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Kappa-carrageenan as an effective cryoprotectant on water mobility and functional properties of grass carp myofibrillar protein gel during frozen storage

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ABSTRACT

The effect of kappa-carrageenan oligosaccharides was analyzed on the gelling abilities of grass carp myofibrillar proteins (MP) during 60 days frozen storage. Four gel treatments were prepared: control without additives (C), a positive control with sucrose: sorbitol (PC), and two batches with 1 and 2% of kappa-carrageenan (KC). All MP gels showed a significant decline in the functional and structural properties. Water holding capacity (WHC) and textural properties of control MP gel was significantly reduced due to oxidative changes, whereas weak cross-linking between amino acid molecules and denaturation of myosin observed. In contrast, PC and KC showed a significant control on the reduction of functional properties due to the enhanced inter/intra-molecular interactions and less formation of ice crystals. Addition of KC (2%) significantly restricted the decline in WHC from 95.45 to 75.47%, which is well associated with high hydrogen proton density. Besides that, low field nuclear magnetic resonance (LF-NMR) analysis exhibited a restricted increase in T_{22} relaxation time in samples added with KC (2%). Overall, MP gel with KC (2%) proved to be an effective alternative in comparison with PC and could be efficient in the production and commercialization of fish and other seafood products.

1. Introduction

Grass carp (*Ctenopharyngodon idella*) is a common freshwater species in China because of its high growth and reasonable price. It is easily available in the surimi processed form, which is the key processing method (Jiang & Wu, 2018; Walayat, Wang, et al., 2021). Myofibrillar proteins (MP) are the important constituent of surimi proteins, which generally comprised of 70–80% of total surimi proteins (Zhang, Fang, dong, juan, & Zhang, 2018). Moreover, MP is the major protein responsible for all the changes in surimi and related products during preservation and processing (Walayat et al., 2020b). Frozen preservation at low temperatures is a commonly used technique for long-term storage of surimi and related products. However, functional properties of surimi may also be reduced during frozen storage due to MP denaturation, such as water holding capacity (WHC), textural properties and gel forming attributes (Lu, Zhang, Li, & Luo, 2017).

Cryoprotectants are typically added in surimi to avoid or to reduce protein denaturation during frozen storage. In the surimi industry, the

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combination of sucrose and sorbitol is commonly used as a cryoprotective mixture (Walayat et al., 2020). Although, this commercial mixture has an outstanding cryoprotective impact on protein denaturation, its higher sweetness and caloric content make this mixture unfavorable for commercial use and unfit for people with diabetics and obesity (Jia et al., 2018). Other cryoprotectants, such as starch, protein hydrolysates, oligosaccharides and polyalcohol have been widely reported in surimi and seafood products during frozen storage, which are not only less in sweetness and caloric values but also have better cryoprotective properties (Walayat, Xiong, Xiong, Moreno, Nawaz, et al., 2020; Zhang, Li, Hong, & Luo, 2020). Carrageenan is an extracted product of red seaweed through enzymatic hydrolysis and is commonly used in the pharmaceutical and food industries. In addition, carrageenan oligosaccharides have been widely renowned as a key ingredient for its role in biological processes such as oxidation, fertilization, immune defense, cell growth, inflammation and cell-cell adhesion (Zhang et al., 2018).

Moreover, carrageenan oligosaccharides have also been reported as effective antioxidants in a cell and *in vitro* (Zhang, Yan, Su, & Chen, 2020). Sun et al. (2015) reported that the antioxidant ability of carrageenan oligosaccharides was remarkably affected by the depolymerization of k-carrageenan, which corresponds to the reduced sugar content. The cryoprotective properties of carrageenan oligosaccharides and other saccharides, such as alginate and trehalose in seafood have been studied (Li, Wu, Chen, & Wu, 2020; Zhang, Yao, Qi, & Ying, 2020). The results demonstrated that carrageenan oligosaccharide inhibited the decline in water holding capacity (WHC), textural and gelling attributes. It is also revealed that the restricted decline in all characteristics could be due to the interaction of proteins with carrageenan oligosaccharide and substitution of the water molecules by hydrogen bonds around the protein surface, thereby resulting in the enhanced MP frozen storage stability (Zhang et al., 2018).

The objective of this research was to analyze the effect of carrageenan oligosaccharides on the gelling abilities of grass carp MP through WHC, textural, rheological analyses and low field nuclear magnetic resonance (LF-NMR), which has not been reported before. Moreover, a comparative study was also performed with a positive control of sucrose and sorbitol. In addition, this research will provide useful evidence regarding the use of oligosaccharides in fish and seafood products for prolonged frozen storage.

2. Material and methods

2.1. Materials

The grass carp (*Ctenopharyngodon idella*) fish (n = 15, weight = 2.5 kg) was purchased from the local market of Huazhong Agricultural University, Wuhan, China. The deceased fish was brought to the College of Food Science and Technology, Huazhong Agricultural University, Wuhan, China. The muscles were separated from the bones and surimi was prepared and further used for MP extraction. k-carrageenan [$C_6H_9O_8SNa$]n was acquired from Seebio Biotech (Shanghai) Co., Ltd., Shanghai, China. All the reagents were of analytical grade.

