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Influence of Konjac oligo-glucomannan as cryoprotectant on physicochemical and structural properties of silver carp surimi during fluctuated frozen storage

Noman Walayat [a,b,c], Wei Tang [a,b,c], Asad Nawaz [d], Yuting Ding [a,b,c], Jianhua Liu [a,b,c,**], Jose Manuel Lorenzo [e,f,*]

[a] College of Food Science and Technology, Zhejiang University of Technology, Hangzhou, 310014, PR China
[b] Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, Hangzhou, 310014, PR China
[c] Ninghai Institute of Ocean Research, Zhejiang University of Technology, Ninghai 315600, PR China
[d] Shenzhen Key Laboratory of Marine Microbiome Engineering, Institute for Advanced Study, Shenzhen University, Shenzhen, 518060, PR China
[e] Centro Tecnolóxico da Carne de Galicia, Rúa Galicia No. 4, Parque Tecnolóxico de Galicia, Ourense, 32900, San Cibrao das Viñas, Spain
[f] Facultade de Ciencias, Universidade de Vigo, Área de Tecnoloxía dos Alimentos, Ourense, 32004, Facultade de Ciencias, Spain

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ABSTRACT

The aim of this study was to analyze the effect of Kanjoc oligo-glucomannan (KOG) on functional and structural changes in silver carp surimi during fluctuated (FLUC) frozen storage. During this study, KOG was prepared and added 1 and 3% in surimi and myofibrillar protein (MP) and compared with a positive control (PC) of sucrose and sorbitol mixture added samples. The results showed that the water holding capacity (WHC), textural and structural properties decreased during FLUC-I and FLUC-II, indicating that it could be due to protein denaturation induced by the formation of irregular crystallizations and recrystallization. Moreover, it was noted that the addition of KOG (3%) enhanced the stability of α-helix content (58–47%) during FLUC-I and (60–50%) during FLUC-II by inhibiting the hydrophobic residue exposure and change in aromatic amino acids. Meanwhile, KOG (3%) enhanced the textural properties by inhibiting the freeze induced protein denaturation. During FLUC frozen storage, the KOG (3%) was found to be a more effective concentration than conventional cryoprotectant mixture (PC). At industrial level, KOG (3%) could be used as a potential alternative to conventional cryoprotectants to attain prolonged commercial and economic values of silver carp.

1. Introduction

In the past few years, surimi and surimi-based products have gained much attention worldwide due to their higher nutritional values and harvesting, which was about 4.79 million tons in 2018 (Fishery & Statistics, 2020). Generally, frozen surimi is used to develop different surimi-based food products, such as fish cakes, sausages, and fish balls (Walayat, Xiong, Xiong, Moreno, Nawaz, et al., 2020). Surimi can be frozen and stored for long periods of time due to the increased consumption and to meet public needs, and it can be transferred to seafood industries via a cold chain to prevent deteriorative changes caused by various microorganisms before being processed into a final product (Jia et al., 2018). Moreover, frozen storage is the ultimate solution, which can restrict the microbial growth, retard the enzymatic reactions, preserve the nutrients and taste of frozen surimi (Su et al., 2019). However, some unavoidable changes, such as protein denaturation, aggregation, crystallization, recrystallization, structural changes, and reduced water holding capacity (WHC), can still take place during freeze thaw cycles and fluctuating frozen storage, which affects the overall quality and consumer suitability of surimi (Zhang, Fang, Hao, & Zhang, 2018).

Temperature fluctuations during transportation or frozen storage of surimi or seafood are often unavoidable and unpredictable that can cause structural damage induced by ice recrystallization, which further leads to reduced surimi quality (Zhang, Zhao, Chen, Zhang, & Wei, 2019). In frozen surimi tissues, the smaller ice crystals are thermodynamically potent in melting and shrinking during changing the

* Corresponding author. Centro Tecnolóxico da Carne de Galicia, Rúa Galicia No. 4, Parque Tecnolóxico de Galicia, Ourense, 32900, San Cibrao das Viñas, Spain.
** Corresponding author. College of Food Science and Technology, Zhejiang University of Technology, Hangzhou, 310014, PR China.
E-mail addresses: jhliu@zjut.edu.cn (J. Liu), jmlorenzo@ceteca.net (J.M. Lorenzo).

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temperature from high to low frozen storage; hence large crystals rise quickly to minimize free energy and surface area during quick frozen storage of samples (Tian, Walayat, Ding, & Liu, 2022; Zhang et al., 2019). Meanwhile, during the frozen temperature fluctuation, the smaller ice crystals melt down successively that water recrystallizes into large ice crystals, which results in the further change in particle shape and size of large ice crystals (Wang et al., 2018). Gao, Hou, and Zeng (2019) reported that the periodic change in temperature resulted in the increased growth and morphology of ice crystals, which caused protein denaturation and resulted in reduced WHC, textural and structural properties. In addition, Zhang et al. (2022) reported that with prolonged temperature fluctuation and frozen storage, the size of ice crystals increases and becomes irregular in shape, making protein molecules more prone to fragmentation and denaturation, resulting in changes in structural and microstructural properties.

In order to reduce functional and structural changes induced by protein denaturation, cryoprotectants are widely added to surimi during frozen storage. The way cryoprotectants protect the surimi from frozen storage damage is through interaction with protein molecules (Walayat, Xiong, Xiong, Moreno, Nawaz, et al., 2020). Generally, a conventional cryoprotective mixture (sucrose and sorbitol) is used by seafood industry to extend the shelf life of surimi and related products. However, the conventional cryoprotectant mixture is not as good enough in cryoprotective properties as polysaccharides due to weak intermolecular bonding interactions. Meanwhile, strong sweetness and caloric values also made the conventional cryoprotectant mixture unfavorable for consumer use (Walayat, Xiong, Xiong, Moreno, Li, et al., 2020). Therefore, to mitigate such challenges, industries are paying attention to better alternatives of conventional cryoprotectants.

