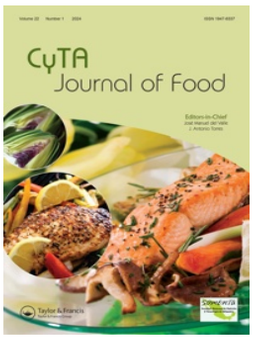

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# Effect of konjac oligosaccharide and Kappa-Carrageenan on oxidative and structural changes in silver carp protein induced by fluctuating storage temperatures

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**ABSTRACT**

Two different low molecular cryoprotectants were analyzed in silver carp surimi proteins during different frozen storage conditions (FTC), and a comparison was performed with a conventional cryoprotectant mixture of sucrose (4%) and sorbitol (4%) as positive control (PC). The results showed that a significant decline was noted in sulfhydryl contents (SH), an increase in carbonyl groups, and a surface hydrophobicity due to myosin and protein oxidation. Meanwhile, FTC also altered the stability of secondary and tertiary structures due to the denaturative changes in myofibrillar proteins (MP). Meanwhile, samples that incorporated KC (3%), KOG (3%), and PC showed significant stability in MP against FTC induced changes. This is due to their strong hydrophilic and hydrogen interactions. MP samples containing KOG (3%) demonstrated improved cryoprotective abilities against oxidative and structural changes. Thus, we can recommend KOG as a potential cryoprotectant for maintaining the shelf-life of fish and related products on an industrial scale.

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**KEYWORDS**
Surimi; oligosaccharides; oxidation; structural changes; myofibrillar proteins

## 1. Introduction

Silver carp is abundantly available freshwater species all over the world. Its higher nutritional values make it important for commercial, economical, and consumption aspects. Silver carp is also well known for producing high-quality surimi and other related products (Jiang & Wu, 2018).

Surimi is a finally chopped mince of fish meat, usually from white-fleshed fish such as pollock, cod, and silver carp. Surimi is rich in concentrated myofibrillar proteins (MP), which contribute to its gel-like texture and firmness. It is frequently utilized to produce surimi-based items like imitation crab sticks, fish cakes, and seafood salads. As mentioned earlier, surimi is comprised of MP, which are responsible for all the quality, functional, and structural changes. MP is a key protein, and its preservation is an important concern for the seafood industry to enhance fish and other seafood quality (Walayat, Tang, Nawaz, et al., 2022; Walayat, Xiong, Xiong, et al., 2022). The seafood industry frequently uses frozen storage methods to preserve and transport seafood products from one place to another. In addition to being stored in frozen conditions, the transportation of surimi and related products can result in a decline in quality and nutritional value due to fluctuating temperatures (Lu et al., 2017). These temperature changes commonly happen during transportation, at retail locations, and during household storage. Consequently, the preservation of the quality and safety of meat-based products becomes a challenging task due to the inevitable degradation of proteins during storage and transportation. Temperature fluctuations can have a detrimental effect on surimi proteins, leading to the formation and reformation of crystals in the protein network. This process is triggered by the presence of free water molecules and can cause significant damage to the secondary and tertiary structures of the proteins.

These changes include, reduced water-holding capacity (WHC), textural, gel strength, and gel forming properties. These changes can be inhibited in surimi and related products using different cryoprotectants such as sucrose and sorbitol, which are typically mixed with surimi to prevent the myosin unfolding and denaturation (Walayat et al., 2020). Even though, these traditional cryoprotectants (sucrose and sorbitol) are on the top list of uses at the industrial level due to their low cost and availability. Meanwhile, their low cryoprotective abilities, higher sweetness, and caloric values made these unfavorable as cryoprotectants in surimi (Jia et al., 2018). Therefore, it is important to replace these traditional cryoprotectants with other cryoprotectants such as, oligosaccharides, polysaccharides, and protein hydrolysates, which are efficient in cryoprotective abilities and have lower sweetness values than sucrose and sorbitol (Zhang, Yan, et al., 2020). Kappa-carrageenan (KC) is an enzymatically hydrolyzed

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product of red seaweed and is widely used in the food and pharmaceutical industries. Moreover, KC has been widely recognized as an efficient cryoprotectant in seafood and related products due to its low molecular weight and strong hydrogen and hydrophilic binding abilities (Walayat, Wang, Liu, et al., 2022; Zhang et al., 2018).

In addition, konjac glucomannan (KGM) is derived from the product of Amorphophallus Konjac C. Koch tubers, with a high molecular weight. Its linear chemical nature, which involves 32 sugar residues associated with $C_3$ mannose through β(1→3) glucoside bond, makes it distinguishable from other polysaccharides (Walayat et al., 2023). Meanwhile, KGM has pronounced gelling, emulsifying, and thickening abilities (Liu et al., 2015). Konjac oligosaccharide (KOG) is an enzymatic hydrolyzed product of KGM, which has efficient cryoprotective abilities, which are well proven by our earlier studies (Liu et al., 2019; Walayat, Tang, Nawaz, et al., 2022).