2.2. Preparation of myofibrillar protein (MP) gel

2.2.1. Extraction of myofibrillar proteins

MP was extracted following the method proposed by Walayat, Wang, et al. (2021). Minced sample (5 g) was homogenized (8000 rpm) with 10 folds of buffer solution (0.05 mmol/L KCl, 20 mmol/L Tris-maleate, pH:7.0). The resulting mixture was centrifuged 10,000×*g* at 4 °C for 15 min. After that, the supernatant was collected, homogenized and centrifuged twice with the same buffer. The obtained sediment was homogenized with the same cold buffer (10 folds) and then centrifuged at 6000×*g* at 4 °C for 15 min. The obtained supernatant was collected as MP. The MP concentration was 81 mg/mL determined through bovine serum albumin as standard.

2.2.2. Preparation of myofibrillar proteins samples

Four myofibrillar proteins were prepared: MP with no additive was referred as control (C), MP samples with positive control (PC) of sucrose (4%) and sorbitol (4%) (1:1), two concentrations (1 and 2%) of k-carrageenan (KC) based on the powder form were added in the MP and mixed properly. All samples were stored at −18 °C and all analyses were performed on 0, 15, 30, 15 and 60 days.

2.2.3. Preparation of MP gel

MP gel was made according to the method of Walayat et al., 2021d. The MP (60 mg/mL) was dissolved into the PBS buffer solution (pH = 7.2). All MP samples were formed in glass beakers (12 mL) and heated in a water bath at 40 °C (20 min) and then transferred to 90 °C (30 min). After that, MP gel samples were placed on ice for 5 min and stored at refrigerated temperature for further use.

2.3. Characterization of MP gel

2.3.1. Water holding capacity

The WHC was determined by thawing loss method by following the protocol of Li, Sun, Ma, Cai, and Li (2019). Samples (5 g) were centrifuged at 5000×*g* at 4 °C for 10 min. WHC (%) was determined as the weight of gel before and after centrifugation, multiplied by 100. All the samples were taken and analyzed in triplicates.

2.3.2. Textural profile properties

The MP gel textural properties were determined using a textural profile analyzer (TA.XT2 plus texture analyzer, Stable Micro System, London, UK). The MP gel samples were made in glass vials and all the parameters (hardness, springiness gumminess and cohesiveness) were determined using the cylindrical probe (flat surface, P/0.5, 12 mm diameter). The displacement and cross head speed were 10 mm and 120 mm/min, respectively. All MP gel samples were prepared and analyzed in triplicates.

2.3.3. Rheological properties

The dynamic rheological properties were determined by DHR rotational Rheometer (TA instruments, Crawley, UK) according to Chen et al. (2018). Samples were loaded on the rheometer and 40 mm probe was lowered to a distance of 1 mm. Stainless steel temperature controller and silicon oil was applied to prevent sample evaporation. The sample was heated from 25 to 90 °C with 0.1 Hz, 1% frequency and strain, respectively. All the dynamic rheological properties were expressed with obtained storage modulus (G') and loss modulus (G").

2.3.4. Low field nuclear magnetic resonance (LFMR)

The LF-NMR T_2 relaxation time of all MP gel samples in glass tubes (d:11, H:30 mm) was measured according to the method of Zhang, Yao, et al. (2020). The parameters applied in the LFNMR (Niumag Electric Company, Shanghai, China) were proton resonance frequency (23.2 Hz), magnetic field strength (0.5 T), Pre-amplifier gain (PGR) = 1, echo number (NECH) = 10,000, Echo time (1 ms), sampling interval time (TW) = 5000 ms and cumulative number (NS) = 4.

2.3.5. Proton density images

The magnetic resonance imaging (MRI) of all MP gel samples was analyzed according to the protocol described by Zhang, Yao, et al. (2020). The MP gel positive control and k-carrageenan samples were determined by LF-NMR. All samples were divided into three layers, being each layer of 1.0 mm in height and with a gap of 0.5 mm. After that, color scale was used to configure the distribution of water molecules in the MP gel samples.

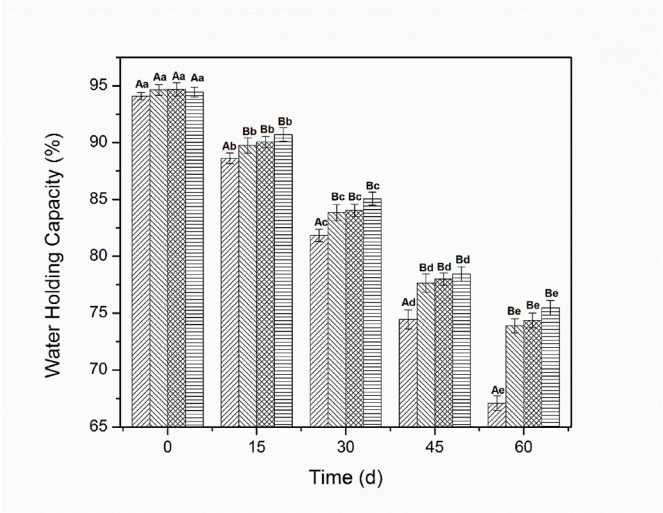

Fig. 1. Effect of kappa-carrageenan on the water holding properties of grass carp MP gels during frozen storage. Where box, represent C PC 1% 2%. Error bars denote the standard deviation (SD) determined from the three values. Letters (a–e) represent the significance difference (P < 0.05) in treatments within the individual treatment at different frozen storage times. Letters (A–D) represent the different treatments at similar times.

