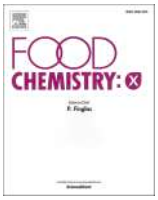
Food Chemistry: X 25 (2025) 102243

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Study on the regulation of tea polyphenols on the structure and gel properties of myofibrillar protein from *Neosalanx taihuensis*

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ARTICLE INFO

Keywords:
Tea polyphenols
N. taihuensis
Myofibrillar protein
Gel modification

ABSTARCT

Neosalanx taihuensis (N. taihuensis) is an important freshwater fish species, rich in nutrients and proteins. However, myofibrillar proteins (MPs), as the major component of muscle, are prone to oxidative denaturation. Tea polyphenols (TPs), as natural antioxidants, have broad applications in the area of aquatic product manufacturing. The research examined the impacts of three typical catechins, namely epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), on the structure and gel characteristics of MP from *N. taihuensis* under oxidative treatment conditions. In comparison to the oxidized group, all three TP compounds notably slowed down the oxidation process of MP and decreased the production of oxidative products. Additionally, TP addition induced changes in the MP structure, with further exposure of hydrophobic regions. Furthermore, TP treatment notably enhanced the functional properties of MP gels, including optimized gel strength, improved water holding capacity (WHC), and altered rheological properties. Among the three TP compounds, EGCG, due to its higher number of phenolic rings, formed complexes with MP that exhibited greater antioxidant activity. The research results indicated that the WHC of the EGCG group had increased by 17.06 % compared to the oxidized group, and the decrease in amino content reached as much as 40.09 %. Finally, molecular docking simulations were performed to explore the ways in which TP and MP interact. This study not only uncovers the relationship between polyphenol types and MP structure, but also confirms the enormous potential of TP as antioxidants in the improvement of *N. taihuensis* surimi products.

1. Introduction

Neosalanx taihuensis (N. taihuensis), characterized by its transparent and edible body, holds great commercial importance as a key aquaculture species in China (Zhou et al., 2023), earning it the nickname "ginseng of fish". Myofibrillar proteins (MPs) are a crucial component of fish muscle, primarily consisting of fibrous proteins formed by the polypeptide chains of actin and myosin. In fish product processing, MP plays an essential part in shaping the quality characteristics. The structure and gelatinous attributes of MP largely dictate meat texture profile. In *N. taihuensis*, MP accounts for about 10 % of the fresh meat weight and 60–75 % of the total protein content (Ryu et al., 2021), making it a key factor influencing the development of the gel matrix structure in fish meat and determining gel strength and elasticity. However, during the stages of meat processing and preservation, MP is highly susceptible to environmental conditions, leading to inevitable oxidative denaturation (Cao et al., 2024). This change ultimately results in a decline in the quality and flavor of meat products.

Tea polyphenols (TPs) are an important class of polyphenols in tea, exhibiting various physiological activities such as antioxidation, antibacterial effects, and immune regulation. Based on their main chemical components, TPs can be categorized into four distinct groups: catechins, flavonoids, anthocyanins, and phenolic acids. The primary polyphenolic compounds in green tea are flavanols, including epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) (Hu et al., 2009). Among these, EC does not contain additional ester functional groups and has a relatively simple structure. EGC and EGCG, on the other hand, have one and two gallate groups introduced to the EC structure, respectively (Du et al., 2012). This structural difference makes EGCG the primary and highly active phenolic compound found in green

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https://doi.org/10.1016/j.fochx.2025.102243
Received 9 December 2024; Received in revised form 16 January 2025; Accepted 27 January 2025
Available online 28 January 2025

tea. A gel is a spatial network structure formed by a sol or polymer under certain conditions, and its formation and properties are affected by numerous factors, including protein configuration and concentration. MP can form a gel under appropriate conditions.

In recent times, TP has been comprehensively studied and utilized in the food sector as a natural antioxidant. Especially in the field of aquatic product processing, TP not only exhibits excellent antioxidant properties but also significantly improves the gel properties of proteins, thereby achieving the production goals of enhancing the standard and durability of aquatic goods. For example, Jin et al. (Jin et al., 2023) found in an in vitro anti-glycation model that TP significantly altered the structure of sturgeon MP and promoted protein crosslinking. Similarly, the research findings of Feng (Feng et al., 2017) confirmed that MP gels treated with an appropriate concentration of EGCG had higher strength and emulsion stability. Moreover, whether it was monophenols, diphenols, or triphenols, the addition of all phenols could significantly induce the unfolding structures and promote crosslinking, enhancing the elasticity of the MP gel network (Guo et al., 2021).

However, there is a paucity of research on the regulation of MP structure and gel properties in *N. taihuensis* by phenolic compounds with different structures. This study aimed to examine the impacts of three typical TP that are structurally related (EC, EGC, EGCG) on MP gels. We investigated the effects of oxidative treatment and the addition of TP on the structural changes, emulsifying properties, water-holding capacity, and gel characteristics of *N. taihuensis* MP. And the binding modes between the three different phenols and MP subunits were revealed utilizing molecular docking technology. At a systemic level, the mechanism of interaction between the two was analyzed, with the aim of expanding the potential applications of the functional food additive TP and the high-quality protein resource *N. taihuensis* in novel food systems. Based on the research results obtained, it was observed that the addition of various phenolic compounds had improved the structure and gelation properties of protein molecules to varying degrees, exerting a profound impact on the functional characteristics of food. This not only provided theoretical reference for the further development of high-quality products derived from *N. taihuensis* and novel gel products. It also offered a new pathway for the application of TP as a natural modifier in meat products, thus facilitating the production of more advanced products.

2. Materials and methods

2.1. Materials and chemicals

The mean weight of fresh *N. taihuensis* is 8.69 ± 0.50 g, and the average length is 9.54 ± 0.43 cm. The fish scales, skin, bones, and internal organs were manually removed, and then stored at −80 °C for freezing. 2,2′-dinitro-5,5′-dithiobenzoic acid (DTNB) and piperazine-N, N′-bis (2-ethanesulfonic acid) (PIPES) were purchased from Zhijiang Yunrui Huaxue Co., Ltd. (Hangzhou, China). Dithiothreitol (DTT) and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Jurui Reagent Co., Ltd. (Hangzhou, China). The remaining reagents, all of analytical grade, were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Extraction of MP

Firstly, an isolation buffer (pH 7.0) was prepared, which included 10 mM Na_2HPO_4/NaH_2PO_4 and 0.1 M NaCl, and was stored at 4 °C for later use. The frozen fish meat was ground using a meat grinder and blended with four volumes of the buffer. A homogenizer (T25, IKA Corporation, Germany) was used to blend the mixture at 10^4 rpm for 30 s. Subsequently, the mixture was subjected to centrifugation for 15 min at 5000 rpm under refrigerated conditions (CF16RN, Tokyo, Japan), with the precipitate retained. Three iterations of the process were conducted. The precipitate was washed twice with 0.1 M NaCl and homogenized for 25 s. Finally, the solution was centrifuged under the same conditions as before to obtain the MP precipitate, which was stored at 4 °C.