The objective of this research was to analyze the effect of KC and KOG against protein oxidative changes such as sulfhydryl, carbonyl contents, surface hydrophobicity, and $Ca^{2+}$-ATPase activity. Meanwhile, KC and KOG (3%) were compared to industrial cryoprotective mixture of sorbitol (4%) and sucrose (4%) as (PC) during different frozen storage conditions (FTC), which had not previously been studied. Moreover, this study also sheds light on the efficient cryoprotective abilities between KC and KOG in silver carp surimi. Furthermore, this study is more productive in the sense of providing the valuable use of KC and KOG in seafood products at industrial level.

## 2. Materials and methods

### 2.1. Materials

Food grade konjac glucomannan (purity 98%) was procured from Hubei Konson Konjac Co., Ltd. (Hubei, P.R. China). Beijing Challenge Bio-Tech Co., Ltd. (Beijing, P.R. China) supplied the β-mannanase with an enzymatic activity of 50,000 U/g. Henan Wang Bang Industrial Co., Ltd. (Henan, P.R. China) supplied the food-grade sucrose and sorbitol.

### 2.2. Preparation of KOG

The KOG was prepared according to the method of our earlier study by Walayat, Tang, Nawaz, et al. (2022). The konjac flour (5 g) was added to the 0.2 M acetate buffer (150 mL) and magnetically stirred at 50°C for 10 minutes. The β-mannanase (0.25 g) was mixed in the above-mentioned solution and heated at 50°C for 4 hours. The β-mannanase reaction was hindered after increasing the heating temperature of the solution at 100°C for 10 min. Then, the rotary evaporator (RE-2000A, Yarong Co., Ltd., Shanghai, China) was used to concentrate the KOG solution. The concentrated KOG was centrifuged three times with 95% ethyl alcohol. After that, the obtained KOG was evenly mixed with distilled water and filtered through a 700-mesh cloth. At the end, the final KOG was obtained after freeze-drying of the KOG solution. The degree of polymerization of the obtained KOG was 5.89 with a molecular weight of approximately 600 Da, as per our earlier studies (Liu et al., 2015).

### 2.3. Preparation of myofibrillar proteins samples

The MP of silver carp surimi was extracted using the method of Walayat, Tang, Nawaz, et al. (2022). Surimi (250 g) was properly homogenized with a (0.05 mol/L) low salt buffer and centrifuged for 10 minutes at 4°C at 8,000 × g; this centrifugation process was repeated twice. The obtained precipitates were mixed to a 0.6 mol/L (high salt buffer) and centrifuged at the above-mentioned parameters. The collected MP was centrifuged after homogenizing with distilled water. The protein concentration was 87.22 mg/mL and analyzed through the method of Gornall et al. (1949). MP samples were divided into four groups: control (C), MP added with KC (3%), samples added with KOG (3%) and samples added with industrial cryoprotective mixture of sucrose (4%) and sorbitol (4%) as PC. All the samples were well mixed with treatments and tightly sealed.

### 2.4. Freeze-thaw cycles

The samples C, KC (3%), KOG (3%), and PC were frozen at 18°C for 24 hours before being transferred to 4°C for 3 hours. The time duration between two different temperatures is called a fluctuation cycle (FTC). All samples (C, PC, KC (3%), and KOG (3%)) were processed through FTC of 0, 2, 4, and 6. All the samples were transferred at 4°C after FTC for experimental analyses.

### 2.5. Carbonyl content

The carbonyl content of silver carp MP was determined using the method of Zhang et al. (2018). The MP was diluted to a protein concentration of 2 mg/mL. TCA and dinitrophenyl hydrazine (DNPH) were added to the concentrated MP in 2 mol/L of HCl before centrifugation at 2,500 × g for 8 minutes. The obtained precipitates were mixed with TCA (20%) and were centrifuged (10 minutes, 2,000 × g). After centrifugation, the sediments were washed using ethyl acetate. The sediments (20 mmol/L) were added with sodium phosphate and 2 mL of guanidine. The sample absorbance was noted at 365 nm and stated in nmol/mg. All the samples were examined in replicates.

### 2.6. Total sulfhydryl content

The sulfhydryl content (SH) of silver carp MP samples was determined by following the protocol by Zhang et al. (2018). Phosphate buffer (0.1 M) was used to concentrate the MP (2 mg/mL). The prepared sample mixture was centrifuged for 10 minutes at 10,000×g with the following solutions: SDS (2%), EDTA (10 mM), and KCl (0.6 M). After centrifugation, 4 mL supernatant was mixed with 0.5 mL of Ellman's reagent. The absorbance of the sample mixture was measured at 412 nm. SH was measured in mmol SH using a 13,600 $M^{-1}$ $cm^{-1}$ molar extinction.

