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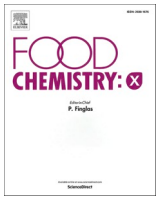

Effect of tea polyphenols on the quality of Mackerel puree (*Scomber scombrus*) during refrigerated storage: Color, oxidative stability and microstructure

Zhifeng Tan [a,b], Xiaoqing Yang [a,b], Zheng Jin [a,b], Lin Han [a,b], Ke Li [c], Sangeeta Prakash [d], Xiuping Dong [a,b,*]

[a] State Key Laboratory of Marine Food Processing and Safety Control, National Engineering Research Center of Seafood, Liaoning Province Collaborative Innovation Center for Marine Food Deep Processing, Dalian Technology Innovation Center for Chinese Prepared Food, Dalian Polytechnic University, Dalian 116034, Liaoning, China

[b] School of Food Science and Technology, Academy of Food Interdisciplinary Science, Dalian Polytechnic University, Dalian 116034, Liaoning, China

[c] Key Laboratory of Cold Chain Food Processing and Safety Control, Ministry of Education, Zhengzhou University of Light Industry, Zhengzhou 450001, PR China

[d] School of Agriculture and Food Sustainability, The University of Queensland, Brisbane, Queensland 4072, Australia

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ABSTRACT

This study investigated the impact of adding tea polyphenols (TP) on the quality of mackerel puree during refrigerated storage. The study used whole mackerel fish and analyzed the sensory characteristics, physicochemical properties, and microstructure of the puree. As storage time increased, significant changes occurred in the puree, including increased levels of thiobarbituric acid value, protein carbonyl, and free radicals. The water holding capacity, whiteness, and pH value of the puree decreased. However, the addition of TP helped to preserve the quality of the mackerel puree, particularly in the later stages of storage. This was evidenced by reduced oxidation of lipids and proteins, minimal color change, and a more compact microstructure. The study also revealed that TP contributes to the development of a gel network within the mackerel puree, enhancing water retention and sensory qualities. These findings suggested that TP treatment has potential for preserving mackerel puree during refrigeration.

1. Introduction

Atlantic mackerel (*Scomber scombrus*) is an economically sustainable fish species in China, mainly found in the Huanghai and Donghai sea regions (Asamoah et al., 2022). Mackerel is a nutrient-rich fish that typically contains both white and dark muscle tissues in its edible parts (Cropotova, Mozuraityte, Standal, & Rustad, 2018). White meat has superior appearance and texture can be consumed directly as a processed product. In contrast, the dark meat is rich in polyunsaturated fatty acids such as DHA and EPA, which can effectively reduce the occurrence of cardiovascular diseases and improve memory (Liu et al., 2023) and is also a valuable raw material for fish oil production. However, due to its dark red color and poor taste, and susceptibility to oxidation during processing, the dark meat is often discarded from processed products, resulting in a lower utilization rate of the whole mackerel and significant nutrient loss.

Mackerel is mainly marketed in direct consumption and processing, with about 30% of mackerel products sold as processed. Refrigerated storage, as one of the most essential storage methods to ensure the quality of the product, prevents irreversible mechanical damage to the muscle tissue caused by the growth of ice crystals during the freezing and thawing process (Wu et al., 2022). However, the deterioration of physicochemical properties in mackerel products during refrigeration limits their use in food processing. Mackerel muscle tissue contains high levels of non-protein nitrogen, fat, and autolytic enzymes. Even after death, fish proteins are broken down by endogenous enzymes along with those from microorganisms. This process not only softens the texture of the fish, affecting its sensory attributes, but also inevitably undergo

* Corresponding author at: State Key Laboratory of Marine Food Processing and Safety Control, National Engineering Research Center of Seafood, Liaoning Province Collaborative Innovation Center for Marine Food Deep Processing, Dalian Technology Innovation Center for Chinese Prepared Food, Dalian Polytechnic University, Dalian 116034, Liaoning, China.
E-mail address: dxiuping@163.com (X. Dong).

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oxidative denaturation by the attack of reactive oxygen species (Cheng, Zhu, & Sun, 2021). The primary reactive oxygen species responsible for oxidative damage to meat proteins is the hydroxyl radical. Hydroxyl radicals are generated through the Fenton reaction, which is catalyzed by iron ions to hydrogen peroxide (H_2O_2) and cytosolic reducing agents. Within the Fenton system, oxidation by hydroxyl radicals leads to modifications in protein side chains, ultimately impacting the functional properties of proteins (Lu, Zhang, Li, & Luo, 2017). Not only that, the bis-allylic carbons in the highly unsaturated fatty acids of mackerel are also susceptible to oxidation to form lipid hydroperoxides, which are further broken down into secondary oxides associated with a number of diseases. Lipid and protein oxidation have interactive effects. Aldehydes produced from lipid oxidation can undergo addition reactions with proteins, impacting the redox stability of proteins. The free radicals generated can also directly contribute to protein oxidation. Additionally, reactive oxygen species resulting from protein oxidation can further promote lipid oxidation (Bayram & Decker, 2023).

Tailored antioxidants incorporated into suitable food packaging materials have the potential to reduce quality degradation resulting from protein and lipid oxidation (Hu, Huyan, Ding, Dong, & Yu, 2020; Taghvaei & Jafari, 2015). Antioxidants are substances that can delay, retard, or prevent oxidation processes. They are widely used due to their applicability and convenience (Halliwell & Gutteridge, 2015). Synthetic antioxidants are often used to extend the shelf-life of aquatic products, but their potential safety risks have prompted an increasing number of researchers to work on the development of natural antioxidants. Natural extracts were found to be effective in slowing down lipid oxidation in aquatic products (Huang, Wang, Cao, Zhou, & Li, 2023). Phenolic compounds exhibit a potent inhibitory effect on MP oxidation through two main mechanisms: chelating metal ions to deactivate non-heme iron pre-oxidation, and scavenging free radicals generated by hydroxyl radicals and iron-mediated Fenton reactions (Estévez & Heinonen, 2010). Tea polyphenols (TP), a general term for polyphenols extracted from tea leaves, appear to have a higher antioxidant potential compared to synthetic antioxidants. Its potent antioxidant activity is mainly attributed to the ability of its phenolic hydroxyl groups and multiple benzene ring structures to provide H-atom donors capable of trapping free radicals and blocking the oxidation process (Qi et al., 2023). Insufficient research has been conducted on the impact of polyphenols on the quality of whole mackerel meat products. Therefore, Addressing the issue of quality oxidation in whole mackerel meat during cold storage and revealing the mechanism of polyphenols in enhancing the quality characteristics of whole mackerel meat is crucial. This will enrich the nutritional value, prolong the shelf-life of processed mackerel products and contribute to the high-quality processing and high-value utilization of mackerel.

In this study, the whole mackerel fish was taken as the research object to study the impact of TP addition on protein and lipid oxidation during refrigerated storage. Sensory changes in mackerel puree during storage were assessed through sensory evaluation. Additionally, protein degradation, free radical content and water distribution of mackerel puree were measured, along with an analysis of its microstructure. Multivariate data analysis methods such as principal component analysis and Pearson correlation analysis were utilized to interpret the intricate findings and establish a connection between quality changes and physicochemical factors. This approach aimed to reveal the mechanism of quality deterioration and provide a theoretical basis for quality control in the storage of frozen aquatic products.

2. Materials and methods

2.1. Materials

The Frozen Atlantic mackerel (*Scomber scombrus*, three mackerel were selected for each set of treatment samples, with an average weight of 911.7 ± 58.2 g) was purchased from local market and stored at −20 °C. TP was extracted from green tea (*Camellia sinensis*) with a purity of 98%. It was obtained as brick red powder with a slightly bitter, purchased from Wanbang Chemical Technology Co., Ltd. (Henan, China). The primary active component in TP was identified as catechins (60% - 80%). Butylated hydroxytoluene (BHT, food grade) was also purchased from Wanbang Chemical Technology Co., Ltd. (Henan, China). All other chemicals were of analytical grade.