2.4. Statistical analysis

Statistical analyses were performed using SPSS (IBM SPSS Statistics, IBM Corp, NY, USA). Normal distribution and homogeneity of variance were previously tested (Shapiro-Wilk). The data were submitted to one-way ANOVA, followed by Duncan's test when the ANOVA was significant at $P < 0.05$. OriginPro 8.5.1 (OriginLab Inc., USA) was used to generate all figures. All samples were analyzed repeatedly and stated in mean ± SD.

3. Results and discussion

3.1. Water holding capacity of MP gel

Water holding capacity (WHC) is the capacity of meat to hold moisture, which is mainly associated with the degree of protein denaturation and proteolysis in fish muscles. The WHC of MP gel treated with sucrose and sorbitol and kappa-carrageenan oligosaccharide is shown in Fig. 1. The WHC of all MP gel samples was significantly ($P < 0.05$) reduced during frozen storage. Initially, no significant changes ($P > 0.05$) were noted, which started to be prominent from the day 15 of frozen storage. MP gel without additives recorded a massive decline in WHC from day 0 (94.08%) to day 60 (67.1%). Meanwhile, PC and MP samples with the carrageenan oligosaccharide (1 and 2%) showed a smaller decrease compared to C samples. Interestingly, PC gel displayed a decreasing trend similar to MP gel with 1 and 2% of carrageenan oligosaccharides. PC and KC (1%) noted a similar decline from day 15 to days 30 (94.63–83.84%) and (94.85–84.03%), respectively. Meanwhile, a slight difference was observed from day 45–60. In contrast, MP gel with KC (2%) also significantly reduced WHC from 94.45 to 75.47% after day 60 of frozen storage, but showed better WHC properties than C, PC and 1% MP gel samples. WHC decline could be due to the alteration of the rigidity of MP structure, which occurs due to the denaturation of protein molecules and the tissue fibers damaged mainly associated with the formation of ice crystals during frozen storage (Nyaisaba et al., 2019). Moreover, it is established that KC also showed cryoprotective properties on frozen MP gel. KC could act as a nucleation inhibitor by

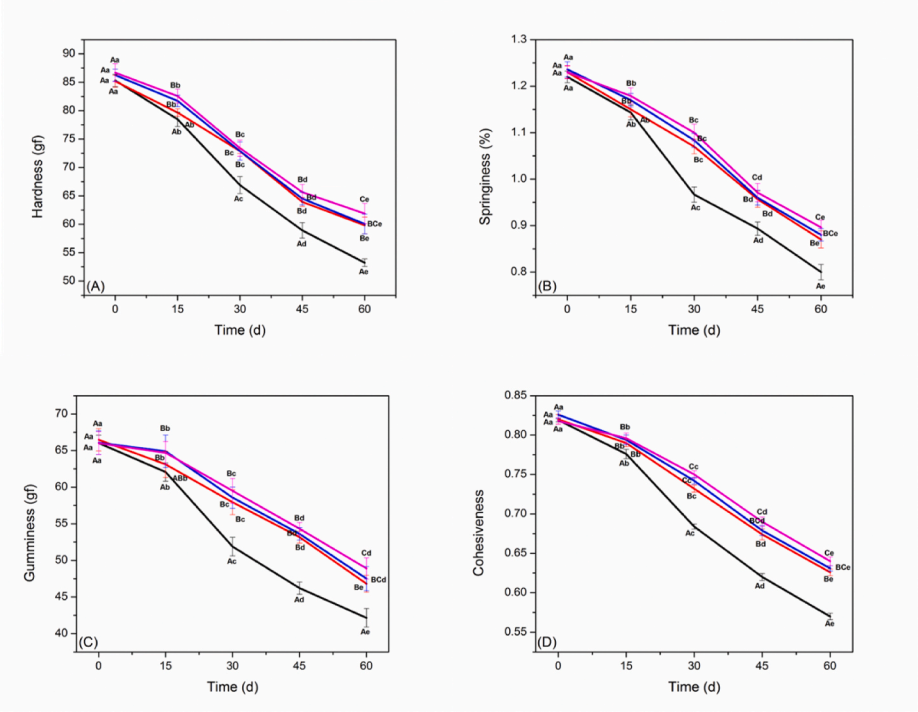

Fig. 2. Effect of kappa-carrageenan on the textural properties of grass carp MP gels during frozen storage. Where lines, represent C PC 1% 2%. Error bars denote the standard deviation (SD) determined from the three values. Letters (a–e) represent the significance difference (P < 0.05) in treatments within the individual treatment at different frozen storage times. Letters (A–D) represent the different treatments at similar times.

