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Effect on myofibrillar protein gelation induced by eugenol modification under oxidative stress

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Abstract

This study aimed to investigate the effects of eugenol (Eu) at different concentrations (0, 10, 50, 100, 200, and 300 μM/g protein) on the gel properties and chemical structure changes of porcine myofibrillar protein (MP). The results showed that Eu inhibited the increase of surface hydrophobicity and carbonyl content but did not prevent the oxidation-induced loss of thiol groups. Moreover, Eu intensified the loss of the α-helical conformation as well as the tertiary structure of MP under oxidative stress. The physicochemical changes at 10 and 50 μM/g Eu resulted in a significant enhancement of the gelling ability of MP and enhanced the positive role of oxidation in building elastic gel networks. Conversely, Eu at the concentrations of 100, 200, and 300 μM/g was not conducive to gelling ability, especially at 300 μM/g, and these concentrations were associated with Eu-induced protein conformational changes.

Keywords: eugenol; myofibrillar protein; gelation; rheology properties; conformation.

Practical Application: Eugenol plays a very important role in traditional meat products in China, which has good antioxidant properties while enhancing flavor. Therefore, this paper studied the effects of different doses of eugenol on the gel properties of pork myofibrin, and selected appropriate dosage to lay a foundation for the application of eugenol in actual meat products processing.

1 Introduction

Myofibrillar protein (MP) is a major component of all muscle proteins (Zhou et al., 2019a), it plays a leading role in the gelation and structure formation of meat products, and is crucial to the quality of meat products (including tenderness, texture, color, etc.). The oxidation of MP readily occurs during the production of meat products, and this causes changes in the functional properties of the resulting proteins, including gel properties and water-holding capacity (WHC), which ultimately leads to a deterioration in the quality of meat and meat-based products (Xiong & Guo, 2020; Zhang et al., 2021). Therefore, the inhibition of MP oxidation has important commercial value.

In recent years, with improvements in consumers' regard for health and food safety, there has been an accompanying increase in concerns about consuming synthetic additives (Xiang et al., 2019). Currently common synthetic antioxidants include $C_{11}H_{16}O_2$ (BHA), $C_{15}H_{24}O$ (BHT) and $C_{10}H_{14}O_2$ (TBHQ), but toxicological and nutritional concerns have limited their use (Al-Hatim et al., 2022), thus natural plant-derived antioxidants have been widely investigated for their safety and health advantages, especially for plant polyphenols (Cheng et al., 2020). Polyphenols are primarily derived from the roots, leaves, skin, and fruits of plants, and are chemically active secondary metabolites with complex structures. They possess useful functional properties that contribute to their use as natural and safe antioxidants in food (Olszewska et al., 2020). For example, onion skin had a positive effect on the sensory properties and storage quality of meat loaf, which could extend the storage period of meat loaf to 9 days (Wang et al., 2022), in addition, oregano essential oil (OE) and rosemary extract (RE) inhibited the oxidation of lipid and protein of chicken stored at different refrigeration time, and improved their sensory quality (Al-Hijazeen, 2022). More and more plant antioxidants are being used in meat products, increasing their commercial value.

Phenols react with proteins, and these interactions can be divided into two categories: non-covalent and covalent. Generally speaking, the former is reversible, while the latter is irreversible. These interactions can change the structure, conformation, and physicochemical properties of proteins, thereby subsequently leading to changes in protein function (Chen et al., 2020; Lv et al., 2021). Within an oxidizing environment, phenolic hydroxyl groups in plant polyphenols generally act as hydrogen donors, whereby they provide hydrogen molecules that combine with free radicals that are generated by protein oxidation, consequently blocking the chain reaction of free radical oxidation, and preventing the further oxidation of proteins; in this process, phenol rings are transformed into quinone molecules (Jia et al., 2017).

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However, the presence of excessive polyphenol can destroy protein structure and limit the gelling capacity of MP (Jia et al., 2019). As indicated by structural alterations, gallic acid, chlorogenic acid, and rosmarinic acid were found to accelerate the loss of thiol groups and produce irreversible protein mutations. Meanwhile, epigallocatechin gallate (EGCG), mulberry polyphenols, and green tea extract have been demonstrated to reduce disulfide cross-linking, which is the key driver of MP gelation via heat treatment, by combining quinone and thiol groups (Cheng et al., 2020; Feng et al., 2017; Jongberg et al., 2011). Low polyphenol concentrations were found to increase MP gel quality, whereas high quantities were found to prevent MP gelation (Cao et al., 2016).