2.2. The preparation of Mackerel puree samples

The frozen mackerel were taken from the refrigerated storage and thawed in running water at room temperature (Liu et al., 2023) until the center temperature reaches - 2 °C. The head and tail were removed, and the fish spines were discarded. The white and dark meat portions of the fish were taken separately. The ratio of the two in each group samples (200 ± 0.5 g) was 87: 13. Samples without or with 0.2 g/kg TP and BHT, respectively, were stirred in a meat grinder for 5 min and then chilled at 4 °C for storage. Each group sample was taken at intervals (0, 2, 4, 6 and 8 d) for further measurement.

2.3. Color analysis

The colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) was calibrated with a white plate according to (Li et al., 2022). Then the L^*, a^*, and b^* values of the samples were measured through an 8 mm viewing area. The whiteness and ΔE were calculated as follows:

$$\text{Whiteness} = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2}\right)\Big]^{1/2}$$

$$\Delta E = \left(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}\right)^{1/2}$$

2.4. Sensory analysis

The sensory evaluation method was carried out according to GB/T 16291.1–2012. The ingredients used in this experiment were purchased in the market and can be safely consumed by consumers without ethical concerns. All evaluators have provided a sensory informed consent prior to testing, as detailed in the supplementary material. The sensory evaluators underwent a screening process prior to being selected for sensory ability training. Following evaluations, 30 assessors were chosen to comprise the sensory evaluation group. Each evaluator carried out individual assessments of heat treatment samples that were presented in coded trays. Attributes were rated on a continuous scale ranging from 0 (not perceived) to 10 (maximum perceived intensity). A preference was unacceptable if it is <5 and acceptable if it is equal to or >5.

2.5. Thiobarbituric acid reactive substances (TBARS)

The TBARS measurements of mackerel puree were based on previous research (Grunwald & Richards, 2006). All the samples (0.5 g) were mixed with 10 mL of TBA-TCA reagent. After heating (95 °C, 20 min), the solution was cooled and centrifuged (8000 rpm, 10 min). Absorbance was measured at 532 and 650 nm, and TBARS concentrations were determined using standard curves for tetraethoxypropane preparation.

The TBARS values was calculated as follows:

$$\text{TBARS} = \frac{\text{C} \times 10}{\text{m}}$$

where C was calculated from the standard curve, m was the sample mass (g).

2.6. Carbonyl content

The carbonyl content of mackerel puree samples was determined by a previous study with slight modification (Zhang et al., 2024). Briefly,

400 μL of the sample was combined with 2 mL of DNPH solution at a concentration of 10 mM. This mixture was then incubated at room temperature for 1 h with intermittent mixing every 10 min. Subsequently, 2 mL of 20% trichloroacetic acid solution was added and centrifuged for 3 min at 11,000 g. The upper waste solution was then discarded, and the precipitate was washed three times with 4 mL of anhydrous ethanol and ethyl acetate in a volume ratio of 1:1. Next, 1.5 mL of 6 mol/L guanidine hydrochloride was added to the precipitate, which was then dissolved by incubating in a water bath at 37 °C for 15 min. The supernatant was obtained through centrifugation at 11,000 *g* for 10 min, followed by measuring the absorbance at 370 nm. Protein content was determined using the biuret test.

2.7. Total volatile basic nitrogen (TVB-N) content

The determination of the TVB-N content of mackerel puree samples was performed according to the National Standard of China (GB 5009.228–2016). Each sample was parallel three times.

2.8. Determination of myofibril fragmentation index (MFI)

The MFI of mackerel puree samples was determined based on a previous method (Li et al., 2012). Briefly, 2 g mackerel puree was mixed with 40 mL of pre-cooled buffer (100 mM KCl, 11.2 mM K_2HPO_4, 8.8 mM KH_2PO_4, 1 mM EGTA, 1 mM $MgCl_2$). After homogenization at 8000 rpm for 2 min, the connective tissue was removed by filtration using two layers of 80-mesh gauze. Then, centrifugation at 4000 ×*g* for 10 min was performed to eliminate the supernatant. Following this, 15 mL of pre-cooled buffer was added to fully suspend the precipitation, which was then centrifuged and this process was repeated twice. The collected precipitate was then mixed with 10 mL of buffer to adjust the protein concentration to 0.5 mg/mL, and the absorbance was measured at 540 nm.

2.9. pH value

The mackerel puree samples (2.0 g) were added to 20 mL of deionized water and homogenized for 15 s by T10 IKA homogenizer (IKA®-Werke, Germany). The pH value of the mixture was then determined by a pH meter (PB-10, Sartorius, Germany).

2.10. Water holding capacity (WHC)

The WHC of mackerel puree samples was conducted using the method described in a previous study (Tan et al., 2024). A cut piece of mackerel puree was initially weighed (M_1). Subsequently, double layer of filter paper was used to tightly wrap the mackerel puree and centrifuged at 10,000 *g* for 10 min, then removed surface water. Finally, the gels were weighed again (M_2). The formula was calculated as follows:

$$\text{WHC}\ (\%) = (M_2/M_1) \times 100$$

2.11. The electron spin resonance spectrum (ESR)

ESR spectrometer A200 (Bruker, Karisruhe, Germany) was used to monitor free radical signals according to the previous research (Chen et al., 2018). In brief, mixed 2 mL of each sample with 200 μL of 56.4 mmol/L N-tert-butyl-α-phenylnitrone (PBN) and incubated in a hot-air-circulating oven at 100 °C for 4 h, with collection every 2 h. Subsequently, the mixture was transferred to an NMR tube and placed in the resonant cavity.

2.12. Low field nuclear magnetic resonance (LF NMR)

The water dynamic of mackerel puree samples was determined based on a previous study (Zhou & Yang, 2020). The mackerel puree samples were cut into uniform squares, weighed and then wrapped in plastic wrap, measured in an NMR tube. The relaxation time (T2) distribution curves for each sample as well as the relaxation parameters of various proton fractions were determined using a low-field NMR analyzer (MesoQMR23-060H, Niumag Co., Ltd., Shanghai, China) and analyzed through specialized software.

2.13. Microstructures of puree

The prepared mackerel puree samples were frozen in liquid nitrogen for 1 min and spray gold plating for 1 min. The cryogenic electron microscope (model SU8000, manufactured by Hitachi, Tokyo, Japan) was used to observe microstructures at a voltage of 10 kV and a magnification of 5 k to observe the microstructure.

2.14. Statistical analysis

Each experiment was conducted three times in parallel, and the displayed data was expressed as mean ± standard deviation of the three measurements. Duncan's test was used in statistical difference analysis (SPSS v. 19.0, SPSS Inc., Chicago, IL, USA). P- values <0.05 were considered statistically significant.

3. Results and discussion

3.1. Color of mackerel puree during storage

Color is a significant visual attribute of mackerel puree (Singh, Benjakul, Zhang, Deng, & Mittal, 2021). The color changes in mackerel puree during storage can be influenced by factors such as pigment, hydration of muscles, the state of myofibrillar and sarcoplasmin (natural or denatured), as well as the extent of lipid and protein oxidation during processing and storage (Guyon, Meynier, & de Lamballerie, 2016). As depicted in Fig. 1A-C that with the increase of storage time, the L^*, a^* and whiteness values of the three types of mackerel puree all decreased with longer storage time, indicating a gradual loss of color in the puree. The shift in a^* value (redness value) of mackerel puree was primarily associated with heme pigment (Zhang et al., 2023). Generally speaking, myoglobin is a kind of hemoglobin, which is one of the direct reasons for affecting the color of puree. During storage, the oxidation of heme iron turns the red oxygenated myoglobin into the maroon high-iron myoglobin, which made the mackerel puree gradually turn dark red as storage time increases. Initially, there were no significant differences in L^*, a^* and whiteness values among the three groups. With the increase in storage time, the values of the mackerel puree with BHT and TP, particularly the TP group, were significantly higher compared to the pure mackerel puree group. This difference may be attributed to BHT and TP binding free radicals produced during oxidation and blocking the reaction of amino acid residues under the action of free radicals (Farvin, Grejsen, & Jacobsen, 2012). The color change of mackerel puree during refrigeration was quantified using ΔE analysis. It was observed that the impact of unadded substances on the color of mackerel puree was more significant compared to the effect of added TP over the same storage period. This suggested the addition of TP led to the highest color retention in the mackerel puree, which might be due to TP and myoglobin having reversible binding through non-covalent interactions such as van der Waals force, hydrophobic action and hydrogen bonding, which inhibited myoglobin oxidation and effectively inhibited the color deterioration of mackerel puree (Prigent, Voragen, Visser, van Koningsveld, & Gruppen, 2007).