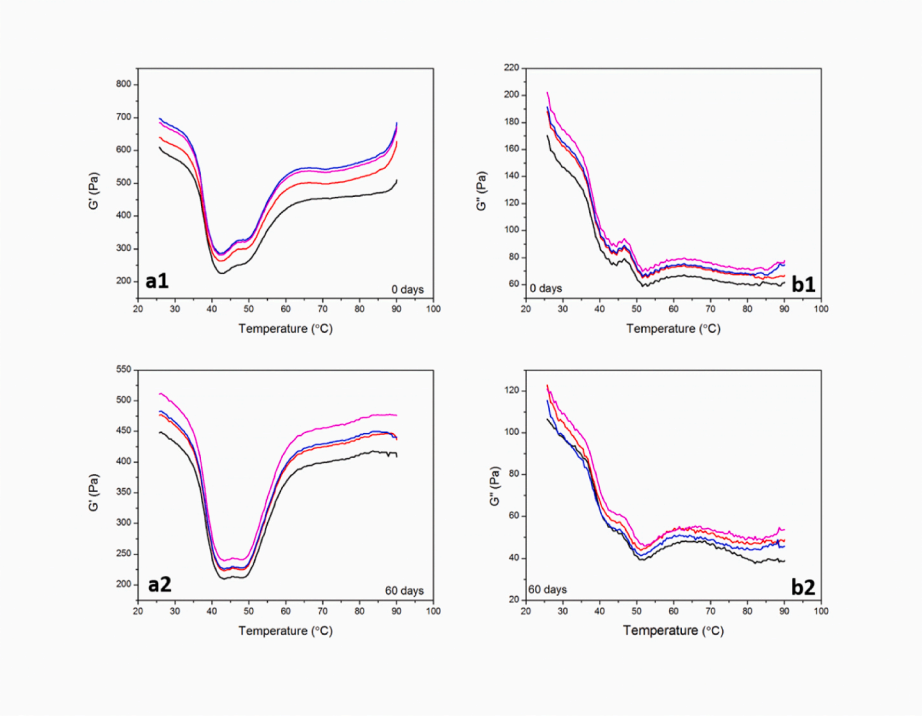

Fig. 3. Changes of storage modulus (a1 and a2) and loss modulus (b1 and b2) of MP gels during frozen storage. Where lines, represent. C PC 1% 2%

interacting with ice surfaces of water molecules (Zhang et al., 2018). Addition of cryoprotectants could result in slight formation of ice crystals due to possible interaction with free water molecules (Zhang, Yao, et al., 2020). The use of polysaccharides as cryoprotectant increased the water binding abilities of MP by reducing the denaturation of protein molecules (Gao, Kang, Zhang, Li, & Zhou, 2015). Walayat et al. (2021d) also reported the addition of egg white protein (EWP) and β-cyclodextrin (βCD) (EWP/βCD) as a potential cryoprotective mixture, which significantly increased the WHC of MP gel during 60 days of frozen storage. Zheng, Beamer, Matak, and Jaczynski (2019) reported that the addition of KC enhanced the WHC of shrimp protein during frozen storage. Thus, our finding suggested that KC (2%) significantly increased the WHC properties of grass carp MP.

3.2. Textural properties of MP gel

Textural attributes are very important to determine the quality characteristics of fish and seafood-based gel products due to their relevance with other functional parameters, such as WHC and sensory properties (Cropotova, Mozuraityte, Standal, Grøvlen, & Rustad, 2019). The textural profile (hardness, springiness, gumminess and cohesiveness) of MP gels is shown in Fig. 2. During the study, a significant ($P < 0.05$) decline was observed in all parameters studied. The MP gel samples without any additive showed a remarkable drop in textural attributes. Meanwhile, the decrease in texture properties was significantly ($P < 0.05$) controlled in MP gel samples with PC and KC (1 and 2%). PC displayed a lower decrease in hardness (85.18–59.81 gf), springiness (1.23–0.87%), gumminess (66.47–46.81 gf) and cohesiveness (0.82–0.61) than the control MP gel sample (C). It is interesting to highlight that the drop in hardness for PC was similar to that observed in KC (1%) from 30 days onwards (Fig. 2a), which narrowed at day 60 of analysis. Besides that, a similar decrease was also observed in springiness, gumminess and cohesiveness (Fig. 2b, c, d). The decreased textural properties could be due to protein denaturation and poorly formed gel matrix, which resulted in reduced WHC with increased frozen storage time (Campo-Deaño, Tovar, Borderías, & Fernández-Martín, 2011). Likewise, a substantial decrease ($P < 0.05$) during storage was also observed in KC (2%), which was more controlled and restricted compared to C, PC and KC (1%) samples. However, no significant differences were obtained between KC (2%) and the other MP treated gels at day 30 frozen storage. Moreover, a slight decrease in springiness and gumminess was analyzed in MP gel treated KC (1 and 2%) and PC treated MP gel after 45 days of storage.

The decline in MP gel textural properties has a major impact on the functional properties like WHC. The decline in these properties can be reduced with the addition of cryoprotectants (Jenkelunas & Li-Chan, 2018; Walayat et al., 2021). Li, Kong, Xia, Liu, and Li (2013) studied that the cryoprotectants prevented the oxidation-induced changes in proteins. The addition of Egg white protein (EWP) and β-cyclodextrin (EWP/βCD) as cryoprotectants effectively restricted the decline in both WHC and textural properties by reducing the changes induced by freezing (Walayat, Xiong, Xiong, Moreno, Li, et al., 2020). Wu, Che, and Chen (2014) also reported that the addition of tea polyphenols reduced the textural properties of MP gel. Kong et al. (2013) demonstrated that the addition of sucrose and sorbitol as an efficient cryoprotectant mixture delayed the change in textural properties by maintaining the functional characteristics and three-dimensional network of surimi gel during freeze thaw cycles.

Overall, KC (2%) exhibited better textural profile characteristics due to its better hydrogen and hydrophilic properties, which prevent the availability of free water molecules for further protein-protein and protein-water interactions. Therefore, it can also be assumed from this study that KC could also be an effective alternative to the traditional cryoprotectants.