Polyphenols, such as tea polyphenols and rosmarinic acid, can eliminate odor, smell and prevent oxidation of meat and meat products (including sausage, canned meat, bacon,etc). Especially in traditional Chinese meat products, spices are essential ingredients and contain many polyphenols, which play a role in enhancing flavor, antioxidant, antibacterial and other functions (Xu et al., 2022). Eugenol (Eu) (4-allyl-2-methoxyphenol) is an active ingredient in many spices such as clove, camphor, and cinnamon. These spices are often added to meat products as flavoring agents, and are widely used in traditional Chinese meat products. For this reason, Eu was chosen as the subject of this study. We investigated the effects of different concentrations of Eu on the gel properties and chemical structure of MP under oxidative conditions of the Fenton system to understand the mechanism of MP-Eu interaction.

2 Materials and methods

2.1 Materials

The fresh pork were purchased from Xinjiang Western Animal Husbandry Co., Ltd. (Shihezi, China). Eugenol (Eu, purity > 95%) was obtained from Macklin Biochemistry Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and obtained from Sigma-Aldrich (Shanghai, China).

2.2 MP extraction and sample preparation

Extraction of MP

Fresh pork samples were sliced into tiny pieces for MP extraction using the technique of Park et al. (2007). The MP pellets can be used up within 24 hours after storage at 4 °C. The concentration of MP pellets was measured using the Biuret technique (Xia et al., 2018) after the final centrifugation, and they were kept on ice before use.

Establishment of oxidation system

MP pellet was dissolved with lysing solution (50 mM phosphate buffer, 0.6 M NaCl, pH 6.0); Eu (0, 10 μM/g, 50 μM/g, 100μM/g, 200μM/g, 300μM/g, Eu was first dissolved in 2 mL ethanol and finally prepared into different concentrations) were dispersed in the MP solution (final concentration 40 mg/mL), then MP-Eu mixture were oxidized in Fenton oxidation system (10 μM $FeCl_3$, 100 μM ascorbic acid, 1 mM H_2O_2). MP suspension with 2mL ethanol solution without Eu was added as the control group. MP suspension of oxidation system without Eu was added as oxidized group. Each treatment groups was placed at 4 °C, the reaction was 12 h, terminated with EDTA (1 mM) reaction.

2.3 Carbonyls

Referring to the Levine et al. (1990) with slightly modified. Mixed 2 mL protein solution and 2 mL HCl solution containing 2,4-dinitrophenylhydrazine (DNPH). The supernatant was discarded after 20% trichloroacetic acid (TCA) was added, agitated, and centrifuged (5000 r/min, 10 min). After three washes with an ethanol/ethyl acetate (1:1) solution, the precipitate was added to a solution of 6 mol/L guanidine hydrochloride. At a wavelength of 370 nm, the absorbance was measured. 22000 $M^{-1}cm^{-1}$ was chosen as the molar extinction coefficient.

2.4 Total thiol content

Referring to the Korchak & Speranskaya (2009) with slightly modified. 1 mL of diluted protein solution was added to 8 mL of Tris-glycine, homogenized, and centrifuged at 5000 r/min for 20 minutes to remove insoluble protein. For 30 minutes in the dark, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to the supernatant. At a wavelength of 412 nm, the absorbance was measured. 13600 $M^{-1}cm^{-1}$ was chosen as the molar extinction coefficient.

2.5 Surface hydrophobicity

Referring to the Chelh et al. (2006) with slightly modified. 1 mL of dilute protein solution was added to 200 μL of 1 mg/mL BPB, centrifuged at 5000 r/min for 20 minutes, 100 L of supernatant was added to 1 mL of water, and the absorbance was measured at 595 nm. The absorbance of the blank control and the sample solution was A_0 and A_1, respectively. Bromophenol blue (BPB) binding was calculated by the following Formula 1:

$$\text{BBP bond}(\mu g) = 200\mu g \times \left(A_0 - A_1\right) / A_0 \quad (1)$$

2.6 Raman spectroscopy

MP samples containing different concentrations of Eu (40 mg/mL) were placed on separate slides and placed into the Raman spectrometer (Bruker, China). Raman spectra were recorded in the range of 800-1800 cm^{-1} (Zhou et al., 2019b).