3.2. Sensory evaluation of mackerel puree during storage

In order to compare different samples of mackerel puree, a detailed assessment was conducted on its appearance, taste, texture, and flavor. The intensity scores shown in Fig. 1E demonstrate that the sensory

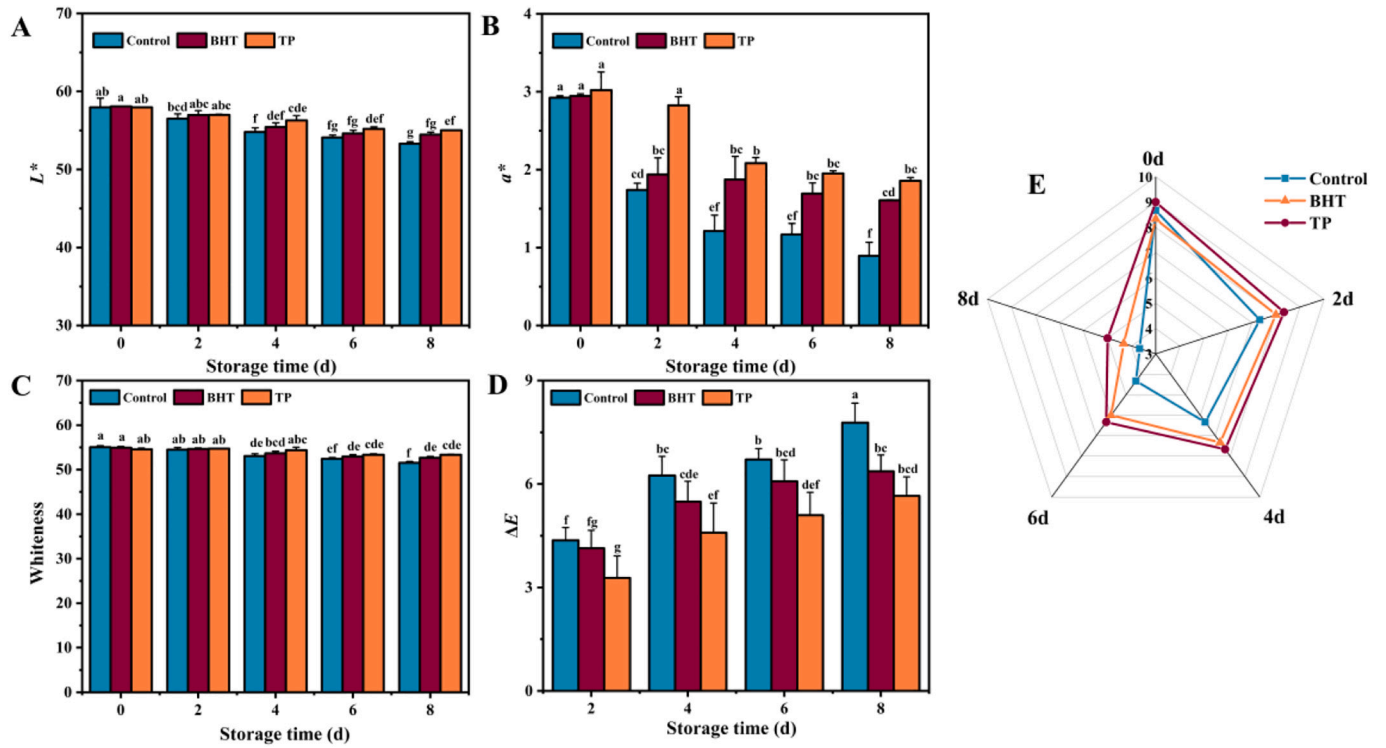

Fig. 1. Effect of TP addition on the L^*(A), a^* (B), whiteness (C), ΔE (D) and sensory score (E) of mackerel puree during refrigerated storage. Different superscripts letters indicate significant differences ($p < 0.05$).

scores of the mackerel puree decreased gradually as the storage days increased, indicating a waning interest in chilled mackerel puree over time. Initially, there was no significant difference in the sensory scores of the three groups of mashed mackerel, suggesting that the addition of TP and BHT did not have a noticeable impact on the appearance, texture, and taste of the mashed mackerel. However, towards the end of the storage period, the sensory scores of mackerel puree with TP addition were significantly higher compared to those with BHT addition and the control group. Mackerel puree, without any antioxidant addition showed clear signs of spoilage and deterioration, such as loose muscle tissue, poor elasticity, slimy meat, and foul odor. Although the TP-added mackerel puree also exhibited some degree of quality deterioration at the end of storage, it was significantly less than that observed in the BHT-added and control mackerel puree groups. The inclusion of TP in the mackerel puree resulted in better overall integrity and texture during refrigerated storage, attributed to its higher inhibition of protein and lipid oxidation. However, the impact of TP addition on the quality changes of mackerel puree during storage cannot be solely assessed through organoleptic evaluation. Therefore, future studies should further investigate the physicochemical indexes of TP-added mackerel puree.

3.3. Lipid oxidation of mackerel puree during storage

TBARS is the main indicator for assessing the oxidation and rancidity of aquatic products. The graph in Fig. 2A illustrates a notable increase in TBARS levels in mackerel puree samples with prolonged storage time ($p < 0.05$), suggesting a progressive oxidation of lipids in all samples. The TBARS levels in samples treated with BHT and TP were significantly lower compared to untreated samples ($p < 0.05$), indicating that both BHT and TP effectively inhibited the oxidation of mackerel puree. By contrast, TP exhibited a higher antioxidant efficacy than BHT, possibly attributed to its antioxidant properties through the donation of a hydrogen atom from ortho- and para-di hydroxy phenolics (Xie et al., 2019). Meanwhile, the activity of pro-oxidant enzymes, such as cyclooxygenase and lipoxygenase, could also be affected by the reaction with polyphenols (Almajano, Delgado, & Gordon, 2007). In addition, TP exhibited a stronger free radical scavenging ability compared to BHT (Fig. 3A-B), which more effectively blocks the free radical chain reaction that triggers lipid oxidation and has a stronger antioxidant ability.

3.4. Carbonyl content of mackerel puree during storage

Protein carbonylation is a type of amino acid modification caused by oxidation, affecting both the backbone and side chain (Turgut, Soyer, & Isikçi, 2016). Monitoring changes in carbonyl content during refrigerated storage can serve as an indicator of protein oxidation and provide valuable insights into the mechanisms of protein damage. Fig. 2B illustrates the changes in the carbonyl content of fish puree over the course of refrigerated storage. Initially, there were no significant differences in carbonyl content among the three groups of mackerel puree. However, carbonyls significantly increased with longer storage times as protein oxidation progressed, in line with the findings of Zhang, Huang, and Xie (2019). Notably, starting from 4d, a substantial difference in carbonyl content was observed. Comparing the puree in the TP and BHT-added groups with the control mackerel puree, it was evident that the addition of TP and BHT reduced the oxidation of mackerel puree proteins and enhanced oxidative stability during refrigerated storage. Furthermore, the carbonyl content of the TP group was lower than that of the BHT group at the same storage time, indicating that the choice of antioxidant influences the rate of protein oxidation and the amount of oxidation products during refrigerated storage (Özen & Soyer, 2018). TP exhibited a higher scavenging capacity for free radicals when compared to BHT. This resulted in the prevention of free radical damage to the amino acid side chain of proteins, reducing protein carbonylation, and slowing down the oxidation process of proteins (Akagawa, 2021).