2.3. TP treatment

The MP precipitate was dissolved in 50 mM PIPES buffer (containing 0.6 M NaCl, pH 6.25) to prepare a viscous sol with a final concentration of 20 mg/mL. Three different phenols (EC/EGC/EGCG) were added to the MP sol to achieve a final concentration of 50 μmol phenolic/g MP. And the oxidation treatment was carried out under a hydroxyl radical system. The system contained 0.1 mM $FeCl_3$, 0.1 mM ascorbic acid, and 3 mM H_2O_2. At 4 °C, the reaction proceeded for 12 h. Finally, to end the reaction, Trolox (1 mM) was added. The unoxidized MP solution was designated as the non-oxidized control group (-OH·). The oxidized MP solution without TP was designated as the oxidized control group (+OH·). The oxidized MP solutions containing different TPs were designated as the EC group, EGC group, and EGCG group, respectively. In total, there were 5 groups of samples.

2.4. Oxidation of MP

2.4.1. Carbonyl content

The carbonyl content of the MP was determined using the DNPH colorimetric method (Rajkovic et al., 2017). The MP was mixed with a DNPH solution, and 20 % trichloroacetic acid (TCA) was added to precipitate it. The precipitate was subjected to three rounds of washing with an equal volume mix of ethanol and ethyl acetate to purge any leftover DNPH. The protein was then redissolved in 6 M guanidine hydrochloride, and the absorbance of the sample was measured at 370 nm (RF-6000, Shimadzu, Japan). The molar absorptivity at 370 nm was 2.2×10^4 $M^{-1}{\cdot}cm^{-1}$. The data were presented as μmol carbonyl/g MP.

2.4.2. Free amino group content

The free amino group content of the MP was determined using the o-phthaldialdehyde (OPA) method (Nuerjiang et al., 2023). 80 mg of OPA was dissolved in 2 mL of methanol, and 5 mL of SDS (2 %), 50 mL of sodium borate buffer (0.1 mol/L), and 200 μL of β-mercaptoethanol were added. The solution was diluted with distilled water to make up to 100 mL. In a 37 °C water bath, 0.2 mL of MP solution (2 mg/mL) was mixed with 4 mL of OPA and allowed to incubate for 5 min. The absorbance of the sample was measured at 340 nm. A standard curve was plotted using L-leucine, and the free amino group content was calculated as μmol/g MP.

2.4.3. Free radical content

The free radical content of the MP was determined using an Electron Spin Resonance (ESR) spectrometer (Bruker, Karisruhe, Germany). The lyophilized MP powder was added to an NMR tube and placed in the resonance chamber for spectrum recording. The free radical signal intensity was computed as the difference between the maximum peak signal and half the value of the minimum peak signal intensity.

2.4.4. Total sulfhydryl (T-SH) content

The T-SH sulfhydryl content of the MP was determined using DTNB. A mixture of 1.5 mL of the MP solution (5 mg/mL) and 10 mL of Tris-Gly buffer (containing 8 mM urea, 4 mM EDTA, 0.5 mL of DTNB, pH 8.0) was prepared and allowed to react at 25 °C for 0.5 h. The mixture was then centrifuged at 4 °C to obtain the supernatant, and the absorbance of the sample was measured at 412 nm.

2.4.5. Protein oxidation products

Due to the fluorescent property of Schiff base compounds (SB), which are oxidation products of proteins, fluorescence spectroscopy can be employed to analyze the degree of oxidation in MP. The emission spectrum of the SB structure was measured in the range of 400–500 nm. In the experiment, a 350 nm excitation wavelength and a 460 nm emission wavelength were used, with slit widths of 10 nm for both.

Images were captured using Image J software, and the fluorescence intensity was calculated.

2.5. MP structure

2.5.1. Fourier transform infrared (FTIR)

FTIR spectroscopy was used to analyze the secondary structure of the MP (Perkin Elmer, Massachusetts, USA), along with peak-fitting analysis. The sample was freeze-dried for 24 h and mixed with KBr powder in a ratio of 1:100 (*w*/w). The mixture was ground using a mortar and pestle to ensure uniformity and then pressed into a thin pellet using a tablet press. The spectrum was recorded using an FTIR spectrometer within a wavenumber range of 4000–400 cm^{-1}, with a total of 32 scans. The FTIR spectral data was subsequently processed and analyzed using OMNIC software.

2.5.2. Intrinsic fluorescence spectroscopy

The intrinsic fluorescence spectrum of the MP was determined using a fluorescence spectrophotometer. Initially, the sample was diluted to a concentration of 0.2 mg/mL with phosphate buffered saline (PBS,10 mM, pH 7.0). With an excitation wavelength of 280 nm, the emission spectrum of the sample was captured over the range of 310–440 nm using a slit width of 5 nm. Additionally, the maximum fluorescence emission wavelength (λ_{max}) was recorded.

2.5.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The cross-linking status of MP samples was analyzed using SDS-PAGE with 5 % stacking gel and 10 % separating gel (Nie et al., 2024). Initially, 1 mL of MP sample was mixed with 2.7 mL of 5 % SDS solution to prepare the MP dilution. After boiling the sample at 95 °C for 5 min and subsequently cooling it to 25 °C, it was centrifuged at 12000 rpm for 10 min. The supernatant was diluted until the protein concentration reached 4 mg/mL and mixed with DTT in a 4:1 volume ratio for reduction electrophoresis. During electrophoresis, stacking was performed at an initial voltage of 80 *V* for 25 min, followed by analysis at 110 V for 1 h. After staining the gel with Coomassie Brilliant Blue R 250 for 2 h, it was subsequently destained to eliminate any excess dye. Finally, gel images were captured using a gel imaging system.

2.5.4. Microstructure observation

The microstructure of MP samples was observed using scanning electron microscopy (SEM, SU8010, HITACHI, Japan). Initially, the samples were freeze-dried and then fixed using double-sided conductive adhesive tape. Excess powder was blown off, and a uniform layer of gold was sputter-coated onto the samples. The processed samples were placed on the observation stage and observed at a magnification of 5000 times under an alternating acceleration voltage of 10 kV. Images were captured to document the morphology.