2.7 Fluorescence spectroscopy

Changes in tryptophan fluorescence were measured by 970CRT spectrofluorometer (Shanghai Precision Instrument Co., Ltd., China). The scanning range was 300-400 nm and excitation wavelength was 295 nm. The excitation interval was 5 nm, the emission interval was 2 nm, and the data of control group were obtained under the same conditions (Geng et al., 2021).

2.8 SDS-PAGE

The protein cross-linking and aggregation of the MP samples were observed by SDS-PAGE in 5% stacking gel and 10% separated gel (Wang et al., 2021).

2.9 MP gel preparation

The MP solutions from the above treatment groups were packed into small test tubes (2 mm × 5 mm) and placed in a water bath. After rising from 20 °C to 80 °C, the samples placed at 80 °C for 10 min, then was removed and cooled in ice water for 30min, Finally the gels were placed in a refrigerator at 4 °C overnight (12 h), pending use.

Gel strength

The gel strength of MP gels was measured by a taxplus structure analyser (TA-XT plus, China) at 25 °C for 30 min, size 1 cm × 1 cm × 1 cm, probe P/0.5.

Water-holding capacity (WHC)

The weighed gel sample (W_1) was centrifuged at 5000 g for 15 min. After centrifugation, take out the centrifuge tube, absorb excess water with filter paper, and weigh it (W_2). The calculation of WHC was as follows (Formula 2):

$$WHC(\%) = (W_1 - W_2) / W_1 \times 100\% \quad (2)$$

Gel whiteness

The L^*, a^* and b^* of the gel were measured by WSC-S chroma meter (Precision Scientific Instrument Co., Shanghai, China). Whiteness was calculated with the Formula 3 below:

$$whiteness = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (3)$$

2.10 Microstructure

The gel samples was cut into uniform pieces, fixed with 2.5% glutaraldehyde for 48 h, and then dehydrated by gradient ethanol solution with volume fraction of 50%, 70%, 80%, 90% and 100%, and then freeze-dried. Platinum was sprayed on samples, then gels were observed by scanning electron microscope (SEM, SU8010, Hitachi, Japan) (Mi et al., 2017).

2.11 Rheological behavior

A rheometer (MCR302, Anton Paar, Austria) was used to measure the rheological behavior of the MP suspension following the Zhao approach (Zhao et al., 2013). Add the sample to the plate, then heated at a rate of 2 °C/min from 20 °C to 80 °C. A strain of 2% and a frequency of 0.1 Hz were used to determine the shear force. The upper and lower plates were sandwiched by 1 mm. The change of storage modulus G' was recorded.

2.12 Statistical analysis

Microsoft Excel 2019 and SPSS Statistics 20.0 were used for statistical analysis. ANOVA was used for significance analysis ($P < 0.05$), and Origin 2018 was used for plotting.

3 Results and discussion

3.1 Carbonyls

In the oxidation reaction, the side chain groups of amino acids are easily oxidized to form carbonyl derivatives. Because of this, carbonyl compounds have been widely employed to evaluate the degree of oxidative modification of proteins (Kehm et al., 2021). Table 1 shows that the carbonyl content of the control and oxidized MP was 0.92 and 1.65 μmol/mg protein, respectively. The carbonyl content of the oxidized group was about twice higher than that of the control group. After adding various concentrations of Eu, the carbonyl content was lowered by at least 10.91% (100 μM/g) and as much as 44.24% (300 μM/g). This was likely due to the antioxidant effect of Eu, which can effectively inhibit the formation of carbonyl compounds, but there was no significant difference elicited among the different concentrations ($P > 0.05$). Moreover, Different concentrations of rosmarinic acid could also effectively inhibit the formation of carbonyl compounds (Tang et al., 2017) and MP derived from Japanese seerfish that was treated with gallic acid (Pan et al., 2020) obtained similar results, Thus, the results of this study indicate that eugenol can effectively resist the effects of oxidation on protein carbonylation and reduce the formation of protein carbonyl derivatives.