Konjac glucomannan (KGM) is a high molecular weight polysaccharide derived from the Amorphophallus Konjac C. Koch tubers, which are composed of β (1 → 4) associated with D-glucose and D-mannose (molar ratio = 1.6:1) (He, Luo, Lin, & Zhang, 2012). It has linear random copolymer structure, in which a single branch chain involved 32 sugar residues with a total length of 3–4 sugar residues and further linked at C_3 mannose through β(1 → 3) glucoside bond. The O-acetyl (-OCOCH^3) linked to sugar units and distributed arbitrarily along the molecule, which rate is 1 in 19 sugar residue at C-6 position (Wang, Zhong, Chen, Li, & Lv, 2011). Apart from this, KGM has great potential as a gelling, thickening and emulsifying agent (Liu et al., 2015). Owing to its astonishing abilities, KGM has been approved by FDA and Europe as food additive (Tester & Al-Ghazzewi, 2016). In addition, KGM is also well-known due to its health promoting abilities, such as reducing cholesterol, improving blood sugar, enhancing intestinal activity and strength the immune system (Zhu, 2018). Besides of all these abilities, KGM has been reported as an excellent cryoprotectant in grass carp proteins by inhibiting the decline in Ca^{2+}-ATPase activity, sulfhydryl content and protein solubility during frozen storage (Liu, Fang, Luo, Ding, & Liu, 2019). Konjac oligo-glucomannan (KOG) is enzymatically derived oligosaccharide from KGM, which is low in molecular weight and contains more acetyl groups and branched chains. The presence of glyosidic bond and acetyl groups make KOG more important as low caloric additive and potential cryoprotectant in seafood products during frozen storage (Liu et al., 2019).

In this study, KOG was prepared to a low molecular weight (average D.P = 5.89), in order to extend the quality of silver carp surimi during different fluctuating frozen storage (fluctuation temperature I and fluctuation temperature II). During this study, different concentrations of KOG 1 and 3% were added in surimi and myofibrillar proteins to analyze which concentration can be a better alternative of conventional cryoprotectants (4% sucrose + 4% sorbitol). Meanwhile, the role of KOG was analyzed in the enhanced functional and structural properties of silver carp surimi proteins during fluctuating frozen storage. This study also provides sufficient evidence that KOG can be used as potential substitute of sucrose and sorbitol to extend the economic and commercial values of silver carp at industrial scale.

2. Materials and methods

2.1. Materials

Konjac glucomannan flour (food grade) was purchased from Hubei Konson Konjac Co., Ltd. (Hubei, P.R. China) with a purity of 98%. The sucrose and sorbitol were purchased from Henan Wang Bang Industrial Co., Ltd. (Henan, P.R. China). Moreover, the β-mannanase enzyme (50000 U/g) was acquired from Beijing Challenge Bio-Tech Co., Ltd. (Beijing, P.R. China). All other reagents and chemicals were bought from Beijing Dingguo Chansheng Bio-Technology Co., Ltd. (Beijing, P.R. China).

2.2. Preparation of KOG

The KOG was prepared following the method of Liu et al. (2015) with slight modifications. Acetate buffer (0.2 M,150 mL) was prepared and added with 0.25 g of β-mannanase (150 U/g) and incubated at 50 °C for 10 min to start the reaction. Konjac powder (5%) was added to the above prepared solutions and heated (water bath) at 50 °C for 3 h with continuous magnetic stirring. After that, the reaction was stopped by boiling the solution for 15 min. The obtained solution was filtered with cotton mesh cloth and rotary evaporated (RE-2000A, Yarong Co., Ltd., Shanghai, China). The concentrated solution was added with ethyl alcohol (95%) and centrifuged at 8000 rpm for 10 min at 4 °C. The KOG was obtained as precipitates, ethyl alcohol (95%) was added and centrifuged, and this process was repeated five times. Then, the KOG was added to distilled water, after being dissolved in distilled water KOG was filtrated through a 700-mesh cloth to remove undegraded KGM and freeze-dried (FD-1-50, Bo Yikang Co. Ltd., Beijing, China). The average degree of polymerization (DP) of the obtained KOG was 5.89 (MW: 600), which is significantly equal to the DP: 5.2 according to our earlier study (J. Liu et al., 2015).

2.3. Preparation of surimi samples

Silver Carp (*Hypophthalmichthys molitrix*) fish (number: 12, weight: 2.8 kg) was acquired from the local market in Hangzhou, Zhejiang, P.R. China. The deceased silver carp fish was stored in ice box and immediately transferred to the Key Laboratory of Marine Fishery Resources Exploitment & Utilization of Zhejiang Province, College of Food Science and Technology, Zhejiang University of Technology, Hangzhou, P.R. China within half an hour. The fish was headed, eviscerated, filleted, minced and surimi was prepared according to the method of An, You, Xiong, and Yin (2018). After that, the surimi was divided into four parts, one part of surimi was added with sucrose (4%) and sorbitol (4%) mixture as a positive control (PC), the second part with KOG (1%), third part with KOG (3%) and surimi added with no cryoprotectants was used as a control (C). All the samples were placed in polythene bags and hermetically sealed.

2.4. Preparation of myofibrillar proteins samples

The Myofibrillar proteins (MP) were extracted from silver carp according to the method of Zhang, Xiong, et al. (2020). The mince (200 g) was mixed with five volume of low salt buffer solution 0.05 mol/L NaCl (20 mmol/L) buffer solution and homogenized (5000 r/min) with a homogenizer (T-25, Digital Ultra Turrax, Guangzhou, China). The homogenized samples were centrifuged (Digicen-21R, Madrid, Spain) at 10,000 × *g* at 4 °C for 10 min. The collected precipitates were added to high salt buffer solution 0.05 mol/L NaCl (20 mmol/L) and centrifuged twice. After that, the precipitates were homogenized with 0.6 mol/L (20 mmol/L) and centrifuged 10,000 × *g* at 4 °C for 10 min. The obtained supernatant was mixed with distilled water ten times and centrifuged under the same conditions. The MP was collected as precipitates and protein concentration was 87.22 mg/mL determined by biuret method

Gornall, Bardawill, and David (1949) using bovine serum albumin. The MP samples were added with sucrose (4%)+sorbitol (4%) as PC, KOG (1%), KOG (3%) and MP added with no cryoprotectant was used as a control MP sample. All the samples were added into sample tubes (50 mL) and tightly capped.

2.5. Frozen storage of samples

2.5.1. Fluctuation-I

First batch of tightly packed samples of C, PC, KOG (1%) and KOG (3%) were stored at −18 °C in the freezer for 24 h. All surimi and MP samples moved to 4 °C for 3 h (−18 °C for 24 h and 4 °C for 3 h, this temperature fluctuation is equal to one fluctuation cycle (FTC)). All samples were placed at 0, 2, 4 and 6 FTC. After that, all the samples were placed at 4 °C for 3 h prior to analyses.

2.5.2. Fluctuation-II

Second batch of tightly packed samples C, PC, KOG (1%) and KOG (3%) were stored at −24 °C for 24 h then moved to −80 °C for 2 days (−24 °C for 24 h and −80 °C for 2 days, this temperature fluctuation is equal to one FTC). All the samples were placed at 0, 2, 4 and 8 FTC. After that, all the samples were placed at 4 °C for 3 h prior to analyses.