3.5. TVB-N content of mackerel puree during storage

TVB-N value is an important indicator for evaluating protein

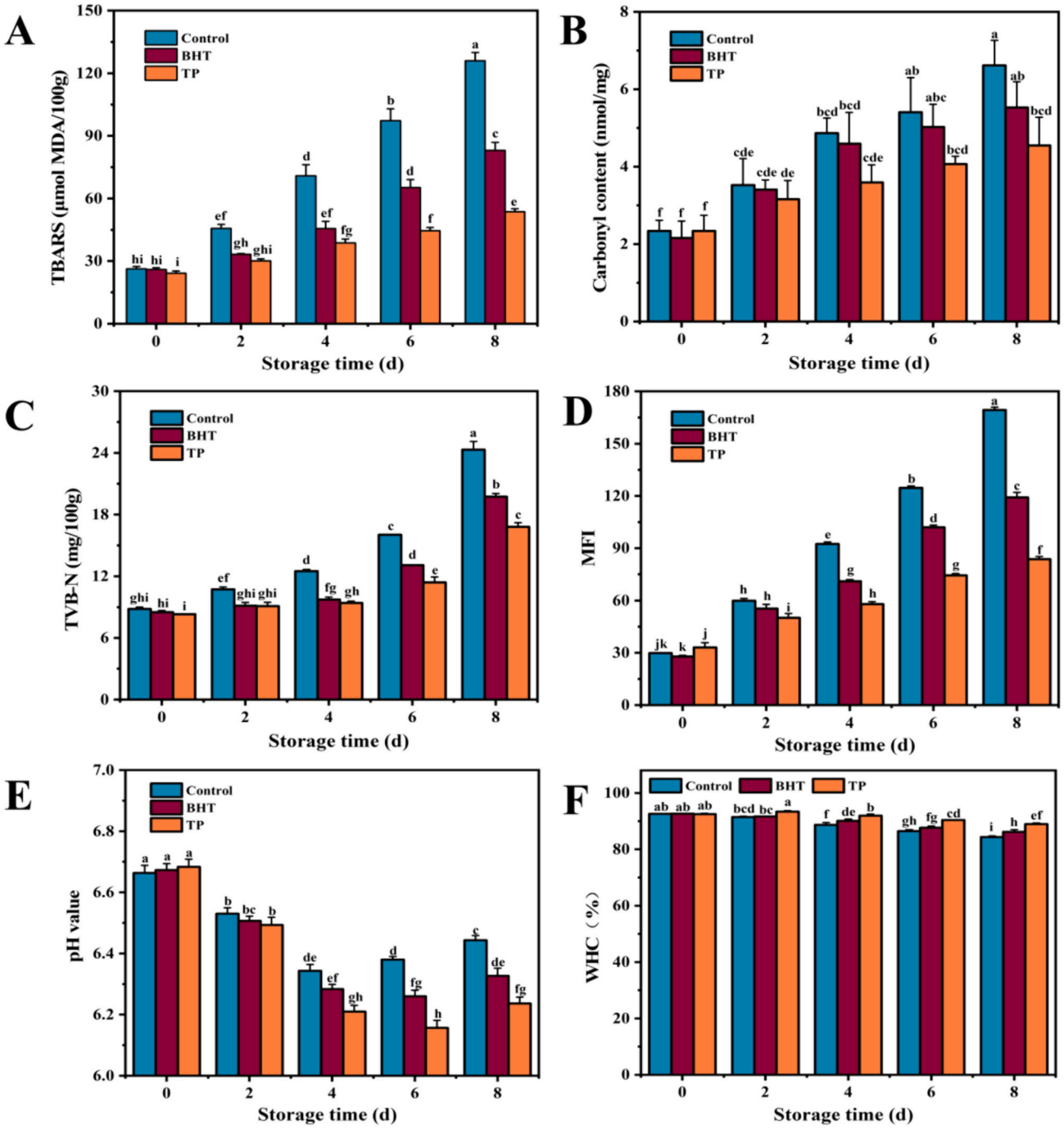

Fig. 2. Effect of TP addition on the TBARS (A), carbonyl content (B), TVB-N content (C), MFI (D), pH value (E) and WHC (F) of mackerel puree during refrigerated storage. Different superscripts letters indicate significant differences ($p < 0.05$).

degradation products in animal-derived food during storage (Ocaño-Higuera et al., 2011). The impact of TP addition on the TVB-N content of mackerel mud during storage is illustrated in Fig. 2C. As the storage duration increased, the TVB-N value of mackerel puree significantly increased in all groups. Nevertheless, the rise in TVB-N value was significantly mitigated in the TP and BHT treatment groups compared to the control group. On the 8 d, the TVB-N value of the fish puree in the control group had reached 24.31 mg/100 g, nearing the permissible limit value of GB 2733–2015. In contrast, the TVB-N value of the mackerel puree in the TP and BHT groups was 16.82 mg/100 g and 19.74 mg/100 g, respectively. These results indicated that TP and BHT treatments effectively suppress the deterioration and oxidation of protein in mackerel puree, with TP treatment showing stronger effects. This could be attributed to the antioxidant and antibacterial properties of TP, which decelerated the rate of oxidative deamination of non-protein substances. TP could also form complexes with proteins, providing stability and inhibiting degradation, as well as inhibiting bacterial growth (Yi et al., 2011). Therefore, TP effectively inhibited the generation of amines during the transformation of meat products from the autolysis stage to the spoilage stage. These findings aligned with the results of this study, which demonstrated that TP effectively inhibited the generation of TVB-N in mackerel puree and slowed down oxidation.

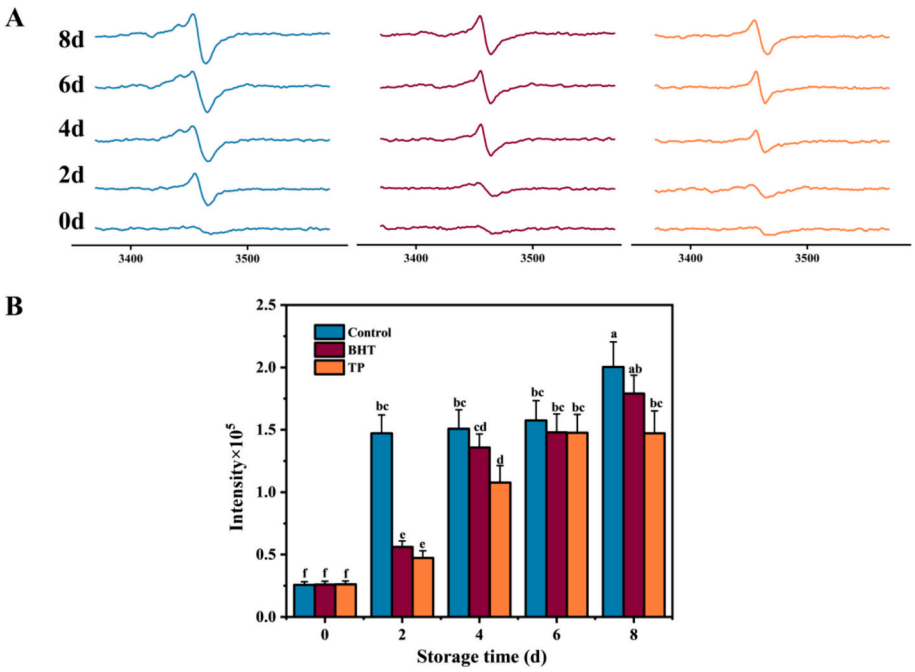

Fig. 3. Effect of TP addition on the ESR spectra (A) and free radical intensity (B) of mackerel puree during refrigerated storage. Different superscripts letters indicate significant differences ($p < 0.05$).