3.2 Thiol groups

Cysteine is an amino acid that is susceptible to oxidation due to its thiol side chain. MP contains a large number of thiol groups, and changes in their content are closely related to changes in protein structure (He et al., 2018). Table 1 shows that, compared with the control group, the content of thiol groups in the oxidized group was decreased ($P < 0.05$). This indicated that oxidation of the protein resulted in the conversion of the thiol groups to a disulfide bond. When the addition of Eu was 10 and 50 μM/g, Eu exerted a protective effect on thiol groups. However, increasing Eu concentration did not seem to inhibit the decrease of thiol groups. On the contrary, high Eu concentration further reduced the content of thiol groups. In fact, the addition of 300 μM/g corresponded with the lowest thiol content at 0.05 μM/mg protein ($P < 0.05$). Numerous studies have found similar results. For example, Lv et al. (2021) found that 2 mM EGCG resulted in a further decrease in the content of thiol groups in MP compared with the oxidative

Table 1. Physicochemical changes in MP isolate upon oxidative stress in the absence or presence of Eu.

Samples	Carbonyl (μmol/mg protein)	Thiol group (μmol/mg protein)	BPB bound (μg)
Control	0.92 ± 0.03^{b}	5.32 ± 0.11^{a}	51.29 ± 2.85^{ab}
Oxidized	1.65 ± 0.05^{a}	0.44 ± 0.32^{c}	57.09 ± 0.95^{a}
10 μM/g	1.47 ± 0.17^{a}	1.67 ± 0.47^{b}	48.79 ± 5.29^{bc}
50 μM/g	1.18 ± 0.24^{b}	1.01 ± 0.35^{bc}	46.80 ± 2.33^{abc}
100 μM/g	1.05 ± 0.20^{b}	0.78 ± 0.52^{c}	42.58 ± 1.96^{cd}
200 μM/g	0.91 ± 0.08^{b}	0.61 ± 0.55^{c}	41.98 ± 0.86^{d}
300 μM/g	0.92 ± 0.09^{b}	0.42 ± 0.37^{c}	41.01 ± 5.58^{d}

Note: Physicochemical changes in MP isolate upon oxidative stress in the absence or presence of Eu. Means with different letters (a–d) in each column differ significantly ($P < 0.05$).

group. Excessive Eu did not protect against the loss of thiol groups, but rather accelerated the loss, probably due to the formation of thiol-quinone adducts between Eu and the thiol groups.

3.3 Surface hydrophobicity

Generally speaking, changes in surface hydrophobicity can be used to measure changes in the spatial structure of MP molecules (Zhang et al., 2018). The surface hydrophobicity of oxidized group significantly increased compared with the control group because the amount of bound BPB increased by 10.16% (Table 1), indicating that oxidation led to the unfolding of MP. The surface hydrophobicity significantly decreased with the addition of Eu, especially at 200 and 300 μM/g ($P < 0.05$). Excessive Eu might increase the degree of MP unfolding, thereby leading to aggregation, hydrophobic amino acids congregating on the surface of MP, and thus a resulting decrease in the surface hydrophobicity of Eu-treated samples. This was consistent with previous findings, Wang et al. (2018) found that with the increase of rosmarinic acid concentration, the surface hydrophobicity of MP was significantly reduced compared with the control group.

3.4 Raman spectrum analysis

Secondary structure refers to the local folding pattern of the peptide skeleton and is stabilized by hydrogen bonds between N-H and C=O groups. Changes in the secondary structure of MP were explained using the Raman spectrum. Proteins have amide I bands ranging from 1600 cm^{-1} to 1700 cm^{-1} and are very sensitive to the strength of hydrogen bonds between C=O and N-H groups. Therefore, in general, the amide I band in Raman spectroscopy is considered to be the most useful band for the analysis of secondary protein structures (Kuhar et al., 2021). The Raman spectra and quantitative analysis results of each treatment groups were shown in Figure 1A and 1B, respectively. After oxidation, the percentage of α-helix structures reduced from 40.94% to 32.15%, while the proportion of β-sheet structures increased from 29.45% to 33.48%. After the addition of Eu, the proportion of α-helices first increased and then reduced, from 32.15% during oxidation, up to 39.10% with 10μM/g, then subsequently followed by a reduction to 29.47% at 200 μM/g, and finally to 30.86% at 300 μM/g. As a result of the interaction between the added Eu and the carbonyl and amino groups of MP, the ability to establish hydrogen bonds between the polypeptide chains may be reduced, thereby changing the α-helix contents of proteins (Zhou et al., 2021). Furthermore, the continued decrease in the fraction of α-helices revealed that the Eu-modified MP caused the further structural unfolding of MP. These results indicate that Eu altered the secondary structure of MP gels, and that this was intimately related to their texture.