### 2.7. Surface hydrophobicity

The surface hydrophobicity (SH) of MP samples was investigated using the protocol of Lu et al. (2017). Different concentrations were prepared at 0, 0.2, 0.3, 0.5, and 1 mg/mL and added with PBS buffer solution (0.2 mol/L) and ANS (2 mmol/L). The prepared samples were heated for 30 minutes at 25°C and analyzed

with fluorescence intensity at 470 nm and excitation at 390 nm. All the treated MP samples were stated in $S_0$ and analyzed in triplicates.

### 2.8. $Ca^{2+}$-ATPase activity

The $Ca^{2+}$-ATPase activity of silver carp MP was analyzed via the protocol of Chen et al. (2022). As a reaction solution, the MP sample (3 mg/mL) was mixed with Tris-maleate (0.01 mol/L). The sample solution was heated for 10 minutes at 25°C. TCA (100 g/L) was added to the sample and centrifuged (3,000×g for 5 minutes). The obtained supernatant (1 mL) was then combined with sulfuric acid in ammonium molybdate (3 mL). Sulfuric acid (1 mL) was incorporated into the obtained supernatant mixture. The $Ca^{2+}$-ATPase activity was analyzed at 700 nm. All the samples were examined in replicates, and the obtained outcomes were stated in mmol (g) of phosphate.

### 2.9. Secondary structural changes

Secondary structural changes of silver carp MP were examined through circular dichroism (CD) following the method of Zhang, Xiong, Lu, et al. (2020). Samples were concentrated to 0.2 mg/mL using 0.5 M KCl. The secondary structural changes were analyzed at given parameters: sensitivity (50 milli mm), spectrum length (190 to 250 nm), resolution (1 nm), and scanning speed was 100 nm/min. The obtained data were expressed in secondary structural attributes such as, α-helix, β-turn, and random values. During this analysis, KCl (0.5 M) was run into CD as a blank, and all MP samples were examined in triplicates.

### 2.10. Tertiary structural changes

Tertiary structural changes in protein samples were analyzed via the procedure of Walayat, Rincón, et al. (2021) with minor changes. Samples were concentrated to 0.5 mg/mL using KCl (0.6 M). The tertiary structural properties were determined using fluorescence spectrophotometry (FI) at wavelength of 300 to 400 nm and a speed of 1200 nm/min. KCl (0.5 M) was run in FI as a blank, and all treated samples were examined in triplicates.

### 2.11. Statistical analysis

The statistical analysis was performed using ANOVA (one way) with mean comparison (Duncan's multiple test). The significance level was set at $p < .05$. In addition, OriginPro 8.5.1 was used to prepare all the figures. All samples were examined in triplicates, and the means and standard deviations were recorded.

## 3. Results and discussion

### 3.1. Carbonyl contents

Carbonyl content is a key analysis to determine the oxidative alterations in fish proteins during frozen storage. During the 6 FTC, the carbonyl content of all samples increased significantly. Notably, there was a significant change observed in all samples after the two FTC due to insufficient time for the KC (3%), KOG (3%), and PC interactions with protein molecules. The samples without any treatment (C) showed a massive increase in carbonyls from 0 to 6 FTC. Meanwhile, the samples treated with KC (3%), KOG (3%), and PC had a lower carbonyl increase at the end of 6 FTC (Figure 1). The samples treated with PC also depicted a significant increase in carbonyls, but the increase was more stable than in the C samples. This increased formation of carbonyls might be due to protein oxidation, leading to protein denaturation, coagulation, and a loss of functional attributes (Özalp Özen & Soyer, 2018). This increase in carbonyls could be attributed to the loss of structural (secondary and tertiary changes), which are vital to maintain the functional properties of proteins. Furthermore, this increase in carbonyls might also be associated with NH and $NH_2$ oxidation, which are known to be sensitive to carbonyl conversion. On the other hand, KC (3%) and KOG (3%) samples showed better stability against oxidative changes and resulting carbonyls after 6 FTC (Figure 1). The increase in carbonyls was greater in the KC (3%) and KOG (3%) treated samples, but the KOG (3%) demonstrated efficient carbonyl formation resistance. This restricted increase in KOG (3%) samples could be due to better hydrogen, ionic, and intermolecular bonding interactions. While KOG interacts with protein functional sites via hydrogen bonding, rendering them unreachable for further interaction with free water molecules (Li, Du, et al., 2021). Walayat, Rincón, et al. (2021) stated that the addition of KC pointedly reduced the formation of carbonyls, owing to its strong ability against free radicals. The chemical structure of KC, which contains sulfate groups, has been shown in studies to contribute to its ability to scavenge free radicals. This interaction with free radicals prevents chain reactions that could lead to significant quality deterioration in surimi. For example, Zhang et al. (2018) discovered that the inclusion of KC in surimi enhanced its antioxidant capacity, resulting in lower levels of peroxide and thiobarbituric acid-reactive substances (TBARS) during frozen storage. Similarly, Shui et al. (2021) conducted research demonstrating that surimi supplemented with KC exhibited improved preservation of MP integrity and reduced lipid oxidation compared to control samples.