3.3. Rheological properties of MP gel

The rheological properties are important to investigate the conversion of MP to MP gel matrix. During the gelation, characterization and formation of MP gel can be evaluated on the basis of the change in

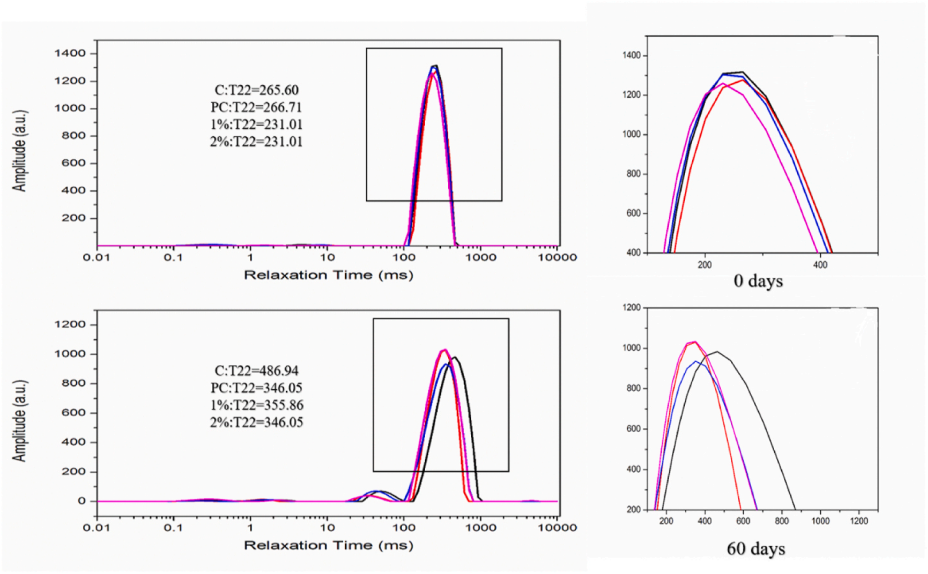

Fig. 4. Changes of LF-NMR T_{22} relaxation time of MP gels during frozen storage, Where lines, represent.

storage and loss modulus (G' and G"). Generally, G' (Pa) indicates the energy used by the MP to transform into a solid. Meanwhile, G" (Pa) represents the viscous portion or dissipated heat (Zhang, Xue, Li, Wang, & Xue, 2015).

The G' (Pa) and G" (Pa) of MP gel with PC and KC (1 and 2%) are shown in Fig. 3. During this cryoprotective study, G' (Pa) and G" (Pa) of all MP gel samples decreased during 60 days frozen storage, which indicates the protein denaturation caused by oxidation (Zhang, Yao, et al., 2020). In addition, a different pattern in MP gelation was found during rheological analysis, which could be described in the following ways: (1) slightly decline from 25 °C to 35 °C, (2) sharp drop from 35 °C to 44 °C indicating gel weakening, (3) further rise in G' (Pa) from 44 °C to 70 °C and, (4) another increase after 70 °C. The MP gel with KC (2%) exhibited improved G' (Pa) than C, PC and KC (1%) gels after 60 days of frozen storage, which was consistent with WHC (Fig. 1) and textural profile attributes (Fig. 2). Surprisingly, PC treated and KC (1%) showed no prominent difference between G' (Pa) and G" (Pa). The decline from 43 °C and 47 °C could be due to the breakdown of protein network caused by protease activity, and the later increase after 55 °C could be related to the formation of an irreversible gel network due to proper cross-linking amino acid side chains. Therefore, the incorporation of KC can be effective in improving the gelling properties of MP gel during freezing.

Chen, Diao, Li, Chen, and Kong (2016) reported that the decline in G' (Pa) at the starting of the heating process could be due to myosin denaturation, dissociated actin and protease activity. Meanwhile, the increase of temperature could also result in the rise of G' (Pa), which indicates the development of a well-established gel network due to interaction and association of protein side chain molecules. Moreover, changes in gelling behavior can also be analyzed by G" (Pa), which showed a similar trend to the G' (Pa) as shown in Fig. 3. The major changes in G" (Pa) were recorded above 50 °C, which represent the continuous cross-linking of amino acid side chains responsible for the proper MP gel network. Moreover, Zhang, Yao, et al. (2020) examined that the oxidative changes resulted in protein unfolding and aggregates, thereby inhibiting protein expansion during heating.

Lu et al. (2017) reported that the addition of cryoprotectants enhanced the viscoelastic properties by improving the three-dimensional network of MP gel. Moreover, a well-established gel network would lead to better WHC properties. Walayat, Xiong, Xiong, Moreno, Niaz, et al. (2020) reported in his study that the addition of EWP and xylooligosaccharides effectively improved the viscoelastic properties of MP during 60 days of frozen storage. The addition of EWP/βCD significantly enhanced the viscoelastic properties of silver carp MP during frozen storage by improving the intermolecular bonding interactions (Walayat et al., 2021d). Thus, it can be suggested from the current results that the addition of KC can also be an effective alternative to traditional cryoprotectants to improve the viscoelastic properties of MP during frozen storage.

3.4. LF-NMR of MP gel

The distribution of water molecules is a key parameter to determine the functional and quality characteristics of food during frozen storage. LF-NMR is the most sophisticated technique, which can be used to analyze the distribution of water. LF-NMR analysis of MP gel treated with different PC and KC (1 and 2%) is shown in Fig. 4. LF-NMR analysis is generally categorized into three peaks, stated as 0–10 ms (T_{2b}), 10–100 ms (T_{21}) and 100–1000 ms (T_{22}). Peak 1 (T_{2b}) denotes bound water joined with hydrophilic groups, peak 2 (T_{21}) immobilized water present in the MP network, and peak 3 (T_{22}) free water present between the fiber bundles (Sánchez-Alonso, Martinez, Sánchez-Valencia, & Careche, 2012).

