values in frozen horse mackerel, especially theaflavins as inner layer glazed material, perhaps due to the synergistic effect of anti-crushing and antioxidant abilities. Therefore, the double-glazing with TF inner layer was more effective in controlling quality changes of frozen horse mackerel.

Introduction
Horse mackerel is an edible fish rich in nutrients and a variety of unsaturated fatty acids, it plays an extremely important position in the world’s marine fisheries.¹ Horse mackerel should be frozen immediately after distant fishing to maximize its shelf life.² However, during the frozen storage process, with the increase of the frozen storage time, the physical and chemical changes of the fish meat will result in discoloration, off-flavors, weight loss, bad taste, and dehydration.³

Ice glazing of aquatic products after frozen can delay the deterioration of quality caused by drying loss, lipid and protein oxidation,⁴,⁵ and improve the commercial value of products by its smooth and beautiful appearance. This method can form a protective ice layer on the surface of the fish body, thereby reducing the oxidation rate and weight loss of fish.⁶ Depending on seafood products, glazing percentage is indeed determined by its surface area-to-volume ratio.⁷ However, considering the quality of fish meat and consumer rights, glazing percentage is typically applied from 4% to 10%.⁸ The commonly used ice coat soaking solution is water or clean seawater, which can prevent the oxidation and discoloration of frozen meat, but it has weak adhesion, and it is prone to crack and fall off during storage and transportation, which lead to lose its protective effect.⁹ Adopting the double-glazing
method can solve the fragile problem of ice glazing, the first layer of ice glazing is a protective ice-coat in the inner layer, which is tightly integrated with the muscles to protect the muscle protein and inhibit recrystallization in the muscle. The second layer of ice glazing in the outer layer forms a protective ice-coat, preventing cracking, microbial contamination, and playing an antioxidant role.\[10\]

Antioxidants have been reported to be successfully used to maintain the quality of frozen fish. However, although synthetic antioxidants can have the effect of antioxidants, it is not conducive to human health.\[11\] In recent years, natural antioxidants have been widely studied as glazing materials to maintain the quality of frozen aquatic products during frozen storage, such as rosemary extract,\[12\] saponin-free quinoa extract,\[13\] chitosan,\[14,15\] bamboo leaf antioxidants,\[16\] and plant essential oil.\[17\] Theaflavin (TF) is a product extracted from the fermentation of black tea and has a variety of potential functions related to human health, such as antioxidants, prevention and treatment of cardiovascular diseases, blood fat reduction, and anti-cancer.\[18\] Polyphenols in theaflavins can prevent the formation of free radicals and inhibit the process of lipid peroxidation.\[19\]

Studies have also shown that the antioxidant properties of theaflavin are higher than that of epigallocatechin gallate (EGCG).\[20,21\]

Therefore, the double-layer glazing with theaflavin was applied as glazing materials for frozen horse mackerel in this experiment. It aimed to study the effect of double-layer glazing combined with natural preservatives on the quality of frozen horse mackerel under the frozen storage.

Materials and methods

Materials and preparation of glazing solution

Fresh house mackerels (weight: 150 ± 20 g, length: 18 ± 2 cm) were captured in the East China Sea and then immediately placed on the shelf of ultra-low temperature cold storage (4 m × 2.5 m × 2.1 m) for quick freezing (−55°C, 50 kg/h) until the center temperature reached below −18°C, and stored on a refrigerator at −30°C for 3 days. After reaching the shore, frozen fishes were packed in insulated boxes and then transported to the laboratory immediately, the transportation time was approximately 8 h. Upon arrival to the laboratory, several frozen fresh house mackerels were separated for analyzing the initial data (week 0), the remaining samples were immediately stored in a refrigerator at −30°C for 1 day to prepare for glazing.

Theaflavin was purchased as dry powders from Shanghai Ruixiang Biological Technology Co., Ltd (purity ≥98%, Shanghai, China); theaflavin was produced from black tea by centrifugal ultrafiltration, extraction, distillation, and freeze-drying. Carrageenan (C) was added for preventing the ice glazing from cracking, it was purchased from Zhejiang Yinuo Biological Technology Co., Ltd (purity ≥99%, Hangzhou, Zhejiang Province, China). Four glazing solutions including (1) distilled water, (2) 0.1% TF (w/v), (3) 0.1% C (w/v), (4) 0.1% TF (w/v) with 0.1% C (w/v) solution were obtained, and the solutions were mixed thoroughly by a magnetic stirrer to complete dissolution. These solutions were cooled down to 1°C in a closed refrigerated chamber, and then used for ice-glazing treatment.