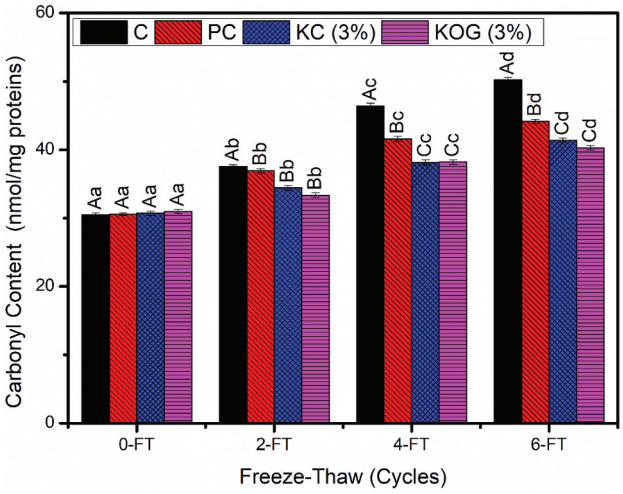


Figure 1. Effect of KC and KOG on the carbonyl contents of silver carp proteins during freeze-thaw cycles. Lowercase letters (a – d) refers to the significant differences in similar treatments within the different storage time ($p < .05$). Capital letters (A-C) refers to the significant difference in the same frozen storage period of different concentrations ($p < .05$).

In the meantime, Walayat, Tang, Nawaz, et al. (2022) also analyzed that the silver carp MP incorporated with KOG (3%) reduced the formation of carbonyls due to its strong hydrophilic interactions.

### 3.2. Sulfhydryl groups

Sulfhydryl groups (SH) are important due to their importance for the functional abilities of proteins. But the sulfhydryl groups are more sensitive to the frozen storage process and after getting exposed to oxidative changes, these can be easily converted into disulfide groups (R. Zhang et al., 2017). The decline in SH groups of silver carp MP samples is shown in Table 1 and Figure 2. SH decreased significantly in (C, PC, KC (3%), and KOG (3%)) samples. There was no decrease in SH after the 0 FTC because there was insufficient time for oxidative changes in MP. Later on, the decline in SH was analyzed after 2 FTC in all samples, but the C samples without additives showed an immense decline in SH after 6 FTC (Table 1). Meanwhile, MP samples added with PC were also noted with significant decline in SH after the 6 FTC but, this decline was more stable than the C samples. In the meantime, MP samples noted with sharp decline in SH groups during FTC, but the decline in SH groups was slighter in comparison to the samples stored during FTC despite treatments (Figure 2). This decline in SH during FTC could be due to the initiation of oxidative alterations in cysteine, resulting in the denaturation of MP and ultimately the formation of disulfides (Jiang & Wu, 2018). Additionally, MP samples treated with KC and KOG (3%) were also examined with a significant decline in SH (total and reactive), but the decline was less than the C and PC samples (Table 1 and Figure 1). The stability against decline in SH in KC (3%) and KOG (3%) was not prominent compared to each other owing to their efficient cryoprotective abilities against oxidation during frozen storage. Meanwhile, the stability of KOG-treated MP samples (3%) was better against the SH decline after 6 FTC, which could be due to their better hydrogen and intermolecular bonding interactions. Xue and He (2021) noted that the addition of polysaccharides to MP can significantly reduce the decrease in SH due to its radical scavenging abilities and better cross-linking between amino acids. Walayat, Tang, Nawaz, et al. (2022) noted that the incorporation of KOG can prevent the decrease in SH by preventing the formation of disulfides and enhancing its hydrogen bonding with protein molecules. These findings are completely consistent with our previous observations of $Ca^{2+}$-ATPase activity (3.4.1) and carbonyl contents (3.4.3). Therefore, it can be concluded from the current results that KOG can prevent the decline in SH by preventing the oxidative and denaturing degradation of myosin during fluctuating frozen storage.