2.6. pH measurement

pH was analyzed according to Walayat, Xiong, Xiong, Moreno, Li, et al. (2020). Briefly, surimi (10 g) was homogenized at a higher speed with 20 mL of water. pH of all samples was determined using a pH meter (FE-28, Metter Toledo, Shanghai, China). For each sample, three replications were used for the analysis and the mean values were noted.

2.7. Surimi gel preparation

The surimi gel was prepared according to the method of Liu et al. (2019). Frozen surimi samples were thawed at 4 °C, chopped 5 min and mixed with 2.5% edible salt. Then the surimi was stuffed in polyvinylidene casing (diameter 3 cm) and both ends were tightly sealed. The surimi stuffed casing was placed in a water bath (HH-4, Shanghai, China) at 40 °C for 1 h and then 90 °C for 30 min. After water bath heating, the surimi gel was cooled in ice slurry and placed in the refrigerator overnight.

2.8. Water holding capacity

The water holding capacity (WHC) (%) was determined according to Zhang, Li, et al. (2020). The surimi gel samples were centrifuged (1000 *g*, 10 min and 4 °C). The weight of surimi gels was recorded before and after centrifugation. The WHC (%) was determined by the following formula:

$$\text{WHC (\%)}: (\text{AB-CD})/\text{AB} \times 100$$

Where; AB indicate gel weight (g) before centrifugation and CD indicate gel weight (g) after centrifugation. Each sample was analyzed in triplicate.

2.9. Whiteness

The whiteness of surimi gel was analyzed by following the procedures of Ruttanapornvareesakul et al. (2006). The colorimeter (Hunter-Lab Color Q, HunterLab, Ltd., Reston, 205 VA, Shanghai, China) was used to determine the L* a* and b* values. The whiteness of surimi gel was calculated using the following formula:

$$\text{Whiteness} = 100\text{-}[(100\text{-L*})2+ \text{a*}2 + \text{b*}2]\ ½$$

Where: L*: lightness, a*: greenness/redness and b*: blueness/yellowness.

2.10. Textural profile analysis

The textural properties of surimi gel were determined according to the method of Zhang, Xiong, et al. (2020) using a texture profile analyser (SMS, Surrey, UK). Gel samples were analyzed with a flat probe (P/36R) using texture profile analyzer. All gel samples were taken in triplicates. Samples were analyzed at pre-test speed (1.0 mm/s), test speed (1 mm/s), trigger type (5 g) and post-test speed (1.0). The obtained data was expressed as hardness (g), springiness (mm), gumminess (mJ) and cohesiveness.

2.11. Ultraviolet spectra

The ultraviolet spectra of silver carp MP was analyzed by following the method of Ekezie, Cheng, and Sun (2019) with minor modifications. MP solution was diluted in 10 mM phosphate buffer to 1 mg/mL (pH:7). The UV spectra was determined using a UV-Spectrophotometer (TU-1900, Beijing, China) at 230–320 (scanning speed). Phosphate buffer (10 mM) was used as a blank. All samples were analyzed in triplicates.

2.12. Fluorescence intensity

Fluorescence intensity (FI) of MP samples were determined by the method of Walayat, Rincón, et al. (2021) with some modifications. All samples were diluted to 0.5 mg/mL in 0.6 M NaCl solution. The FI of diluted samples was analyzed at excitation wavelength (295 nm), wavelength (300–450 nm), scanning speed (1200 nm/min) with 10 nm excitation and emission of slit. All FI of all MP samples analyzed in triplicates using 0.6 M NaCl as blank.

2.13. Circular dichroism spectrum

Circular dichroism (CD) spectra of MP samples were determined using the protocol of Walayat, Wang, et al. (2021) with slight modifications. MP samples were diluted to 0.2 mg/mL in 10 mM phosphate buffer solution. The CD spectra was analyzed at resolution (1 nm), spectrum (190–250 nm), response time (2 s), scanning speed (100 nm/min) and sensitivity (50 milli mm). The generated data was obtained in percentages of α-helix, β-turn and random values. Phosphate buffer (10 mM) was used as a blank. All samples were analyzed in triplicate in order to calculate the mean value.

2.14. Statistical analysis

Each analysis was repeated in triplicates and the results were expressed as mean value ± standard deviation (SD). One-way ANOVA was used to analyze all the data by DUNCAN'S test with significance values of $P < 0.05$. All figures were designed using Origin Pro 9 (Origin Lab Inc., USA).

3. Results and discussion

3.1. pH

The pH of all surimi samples treated with C, PC, KOG (1%) and KOG (3%) during fluctuated frozen storage is shown in Fig. 1 (a, b). The pH values of both fluctuation temperatures did not show any significant difference ($P > 0.05$), which were ranged from 6.86 to 6.90. It has been reported by Kaewprachu, Osako, Benjakul, Suthiluk, and Rawdkuen (2017) that the surimi with pH from 6.90 to 7.0 has better gelling and textural properties, which is most possibly due to better cross-linking and interaction of myosin chains in this range of pH. Moreover, Walayat, Xiong, Xiong, Moreno, Li, et al. (2020) also verified the

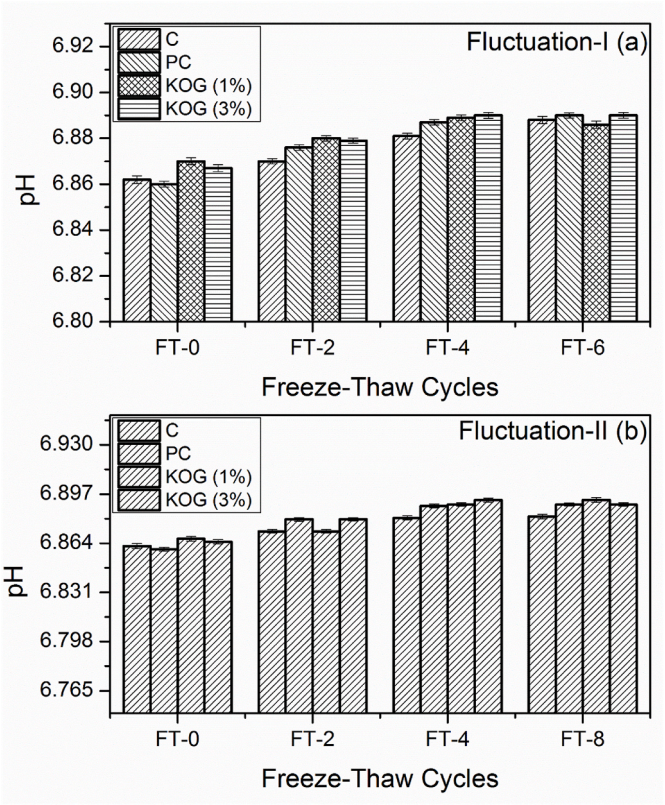

Fig. 1. pH of surimi treated with PC, KOG (1%) and KOG (3%) during different fluctuated frozen storage. Error bars show the standard deviation (SD) of three replicate measurements.