At the beginning of frozen storage, no significant differences were observed between MP gel samples, probably because there is not enough time to change the water distribution. In contrast, at the end of storage all MP gel samples exhibited a prominent change in T_{22} relaxation time, which was lower in the MP gel samples with PC and KC (1 and 2%). Therefore, the addition of cryoprotectants in MP gel reduced the T_{22} relaxation time compared to the control sample (C).

The increase in relaxation time T_{22} observed in MP gel without any additives during storage means the increase in the water mobility, which corresponds to the reduction of WHC. These results are well associated with the decline of WHC (Fig. 1.) and textural properties (Fig. 2.). From the results, PC and KC(2%) had same increase in T_{22} after 60 days, which indicates that the reduction of functional properties is due to protein denaturation, resulting in the decrease of inter/intra molecular interactions. Meanwhile, MP gel with KC (2%) significantly reduced the changes in T_{22} relaxation time during frozen storage. The lesser increase in water mobility could be due to proper cross-linking of amino acids and inhibited denaturation of myosin. Zhang, Yao, et al. (2020) reported that the addition of xylitol and mannitol addition increased the T_{21} and T_{22} relaxation time of shrimp protein during frozen storage. Wang, Zhang, Bhandari, and Gao (2016) suggested that the addition of

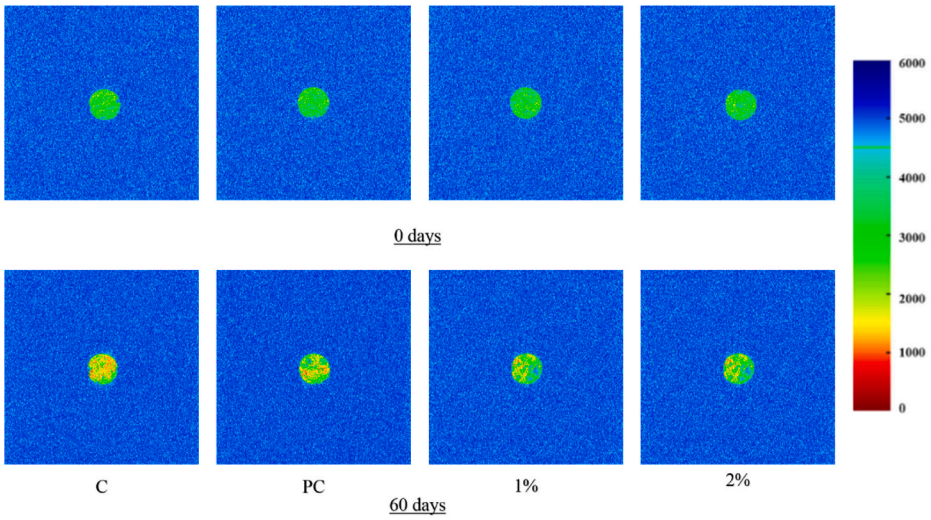

Fig. 5. Changes of proton density image of MP gels during frozen storage.

malondialdehyde to MP gel significantly increased the T_{22} relaxation time, which indicated more protein-water interaction and less availability for oxidative changes. Zhang, Yao, et al. (2020) also reported that the oxidative changes in the MP could also affect the water mobility in protein molecules due to myosin denaturation. Therefore, it can be recommended from the current results that the addition of KC could reduce the change in MP water activity corresponding to the oxidative changes, resulting in weaker gel matrix and cross-linking of protein molecules.

3.5. Proton density map of MP gel

The proton density analysis indicates the water mobility in the MP gel samples. The proton density images of MP gel samples with PC and KC during frozen storage are shown in Fig. 5. In the images, the brighter color exhibited the high density of hydrogen protons in the MP gel samples, easily accessed from the pseudo color images (Fig. 5). The signal intensity (dark or blue areas) specified relatively free water, while the signal intensity (bright or red) reflected immobile or bound water associated with macromolecules.

At the beginning of frozen storage, all MP gel samples showed similar color pattern, which indicates a low water mobility. Meanwhile, after 60 days of storage, control MP gel displayed a significant change in color, which is generally associated with the reduction of WHC (Fig. 1), textural properties (Fig. 2) and increased water mobility (Fig. 4). The MP gel with PC and KC (1%) displayed a similar increase of hydrogen protons to that observed in MP gel with KC (2%), which showed a more restricted increase in water mobility during frozen storage (As analyzed from the proton density scale). Besides that, the MP gel samples with PC and KC exhibited the slightest change in color, indicating the low hydrogen proton density, which proves the cryoprotective effect of KC on MP gelling properties during frozen storage. In this regard, Zhang, Yao, et al. (2020) stated that oxidation could change the structural and functional attributes of MP, thereby affecting the WHC, textural attributes and gel formation.

4. Conclusion

It can be concluded from the current results that the addition of KC improved the functional and gelling abilities of grass carp MP gel during frozen storage of 60 days, which is the main reason for protein oxidation and denaturation. During the frozen storage, KC effectively controlled the water mobility in the protein molecules. Moreover, KC had a better cryoprotective effect than the conventional mixture of sucrose and sorbitol. In addition, KC enhanced the viscoelastic properties of MP gel by prompting the proper cross-linking of protein molecules. Thus, KC has the potential to be used in the fish and aquatic food based industries. Moreover, this study also proved that KC could be used as an effective alternative to other commercially available cryoprotectants higher in sweetness and caloric values. This study might also be used for the extended application of KC in fish and other seafood commercialization.