### 3.3. Surface hydrophobicity

Surface hydrophobicity is important to determine the structural changes associated with protein oxidation. The surface hydrophobicity of all samples treated with PC, KC (3%), (KOG 3%) and without any treatment (C) is shown in Figure 3. At 0 FTC, all samples showed no significant change in surface hydrophobicity. The change in surface hydrophobicity in all samples began after 2 FTC. Except for the PC, KC (3%), and KOG (3%), all samples in this FTC study showed a significant increase in surface hydrophobicity. The samples without any treatment exhibited an immense increase in surface hydrophobicity after 6 FTC. This increase could be associated with protein denaturation and the exposure of hydrophobic molecules to the microenvironment (Zhang, Li, et al. (2020)). Meanwhile, this increase in surface hydrophobicity could be due to the disintegration of amino acid molecules, resulting in oxidative changes (Lu et al., 2017). On the other hand, the samples treated with PC (conventional cryoprotective mixture) recorded with a substantial increase in surface hydrophobicity as well, but the decline was restricted compared to the C samples (Figure 3). Furthermore, after 6 FTC, the samples incorporated with KC (3%) and KOG (3%) showed an increase in surface hydrophobicity (Figure 3). The increase in KC (3%) and KOG (3%) was more restricted as compared to the increases in C and PC. Moreover, the increase in surface hydrophobicity in KC (3%) and KOG (3%) treated samples showed a similar trend as can be seen in Figure 3. The increases in KC (3%) and KOG (3%) were sharper to each other, while the KOG (3%) samples showed efficient restraint against the oxidative changes and surface hydrophobicity increase. The limited increase in KOG (3%) samples might be due to interactions between the functional sites of amino acids and KOG better hydrophilic–hydrophilic binding sites. Furthermore, Walayat, Tang, Wang, et al. (2022) noted that the addition of KOG (3%) to silver carp surimi proteins restricted the increase in surface hydrophobicity by inhibiting the oxidative changes during different fluctuated frozen storage conditions. Zhang et al. (2018) also studied that the oligosaccharide incorporation into seafood proteins significantly prevented MP denaturation and oxidative changes in myosin, resulting in a reduced increase in surface hydrophobicity. Moreover, Walayat, Wang, Nawaz, et al. (2021) stated that the addition of ovalbumin and KC mixture to fish MP proteins can reduce the rise in surface hydrophobicity by enhancing hydrogen binding and intermolecular binding interactions. Previous articles also noted that the addition of alginate improved the ionic, hydrophilic, and hydrogen interactions between protein molecules due to its low molecular weight, leading to a limited increase in surface hydrophobicity (B. Zhang et al., 2019; Zheng et al., 2019). Therefore, from the current results, it can be suggested that the surimi treatment with

Table 1. Total sulfhydryl content of myofibrillar proteins treated with KC and KOG during different freeze-thaw cycles.

| | Total Sulfhydryl Content (mmol/kg) | | | |
|---|---|---|---|---|
| Freeze-Thaw (Cycles) | Control (C) | PC | KC (3%) | KOG (3%) |
| 0-FT | $48.63 \pm 0.62^{Aa}$ | $48.61 \pm 0.51^{Aa}$ | $48.71 \pm 0.77^{Aa}$ | $48.68 \pm 0.39^{Aa}$ |
| 2-FT | $44.87 \pm 0.71^{Ab}$ | $45.61 \pm 0.47^{Bb}$ | $44.91 \pm 0.44^{Cb}$ | $45.43 \pm 0.42^{Db}$ |
| 4-FT | $32.41 \pm 0.45^{Ac}$ | $37.66 \pm 0.53^{Bc}$ | $36.81 \pm 0.62^{Cc}$ | $39.44 \pm 0.64^{Dc}$ |
| 6-FT | $28.06 \pm 0.53^{Ad}$ | $31.82 \pm 0.75^{Bd}$ | $31.21 \pm 0.66^{Cd}$ | $35.51 \pm 0.52^{Dd}$ |

Capital letters (A – D) refers to the significant differences in similar treatments within the different storage time ($p < .05$). Lowercase letters (a-d) refers to the significant difference in the same frozen storage period of different concentrations ($p < .05$).

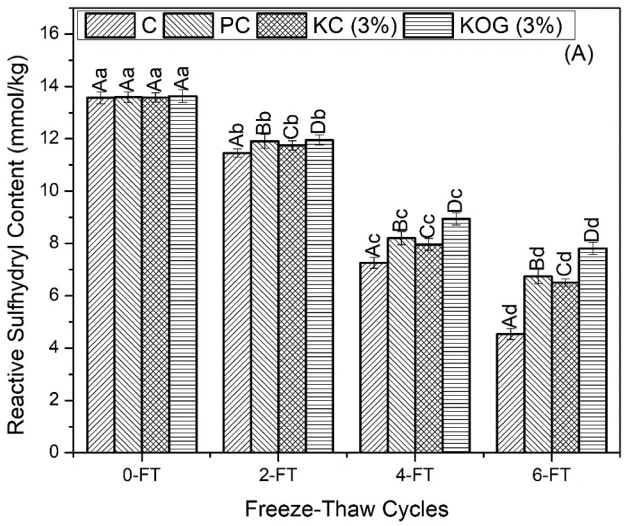


Figure 2. Effect of KC and KOG on the reactive sulfhydryl content of silver carp proteins during freeze-thaw cycles. Lowercase letters (a – d) refers to the significant differences in similar treatments within the different storage times ($p < .05$). Capital letters (A – D) refers to the significant difference in the same frozen storage period of different concentrations ($p < .05$).