3.6. MFI of mackerel puree during storage

The MFI could reflect the degradation of myofibrillar proteins of mackerel puree macroscopically (Lametsch, Knudsen, Ertbjerg, Oksbjerg, & Therkildsen, 2007). The changes in the MFI of mackerel puree during storage were shown in Fig. 2D. The MFI of mackerel puree increased with refrigerated storage time, and mackerel puree with added TP exhibited a significantly lower MFI compared to the control group at the same storage time. Furthermore, mackerel puree with TP showed the slowest increase in MFI. Overall, TP had a more pronounced retardation effect on MFI increase compared to BHT. Previous studies have also indicated that protein oxidation-induced textural changes occur at the myofiber level, leading to an increase in MFI (Lund, Christensen, Fregil, Hviid, & Skibsted, 2008). The above results confirmed that TP effectively inhibited muscle myofibril degradation caused by protein oxidative degradation during mackerel puree storage.

3.7. pH value of mackerel puree during storage

The pH value reflected the concentration of free H^+ and OH^- in the muscle, as an indicator of the quality of mackerel puree. As shown in Fig. 2E, the aqueous extracts of mackerel puree were weakly acidic. The pH values in each group generally followed a pattern of decreasing and then increasing as the storage time progressed. The untreated groups reached the lowest pH value after 4d storage at 6.34, while the BHT and TP-treated groups reached the lowest value after 6d storage at 6.26 and 6.15, respectively. This phenomenon could be attributed to mackerel being migratory red fishes with a higher glycogen content in their muscles compared to lower-dwelling fishes (Zhao et al., 2021). The glycogen in mackerel puree undergoes anaerobic glycolysis, leading to the accumulation of lactic acid and subsequently causing a decrease in pH value. Similar findings were observed in a study investigating the impact of TP on the physicochemical properties of tilapia surimi during refrigerated storage (Wu et al., 2022). The increase in pH value at the end of storage is probably due to the microorganisms in the mackerel puree interacting with endogenous enzymes to break down proteins, producing more ammonia amines and alkaline substances than the production of lactic acid. Samples treated with TP consistently exhibited significantly lower pH values during storage compared to untreated and BHT-treated samples, demonstrating the effectiveness of TP in inhibiting the growth of spoilage microorganisms. Furthermore, the lower pH value in fish products contributed to better firmness, flavor and slice ability (Trabelsi et al., 2019). This suggests that the lower pH value caused by TP treatment might help improve the gel quality and shelf-life of mackerel puree.

3.8. WHC of mackerel puree during storage

WHC is an important index of water binding ability, which determines the stability of mackerel puree. As can be seen from Fig. 2F, the WHC of mackerel puree decreased over time during refrigeration, with mackerel puree containing TP showing higher WHC compared to those without or with BHT at the same storage duration. This difference can be attributed to the pivotal role of muscle protein structure in water distribution (Straadt, Rasmussen, Andersen, & Bertram, 2007). Oxidation of mackerel puree during storage led to the breakdown of myofibrillar protein, reducing water retention (Dublán-García, Cruz-Camarillo, Guerrero-Legarreta, & Ponce-Alquicira, 2006). Interestingly, the addition of TP slightly increased WHC before storage, likely due to structural changes induced by protein oxidation that caused myofibril swelling and facilitated the formation of a water-binding network (Lu, Zhang, et al., 2017). Moreover, polyphenols, in addition to their antioxidant effects, influenced the structure and gel-forming ability of meat proteins, resulting in improved water distribution within the protein gel matrix, enhancing physical stability and WHC (Jongberg, Terkelsen, Miklos, & Lund, 2015).

3.9. ESR of mackerel puree during storage

The signal intensity of free radicals of mackerel puree samples assessed by ESR spectroscopy during storage is shown in Fig. 3A. Throughout the storage period, free radical intensities in all mackerel puree samples increased, suggesting a gradual generation of free radicals. After 8 d of storage, the BHT-treated and TP-treated samples had significantly lower free radical intensities than the untreated samples. It showed that both BHT and TP in mackerel puree samples significantly

inhibited the production of free radicals. Throughout the whole storage process, the free radical intensity of TP in mackerel puree samples was always at the lowest level. It showed that TP in mackerel puree samples could inhibit the generation of free radicals to the greatest extent. During the refrigerated storage of mackerel puree, the disruption of hemoglobin complex led to the release of free iron, which catalyzed the lipid peroxidation process in the muscle. The lipid oxidation process involved a chain reaction of various free radicals, such as allylic, lipid peroxyl and alkoxyl radicals, which played crucial roles as intermediates and initiators in the radical chain reaction propagation (Shahidi & Zhong, 2010). Previous studies have shown that the addition of TP absorbed oxidation to produce free radicals, converting them into stable products and blocking free radical chain reactions (Maqsood, Benjakul, Abushelaibi, & Alam, 2014). Consequently, TP was found to reduce the intensity of carbon-centered radicals in mackerel puree samples, providing enhanced protection against oxidation.

3.10. Water dynamic of mackerel puree during storage

The oxidation of mackerel puree can affect the state and distribution of water. LF-NMR was used to study the effect of TP on the water phase state of mackerel puree during refrigerated storage. In general, a shorter T2 relaxation time suggests tighter binding of water molecules to macromolecules, resulting in decreased water mobility. Three peaks of mackerel puree labelled as 0–10 ms (T_{21}), 10–100 ms (T_{22}), and 100–1000 ms (T_{23}) were identified in Fig. 4. These peaks represent water molecules in different molecular states, T_{21} as bound water bound to proteins and other macro-molecules, T_{22} as immobilized water distributed between tissues and fixed in the protein network space without strong interaction with components, and T_{23} as free water inside puree (Møller et al., 2011). The results showed that the water phase composition of mackerel puree was significantly different with different oxidation degrees during the refrigerated storage. As can be seen from the figure, the water in the mackerel puree was predominantly immobile, playing a key role in the overall water composition changes. This observation aligns with findings from prior research. (He et al., 2021). As the refrigerated storage time extended, the three peaks in all mackerel puree sample groups showed a tendency to shift towards longer relaxation times to varying degrees, and the water gradually changed to the unbound direction. In addition, the immobilized water content of mackerel puree increased, the bound water content decreased, and the free water content basically remained unchanged. This might be due to the oxidation of the mackerel puree leading to the destruction and gradual dissolution of the protein molecules-the degree of water binding decreased (Lu, Zhang, et al., 2017). Compared with the control group, there were no significant differences in mackerel puree added with TP during storage, except for a slight blue shift in the T_{21} relaxation time. It was suggested that TP promoted its interactions with fish proteins, improved the stability and ability to bind water in the mackerel puree by inhibiting changes in the protein structure, thereby reduced the mobility of water in the puree.

3.11. Microstructure of mackerel puree during storage

The quality characteristics of aquatic products can be reflected and determined by their microstructure (Alabi, Zhu, & Sun, 2020). Fig. 5 showed the microstructural changes of mackerel puree samples at the beginning, middle and end of the storage period. The mackerel puree samples without and with added BHT and TP exhibited a dense gel network structure on 0 d that was uniform and evenly distributed. Over time, the gel network became less compact, the surface became rougher, and the pore size increased. There were distinct differences in micromorphology among the three groups of mackerel puree samples. The untreated group showed a loss of flatness and smoothness on the surface after 4 d of storage, with pores appearing more open. By the end of the storage period, the network structure had already ruptured, leading to a significant decrease in water retention capacity of the mackerel puree samples. The mackerel puree samples with added BHT maintained the network structure compared to the untreated group, and the surface was also smoother. However, when compared to mackerel puree samples with added TP, the network arrangement appeared looser, indicating a decline in texture and quality. Changes in protein structure, such as myosin degradation, often lead to alterations in the microstructure of aquatic muscle products. Previous research has shown that thermal denaturation of myofibrillar proteins and collagen contraction can lead to a looser microstructure in muscle tissue (Chang et al., 2011). Based on the experimental results, it could be inferred that oxidative protein degradation may be responsible for these microstructural changes. Furthermore, the incorporation of TP into the mackerel puree was observed to enhance the cross-linking of the network structure, possibly due to non-covalent or covalent interactions between phenolics and myofibrillar proteins in the puree. When the polyphenol content was at an appropriate level, the oxidation of polyphenols led to the formation of quinone, which in turn facilitated the conversion of sulfhydryl groups in myofibrillar proteins into disulfide bonds. This process acted as a cross-linking agent, covalently combining with various nucleophilic groups to form an organized gel network structure. These findings demonstrated that TP treatment had a positive impact on the gel network structure and effectively slowed down the deterioration of mackerel puree quality during refrigerated storage.