CRediT authorship contribution statement

Noman Walayat: Writing – original draft. **Xiukang Wang:** Data curation. **Jianhua Liu:** Supervision, Investigation. **Asad Nawaz:** Conceptualization. **Zhongli Zhang:** Methodology. **Ibrahim Khalifa:** Visualization. **Miguel Ángel Rincón Cervera:** Investigation. **Mirian Pateiro:** Writing – review & editing. **José M. Lorenzo:** Writing – review & editing. **Mehdi Nikoo:** Validation. **Shahida Anusha Siddiqui:** Conceptualization.

Declaration of competing interest

Authors declare no conflict of interest.

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References

Campo-Deaño, L., Tovar, C. A., Borderías, J., & Fernández-Martín, F. (2011). Gelation process in two different squid (Dosidicus gigas) surimis throughout frozen storage as affected by several cryoprotectants: Thermal, mechanical and dynamic rheological properties. *Journal of Food Engineering, 107*(1), 107–116.

Chen, H., Diao, J., Li, Y., Chen, Q., & Kong, B. (2016). The effectiveness of clove extracts in the inhibition of hydroxyl radical oxidation-induced structural and rheological changes in porcine myofibrillar protein. *Meat Science, 111*, 60–66. https://doi.org/10.1016/j.meatsci.2015.08.017

Chen, X., Xu, X., Liu, D., Zhou, G., Han, M., & Wang, P. (2018). Rheological behavior, conformational changes and interactions of water-soluble myofibrillar protein during heating. *Food Hydrocolloids, 77*, 524–533. https://doi.org/10.1016/j.foodhyd.2017.10.030

Cropotova, J., Mozuraityte, R., Standal, I. B., Grøvlen, M. S., & Rustad, T. (2019). Superchilled, chilled and frozen storage of Atlantic mackerel (*Scomber scombrus*) fillets – changes in texture, drip loss, protein solubility and oxidation. *International*

Journal of Food Science and Technology, 54(6), 2228–2235. https://doi.org/10.1111/ijfs.14136

Jenkelunas, P. J., & Li-Chan, E. C. Y. (2018). Production and assessment of Pacific hake (*Merluccius productus*) hydrolysates as cryoprotectants for frozen fish mince. *Food Chemistry, 239*, 535–543. https://doi.org/10.1016/j.foodchem.2017.06.148

Jia, R., Eguchi, M., Ding, W., Nakazawa, N., Osako, K., & Okazaki, E. (2018). Quality changes of commercial surimi-based products after frozen storage. *Transactions of the Japan Society of Refrigerating and Air Conditioning Engineers, 35*(3), 205–210. https://doi.org/10.11322/tjsrae.18-15FB_OA

Jiang, L., & Wu, S. (2018). Pullulan suppresses the denaturation of myofibrillar protein of grass carp (*Ctenopharyngodon idella*) during frozen storage. *International Journal of Biological Macromolecules, 112*, 1171–1174. https://doi.org/10.1016/j.ijbiomac.2018.02.094

Kong, B., Guo, Y., Xia, X., Liu, Q., Li, Y., & Chen, H. (2013). Cryoprotectants reduce protein oxidation and structure deterioration induced by freeze-thaw cycles in common carp (*Cyprinus carpio*) surimi. *Food Biophysics, 8*(2), 104–111. https://doi.org/10.1007/s11483-012-9281-0

Li, Y., Kong, B., Xia, X., Liu, Q., & Li, P. (2013). Inhibition of frozen storage-induced oxidation and structural changes in myofibril of common carp (*Cyprinus carpio*) surimi by cryoprotectant and hydrolysed whey protein addition. *International Journal of Food Science and Technology, 48*(9), 1916–1923. https://doi.org/10.1111/ijfs.12171

Li, X., Sun, P., Ma, Y., Cai, L., & Li, J. (2019). Effect of ultrasonic thawing on the water-holding capacity, physicochemical properties and structure of frozen tuna (*Thunnus tonggol*) myofibrillar proteins. *Journal of the Science of Food and Agriculture, 99*(11), 5083–5091. https://doi.org/10.1002/jsfa.9752

Li, M., Wu, J., Chen, Z., & Wu, T. (2020). Improving the solubility of myofibrillar proteins (MPs) by mixing with sodium alginate: Effects of pH, mixing ratios and preheating of MPs. *Food Biophysics, 15*(1), 113–121. https://doi.org/10.1007/s11483-019-09605-y

Lu, H., Zhang, L., Li, Q., & Luo, Y. (2017). Comparison of gel properties and biochemical characteristics of myofibrillar protein from bighead carp (*Aristichthys nobilis*) affected by frozen storage and a hydroxyl radical-generation oxidizing system. *Food Chemistry, 223*, 96–103. https://doi.org/10.1016/j.foodchem.2016.11.143

Nyaisaba, B. M., Liu, X., Zhu, S., Fan, X., Sun, L., Hatab, S., … Deng, S. (2019). Effect of hydroxyl-radical on the biochemical properties and structure of myofibrillar protein from Alaska pollock (*Theragra chalcogramma*). *LWT, 106*, 15–21. https://doi.org/10.1016/j.lwt.2019.02.045