2.6. Functional properties of MP

2.6.1. Emulsifying properties

The emulsifying properties of the MP, including emulsifying activity index (EAI) and emulsifying stability index (ESI), were determined using the traditional turbidity method (Fan et al., 2021). Initially, the sample was dissolved in PBS (pH 7.0) and soybean oil was added. The mixture was then homogenized at 10^4 rpm for 5 min to ensure thorough mixing. Subsequently, 50 μL of the emulsion was added to 5 mL SDS solution (0.1 %, *w/v*). Finally, emulsions were collected from the bottom at 0 min and 10 min, and their absorbance values were measured at 500 nm, recorded as A_0 and A_{10}, respectively.

$$\mathrm{EAI}\ (\mathrm{m2/g}) = \frac{2 \times 2.303 \times A_0 \times d}{c \times \varnothing \times 10^{4.}}$$

$$\mathrm{ESI}\ (min) = \frac{A_0}{A_0 - A_{10}} \times 10$$

here, *d* refers to the dilution factor, *c* represents the protein concentration before emulsification (mL), and ∅ denotes the volume fraction of the oil phase (*v*/v).

2.6.2. WHC

To determine the WHC, 2 g of the sample was placed in a centrifuge tube equipped with an ultrafiltration membrane (MWCO = 100 kDa). The sample was centrifuged at 6000 rpm for 15 min at 4 °C. The supernatant was discarded, and the mass of the remaining gel was measured. WHC was calculated as $\mathrm{WHC} = \frac{m_1}{m_0} \times 100\%$, where m_1 represents the mass of the gel after centrifugation and m_0 represents the mass of the gel before centrifugation.

2.6.3. Surface hydrophobicity (H_0)

The surface hydrophobicity of the protein was determined by measuring the binding capacity of MP to bromophenol blue (BPB) (Wu et al., 2024). A protein solution was prepared from the sample to a concentration of 5 mg/mL. The MP solution was then uniformly mixed with 200 μL of BPB solution (1 mg/mL). The mixture was oscillated at room temperature for 10 min to ensure thorough mixing. The sample was centrifuged at 4000 rpm for 10 min at 4 °C, and the supernatant was retained. The supernatant was diluted 10 times with PBS, and the absorbance value of the sample at 595 nm was measured, recorded as A_2. PBS was used as a control and its absorbance value was recorded as A_1. $\mathrm{BPB\ bound}\ (\mu g) = 200\ \mu g \times (A_1 - A_2)/A_1$.

2.6.4. Gel strength

The gel strength was analyzed using a texture analyzer (TA. XT. Plus, Stable Micro Systems, UK) with a cylindrical probe (P/0.5). The pre-test and post-test speeds were set to 1 mm/s, and the test speed was set to 0.5 mm/s. The trigger force was 5 g, the compressive strain was 50 %, and the data acquisition rate was 200 points per second (pps). The probe was then used to perforate the center of the sample. The gel strength was determined as the maximum force sensed during the downward movement of the probe (Liang et al., 2024).

2.6.5. Rheological properties

The rheological properties of native MP and the MP/TP combination system were determined using a rheometer. The sample was placed between the parallel plate measurement system (with a gap of 1 mm). To prevent evaporation, the edges of the sample were coated with paraffin oil. The experimental temperature was set to 25 °C. Dynamic tests, including strain and frequency scans, were conducted to ensure that the measurements were within the linear viscoelastic region (LVR). A temperature sweep experiment was conducted within the LVR, with a heating range of 20–70 °C and a heating rate of 1 °C /min. During the measurement, the changes in storage modulus (G') were recorded to indicate the effects of different TP additions and oxidation treatments on the gelation of MP.

2.7. Molecular docking

To further understand the interaction mechanisms between three types of TP (putative phenolic compounds) and MP, myosin was selected for molecular docking studies. Firstly, a three-dimensional model of myosin was built using the Uni-Fold online server. Then, Chem3D (PerkinElmer, Waltham, USA) was utilized to illustrate and minimize the chemical structures of EC, EGC and EGCG. Finally, molecular docking simulations between MP and the phenolic compounds were performed using BIOVIA Discovery Studio 4.5 software (Leng et al., 2024).

2.8. Data analysis

All experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). SPSS Statistics 27 (IBM, Chicago, USA) was utilized to perform one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests to determine the significance of differences. Statistical significance was considered at $p < 0.05$. Graphs were generated using Origin Software 2021 (OriginLab Corp., MA, USA).

3. Results and discussion

3.1. Oxidation of MP

3.1.1. Carbonyl groups and free amino groups

The -NH or -NH_2 groups located on the side chains of amino acids within proteins are prone to oxidation, leading to their conversion into carbonyl derivatives. Consequently, the carbonyl content measurement is a key indicator for assessing oxidative damage to proteins (Fan et al., 2025). Generally, an elevation in carbonyl content signifies a greater level of protein oxidation. The changes in carbonyl content are shown in the Fig. 1A. The oxidized MP group without TP addition had the highest carbonyl content, reaching 0.94 μmol/g. With EC, EGC, and EGCG included, the carbonyl content significantly decreased, indicating that TP can effectively inhibit protein oxidation. Among them, the EGCG group showed the greatest reduction in carbonyl content, followed by EGC and EC. This was mainly because EGCG possessed multiple phenolic rings, thus enabling it to acquire strong free radical scavenging ability (Fan et al., 2024). On one hand, the conjugated structure of the phenolic rings stabilized the intermediates formed during oxidation, thereby enhancing the antioxidant effect. On the other hand, the electron-donating ability of the phenolic rings facilitated redox reactions and promoted free radical scavenging. Correspondingly, as observed in the Fig. 1B, the free amino group content of unoxidized MP was 10.81 μmol/g, and the free amino group content of the oxidized group decreased by 27.32 %. This might be a result of the oxidation of amino groups on side chain amino acids to form carbonyl groups. Furthermore, the addition of TP further promoted the decreasing trend of amino groups, with the EGCG group showing the most significant effect. This might be because TP was oxidized to generate electrophilic products, such as quinones, which could bind to -NH_2 to form complexes (Liu et al., 2021). Chen et al. also reported a similar phenomenon, observing a persistent decline in free amino group content after binding different polyphenols to quinoa protein (Chen, Li, et al., 2024; Chen, Yang, et al., 2024).

3.1.2. Free radicals

The oxidation process of proteins often involves the generation of free radicals, which can generally be viewed as a free radical-mediated chain reaction (Li et al., 2024). As illustrated in Fig. 1C, in comparison to the group that remained unoxidized, the free radical signal intensity of the oxidized MP group significantly increased. The generation of a large number of free radicals not only disrupted the normal function and structure of amino acids but might also react with proteins to produce products, decompose, or generate new free radicals, further exacerbating oxidative damage. After the addition of the three phenolics, the signal intensity decreased in all cases, indicating that TP can