Glazing of house mackerel and frozen storage

The frozen house mackerels were randomly divided into five groups: (1) CK (unglazed), (2) WG (distilled water-single glazed), (3) TFG+CG (0.1% TF with 0.1% C-single glazed), (4) TFG-CG (inner layer is 0.1% TF, outer layer is 0.1% C-double glazed), (5) CG-TFG (inner layer is 0.1% C, outer layer is 0.1% TF-double glazed). The frozen fish was put into the prepared glazing solution for 20 s to achieve a glazing percentage (10%±1%) for the single glazed group. In the double-glazed group, the frozen fish was dipped into a glazing solution for 5 s to make it coated with the inner layer of glazing, and then it was frozen in the refrigerator, after freezing to −30°C, the fish was removed and dipped into another glazing solution for 10 s, the total amount of glazing percentage was the same as that of the single-
Weight loss, drip loss, and water-holding capacity (WHC)

Weight loss of horse mackerel was calculated by following the next equation (1). \( W_1 \) indicates the initial weight of horse mackerels without glazing. After frozen storage, \( W_2 \) indicates the weight of horse mackerel that its surface ice glazing was stripped. Drip loss was calculated by following the next equation (2) where \( W_3 \) represented the weight of thawed horse mackerel samples.

\[
\text{WeightLoss}(\%) = \frac{W_1 - W_2}{W_1} \times 100
\]

(1)

\[
\text{DripLoss}(\%) = \frac{W_1 - W_3}{W_1} \times 100
\]

(2)

After thawing, the fish sample was cut into blocks about 2 g (\( W_4 \)) and wrapped with a layer of filter paper and placed in 50 ml centrifuge tube, then centrifuged for 10 min (5000 r/min, 4°C), the sample blocks were re-weighed (\( W_5 \)). The WHC was calculated as the equation (3):

\[
\text{WHC}(\%) = \frac{W_5}{W_4} \times 100
\]

(3)

Determination of total volatile basis nitrogen (TVB-N)

The TVB-N values were determined by the microtitration method.\(^{[22]}\) The result was expressed as TVB-N mg/100 g sample. The samples were measured in triplicate.

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

The LF-NMR measurement was performed according to the method\(^{[23]}\) with the following modifications. The pieces of 2 cm × 2 cm × 1 cm were cut from the back muscles of horse mackerel and wrapped in plastic wrap to prevent water evaporation during the measurement; the transverse relaxation time (\( T_2 \)) was measured by a LF-NMR analyzing system (Meso MR23-060H-I, Niumag Corporation, Shanghai, China), the measurement collected decay signals by the Carr–Purcell–Meiboom–Gill (CPMG) sequences. Then proton density weighted images of fish were obtained by uniformly mapped and pseudo-color imaged.

Preparation of the myofibrillar proteins

The extraction of myofibrillar protein was performed according to \(^{[24]}\) with slight modifications. 2 g minced horse mackerel muscle was mixed and homogenized with 20 mL Tris-maleic acid buffer (20 mmol/L Tris-maleic containing 0.05 mol/L KCl), and then the homogenate was centrifuged (4°C) at 10000 r/min for 15 min. After discarding supernatant, the resulting was added with the same buffer and extracted again. The obtained sediment was mixed and homogenized with a 20 mL Tris-maleic acid buffer (20 mmol/L Tris-maleic containing 0.6 mol/L KCl) and extracted at 4°C for 2 h after homogenizing, then centrifuged (4°C) at 10000 r/min for 15 min. The resulting supernatant was the myofibrillar protein solution. Myofibrillar protein content (g/L) was measured by the protein detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).
Total sulfhydryl (SH) content

The total sulfhydryl content (umol/g) of myofibrilar protein in horse mackerel was determined by the total sulfhydryl test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Intrinsic fluorescence intensity

Intrinsic fluorescence intensity was monitored by the method of [25] with slight modification. Fluorescence spectrophotometer (F-7100, Hitachi, Tokyo, Japan) was used to measure the intrinsic fluorescence emission spectrum of myofibrillar protein solution. The excitation wavelength was 295 nm, the slit width was 5 nm, and the emission spectra range was 310 ~ 400 nm.