3.12. Correlation analysis of physicochemical properties and quality of mackerel puree during storage

During refrigerated storage, the quality of mackerel puree samples from different treatment groups can deteriorate to varying degrees. To visualize this, principal component analysis was utilized to assess the physicochemical properties and sensory characteristics. As shown in

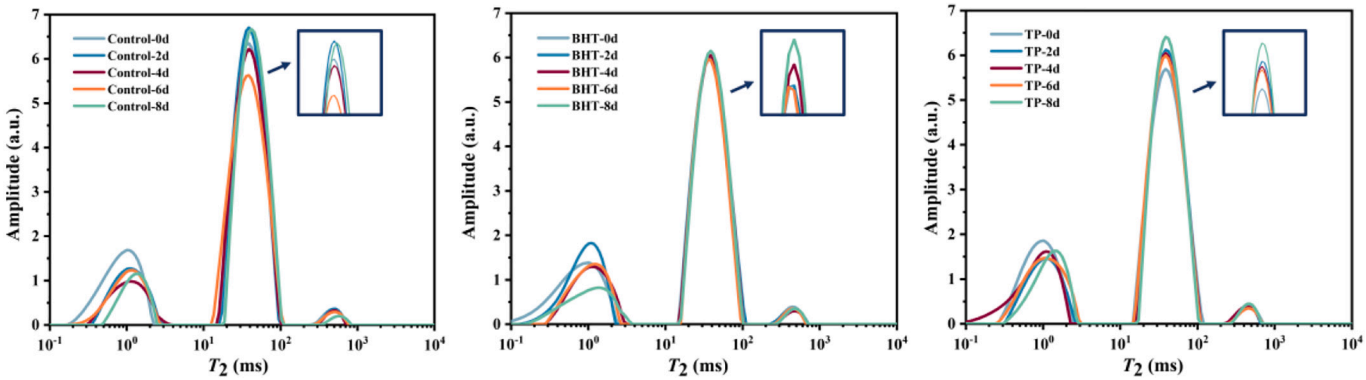

Fig. 4. T2 relaxation spectra of mackerel puree during refrigerated storage.

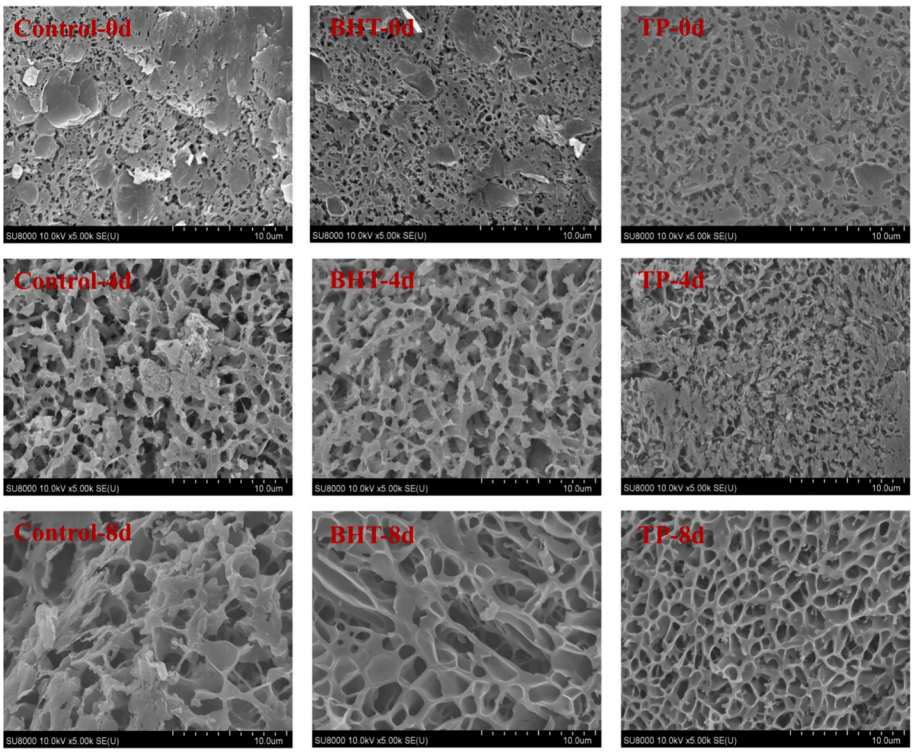

Fig. 5. Cryo-SEM images of mackerel puree during refrigerated storage (Scale bars = 10 μm).

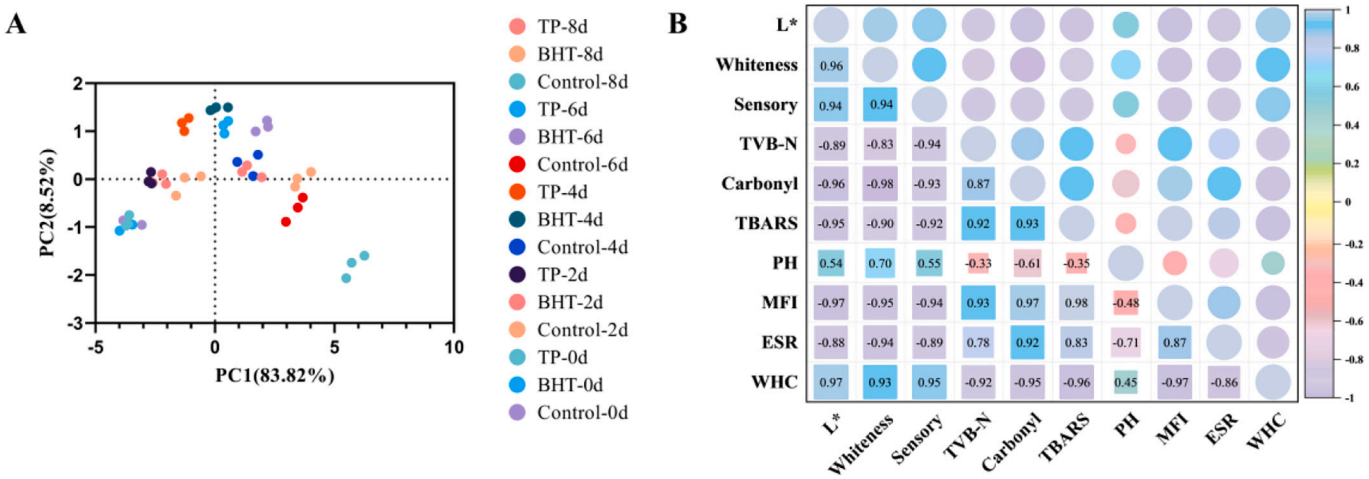

Fig. 6. Correlation between physicochemical and quality characteristics of mackerel puree during refrigerated storage after treatment with different antioxidants. Similarity analysis of different groups at different storage day by PCA (A) and clustering heatmap based on Pearson's correlation tests between physicochemical properties and quality characteristics of mackerel puree (B).

Fig. 6A, two principal components (PCs) explained 92.34% of the overall situation (83.82% and 8.52% for PC1 and PC2, respectively). The physicochemical and sensory properties of the mackerel puree from the untreated group differed significantly from the other groups on 6 d and 8 d. This illustrated the difficulty of untreated mackerel puree in maintaining its original characteristics, which reduced its sensory acceptability to consumers. Conversely, the physicochemical properties of TP-treated mackerel puree after 8 d of refrigeration closely resembled those of untreated mackerel puree after 2 d. These results demonstrated that the addition of TP played a positive role in maintaining the quality of mackerel puree during refrigerated storage.