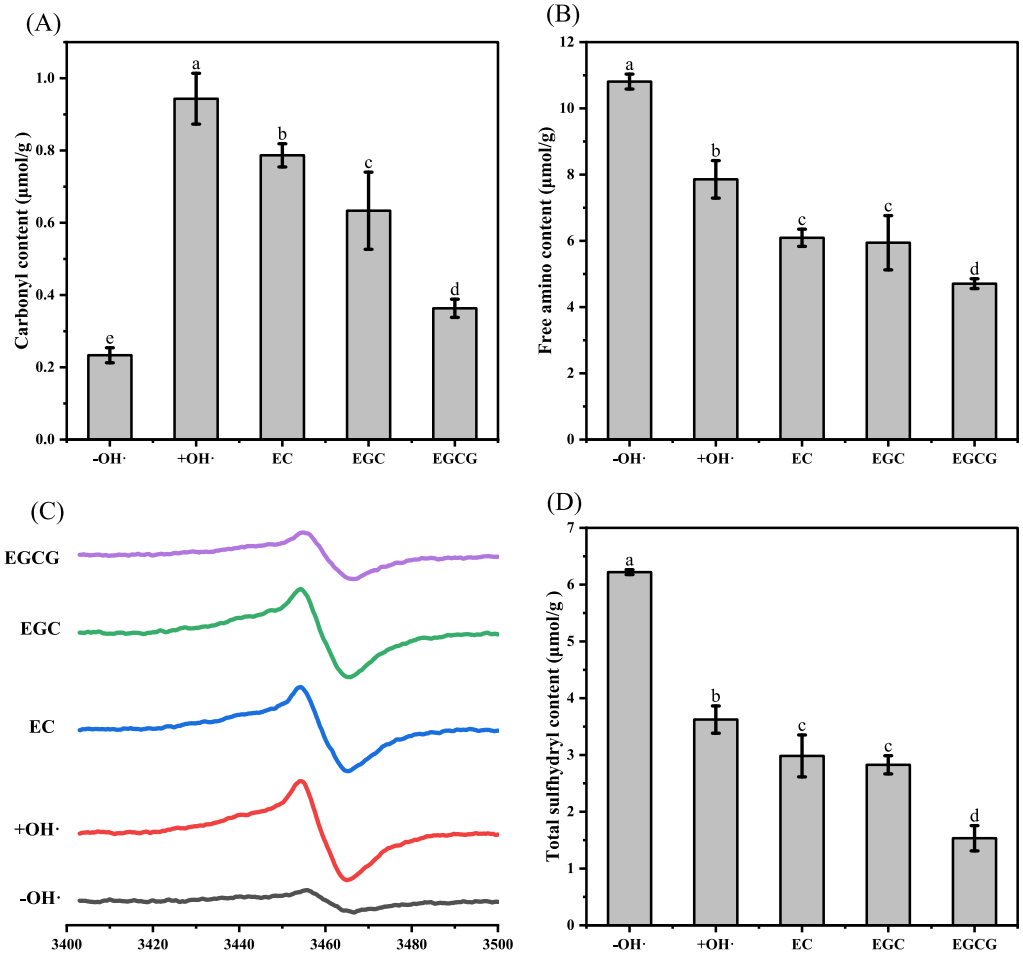

Fig. 1. Carbonyl (A), free amino (B), free radical (C), and total sulfhydryl content (D) before and after myofibrillar protein (MP) oxidation, as well as after modification with different tea polyphenols (TPs). Different lowercase letters in each column indicate statistically significant differences ($p < 0.05$).

significantly inhibit the production of free radicals during the oxidation. Among them, EGCG exhibited the best antioxidant effect. This was mainly attributed to TP's ability to directly capture and neutralize free radicals, undergoing a coupling process that converted them into ferulic acid radicals. TP inhibited the activation of pro-oxidant enzymes such as xanthine oxidase, indirectly reducing the number of free radicals. Conversely, it regulated the expression of enzymes and genes related to the antioxidant enzyme system, enhancing the ability to scavenge free radicals. The 3′,4′-dihydroxy groups on the catechin structure chelated with free metal ions, such as $Fe^{3+}$and Cu^{2+}, which catalyze the production of free radicals, rendering them catalytically inactive. This delay could effectively mitigate the denaturation and deterioration of protein-related products (Li et al., 2025).

3.1.3. T-SH groups

During oxidation, sulfhydryl groups are vulnerable to attack by free radicals, resulting in their transformation into either intramolecular or intermolecular disulfide bonds, which can lead to protein crosslinking. In myosin, sulfhydryl groups are mainly concentrated in the head region, playing a crucial role in the protein structure (Zhu et al., 2023). The changes in the T-SH content of MP after oxidation treatment and the addition of TP are shown in Fig. 1D. It could be seen from the Fig. 1D that the T-SH content of MP significantly decreased after oxidation, dropping from 6.22 μmol/g protein to 3.62 μmol/g protein. Upon the addition of TP, the sulfhydryl content continued to decrease, with EGCG having the most pronounced effect, reducing it to nearly a quarter of the unoxidized group. This might be because TP was initially oxidized to semiquinone or quasiquinone, which then formed a relatively stable thiol-quinone adduct with sulfhydryl groups through a Michael reaction, further reducing the T-SH content (Cao et al., 2018). This was consistent with the results obtained by Wu et al. (Wu et al., 2022), who concluded that it was related to the covalent interaction between polyphenols and sulfhydryl groups. This covalent binding formed stable compounds, consuming a part of sulfhydryl groups or inactivating them. In addition, non-covalent interactions such as hydrophobic interactions and hydrogen bonds existed between tea polyphenols (TP) and proteins. These interactions altered the spatial conformation of myosin protein (MP) molecules, leading to a decrease in the activity or masking of some sulfhydryl groups, and consequently reducing the total content.

3.1.4. Protein oxidation products

The protein oxidation product, SB, is capable of emitting fluorescence. The impact of oxidation and TP treatment on SB content in *N. taihuensis* is illustrated in Fig. 2A. After MP oxidation, the strongest fluorescence intensity was observed, it was approximately three times as high in comparison to the unoxidized group. This might be because of the generation of a large amount of reactive oxygen species (ROS) when proteins underwent oxidative stress. These ROS could react with amino acid side chains containing easily oxidizable functional groups, converting them into aldehydes or ketones, and further interacting with the -NH_2 groups of other amino acids in proteins (such as the ε-NH_2 of lysine) to form SB (Utrera et al., 2014). The addition of the three types of TP significantly reduced the fluorescence intensity, indicating their notable inhibitory effect on protein oxidation, particularly EGCG. This was consistent with the aforementioned results regarding changes in carbonyl and thiol group content. TP not only directly or indirectly scavenged free radicals to reduce the production of SB, but also effectively inhibited lipid peroxidation. These peroxidized lipids generated a series of ROS. By chelating metal ions, TP inhibited the occurrence of peroxidation reactions, ultimately mitigating the oxidative damage to proteins caused by their products. Tian (Tian et al., 2021) also reported that the addition of TP reduced the formation of SB in whey protein.