Malondialdehyde (MDA) value

Two grams minced fish sample was added with nine times normal saline, and mechanically homogenized under ice bath, then the homogenate was centrifuged (4°C) at 5000 r/min for 10 min. The supernatant was used to measure the MDA value (nmol/mg) of horse mackerel sample by the MDA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Each group of triplicate is executed.

Free amino acid (FAA)

Free amino acid of samples was analyzed by using the method of [26]. Two grams minced fish sample was prepared by homogenizing in 5% 10 mL, trichloroacetic acid solution, then centrifuged (4°C) at 10000 r/min for 15 min. The previous operation was repeated. The two supernatants were combined and made up to 25 mL, then filtered through 0.22 μm filters for using an automatic amino acid analyzer (L-8800, Hitachi, Tokyo, Japan) to measure FAA.

Statistical analysis

Experimental data were statistically analyzed by using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) to perform analysis of variance (ANOVA). Statistical significance was reported at a level of $P < .05$. All the curves in this paper were generated by Origin Pro 2016 (OriginLab, Northampton, MA, USA).

Results and discussion

Change in TVB-N

TVB-N is an important indicator for evaluating the freshness of fish product. Changes in TVB-N values of horse mackerel samples during frozen storage were presented in Figure 1. The initial TVB-N value of fresh sample was 7.87 mg/100 g, all groups both showed an increase of TVB-N during the whole frozen storage, at week 28, the TVB-N value of the CK group increased to 23.26 mg/100 g, which was the closest to the safe limit (30 mg/100 g), TVB-N value of all glazed groups was significantly lower than that of the CK group. It showed that glazing may inhibit endogenous enzymatic activity and decomposition of proteins, thereby reducing the production of nitrogen-containing volatile substances in frozen storage. [6,27] In addition, the TVB-N value of the double-glazing groups was lower than those of the WG and TFG+CG groups, especially in TFG-CG group, indicating that the double-glazing is not easy to break, and it can more effectively inhibit the degradation of nitrogen-containing macromolecular components. [28]

Changes in the WHC, drip loss and weight loss

During the period of frozen storage, the sublimation of ice crystals on food surface leads to drying loss with the storage time. [29] Weight loss can be used to characterize the drying loss of food. As shown in
Figure 1. Changes in the total volatile basic nitrogen (TVB-N) value of horse mackerel in different groups during frozen storage.

Figure 2. The weight loss (a), water-holding capacity (WHC) (b) and drip loss (c) of horse mackerel in different groups during frozen storage.

**Figure 2a**, weight loss of all fish sample group increased slowly at the first 8 weeks of storage and then increased sharply. The increase of weight loss in all glazed groups was lower than that of unglazed (CK group), this is similar to the result of [30]. This was may be because ice glazing can prevent water evaporation in tissues until ice glazing was sublimated out. [31]

WHC, drip loss is an important indicator to evaluate the water retention of aquatic products. [32] During frozen storage, ice crystals in frozen food will continue to grow and cause mechanical damage
to tissue cells, which reduce the water-holding capacity of protein and other substances in fish samples, and increase the drip loss of samples after thawing.\textsuperscript{[35]} Figures 2b and Figures 2c, respectively, show the changes of WHC and drip loss in all treated samples during frozen storage. As seen, the drip loss increased and the WHC decreased during the frozen storage for all samples. The changes could be due to myofibrillar protein denaturation, which reduce the amount of water retained inside the tissue voids.\textsuperscript{[32]} After 28 weeks of storage, the WHC of CK group dropped to 71.12%, which was much lower than those of all glazed groups, and the glazed groups containing theaflavin and carrageenan were higher than that of WG group (Figure 2b). Wang et al.\textsuperscript{[16]} also proved that the antioxidant glazing with CMC-Na can significantly maintain the water-holding capacity of tuna. After 28 weeks, the drip loss of the CK group increased by 6.3%, the increase of the glazed group was lower than that of the CK group, and the double-glazed group only increased by 2.9%. High drip loss at the end of storage may be caused by protein aggregation.\textsuperscript{[34]} In summary, these results illustrated that the double-glazed containing theaflavin can effectively reduce the damage of the large ice crystals to the myofibril structure, thereby decreasing moisture loss of the horse mackerel.