Sánchez-Alonso, I., Martinez, I., Sánchez-Valencia, J., & Careche, M. (2012). Estimation of freezing storage time and quality changes in hake (*Merluccius merluccius, L.*) by low field NMR. *Food Chemistry, 135*(3), 1626–1634. https://doi.org/10.1016/j.foodchem.2012.06.038

Sun, Y., Yang, B., Wu, Y., Liu, Y., Gu, X., Zhang, H., … Wang, Z. (2015). Structural characterization and antioxidant activities of κ-carrageenan oligosaccharides degraded by different methods. *Food Chemistry, 178*, 311–318. https://doi.org/10.1016/j.foodchem.2015.01.105

Walayat, N., Rincón, M.Á., Niaz, S., Nawaz, A., Niaz, N., Zahid Farooq, M., … Zhang, Z. (2021b). Egg white proteins and β-cyclodextrin: Effective cryoprotectant mixture against oxidative changes in the myofibrillar proteins of *Culter alburnus*. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.15021. ijfs.15021.

Walayat, N., Wang, X., Nawaz, A., Zhang, Z., Abdullah, A., Khalifa, I., … Lorenzo, J. M. (2021a). Ovalbumin and kappa-carrageenan mixture suppresses the oxidative and structural changes in the myofibrillar proteins of grass carp (*Ctenopharyngodon idella*) during frozen storage. *Antioxidants, 10*(8), 1186.

Walayat, N., Xiong, Z., Xiong, H., Moreno, H. M., Li, Q., Nawaz, A., … Niaz, N. (2020e). The effectiveness of egg white protein and β-cyclodextrin during frozen storage: Functional, rheological and structural changes in the myofibrillar proteins of *Culter alburnus*. *Food Hydrocolloids, 105*, 105842.

Walayat, N., Xiong, Z., Xiong, H., Moreno, H. M., Nawaz, A., Niaz, N., … Khalifa, I. (2021d). The effect of egg white protein and β-cyclodextrin mixture on structural and functional properties of silver carp myofibrillar proteins during frozen storage. *LWT, 135*, 109975. https://doi.org/10.1016/j.lwt.2020.109975

Walayat, N., Xiong, H., Xiong, Z., Moreno, H. M., Nawaz, A., Niaz, N., et al. (2020c). Role of cryoprotectants in surimi and factors affecting surimi gel properties: A review. *Food Reviews International*, 1–20.

Walayat, N., Xiong, Z., Xiong, H., Moreno, H. M., Niaz, N., Ahmad, M. N., … Wang, P. K. (2020f). Cryoprotective effect of egg white proteins and xylooligosaccharides mixture on oxidative and structural changes in myofibrillar proteins of *Culter alburnus* during frozen storage. *International Journal of Biological Macromolecules, 158*, 865–874. https://doi.org/10.1016/j.ijbiomac.2020.04.093

Wang, L., Zhang, M., Bhandari, B., & Gao, Z. (2016). Effects of malondialdehyde-induced protein modification on water functionality and physicochemical state of fish myofibrillar protein gel. *Food Research International, 86*, 131–139.

Wu, L., Che, L., & Chen, X. D. (2014). Antiretrogradation in cooked starch-based product application of tea polyphenols. *Journal of Food Science, 79*(10), E1984–C1990.

Zhang, B., Fang, C., dong, H., juan, G., & Zhang, Y. yang (2018). Effect of kappa-carrageenan oligosaccharides on myofibrillar protein oxidation in peeled shrimp (*Litopenaeus vannamei*) during long-term frozen storage. *Food Chemistry, 245*, 254–261. https://doi.org/10.1016/j.foodchem.2017.10.112

Zhang, L., Li, Q., Hong, H., & Luo, Y. (2020). Prevention of protein oxidation and enhancement of gel properties of silver carp (*Hypophthalmichthys molitrix*) surimi by addition of protein hydrolysates derived from surimi processing by-products. *Food Chemistry, 316*, 126343. https://doi.org/10.1016/j.foodchem.2020.126343

Zhang, T., Xue, Y., Li, Z., Wang, Y., & Xue, C. (2015). Effects of deacetylation of konjac glucomannan on Alaska Pollock surimi gels subjected to high-temperature (120°C) treatment. *Food Hydrocolloids, 43*, 125–131. https://doi.org/10.1016/j.foodhyd.2014.05.008

Zhang, B., Yan, H. B., Su, L. J., & Chen, X. N. (2020). Kappa-carrageenan oligosaccharides retard the progression of protein and lipid oxidation in mackerel (*Scomber japonicus*) fillets during frozen storage. *RSC Advances, 10*(35), 20827–20836. https://doi.org/10.1039/d0ra03431b

Zhang, B., Yao, H., Qi, H., & Ying, X.-g. (2020). Cryoprotective characteristics of different sugar alcohols on peeled Pacific white shrimp (Litopenaeus vannamei) during frozen storage and their possible mechanisms of action. *International Journal of Food Properties, 23*(1), 95–107.

Zheng, H., Beamer, S. K., Matak, K. E., & Jaczynski, J. (2019). Effect of κ-carrageenan on gelation and gel characteristics of Antarctic krill (*Euphausia superba*) protein isolated with isoelectric solubilization/precipitation. *Food Chemistry, 278*, 644–652. https://doi.org/10.1016/j.foodchem.2018.11.080