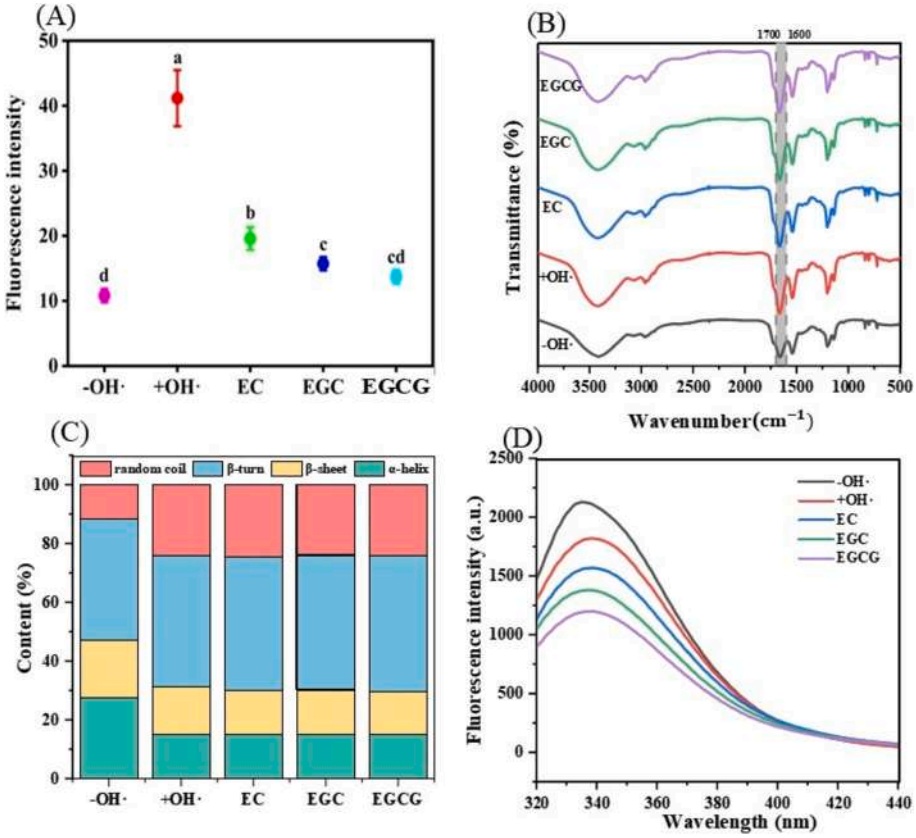

Fig. 2. The fluorescence intensity of oxidation products (A), Fourier Transform Infrared (FTIR) spectra (B), secondary structure content (C), and intrinsic fluorescence spectra (D) before and after oxidation, as well as after modification with different tea polyphenols (TPs).

3.2. MP structure

3.2.1. FTIR and secondary structure

The secondary structure of proteins is closely related to the strength and density of gels. FTIR can be employed to detect alterations in the content of protein secondary structures, as shown in Fig. 2B. The amide I band (1600–1700 cm^{-1}) is most sensitive to changes in secondary structure and can be used to monitor the vibrational stretching of the peptide bond C═O (Wang, Ji, et al., 2024; Wang, Wang, et al., 2024). Therefore, peak fitting analysis can be conducted using OMNIC and Peakfit software to determine the content of each secondary structure, with the results shown in Fig. 2C. When compared to the unoxidized group, the content of α-helix decreased from 27.5 % to 14.9 % after oxidation. Conversely, with the progression of oxidation treatment and the addition of TP, the content of β-turn and random coil increased significantly. α-Helix is mainly maintained by hydrogen bonds. This indicated that oxidation facilitated the disruption of α-helix and its conversion to β-turn and random coil (Zhang et al., 2025). The protein structure became looser and more flexible, with the molecular conformation shifting from relatively ordered to disordered. Furthermore, the content of each secondary structure did not show significant differences after the addition of EC. However, the incorporation of EGC and EGCG slightly increased the content of α-helix, while the content of β-sheet and random coil decreased. This suggested that EGC and EGCG could reduce the breakage of hydrogen bonds between MPs and the conversion of α-helix to some extent, inhibiting structural unfolding and enhancing stability. The primary cause of this discrepancy was that the binding between EC and proteins was relatively weak, while EGC and EGCG bound to MPs through hydrogen bonds and hydrophobic interactions. This interaction could lead to the loosening of peptide chains, thereby increasing the content of α-helix.

3.2.2. Intrinsic fluorescence spectroscopy

Protein fluorescence primarily relies on the absorption spectrum generated by chromophores absorbing ultraviolet light, particularly tryptophan, which is often used as an endogenous fluorescent probe to assess changes within the amino acid microenvironment (Estévez et al., 2008). The research results are shown in Fig. 2D. The λ_{max} of MP is around 330 nm. The addition of the three types of TP caused a red shift in the λ_{max} (Huang et al., 2024). Meanwhile, with the progression of oxidation treatment and the binding of TP, the fluorescence intensity of MP gradually decreased. These results all demonstrated that the tertiary structure of the protein underwent unfolding. The change resulted in the exposure of tryptophan, originally within the hydrophobic core, to the exterior hydrophilic surroundings, increasing the surrounding polarity and causing a red shift in λ_{max} (Huang et al., 2024). The reduction in fluorescence intensity suggested that tryptophan was oxidized or free radicals were generated, leading to fluorescence quenching. The exposure of tryptophan in a polar environment also affected its fluorescent properties, further reducing the fluorescence intensity. Comparable fluorescence quenching phenomena have been documented for the interaction between MP and theaflavins (Nie et al., 2024).

3.2.3. SDS-PAGE

The protein composition and soluble protein patterns of MP, which had undergone oxidation and TP treatment, were determined through SDS-PAGE in order to more clearly reveal how TP had affected the protein cross-linking status. This was done to further advance the research into the specific mechanisms of action of polymers during the protein gelation process. The results were shown in Fig. 3A. Upon observing the bands, it was found that MP primarily consisted of myosin heavy chain (MHC), paramyosin, actin, and tropomyosin (Li et al., 2020). Compared to the unoxidized group (lane 3), the myosin heavy chain bands had exhibited a decrease in intensity to a certain extent after oxidation, with the band for the oxidized MP without TP addition appearing the faintest. A similar trend in band intensity changes was also observed for the actin bands. This evidenced that oxidation had caused a relative loss of myosin heavy chain and actin, promoting aggregation of the soluble proteins. Notably, TP had significantly inhibited the decrease in protein band intensity, indicating an interaction between