**Low-field nuclear magnetic resonance (LF-NMR) relaxation time \((T_2)\) and moisture distribution**

Figure 3a shows the \(T_2\) transverse relaxation time spectra of horse mackerel samples stored after 0, 8 and 28 weeks. There are three peaks in each curve in this figure. \(T_{21}\) represents the bound water tightly combined with macromolecules, \(T_{22}\) represents the non-flowing water fixed in the myofibrils, and \(T_{23}\) represents the free water outside the myofibrils.\textsuperscript{[35]} The amplitude of \(T_2\) reflects the bonding strength between water and muscle tissue, and a large signal density indicates high water mobility.\textsuperscript{[16]} It can be seen from the figure that with the extension of storage time, \(T_{21}\) remained basically unchanged, \(T_{22}\) decreased significantly, and \(T_{23}\) increased significantly. These illustrated that the non-flowing water in the fish tissues would migrate out of the tissues. Among them, the trend that \(T_{22}\) of the CK group changed to \(T_{21}\) was obvious. After 28 weeks, the changes in \(T_{22}\) and \(T_{23}\) of double-glazing groups were the smallest. There is a certain correlation with the fact that double-glazing samples have significantly higher water-holding capacity and lower drip loss from the above water retention research results. This may be because double-glazing can reduce the influence of temperature fluctuation on the fish samples, and then inhibit the destruction of the cell membrane caused by the dissolution and reformation of ice crystals.\textsuperscript{[14]} These results indicated that the double-glazing can effectively delay water migration and maintain tissue water retention.

Magnetic resonance imaging (MRI) can visually observe the water distribution and migration of the internal tissues of fish during processing.\textsuperscript{[36]} The corresponding pseudo-color image is shown in Figure 3b, where red indicates high proton signal density and blue indicates low proton signal density. The higher the image brightness (tends to red) was the less water loss.\textsuperscript{[37]} As shown in Figure 3b, the intensity of the red signal became weaker and weaker during frozen storage, which indicated the continuous loss of water in the fish fiber. The brightness of all glazed groups was significantly higher than that of CK group (unglazed), especially the TFG-CG group had the most obvious water retention, which demonstrated that theaflavin as the inner layer can effectively maintain the fish tissue structure and reduce water migration.

**Changes in protein and lipids**

Sulphydryl (SH) is an important active group in aquatic protein, which can stabilize the spatial structure of myofibrillar protein. During the freezing storage process, if the sulphydryl content was oxidized, the protein molecules will be cross-linked to form disulfide bonds, thereby reducing the sulphydryl content.\textsuperscript{[38]} Figure 4a showed the changes in sulphydryl content of horse mackerel during frozen storage for 28 weeks. The SH content of all horse mackerel samples significantly \((P < .05)\) decreased during the entire frozen storage because the formation of disulfide bonds caused by the conformational changes of protein in the freezing process.\textsuperscript{[39]} After 28 weeks, the SH of TFG+CG,
TFG-CG and CG-TFG groups was obviously ($P < .05$) higher than that of CK and WG groups, which may be attributed to the antioxidant properties of polyphenols in theaflavins and the anti-crushing ability of carrageenan. Lin et al.\cite{40} and Srijanani et al.\cite{41,42} also reported that the anti-oxidative effect of tea extract on coated bonito fillets and shrimp during frozen storage. Tan et al.\cite{9} have also proved that glazing with sodium polyacrylate as thickener effectively maintained the quality of squid during frozen storage. These phenomena demonstrated that horse mackerel glazed with TF and C can effectively delay the oxidation of protein during frozen storage.