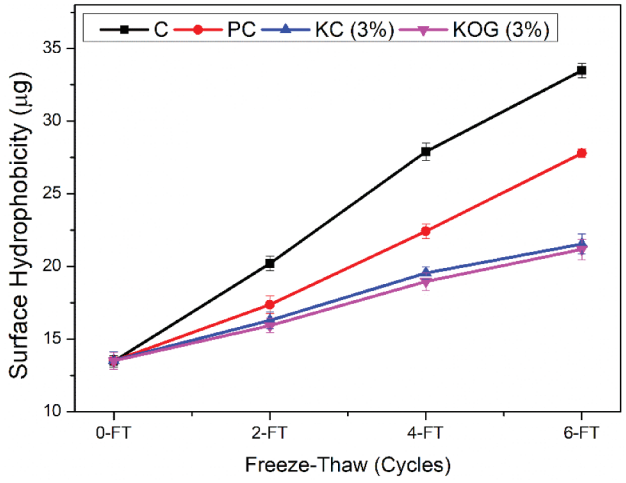


Figure 3. Effect of KC and KOG on the surface hydrophobicity of silver carp proteins during freeze-thaw cycles.

KOG might prevent the increase in surface hydrophobicity due to its better bonding interaction with functional sites. The interaction mechanism between KOs and surimi proteins primarily involves hydrogen bonding. The hydroxyl groups found in KOG create hydrogen bonds with the amino and carboxyl groups of proteins, which helps stabilize the protein structure and prevent aggregation and denaturation. This bonding is crucial for maintaining the integrity of the protein network, which is essential for the gelation and water-holding capacity of surimi (Walayat et al., 2023). Walayat, Tang, Nawaz, et al. (2022) demonstrated that KOG effectively preserved the gel strength of surimi during frozen storage by protecting MP from denaturation through the formation of hydrogen bonds.

### 3.4. Secondary structural changes

The structural changes in MP have a direct relationship with protein oxidation and denaturation. The structural change of silver carp protein samples treated with PC, KC (3%), KOG (3%), and samples with no treatment (C) is shown in Figure 4(A-D). A substantial decline was noted in all samples during FTC irrespective of treatments. The C samples with no cryoprotectant noted a major decline α-helix (59% to 39%), increase in β-sheets (19% to 34%), decline in β-turns (10% to 8%) and an increase in random coils (12% to 18%) after 6 FTC (Figure 4A-D). This significant decrease in α-helix content denotes a change in myosin and exposure to hydrophobic interactions. In addition, this increase could be associated with an increase in surface hydrophobicity, which is important to specify the oxidative changes and formation of disulfide groups (Zhang, Yan, et al., 2020). Moreover, samples treated with PC displayed major changes in all contents, such as α-helix, β-sheets, β-turns, and random coils, but the decline in α-helix was lesser than the C samples, indicating that the addition of conventional cryoprotectants might reduce the oxidative changes but not efficiently. In comparison to the C and PC added samples, MP samples incorporated with KC (3%) and KOG (3%) reduced secondary structural changes due to their efficient cryoprotective abilities (Figure 4A-D). This significant limitation in secondary structural changes could be attributed to decreased oxidative changes in myosin, which protects the α-helix from freeze-induced changes. This significant limitation in secondary structural changes could be attributed to decreased oxidative changes in myosin, which protects the α-helix from freeze-induced changes. The addition of KC (1%) reduced the -helix decline from 59% to 46.5%, while KOG (3%) reduced the decline from 59% to 48%) (as shown in Figure 4A-D). The incorporation of KOG (3%) into MP is more efficient owing to its better cryoprotective abilities against myosin denaturation and crystallization during fluctuating frozen storage. It is also expected that KOG could link up with the functional sites of amino acids, deterring the free water molecules (Li, Du, et al., 2021). Furthermore, the lower α-helix decline in KOG (3%) added samples may be due to fewer crystals forming and a faster recrystallization process during FTC. Meanwhile, KOG also improved the secondary structural constancy of proteins because of its abundant hydrophilic interactions (Walayat, Tang, Nawaz, et al., 2022). Research has indicated that *Flammulina velutipes* polysaccharides, specifically, are successful in conserving the α-helix composition and inhibiting the creation of clusters that result in β-sheet configurations, commonly linked to protein degradation (Ling et al., 2023). Overall, it can be assumed from these outcomes that the KOG (3%) could prevent the formation of ice crystals and protein denaturation, resulting in reduced disulfide binding and protein–water interactions.