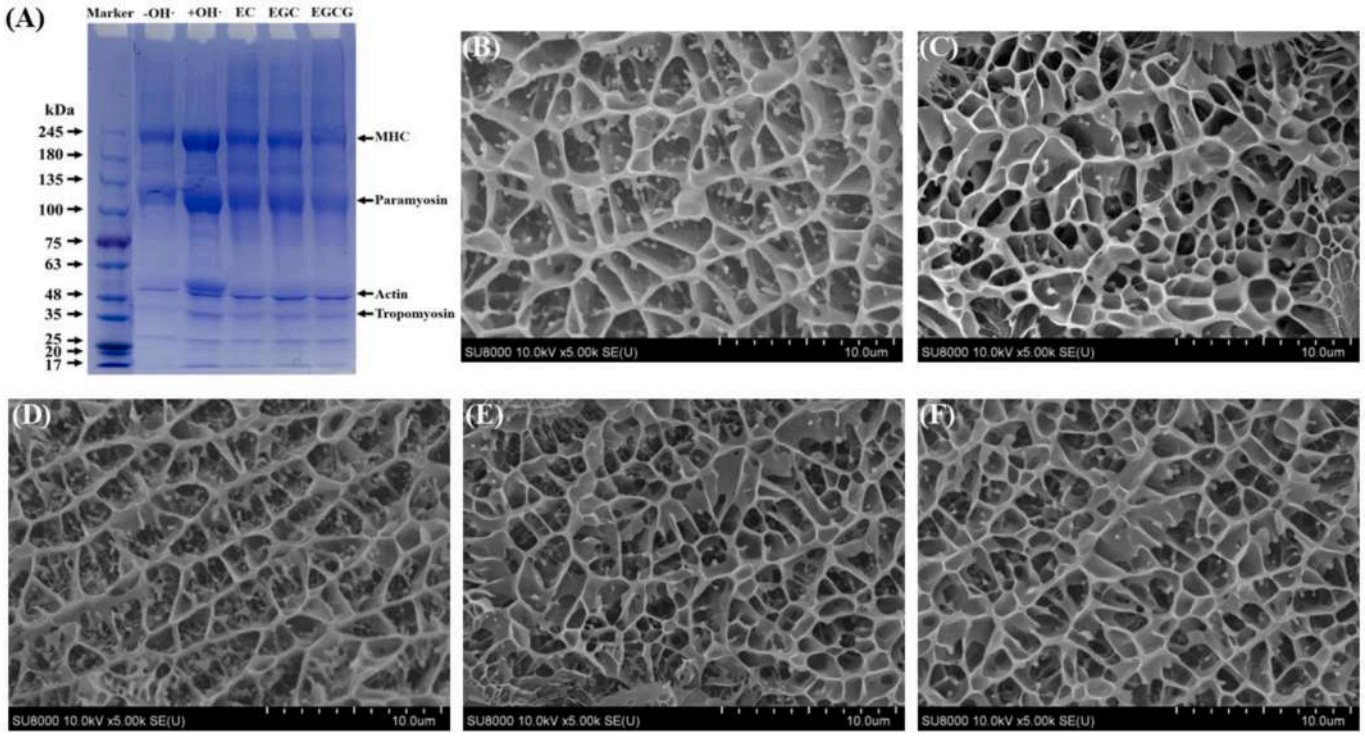

Fig. 3. Electrophoresis bands (A) and microstructure (B—F) of myofibrillar protein (MP) before and after oxidation, as well as after modification with different tea polyphenols (TPs). B: -OH·; C: +OH·; D: Epicatechin (EC); E: Epigallocatechin (EGC); F: Epigallocatechin gallate (EGCG).

the protein and TP. This indicated that TP had effectively inhibited, to a certain level, the cross-linking and aggregation that resulted from protein oxidation.

3.2.4. SEM

The microstructure of MP with different TP additions under oxidation conditions is shown in Fig. 3B-F. As observed, the unoxidized protein formed a three-dimensional network structure with numerous voids, appearing as thick filaments. After oxidation, the protein developed more cross-links, resulting in a rougher surface with many smaller pores or irregular structures. With the addition of the three types of TP, the void areas in the gel further decreased, and the network structure became denser, with the MP appearing as clear, fine filaments. This could be due to the interactions involving cross-linking between the reactive hydroxyl groups in TP and various groups in the protein, leading to a more ordered and uniform gel structure with reduced local aggregation. The SEM findings suggested that incorporating TP enhanced the stability and elasticity of the gel. Similar phenomena were observed in the study by Han et al. (Han et al., 2020).

3.3. Functional properties of MP

3.3.1. EAI and ESI

The emulsifying properties of proteins refer to their ability to adsorb at the oil-water interface, forming and stabilizing oil-in-water mixtures (emulsions). These properties are typically evaluated through EAI and ESI, which measure the protein's adsorption capacity at the oil-water interface and the stability of the emulsion during a specific timeframe, respectively (Shokri et al., 2022). The EAI and ESI values after TP treatment are shown in Fig. 4A-B. It was observed that oxidation caused a decline in the emulsifying performance. Specifically, EAI decreased by approximately 26.57 %, and ESI decreased by about 29.38 %. After adding different TP compounds, EAI and ESI further decreased to varying degrees. This might be the result of phenolic hydroxyl groups from TP binding to MP, which decreased H_0. This weakening of the adsorption interaction between MP and oil droplets ultimately resulted in a decline in emulsifying performance (Wang, Ji, et al., 2024; Wang, Wang, et al., 2024). Additionally, the aggregation of MP molecules was also a significant factor contributing to the reduced emulsifying properties. The EGC-treated group exhibited a higher ESI compared to the other two types of TP, possibly because its binding affinity was relatively weaker and its interaction with the system was more harmonious. This facilitated the formation of a more stable binding between EGC and the protein, thereby more effectively stabilizing the emulsion system.

3.3.2. WHC

WHC is an important quality attribute of meat, directly influencing the quality and nutritional value. Furthermore, WHC can reflect the structure of the gel to a certain extent; a higher WHC generally indicates a more uniform and dense gel structure. During the establishment of the MP gel network framework, hydrogen bonds and hydrophobic interactions are the primary chemical forces affecting the WHC of proteins. As shown in Fig. 4C, the WHC of MP substantially reduced after oxidation treatment. Oxidation primarily altered the protein structure, causing more hydrophobic groups to be exposed and consequently weakening the water-holding ability (Yolandani et al., 2023). This aligned with the discoveries of Chen et al.(Chen, Li, et al., 2024; Chen, Yang, et al., 2024), who also observed a significant increase in centrifugal loss of pork with increasing oxygen concentration, indicating a notable decrease in WHC. Upon the addition of the three types of TP, the WHC improved, with the degree of improvement ranked as follows: EGCG > EGC > EC.