Protein denaturation causes damage to the secondary and tertiary structure of the protein. Fluorescence spectroscopy is widely used to determine the exposure index of tryptophan and other

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Transverse relaxation time (a) and magnetic resonance imaging (MRI) (b) of horse mackerel in different groups during frozen storage.}
\end{figure}
aromatic amino acids to monitor the conformational changes of myofibrillar protein. Lower fluorescence intensity is a sign of the expansion of the tertiary structure of myofibril protein. The inherent fluorescence intensity of myofibrillar protein in all horse mackerel groups at 0, 8, and 28 weeks are shown in Figure 4b. With the extension of storage time, the IFI of all groups had a downward trend. After 28 weeks of storage, the IFI of all glazed groups was significantly higher than that of the CK group (unglazed). This indicated that glazing can inhibit the exposure of buried tryptophan residues and tertiary structure changes of myofibrillar protein. This result was consistent with [12]. In addition, the IFI of the TFG-CG group was the highest at the same period. In summary, it can be explained that theaflavin as the inner layer can more effectively prevent the oxidation and denaturation of fish protein and maintain the quality of frozen horse mackerel.

Horse mackerel has a high content of unsaturated fatty acids that can remain liquid even at very low temperatures. During the freezing storage process, the pressure of ice crystals transfers fatty acids to the surface, making it more susceptible to oxidation and producing harmful substances. Malondialdehyde (MDA) is one of the secondary products of fatty oxidation, and its content can be used to evaluate the degree of fatty oxidation. Figure 4c showed that the MDA values of all horse mackerel groups at 0, 8, 20 and 28 weeks, respectively. As the storage time continued, the MDA value of each group continued to increase, and the degree of lipid oxidation gradually deepened. After 28 weeks of frozen storage, the MDA values of the CK group and the WG group were significantly higher than those of the other groups. TFG-CG had the lowest MDA value, which was attributed to this glazed method that can enhance the antioxidant capacity of ice coating by isolating the fish from the oxygen in the environment. The change trend of the MDA value in all treated group was consistent.
| storage time | Groups   | Asp  | Thr  | Ser  | Glu  | Gly  | Ala  | Cys  | Val  | Met  |
|--------------|----------|------|------|------|------|------|------|------|------|------|
| week 0       | CK       | 0.5  | 13.51| 3.19 | 34.83| 23.22| 12.53| 6.08 | 4.8  | 2.33 |
|              | WG       | 0.69 | 9.32 | 5.37 | 33.37| 23.82| 21.57| 8.38 | 4.21 | 0.67 |
|              | TFG+CG   | 0.54 | 9.91 | 7.02 | 23.39| 22.02| 21.57| 8.38 | 4.21 | 0.67 |
|              | TFG-CG   | 0.55 | 9.07 | 7.6  | 16.71| 13.15| 11.26| 4.69 | 2.41 | 1.17 |
|              | CG-TFG   | 0.66 | 10.48| 9.07 | 20.49| 17.99| 15.11| 5.7  | 2.66 | 1.17 |
| week 8       | CK       | 1.93 | 16.86| 9.12 | 63.64| 49.08| 61.39| 15.88| 19.56| 3.85 |
|              | WG       | 1.59 | 13.04| 6.69 | 43.45| 36.52| 40.34| 9.85 | 16.17| 2.37 |
|              | TFG+CG   | 1.39 | 12.86| 8.81 | 40.14| 33.31| 29.13| 8.51 | 13.64| 2.66 |
|              | TFG-CG   | 0.67 | 10.11| 4.57 | 34.92| 20.61| 19.57| 5.98 | 8.03 | 1.54 |
|              | CG-TFG   | 0.8  | 11.34| 5.16 | 44.53| 27.47| 23.9 | 6.89 | 14.26| 4.56 |
| week 28      | CK       | 2.08 | 5.28 | 4.78 | 12.3 | 155.55| 3.19 | 2.7  | 233.92| 14.65 |
|              | WG       | 2.09 | 8.62 | 6.7  | 23.16| 44.16| 6.14 | 2.76 | 633.56| 89.74 |
|              | TFG+CG   | 1.94 | 9.24 | 9.16 | 20.28| 38.75| 4.8  | 2.6  | 611.44| 65.43 |
|              | TFG-CG   | 3.6  | 9.44 | 9.88 | 17.67| 197.71| 4.08 | 1.75 | 537.11| 79.26 |
|              | CG-TFG   | 5.7  | 10.49| 10.91| 21.69| 310.6| 7.3  | 2.41 | 317.79| 54.12 |
| storage time | Groups   | Ile  | Leu  | Tyr  | Lys  | FAA  | His  | Arg  | Pro  | total|
| week 0       | CK       | 2.08 | 5.28 | 4.78 | 12.3 | 155.55| 3.19 | 2.7  | 233.92| 14.65 |
|              | WG       | 2.09 | 8.62 | 6.7  | 23.16| 44.16| 6.14 | 2.76 | 633.56| 89.74 |
|              | TFG+CG   | 1.94 | 9.24 | 9.16 | 20.28| 38.75| 4.8  | 2.6  | 611.44| 65.43 |
|              | TFG-CG   | 3.6  | 9.44 | 9.88 | 17.67| 197.71| 4.08 | 1.75 | 537.11| 79.26 |
|              | CG-TFG   | 5.7  | 10.49| 10.91| 21.69| 310.6| 7.3  | 2.41 | 317.79| 54.12 |
| week 8       | CK       | 17.91| 15.9 | 17.93| 68.14| 618.58| 9.16 | 6.49 | 996.27| 105.75|
|              | WG       | 15.82| 10.47| 8.99 | 45.14| 512.64| 6.61 | 3.38 | 772.62| 79.58 |
|              | TFG+CG   | 11.63| 10.53| 7.97 | 21.63| 464.96| 4.91 | 3.68 | 672.86| 53.29 |
|              | TFG-CG   | 9.28 | 10.73| 7.97 | 19.6 | 323.18| 3.39 | 2.81 | 483.02| 48.76 |
|              | CG-TFG   | 8.63 | 11.33| 8.91 | 21.38| 385.9 | 3.01 | 2.95 | 577.7 | 31.22 |