This study examined the correlation between physicochemical properties and sensory attributes of mackerel puree through Pearson correlation analysis. The results showed that the L^* value, whiteness, sensory score, and pH value were positively correlated with WHC and negative correlations with TVB-N content, carbonyl content, TBARS value, MFI value and ESR content (Fig. 6B). Additionally, various treatments applied to mackerel puree had a significant effect on its quality changes during refrigerated storage. A study conducted earlier showed that there was a significant linear relationship between protein oxidative degradation parameters and WHC of fish fillets (Lu, Wang, & Luo, 2017). Excessive cross-linking in oxidized myofibrillar proteins might weaken hydrogen bonding within the protein matrix, leading to the disruptions in protein-protein and protein-water interactions (Huff-Lonergan & Lonergan, 2005; Li, Kong, Xia, Liu, & Diao, 2013). This study observed a gradual decrease in WHC of mackerel puree during

refrigerated storage, attributed to microstructural changes from oxidative protein degradation. Furthermore, the alteration in whiteness of the mackerel puree was influenced by the extent of protein denaturation. (Zhou, Zhao, Zhao, Sun, & Cui, 2014). Therefore, it was hypothesized that the oxidation and degradation of proteins during storage of mackerel puree led to the reduction of WHC and the destruction of the microstructure, ultimately leading to a decline of its organoleptic quality.

4. Conclusion

In this study, the effect of TP on the quality of mackerel puree during refrigeration was evaluated. This was demonstrated by a significant increase in TBARS value, carbonyl content, and free radical content, and a decrease in pH value initially followed by an increase. After 8 days of refrigerated storage, the protein and lipid molecules were broken down, leading to a change in the state of the aqueous phase of mackerel puree. This resulted in a significant decrease in sensory acceptance. However, the addition of TP effectively delayed the oxidation of lipids and proteins in mackerel puree, while also influencing the structural changes of mackerel protein. This led to a relatively dense and stable microstructure being maintained, resulting in improved quality characteristics of mackerel puree products during storage. Pearson correlation analysis revealed a significant association between the physicochemical characteristics of mackerel puree and its quality properties during refrigerated storage. This suggests that the improvement in color and WHC in mackerel puree by TP was primarily due to the suppression of protein and lipid oxidation and degradation. In conclusion, this study establishes a research foundation for reducing nutritional loss in whole fish products and enhancing quality in food processing.

CRediT authorship contribution statement

Zhifeng Tan: Writing – original draft, Methodology, Data curation. **Xiaoqing Yang:** Investigation. **Zheng Jin:** Methodology, Data curation. **Lin Han:** Methodology, Investigation. **Ke Li:** Writing – review & editing. **Sangeeta Prakash:** Investigation, Conceptualization. **Xiuping Dong:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101480.

References

Akagawa, M. (2021). Protein carbonylation: Molecular mechanisms, biological implications, and analytical approaches. *Free Radical Research, 55*(4), 307–320.

Alabi, K. P., Zhu, Z. W., & Sun, D. W. (2020). Transport phenomena and their effect on microstructure of frozen fruits and vegetables. *Trends in Food Science & Technology, 101*, 63–72.

Almajano, M. P., Delgado, M. E., & Gordon, M. H. (2007). Changes in the antioxidant properties of protein solutions in the presence of epigallocatechin gallate. *Food Chemistry, 101*(1), 126–130.

Asamoah, E. K., Nunoo, F. K. E., Addo, S., Nyarko, J. O., Acquah, S. A., & Hyldig, G. (2022). Effect of smoking and gamma irradiation on the nutritional and sensory quality of Atlantic chub mackerel in Ghana. *Radiation Physics and Chemistry, 201*, Article 110458.

Bayram, I., & Decker, E. A. (2023). Underlying mechanisms of synergistic antioxidant interactions during lipid oxidation. *Trends in Food Science & Technology, 133*, 219–230.

Chang, H. J., Xu, X. L., Li, C. B., Huang, M., Liu, D. Y., & Zhou, G. H. (2011). A comparison of heat-induced changes of intramuscular connective tissue and collagen of beef semitendinosus muscle during water bath and microwave heating. *Journal of Food Process Engineering, 34*(6), 2233–2250.

Chen, Q. M., Xie, Y. F., Xi, J. Z., Guo, Y. H., Qian, H., Cheng, Y. L., … Yao, W. R. (2018). Characterization of lipid oxidation process of beef during repeated freeze-thaw by electron spin resonance technology and Raman spectroscopy. *Food Chemistry, 243*, 58–64.

Cheng, L. N., Zhu, Z. W., & Sun, D. W. (2021). Impacts of high pressure assisted freezing on the denaturation of polyphenol oxidase. *Food Chemistry, 335*, Article 127485.

Chinese Standard GB 2733. (2015). *National food safety standards for fresh and frozen animal aquatic products*. Beijing: National Health Commission of the PRC.

Chinese Standard GB 5009.228. (2016). *National food safety standard - determination of volatile base nitrogen in food*. Beijing: National Health Commission of the PRC.

Chinese Standard GB/T 16291.1. (2012). *General guidelines for selection, training and management of assessors for sensory analysis part 1: Selection of assessors*. Beijing: General Administration of Quality Supervision, Inspection and Quarantine of PRC.

Cropotova, J., Mozuraityte, R., Standal, I. B., & Rustad, T. (2018). A non-invasive approach to assess texture changes in sous-vide cooked Atlantic mackerel during chilled storage by fluorescence imaging. *Food Control, 92*, 216–224.

Dublán-García, O., Cruz-Camarillo, R., Guerrero-Legarreta, I., & Ponce-Alquicira, E. (2006). Effect of refrigerated storage on proteolytic activity and physicochemical and microstructural properties of giant squid (*Dosidicus gigas*) mantle muscle. *Journal of Muscle Foods, 17*(3), 291–310.

Estévez, M., & Heinonen, M. (2010). Effect of phenolic compounds on the formation of α-aminoadipic and γ-glutamic semialdehydes from myofibrillar proteins oxidized by copper, iron, and myoglobin. *Journal of Agricultural and Food Chemistry, 58*(7), 4448–4455.

Farvin, K. H. S., Grejsen, H. D., & Jacobsen, C. (2012). Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (*Trachurus trachurus*): Effect on lipid and protein oxidation. *Food Chemistry, 131*(3), 843–851.

Grunwald, E. W., & Richards, M. P. (2006). Mechanisms of heme protein-mediated lipid oxidation using hemoglobin and myoglobin variants in raw and heated washed muscle. *Journal of Agricultural and Food Chemistry, 54*(21), 8271–8280.

Guyon, C., Meynier, A., & de Lamballerie, M. (2016). Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science & Technology, 50*, 131–143.

Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. USA: Oxford University Press.

He, X., Lin, R., Cheng, S., Wang, S., Yuan, L., Wang, H., Wang, H., & Tan, M. (2021). Effects of microwave vacuum drying on the moisture migration, microstructure, and rehydration of sea cucumber. *Journal of Food Science, 86*(6), 2499–2512.

Hu, K., Huyan, Z., Ding, S., Dong, Y., & Yu, X. (2020). Investigation on food packaging polymers: Effects on vegetable oil oxidation. *Food Chemistry, 315*, Article 126299.

Huang, Z., Wang, Q., Cao, J., Zhou, D., & Li, C. (2023). Mechanisms of polyphenols on quality control of aquatic products in storage: A review. *Critical Reviews in Food Science and Nutrition*, 1–20.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 71*(1), 194–204.

Jongberg, S., Terkelsen, L. D., Miklos, R., & Lund, M. N. (2015). Green tea extract impairs meat emulsion properties by disturbing protein disulfide cross-linking. *Meat Science, 100*, 2–9.

Lametsch, R., Knudsen, J. C., Ertbjerg, P., Oksbjerg, N., & Therkildsen, M. (2007). Novel method for determination of myofibril fragmentation post-mortem. *Meat Science, 75*(4), 719–724.