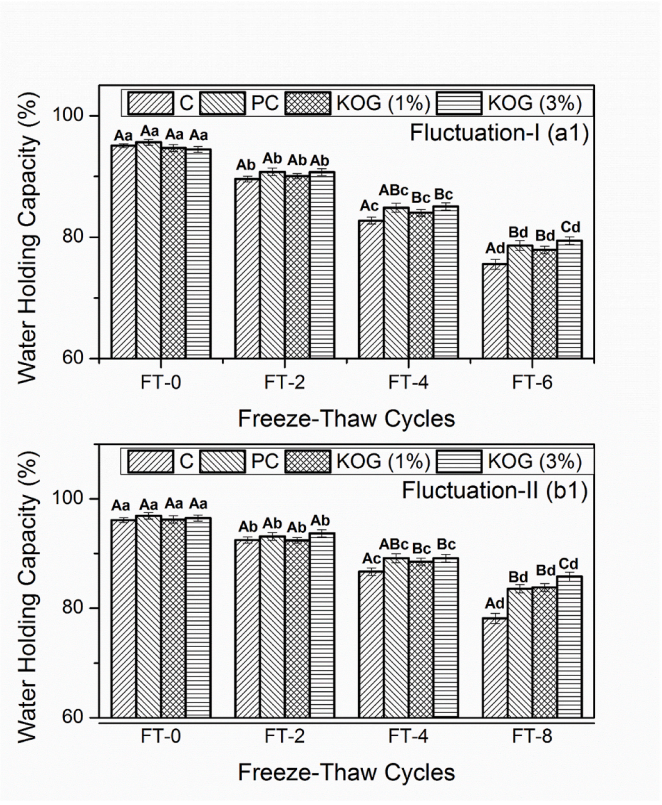

Fig. 2. Water holding capacity of surimi gel treated with PC, KOG (1%) and KOG (3%) during different fluctuated frozen storage. Error bars show the standard deviation (SD) of three replicate measurements. Upper case letters (A–D) on error bars show significant differences ($P < 0.05$) in different treatment within the same freeze thaw cycle. Lower case letters (a–d) show the freeze thaw cycle of same treatment.

previous studies that the pH range from 6.8 to 6.9 showed no negative effects on the structural and physicochemical properties of topmouth culter MP during frozen storage of 60 days. Torrieri et al. (2011) also analyzed no significant change in pH of Bluefin surimi during frozen storage of two weeks; hence, pH cannot be used as a key indicator to analyze the physicochemical and functional characteristics of surimi and related products during frozen storage. The decrease in pH during frozen storage of surimi from 6.94 to 6.91 could be due to unnecessary lactic acid production during the glycolysis process (Liu, Liang, Xia, Regenstein, & Zhou, 2013; Sultanbawa & Li-Chan, 2001). Meanwhile, Lorenzo, Batlle, and Gómez (2014) analyzed that the trimethylamine and ammonia formation in muscle proteins prompted by microbial and autolytic reactions can also be factors of increased pH.

3.2. Water holding capacity

Water holding capacity (WHC) is an important parameter in determining the water binding abilities of surimi gel. The potential inter cross-linking of myosin chains during the gelation process, which ultimately results in a better surimi gel with a three-dimensional network and assists surimi gel to entrap more water molecules. The WHC of surimi gel (C, PC, KOG 1% and KOG 3%) during FLUC-I and FLUC-II is shown in Fig. 2 (a1 and b1). The incorporation of cryoprotectants (PC, KOG 1% and KOG 3%) restricted the decline in WHC of surimi gel. Despite the addition of PC and KOG treatments, there was no significant difference ($P > 0.05$) analyzed at freeze-thaw cycle 0 (FTC-0) during both FLUC-I and FLUC-II, which was followed by a substantial drop ($P < 0.05$) from 95.07 to 75.54% in control samples after FTC-6 (FLUC-I). Meanwhile, after FTC-8 of FLUC-II, significant decline ($P < 0.05$) from 96.12 to 78.17% in C samples was analyzed. Apart from this, addition of PC and KOG (1 and 3%) significantly reduced the decline in WHC of surimi gel during both FLUC-I and FLUC-II. It was interesting to note that PC treated samples showed better stability against decline in WHC than the gel samples treated with KOG (1%) after FTC-2 and FTC-4 (FLUC-I) and the same trend was noted in FLUC-II. Sun, Huang, Hu, Xiong, and Zhao (2014) examined that the addition of modified starch and polysaccharides mixture in surimi can reduce the decline in WHC induced by structural changes and protein denaturation during frozen storage. In addition, incorporation of KOG (3%) significantly reduced ($P < 0.05$) the decline in WHC during FLUC-I. Meanwhile, the WHC of C samples during FLUC-II was reduced from 96.48 to 85.78, which is better than the PC and KOG (1%). The FLUC-I gel samples exhibited a more major decline ($P < 0.05$) in WHC than the FLUC-II samples. It could be due to the formation of large, irregular in shape and size of ice crystals, which results in protein denaturation, structural change and weak cross-linking between myosin chains. KOG (3%) could reduce these alterations by interacting with the protein molecules, entrapping the free water molecules that are available in the MP network. Consequently, this leads to KOG and protein interactions. Walayat, Xiong, et al. (2021) also stated that the egg white protein/β-cyclodextrin (EWP/βCD) can significantly increase the WHC of silver carp MP during frozen storage at −18 °C for 60 days. Thus, it can be established from the present outcomes that the incorporation of KOG (3%) prevents the decline in WHC probably by binding with functional groups of proteins or might enhancing the inter-cross-linking of amino acid side chains and hydrogen bonding between the protein molecules.