Note: Different lowercase letters represent the significant difference (p < 0.05) between treatment groups at the same storage time.
with the change trends of the sulfhydryl and protein fluorescence intensity since the oxidation products of lipid decomposition can promote protein oxidation and denaturation. \[46\]

**Changes in free amino acids (FAAs)**

Table 1 shows the FAAs changes of all samples after 0, 8 and 28 weeks of frozen storage. It is worth noticing that the most abundant FAA in horse mackerel is histidine, followed by glutamic acid, alanine and lysine, which has a similar finding in mackerel by \[47\]. A large amount of histidine will cause bitter taste in fish meat, and it is easy to generate histamine by the microbial decarboxylation, which may cause food safety hazards. \[48\] With the storage time, the total FAAs of all groups showed an upward trend, the total FAAs in glazed groups with theaflavin were significantly \((P < .05)\) lower than that of unglazed and water-glazed. The total FAAs of unglazed (CK) increased rapidly from an initial value of 233.92 mg/100 g to a high value of 996.27 mg/100 g, which indicated the extensive hydrolysis of protein. While for TFG-CG and CG-TFG, the total FAAs increased to 483.02 and 577.5 mg/100 g, respectively, after 28 weeks of storage. The content of histidine had similar change properties. It is obvious that glazing material containing TF can significantly inhibit protein degradation, and the effect of double-glazing was more significant since the double-glazing can make the protective film more difficult to break, the glazing isolated the fish meat from the air, prevented the protein from being degraded, reduced the production of nitrogen-containing substances, and effectively maintained the flavor of horse mackerel.

**Conclusion**

This study has demonstrated that the effects of different glazed methods on the quality of frozen horse mackerel and found that all glazed groups containing theaflavins can significantly inhibit the oxidation of protein and lipid in horse mackerel, and the application of double-glazing had more positive effect than that of single-glazing, which was attributed to combine the anti-oxidation of theaflavins with the protection of double-glazing. After frozen storage of 28 weeks, TFG-CG showed the best acceptability with the lowest TVB-N, the lowest free amino acids content and the best water retention. Therefore, the double-glazing with TF inner layer was more suitable for long-term storage of frozen horse mackerel.

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**ORCID**

XueSong Wang [http://orcid.org/0000-0001-6974-943X](http://orcid.org/0000-0001-6974-943X)

Jing Xie [http://orcid.org/0000-0002-3194-9273](http://orcid.org/0000-0002-3194-9273)

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