Li, D. Y., Li, N., Dong, X. H., Tan, Z. F., Na, X. K., Liu, X. Y., & Zhou, D. Y. (2022). Effect of phytic acid combined with lactic acid on color and texture deterioration of ready-to-eat shrimps during storage. *Food Chemistry, 396*, Article 133702.

Li, K., Zhang, Y. M., Mao, Y. W., Cornforth, D., Dong, P. C., Wang, R. H., … Luo, X. (2012). Effect of very fast chilling and aging time on ultra-structure and meat quality characteristics of Chinese yellow cattle *M. longissimus* lumborum. *Meat Science, 92*(4), 795–804.

Li, Y. Q., Kong, B. H., Xia, X. F., Liu, Q., & Diao, X. P. (2013). Structural changes of the myofibrillar proteins in common carp (*Cyprinus carpio*) muscle exposed to a hydroxyl radical-generating system. *Process Biochemistry, 48*(5–6), 863–870.

Liu, Q. Y., Lin, J. J., Zhao, W. H., Lei, M. L., Yang, J., & Bai, W. D. (2023). The dynamic changes of flavors and UPLC-Q-Exactive-Orbitrap-MS based lipidomics in mackerel (*Scomberomorus niphonius*) during dry-cured processing. *Food Research International, 163*, Article 112273.

Liu, Y., Huang, Y., Wang, Y., Zhong, J., Li, S., Zhu, B., & Dong, X. (2023). Application of cod protein-stabilized and casein-stabilized high internal phase emulsions as novel fat substitutes in fish cake. *LWT - Food Science and Technology, 173*, Article 114267.

Lu, H., Wang, H., & Luo, Y. K. (2017). Changes in protein oxidation, water-holding capacity, and texture of bighead carp (*Aristichthys Nobilis*) fillets under chilled and partial frozen storage. *Journal of Aquatic Food Product Technology, 26*(5), 566–577.

Lu, H., Zhang, L. T., Li, Q. Z., & Luo, Y. K. (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.

Lund, M. N., Christensen, M., Fregil, L., Hviid, M. S., & Skibsted, L. H. (2008). Effect of high-oxygen atmosphere packaging on mechanical properties of single muscle fibres from bovine and porcine *longissimus dorsi*. *European Food Research and Technology, 227*(5), 1323–1328.

Maqsood, S., Benjakul, S., Abushelaibi, A., & Alam, A. (2014). Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: A detailed review. *Comprehensive Reviews in Food Science and Food Safety, 13*(6), 1125–1140.

Møller, S. M., Grossi, A., Christensen, M., Orlien, V., Søltoft-Jensen, J., Straadt, I. K., … Bertram, H. C. (2011). Water properties and structure of pork sausages as affected by high-pressure processing and addition of carrot fibre. *Meat Science, 87*(4), 387–393.

Ocaño-Higuera, V. M., Maeda-Martínez, A. N., Marquez-Ríos, E., Canizales-Rodríguez, D. F., Castillo-Yáñez, F. J., Ruíz-Bustos, E., … Plascencia-Jatomea, M. (2011). Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. *Food Chemistry, 125*(1), 49–54.

Özen, B., & Soyer, A. (2018). Effect of plant extracts on lipid and protein oxidation of mackerel (*Scomber scombrus*) mince during frozen storage. *Journal of Food Science and Technology-Mysore, 55*(1), 120–127.

Prigent, S. V., Voragen, A. G., Visser, A. J., van Koningsveld, G. A., & Gruppen, H. (2007). Covalent interactions between proteins and oxidation products of caffeoylquinic acid (chlorogenic acid). *Journal of the Science of Food and Agriculture, 87*(13), 2502–2510.

Qi, C., Liu, G., Ping, Y., Yang, K., Tan, Q., Zhang, Y., Chen, G., Huang, X., & Xu, D. (2023). A comprehensive review of nano-delivery system for tea polyphenols: Construction, applications, and challenges. *Food Chemistry: X, 17*, Article 100571.

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews, 39*(11), 4067–4079.

Singh, A., Benjakul, S., Zhang, B., Deng, S. G., & Mittal, A. (2021). Effect of squid pen chitooligosaccharide in conjugation with different modified atmospheric packaging conditions on color and storage stability of tuna slices. *Food Control, 125*, Article 108013.

Straadt, I. K., Rasmussen, M., Andersen, H. J., & Bertram, H. C. (2007). Aging-induced changes in microstructure and water distribution in fresh and cooked pork in relation to water-holding capacity and cooking loss - A combined confocal laser scanning microscopy (CLSM) and low-field nuclear magnetic resonance relaxation study. *Meat Science, 75*(4), 687–695.

Taghvaei, M., & Jafari, S. M. (2015). Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. *Journal of Food Science and Technology-Mysore, 52*(3), 1272–1282.

Tan, Z., Yang, X., Wang, Z., Chen, Z., Pan, J., Sun, Q., & Dong, X. (2024). Konjac glucomannan-assisted fabrication of stable emulsion-based oleogels constructed with pea protein isolate and its application in surimi gels. *Food Chemistry, 443*, Article 138538.

Trabelsi, I., Ben Slima, S., Ktari, N., Triki, M., Abdehedi, R., Abaza, W., Moussa, H., Abdeslam, A., & Ben Salah, R. (2019). Incorporation of probiotic strain in raw minced beef meat: Study of textural modification, lipid and protein oxidation and color parameters during refrigerated storage. *Meat Science, 154*, 29–36.

Turgut, S. S., Soyer, A., & Isikçi, F. (2016). Effect of pomegranate peel extract on lipid and protein oxidation in beef meatballs during refrigerated storage. *Meat Science, 116*, 126–132.

Wu, J., Li, C. S., Li, L. H., Yang, X. Q., Wang, Y. Q., & Zhou, W. G. (2022). Improved physicochemical properties and product characteristics of tilapia surimi by tea polyphenols during chilled storage. *LWT - Food Science and Technology, 167*, Article 113822.

Xie, H. K., Zhou, D. Y., Liu, Z. Y., Li, D. Y., Tan, Z. F., Dong, X. F., … Zhu, B. W. (2019). Effects of natural phenolics on shelf life and lipid stability of freeze-dried scallop adductor muscle. *Food Chemistry, 295*, 423–431.

Yi, S. M., Li, J. R., Zhu, J. L., Lin, Y., Fu, L. L., Chen, W., & Li, X. P. (2011). Effect of tea polyphenols on microbiological and biochemical quality of *Collichthys* fish ball. *Journal of the Science of Food and Agriculture, 91*(9), 1591–1597.

Zhang, K., Li, N., Wang, Z., Feng, D., Liu, X., Zhou, D., & Li, D. (2023). Recent advances in the color of aquatic products: Evaluation methods, discoloration mechanism, and protection technologies. *Food Chemistry, 434*, Article 137495.

Zhang, K., Wang, Y., Fan, X., Li, N., Tan, Z., Liu, H., Liu, X., Zhou, D., & Li, D. (2024). Effects of calcium chloride on the gelling and digestive characteristics of myofibrillar protein in *Litopenaeus vannamei*. *Food Chemistry, 441*, Article 138348.

Zhang, X. C., Huang, W. B., & Xie, J. (2019). Effect of different packaging methods on protein oxidation and degradation of grouper (*Epinephelus coioides*) during refrigerated storage. *Foods, 8*(8), 325.

Zhao, Y., Wang, Y. Q., Li, C. S., Li, L. H., Yang, X. Q., Wu, Y. Y., … Zhao, Y. Q. (2021). Novel insight into physicochemical and flavor formation in naturally fermented tilapia sausage based on microbial metabolic network. *Food Research International, 141*, Article 110122.

Zhou, F. B., Zhao, M. M., Zhao, H. F., Sun, W. Z., & Cui, C. (2014). Effects of oxidative modification on gel properties of isolated porcine myofibrillar protein by peroxyl radicals. *Meat Science, 96*(4), 1432–1439.

Zhou, Y. G., & Yang, H. S. (2020). Enhancing tilapia fish myosin solubility using proline in low ionic strength solution. *Food Chemistry, 320*, Article 126665.