3.3. Whiteness

The whiteness is an important parameter that is associated with the color and sensory attributes of surimi (Chen et al., 2013). The whiteness

of all surimi gel samples treated with PC and KOG (1 and 3%) is presented in Table 1. The whiteness of all surimi gel samples was reduced significantly during FLUC-I and FLUC-II. There was no significant difference ($P > 0.05$) noted in all samples despite cryoprotectants addition during FLUC-I and FLUC-II. The significant difference ($P < 0.05$) in whiteness started after FTC-2 during both FLUC-I and FLUC-II, which could be due to the initiation of protein changes. The whiteness of C gel samples showed a significant decline during all FTC, which was 86.12 to 71.82% during FLUC-1 and 83.22 to 73.67% during FLUC-II, respectively. The decline in whiteness could be due to lipid and amide groups oxidation and non-enzymatic reactions as well, which is reported by Chen et al. (2013). Moreover, the formation of carbonyls might also be a chief factor in reduced whiteness, which can lead to protein denaturation and aggregation (Kong et al., 2013). During the whiteness analysis, PC treated surimi gel showed significantly ($P < 0.05$) higher whiteness than the gel samples treated with KOG (1%) and remained unchanged after the FTC-6 during FLUC-I and FTC-8 during FLUC-II. Overall, KOG (3%) presented the best stability in surimi gel whiteness in contrast with PC and KOG (1%) treated surimi gel samples, which was 85.93 (FTC-0) to 77.33% (FTC-6) during FLUC-I and 83.76 (FTC-0) to 77.16% (FTC8) during FLUC-II. J. Liu et al. (2019) reported that the addition of KOG showed better stability in the whiteness of red gurnard surimi gel during frozen storage of 50 days than the sucrose and sorbitol mixture added surimi gel. Walayat et al. (2020f) also examined that EWP/βCD mixture has a potential cryoprotective effect and inhibited the decline in *Culter alburnus* MP gel whiteness during 60 days of frozen storage. Hence, from these results, it can be suggested that the KOG could be used an alternative to conventional cryoprotectants to prevent the drop in whiteness by significantly preventing the lipids and protein oxidation.

3.4. Textural profile analysis

Texture profile analysis (TPA) is an important technique to analyze the quality and sensory characteristics of any product. The TPA of all surimi gel samples incorporated with PC, KOG (1 and 3%) during FLUC-I and FLUC-II is shown in Fig. 3. During the FLUC frozen storage, a significant decline ($P < 0.05$) was noted in all the surimi gel samples. There was no significant difference ($P > 0.05$) was recorded in all surimi gel samples at FTC-0 during FLUC-I, whereas slight difference was observed in FLUC-II. The C surimi gel samples showed a remarkable decline in all TPA properties during FLUC-I, including hardness (638–561 g), springiness (0.94–0.62 mm), gumminess (539–456 mJ) and cohesiveness (0.75–0.41). In addition, during FLUC-II the hardness of C surimi gel significantly ($P < 0.05$) decreased from (645–585 g), springiness (0.96–0.71 mm), gumminess (547–485 mJ) and cohesiveness (0.81–0.53). The results of decline in textural properties are totally coincide with WHC results (as shown in Fig. 2). This significant ($P < 0.05$) decline in all textural parameters could be due to protein denaturation induced by fluctuated frozen storage, which results in oxidative changes of protein molecules and weak cross-linking, which consequently leads to a poor and coarser gel network (Nikoo, Benjakul, & Xu, 2015). Meanwhile, surimi gel samples added with PC and KOG (1%) showed a slight difference in hardness, springiness, gumminess and cohesiveness during both FLUC-I and FLUC-II. On the other hand, surimi gel treated with KOG (3%) showed significant stability in all textural parameters regardless of FLUC-I and FLUC-II. The increase in textural properties after adding KOG (3%) to surimi gel is possibly due to better cross-linking between amino acid side chains and strong disulfide bonding by retarding the proteins' denaturation and aggregation, which inhibit the alterations in intermolecular interactions and spatial structure, resulting in less decline in protein solubility (Liu et al., 2013). Our results also coincide with previous study by Liu et al. (2019) that found the addition of KOG significantly improved the gel strength of red gurnard surimi gel by enhancing the intermolecular bonding interactions and cross-linking of myosin molecules. Walayat, Xiong, et al. (2021) also reported the EWP/βCD mixture as potential substitute of conventional cryoprotectants to enhance the textural attributes of surimi gel. Hence, from these results, it can be confirmed that the KOG (3%) could be used as an effective cryoprotectant to prevent the texture loss of seafood and other products during frozen storage.

3.5. Secondary structural analysis

The alterations in secondary structural properties of all MP samples C, PC, KOG (1 and 3%) during FLUC-I and FLUC-II are shown in Fig. 6 (a1, a2, a3 and a4) and (b1, b2, b3 and b4). The decline in secondary structural properties was significant ($P < 0.05$) in all MP samples during FLUC-I and FLUC-II, regardless of incorporation with PC and KOG (1 and 3%). The C MP samples added with no cryoprotectant showed a remarkable decline in α-helix (58–39%), β-sheets (35–20%) and a slight increase in β-turns (9–7%) after FTC-6 during FLUC-I. Meanwhile, a significant increase was noted in random coils from 13 to 19% as shown in Fig. 6 (a1 to a4). The decline in α-helix indicates the change in myosin rod portion occurring due to hydrophobic residue exposure (Yi et al., 2020). Furthermore, the formation of disulfide bonds could also be a key reason for oxidation in the myosin rod portion, which results in secondary structural changes (Zhang, Xiong, et al., 2020). Furthermore, the MP samples added with PC and KOG (1 and 3%) recorded with significant stability in secondary structural properties, indicating the addition of cryoprotectants could prevent myosin damage by forming complexes with α-helix. During the FLUC-I, it was found that the addition of KOG (3%) in MP samples increased the stability of α-helix content from 58 to 47% (Fig. 6 a1 and a4). The addition of KOG in MP is also effective because of its role against protein denaturation induced by ice crystallization. It is possible that the KOG interacts at the functional sites of protein molecules, hindering the interaction with free water molecules (Li et al., 2021). In the meantime, the increase in random coils was in a similar pattern to FLUC-I. However, the decline was examined during

Table 1
Whiteness of surimi gel treated with PC, KOG (1%) and KOG (3%) during different fluctuated frozen storage.

Fluctuation-I					Fluctuation-II				
Whiteness									
Freeze-Thaw Cycles	**C**	**PC**	**KOG (1%)**	**KOG (3%)**	**Freeze-Thaw Cycles**	**C**	**PC**	**KOG (1%)**	**KOG (3%)**
0-FT	86.16 ± 1.17^{Aa}	84.93 ± 0.34^{Aa}	84.48 ± 0.52^{Aa}	85.93 ± 0.69^{Aa}	**0-FT**	83.22 ± 0.72^{Aa}	84.43 ± 0.67^{Aa}	83.37 ± 0.55^{Aa}	83.76 ± 0.81^{Aa}
2-FT	82.45 ± 0.96^{Ab}	83.67 ± 0.62^{Ab}	82.14 ± 0.44^{Ab}	83.44 ± 0.95^{Ab}	**2-FT**	81.47 ± 0.48^{Ab}	83.39 ± 0.61^{ABb}	82.60 ± 0.43^{ABb}	82.91 ± 0.36^{Bb}
4-FT	77.69 ± 0.57^{Ac}	80.24 ± 0.82A^{Bc}	79.51 ± 0.58^{Bc}	80.72 ± 0.49^{Bc}	**4-FT**	78.52 ± 0.91^{Ac}	81.72 ± 0.54^{ABc}	80.23 ± 0.76^{ABc}	81.33 ± 0.41^{Bbc}
6FT	71.82 ± 0.65^{Ad}	74.43 ± 0.31^{Bd}	74.25 ± 0.71^{Bd}	77.33 ± 0.32^{Cd}	**8-FT**	73.67 ± 0.79^{Ac}	75.31 ± 0.36^{ABc}	74.46 ± 0.44^{ABc}	77.16 ± 0.62^{Bc}

Upper case letters (A-D) show significant differences ($P < 0.05$) in different treatment within the same freeze thaw cycle. Lower case letters (a-d) show the freeze thaw cycle of same treatment.