### 3.5. Tertiary structural changes

Fluorescence spectrophotometry was used to determine the tertiary structural changes of all MP samples (C, PC, KC (3%), and KOG (3%)). The tertiary structural changes of all MP samples (C, PC, KC (3%), and KOG (3%)) are shown in Figure 5(A-D). During this FTC study, all samples (C, PC, KC (3%), and KOG (3%)) had a significant decrease in fluorescence intensity (FI), indicating tertiary structural changes. The decline in all samples depicts the denaturation of tryptophan (Trp) residues. Trp residues are more prone to oxidative changes and can easily be exposed to the microenvironment, resulting in reduced FI (Stănciuc et al., 2017). During FI analysis, C samples showed a major decline

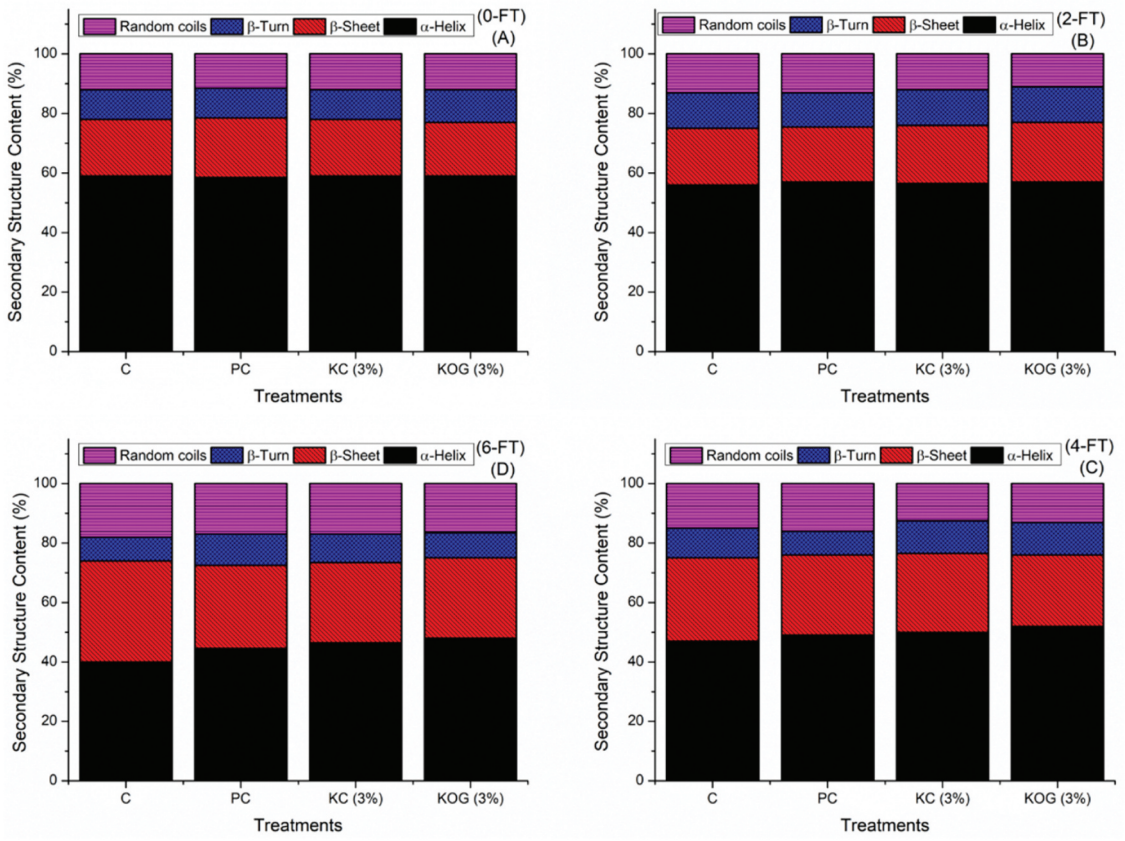


Figure 4. Effect of KC and KOG on the secondary structural changes of silver carp proteins during freeze-thaw cycles. Where, A, B, C and D represent the different freeze thaw cycles (FT).