3.3.3. Surface hydrophobicity (H_0)

H_0 indicates the actual arrangement of hydrophobic amino acid residues across the protein surface and is an important physicochemical property manifested as water molecule repulsion (Yolandani et al., 2023). Using the amount of BPB binding as an indication of protein surface hydrophobicity, the experimental results are shown in Fig. 5A. After oxidation treatment, the H_0 exhibited a significant downward trend. This might result from the partial cross-linking and aggregation of proteins, decreasing the accessibility of hydrophobic residues on the molecular surface. With the introduction of TP, there was a further reduction in H_0. This indicated that the affinity between the MP surface and water molecules continued to increase, possibly due to the interaction between TP and hydrophobic amino acids (Wang, Ji, et al., 2024; Wang, Wang, et al., 2024). At the same time, the binding of TP reduced the conversion of protein α-helices, resulting in reduced exposure of hydrophobic groups. Additionally, phenol-mediated aggregation of MP could delay the unfolding of hydrophobic amino acids. In this study, MP treated with EGCG had the lowest H_0, at 102.67 μmol/g, compared to EC and EGC. However, there was no notable distinction between the EC and EGCG treatment groups. The main reason lied in the unique chemical structure of EGCG and its mode of interaction with proteins. EGCG possessed multiple phenolic hydroxyl groups and primarily binds to the hydrophobic cavities of proteins, forming stronger interactions with the hydrophobic regions in MP (Botten et al., 2013).

3.3.4. Gel strength

In this study, the gel strength under varying treatment conditions was also measured, including standalone oxidation treatment and

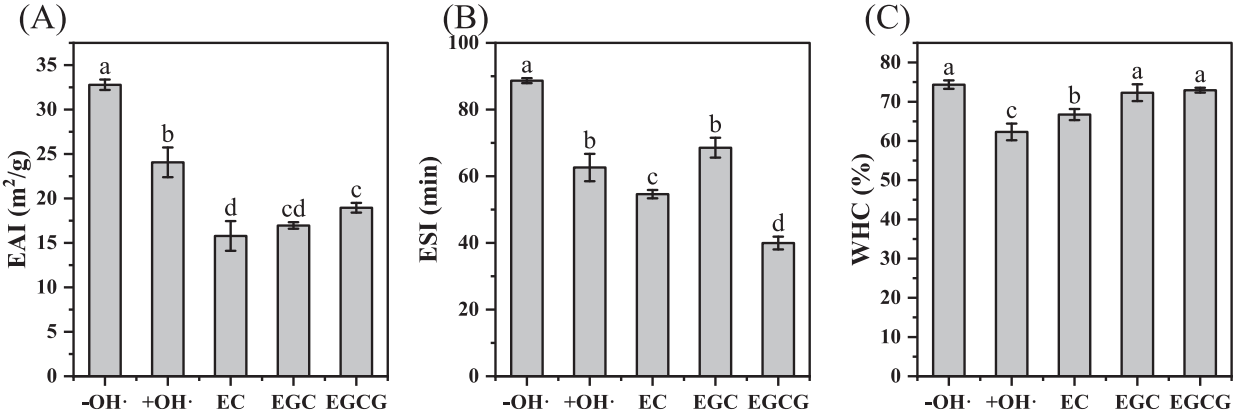

Fig. 4. Emulsifying activity index (EAI) (A), emulsifying stability index (ESI) (B), and water holding capacity (WHC) (C) of myofibrillar protein (MP) before and after oxidation, as well as after modification with different tea polyphenols (TPs). Different lowercase letters in each column indicate statistically significant differences ($p < 0.05$).

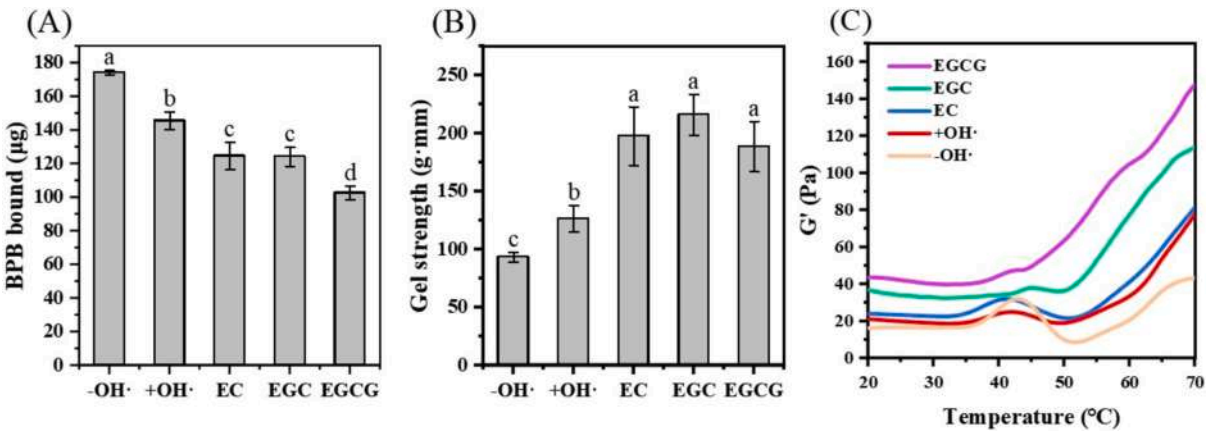

Fig. 5. Surface hydrophobicity (A), gel strength (B), and rheological properties (C) of myofibrillar protein (MP) before and after oxidation, as well as after modification with different tea polyphenols (TPs). Different lowercase letters in each column indicate statistically significant differences ($p < 0.05$).

combined treatment with oxidation and TP, as illustrated in Fig. 5B. Gel strength, which indicates the initial pressure necessary to pierce through the gel, reflects the firmness of the gel structure formed by proteins and is a crucial indicator for assessing gel performance (Chang et al., 2024). As indicated in Fig. 5B, after the oxidation process, the gel strength significantly increased. Upon subsequent addition of TP, the strength further heightened. This indicated that oxidation facilitated the interaction between amino acids in proteins, thereby bolstering the overall gel strength and promoting gel formation. Firstly, TP, serving as a crosslinking agent, bound with different nucleophilic groups in proteins, which facilitated the formation of more crosslinking points between protein molecules. Secondly, during the process of gel formation, TP reduced the degradation of network proteins, contributing to the maintenance of the structural integrity of the network. Similarly, Fan et al. (Fan et al., 2025) also demonstrated that appropriate denaturation can enhance the rigidity of the gel network structure and improve its strength. When comparing the outcomes after treating with the three types of TP, it was evident that the gel strength in the EGCG group experienced a decline. This could be due to the fact that EGCG is more prone to forming covalent conjugates with certain amino acid residues in proteins, thereby altering the secondary structure of the proteins and making them relatively unstable. Consequently, this structural modification ultimately resulted in a reduction in gel strength.

3.3.5. *Rheological properties*

Fig. 5C depicts the G' spectra of MP gels under oxidation and combination treatments with three different TPs. As shown in Fig. 5C, all MP samples exhibited distinct rheological patterns. The unoxidized MP group displayed a typical rheological transition peak between 40 and 45 °C, which was linked to temporary hydrophobic interactions and structural reorganization of the myosin heads (Cao et al., 2016). After oxidation and the addition of TP, the transition peaks of MP decreased to varying degrees, suggesting that the myosin's interactions in a head-to-head configuration were suppressed. The addition of EC, EGC, and EGCG all resulted in a rise in the G' values, suggesting that the presence of TP further enhanced the gelling ability of the proteins. This could be because the multiple phenolic hydroxyl groups of TP covalently bound with sulfhydryl groups and amino groups on the side chains of MP, forming "thiol-quinone" and "amino-quinone" adduct products. This significantly increased the degree of protein crosslinking, enhancing the gel network structure and increasing G'. This was consistent with the observations from SEM as well.