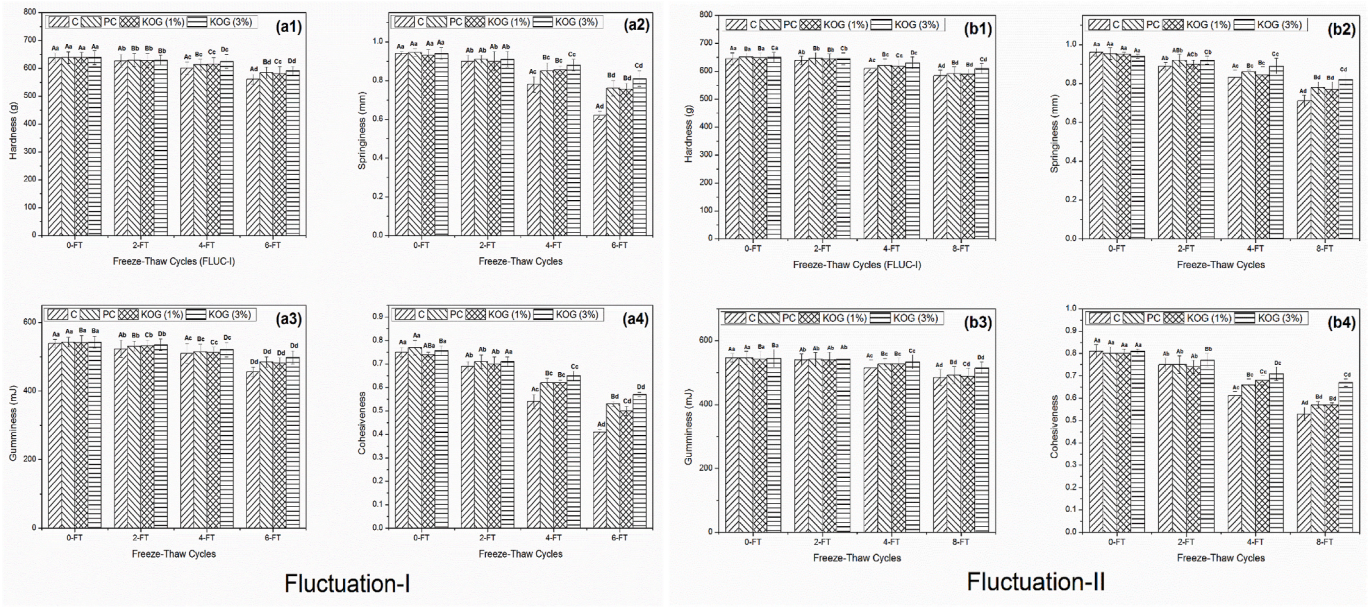

Fig. 3. Textural properties of surimi gel treated with PC, KOG (1%) and KOG (3%) during different fluctuated frozen storage. Error bars show the standard deviation (SD) of three replicate measurements. Upper case letters (A–D) on error bars show significant differences ($P < 0.05$) in different treatment within the same freeze thaw cycle. Lower case letters (a–d) show the freeze thaw cycle of same treatment.

both FLUC-I and FLUC-II. The decrease in α-helix (61–44%) and other secondary structural properties was less pronounced than in FLUC-I due to fluctuating frozen storage at −80 °C and −24 °C, which indicates the formation of more regular and shaped ice crystals than the FLUC-I (fluctuated between −18 °C and 4 °C). The KOG could increase the structural stability of MP due to the abundant availability of hydrophilic interactions of KOG with protein molecules. Our results are in agreement with our previous results of WHC (Fig. 2), textural properties (Fig. 3) and UV absorption spectra (Fig. 4) that the addition of KOG could reduce the freeze induced protein denaturation by inhibiting the irregular formation of ice crystals, disulfide bindings, protein-protein and protein-water interactions.

3.6. UV absorption spectra

The MP tertiary structural changes added with PC and KOG (1 and 3%) during frozen storage are analyzed using a UV absorption spectra. The change in UV absorption spectra of all MP samples added with PC and KOG (1 and 3%) during FLUC-I and FLUC-II is shown in Fig. 4 (a1, a2, a3 and a4) and (b1, b2, b3 and b4). The UV absorption spectra of all silver carp MP samples decreased during FLUC-I and FLUC-II despite the incorporation of PC and KOG. The absorption peak at 275 nm indicates the presence of tryptophan (Trp) and tyrosine residues (Tyr), which increased in samples treated with PC and KOG (1 and 3%). During the FLUC-I, almost all peaks showed a similar pattern of peak around 275 nm and there was no difference ($P > 0.05$) in absorption spectra at FTC-0 (Fig. 4, a1) was observed, which could be due to insufficient time for