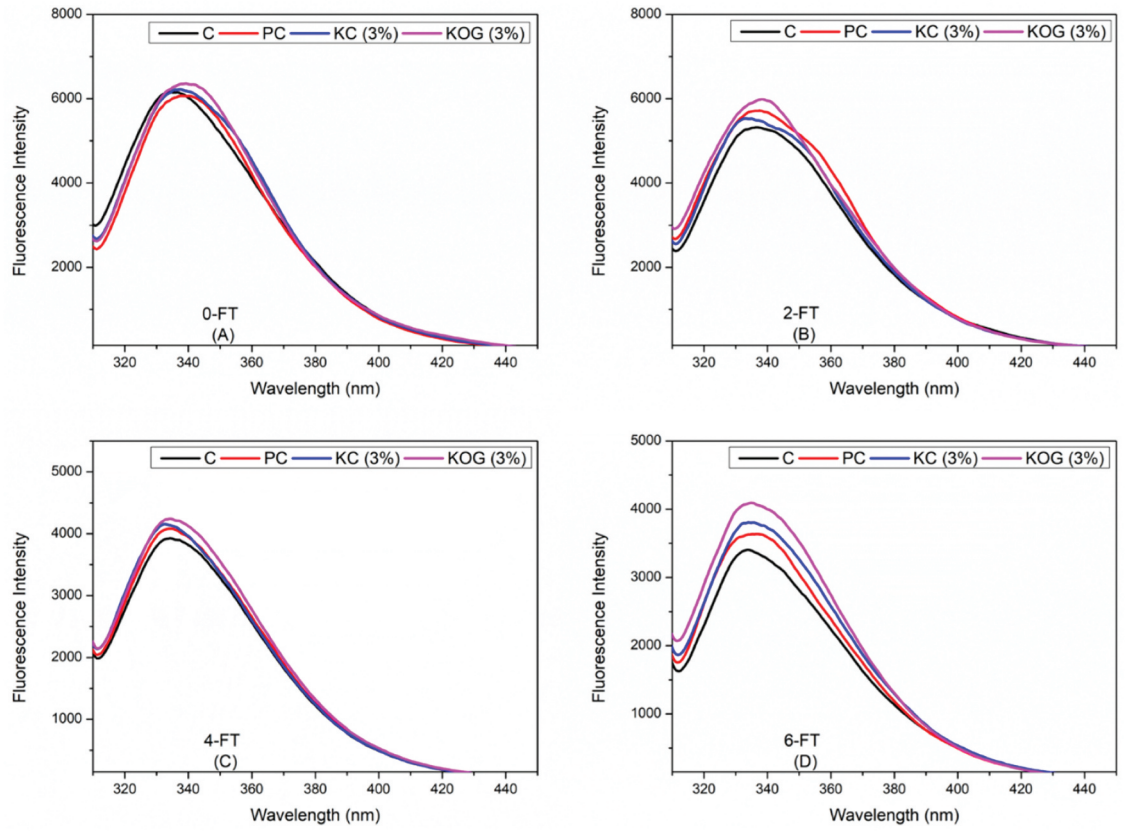


Figure 5. Effect of KC and KOG on the tertiary structural changes of silver carp proteins during freeze-thaw cycles. Where, A, B, C and D represent the different freeze thaw cycles (FT).

in FI from 0 to 6 FTC with a minor red shift in FI. This red shift in FI represents an alteration in Trp residues being oxidized after exposure to the microenvironment. Furthermore, increased steric interference may be another factor for decreased FI, which increases protein aggregation (Yi et al., 2020). MP samples containing PC, KC, and KOG (3%) demonstrated a more limited decline in FI after 6 FTC than C samples. If we talk about the PC treated samples, the FI was also decreased, but less than in the C samples. Meanwhile, samples with KC (3%) and KOG (3%) showed a greater decline than the others. However, the decline in samples treated with KOG (3%) was more stable than in samples treated with KC (3%) (Figure 5A–D). It is possible that the addition of KOG inhibits the exposure of Trp indole side chains to oxidative processes. Additionally, Walayat, Tang, Nawaz, et al. (2022) reported that the addition of KOG (3%) to the silver carp surimi protein enhanced its stability by protecting the myosin during fluctuated frozen storage. Studies have shown that KC has also proven to be successful in retaining the tertiary configuration of fish MP. Research suggests that KC has the capability to hinder the aggregation of proteins and uphold their solubility when stored in freezing conditions. This outcome is due to the potential ability of KC to create a shielding barrier around the protein molecules, thereby preventing their interaction and subsequent clustering (Zhang, Yan, et al., 2020). The addition of xylooligosaccharide (XO) prevented tertiary structural changes by providing a protective shield to myosin, which is responsible for Trp residue stability (Z. Zhang et al., 2021). Meanwhile, Z. Zhang et al. (2021) also stated that the addition of XO prevented the decrease in FI by replacing the free water molecules around the myosin surface, therefore stabilized tertiary structural properties during frozen storage.

## 4. Conclusion

A comparative study was performed among PC, KC (3%), and KOG (3%) in silver carp surimi MP during different freeze-thaw cycles. During this study, a significant increase in carbonyl content and surface hydrophobicity was analyzed. Meanwhile, there was a significant decrease in sulfhydryl contents (SH) and structural changes during 6 FTC. All these changes are well associated with protein oxidation and denaturation. The addition of PC, KC, and KOG (3%) significantly reduced the increase in carbonyl and surface hydrophobicity by protecting myosin and binding the free water molecules. The PC samples showed a restricted decline in oxidative and structural changes but were better than the C samples. Most interestingly, KC (3%) and KOG (3%) represented an almost similar trend against decline in SH, secondary and tertiary structural changes by enhancing hydrogen binding interactions and protecting the Trp residues. Overall, KOG (3%) proved to be an efficient cryoprotectant against oxidative and structural changes during FTC due to its stronger hydrophilic, ionic, and hydrogen bonding interactions with amino acids than KC (3%) and PC. Therefore, from the current results, we can assume that the addition of KOG could be an effective cryoprotectant for the prolonged shelf-life and commercial and economical values of seafood.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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