3.4. *Molecular docking*

The above studies indicated that the interactions between TP and MP played a crucial role in maintaining and improving protein properties. The subunits of EC, EGC, and EGCG molecules were docked with myosin to reveal their binding sites and interaction modes, as illustrated in Fig. 6. The three-dimensional view of the docking results showed that TP had multiple sites capable of binding with phenols, primarily within the hydrophobic pockets of myosin. Furthermore, the docking results revealed that the main binding forces forming the TP-MP complexes were van der Waals forces, hydrogen bonds, and hydrophobic interactions. Among these, the formation of hydrogen bonds significantly enhanced the stability of the complexes (Han et al., 2022). Ile716, Lys147, and Leu773 interacted with EC through hydrophobic interactions, while Gln149, Tyr725, and Glu777 interacted with EC via hydrogen bonds. In the case of EGC, hydrogen bonds were formed with Ser714 and Gln149, and hydrophobic interactions occurred with Pro713. Similarly, the flavonoid structure of EGCG engaged with MP via hydrogen bonding and hydrophobic associations. Additionally, it was observed that Pi-Pi stacking interactions existed between EGCG and MP, which enabled EGCG to more effectively inhibit protein aggregation and prevent the development of β-sheet structures. In summary, the interactions between MP and TP blocked accessible exposed hydrophobic sites (Guo et al., 2021), leading to a reduction in protein surface hydrophobicity and ultimately affecting emulsifying properties. Meanwhile, under the action of multiple binding modes, the two formed a complex, which wrapped or concealed the active groups of the protein. This effectively reduced the direct contact between the molecules and free radicals, making the structure more compact or stable, thereby enhancing the antioxidant capacity and reducing the likelihood of free radical formation.

4. Conclusion

This study focused on the myofibrillar protein (MP) of *N. taihuensis* and delved into the regulatory effects of tea polyphenols (TP), particularly three typical catechins: epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), on the structure and gel properties of MP under oxidative conditions. The results indicated that TP effectively delayed the oxidation process of MP and significantly reduced the generation of oxidation products. Moreover, it enhanced the stability and ductility of the gel conformation of MP by altering its structure. Notably, due to its unique chemical structure with more phenolic rings, EGCG formed complexes with MP that exhibited higher antioxidant activity. Furthermore, the addition of TP significantly improved the functional properties of MP gels, such as increased gel strength, water-holding capacity, and altered rheological properties. Molecular docking simulations further revealed the interaction mechanisms between TP and MP. In summary, this study not only provided new insights into the

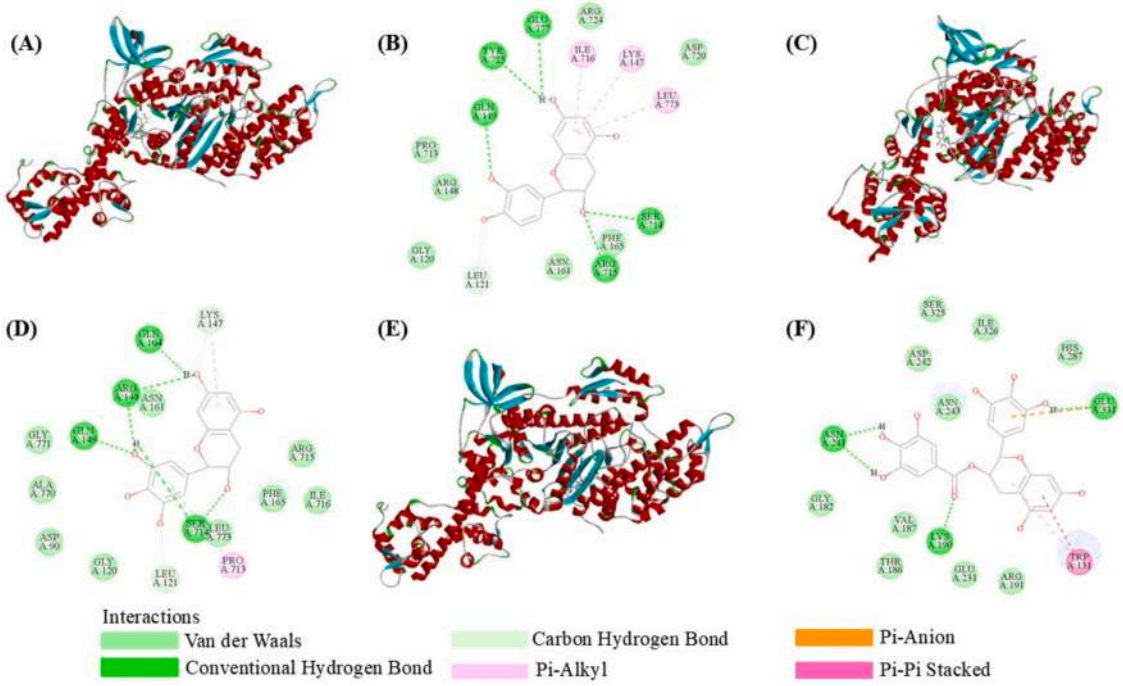

Fig. 6. Molecular docking results of the interaction between different tea polyphenols (TPs) and myosin. A, C, E: 3D structures of myosin interacting with epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) respectively; B, D, F: 2D diagrams of molecular docking analysis between myosin and epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG).

relationship between polyphenol types and MP structure but also confirmed the great potential of TP as a natural antioxidant in modifying *N. taihuensis* surimi products, offering theoretical and technical support for the development of high-quality aquatic products. However, the specific process of improving the gel state to obtain proteins with optimal performance still required further investigation and determination.

CRediT authorship contribution statement

Xiangxiang Ni: Writing – original draft, Investigation. **Mengting Li:** Writing – original draft, Investigation. **Zhiwei Huang:** Software, Formal analysis, Data curation. **Yinyin Wei:** Validation, Methodology. **Chaoyi Duan:** Visualization, Software. **Ruixi Li:** Resources. **Yajie Fang:** Methodology, Investigation. **Xiu Wang:** Funding acquisition, Conceptualization. **Mingfeng Xu:** Writing – review & editing, Conceptualization. **Rongrong Yu:** Project administration, Funding acquisition.

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.

Acknowledgments

This work was financially supported by Jiaxing Nanhu University's Scientific research start funds (No: 70500000/041) and Basic Research Project of Wenzhou Science and Technology Bureau (No: Y2020076).

Data availability

Data will be made available on request.

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