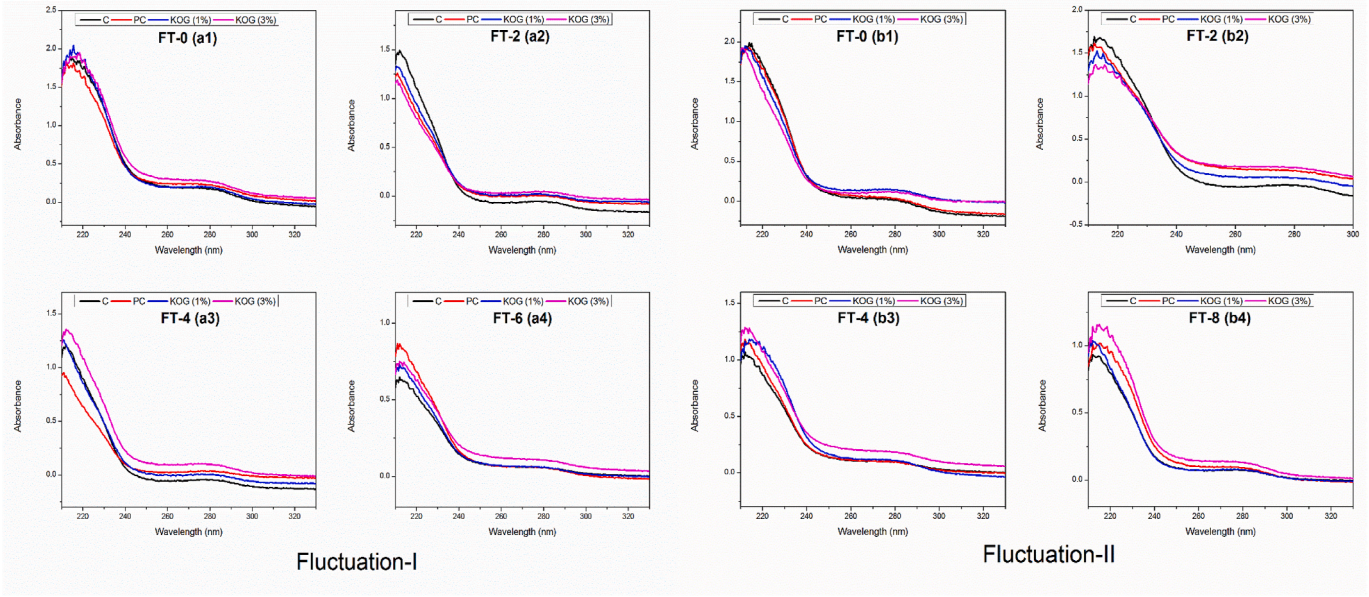

Fig. 4. UV absorption spectra of MP samples treated with PC, KOG (1%) and KOG (3%) during different fluctuated frozen storage.

PC and KOG to interact with myosin molecules. The actual decline in absorption of C samples was recorded at FTC-2, which remained prominent until FTC-6. The C samples also showed a red shift from 278 nm to 283 nm, which indicates a significant effect of fluctuated frozen storage on aromatic amino acids and exposure of Trp and Tyr to a hydrophilic environment. This might be due to the spatial distribution of KOG and the availability of hydrophilic groups. With these features, KOG generates steric hindrance, which results in the better stability of protein molecules (Walayat et al., 2022, p. 107761). The decrease in UV spectra absorption during FLUC-I and FLUC-II could be due to oxidative and denaturation changes in protein molecules (Qiu, Xia, & Jiang, 2014). Meanwhile, silver carp MP samples treated with PC and KOG (1 and 3%) showed a sharper decline in absorption spectra than the C samples, the better stability in these samples could be due to better cross-linking between protein molecules. The samples treated with KOG (3%) showed a slight change in red shift, which indicates the addition of KOG has no negative effect on the aromatic amino acids (Trp and Tyr). On the other hand, almost similar trend of decline was observed in all MP samples during FLUC-II (Fig. 4, b1, b2, b3 and b4). The C samples noted with substantial decline in absorption spectra from FTC-0 to FTC-8 during FLUC-II, which signifies the protein denaturation and ultimate consequences on Trp and Tyr. Wang, Zhao, Cai, Wang, and Xiao (2019) reported that the decline in UV absorption spectra of shrimp protein could be due to oxidation and denaturation of protein molecules after subjecting to freezing process. Meanwhile, addition of PC and KOG (1 and 3%) remarkably restricted the decline in UV absorption spectra. In this case, PC added MP samples showed better stability in UV spectra as compared to MP samples added with KOG (1%), the difference between PC and KOG (1%) was not as prominent at FTC-4 and FTC-8 during FLUC-II. Overall, the MP samples incorporated with KOG (3%) showed the highest stability in UV spectra during FLUC-I and as well as FLUC-II. Zhao, Zou, Sun, and Yu (2020) reported that the addition of sodium alginate in myofibrillar protein increased the stability in Trp residues, its might be due polysaccharides-protein interactions. Thus, it can be concluded from the current results that the addition of KOG (3%) significantly reduces the changes in aromatic amino acids and can extend the stability of UV absorption spectra.

3.7. Fluorescence intensity

Fluorescence intensity (FI) intensity of all MP samples C, PC and KOG (1 and 3%) during FLUC-I and FLUC-II is shown in Fig. 5. (a1, a2, a3 and a4) and (b1, b2, b3 and b4), respectively. All samples showed a broad spectrum peak at 336 nm during FLUC-I and FLUC-II. During the analysis of FLUC-I and FLUC-II, the FI of all samples decreased, which indicates the denaturation of Trp indole side chains after being exposed to the microenvironment. Trp residues are very sensitive to polar or microenvironment due to their availability on head and rod regions, which causes protein aggregation and conformational changes (Stănciuc et al., 2017). The C samples showed a remarkable decline in FI and a slight red shift during FLUC-I and FLUC-II. The red shift in FI indicates the change in Trp during exposure to the microenvironment and then oxidized. Moreover, increased steric hindrance could also be the reason for decreased FI, which further cause the enhanced hydrophobic interactions and protein aggregation (Yi, Ye, et al., 2020). Meanwhile, MP samples with PC and KOG (1 and 3%, respectively) demonstrated greater FI stability than C samples. During FLUC-I and FLUC-II, the quenching in the FI of PC-treated samples was very similar to that of MP samples treated with KOG (1%). Instead of others, MP samples incorporated with KOG (3%) showed significantly more stability in FI than the samples treated with PC and KOG (3%); it is most probably that the addition of KOG prevents Trp exposure to the microenvironment. Moreover, the addition of xylooligosaccharides and EWP mixture enhanced the stability in FI by changing the microenvironment of myosin, which specifies the presence of Trp residues in a more hydrophilic environment (Zhang et al., 2021). Walayat, Xiong, et al. (2021) examined that the addition of EWP/βCD in silver cap MP increased the FI by preventing the changes in indole side chains of Trp residues during frozen storage for 60 days. Zhang et al. (2021) also reported that the addition of xylooligosaccharides inhibited the decline in FI by substituting the water molecules around the surface of myosin molecules, hence stabilizing the myosin during freeze thaw cycles. Our results are consistent with results of UV-absorption spectra (Fig. 4), which showed the addition of KOG (3%) significantly reduced the tertiary structural changes induced during fluctuated frozen storage.

4. Conclusion

In this study, a significant decline was observed in the functional and structural attributes of silver carp surimi during fluctuated frozen storage. The fluctuated frozen storage showed a substantial decline in the water binding and textural properties of silver carp surimi gel. Furthermore, the secondary and tertiary structural properties of MP were reduced as a result of the changes in the myosin rod portion and aromatic amino acids. Meanwhile, the effective role of KOG as a potential cryoprotectant was analyzed in comparison with a conventional

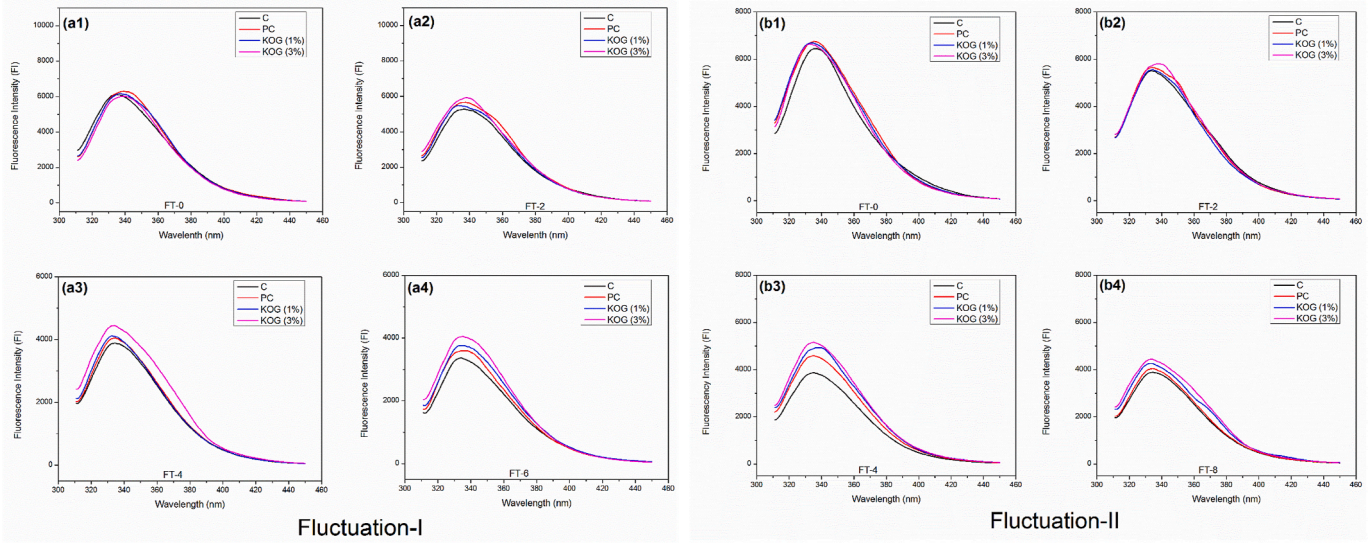

Fig. 5. Fluorescence intensity of MP samples treated with PC, KOG (1%) and KOG (3%) during different fluctuated frozen storage.

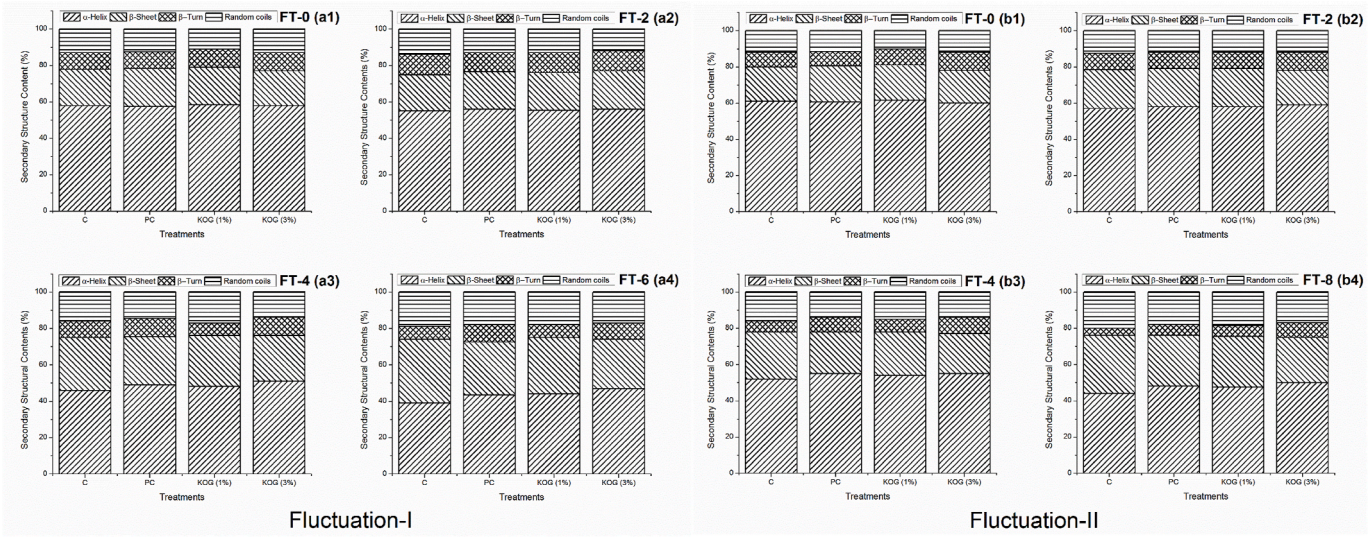

Fig. 6. Secondary structural properties of MP samples treated with PC, KOG (1%) and KOG (3%) during different fluctuated frozen storage.

cryoprotectant mixture (sucrose and sorbitol) in the silver carp surimi. KOG incorporated samples showed better stability in aromatic amino acids and α-helix content owing to their hydrophilic abilities, resulting in stable structural properties. Moreover, the addition of KOG (3%) improved the gelling abilities by inhibiting protein denaturation and aggregation. Furthermore, samples containing 1% KOG demonstrated lower cryoprotective abilities than PC in some indices. It could be due to a lack of sufficient concentration of KOG. Overall, it was determined that KOG (3%) effectively improved the functional and structural properties of silver carp surimi proteins during FLUC-I and FLUC-II. Therefore, it can be established from the existing findings that the KOG could be used as a potential alternative to the conventional cryoprotectants (sucrose + sorbitol) mixture for improved commercial and economic values of silver carp surimi.

CRediT authorship contribution statement

Noman Walayat: Formal analysis, writing of initial manuscript and review the final draft. **Wei Tang:** Formal analysis. **Asad Nawaz:** Formal analysis, Review the final draft. **Yuting Ding:** contributed to review the final draft. **Jianhua Liu:** Conceptualization, Review the final draft, Supervision. **Jose Manuel Lorenzo:** Conceptualization, review the final draft.

Declaration of competing interest

The authors declare no conflict of interest.

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