3.5 Fluorescence spectroscopy

MP possess endogenous fluorescence properties. At certain excitation wavelengths, the aromatic amino acids present in MP can produce fluorescence, among which, tryptophan has

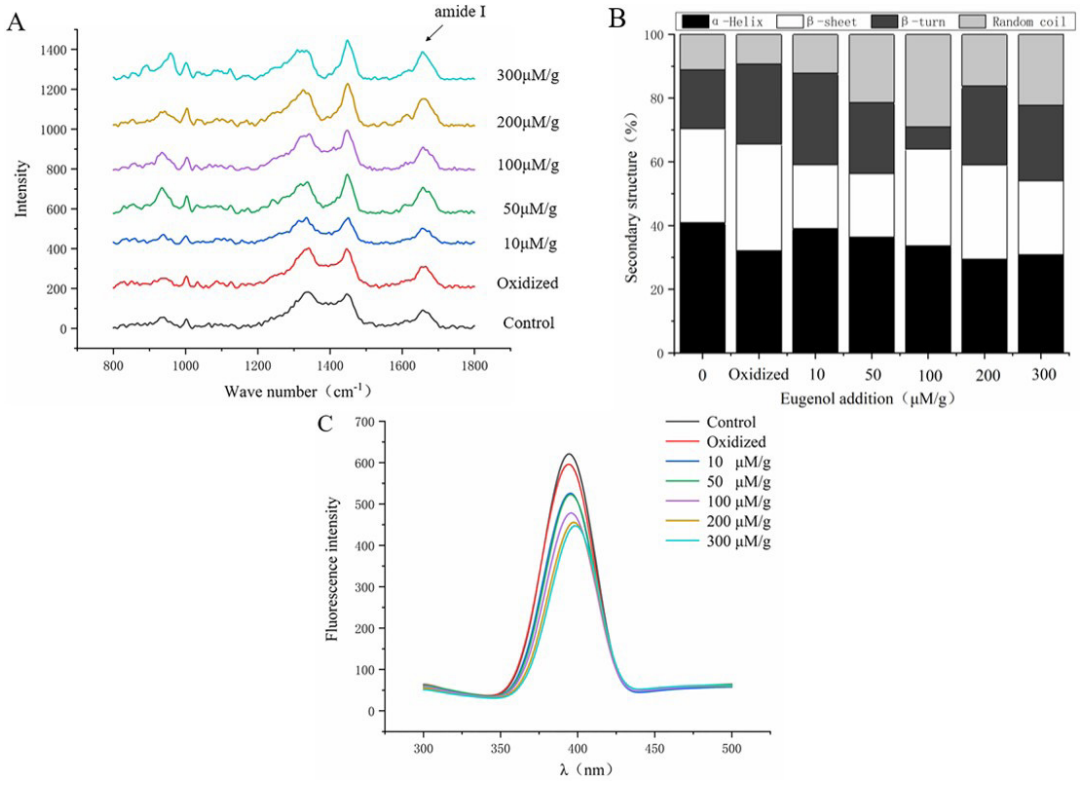

Figure 1. (A) Raman spectrum between 800 -1800cm^{-1} of MP with various concentrations of eugenol (Eu) under oxidative stress; (B) Relative proportion of secondary structure with various concentrations of Eu under oxidative stress; (C) Tryptophan fluorescence of MP with various concentrations of Eu under oxidative stress.

the strongest fluorescence. Tryptophan is highly sensitive to the microenvironment. When a protein is unfolded, any contained tryptophan is then exposed, thereby leading to fluorescence quenching. Therefore, the fluorescence spectrum can reflect the changes in the tertiary structure of a given protein.

In Figure 1C, the fluorescence intensity of the oxidized group was lower than that of the control group, indicating that the structure of MP was further expanded. The addition of Eu reduced the fluorescence intensity even further, particularly at high Eu concentrations, indicating further unfolding and increased interaction between Eu and the tryptophan residues, the maximum fluorescence intensity of endogenous tryptophan decreased from 525.94 at 10 μM/g to 447.27 at 300 μM/g. Eu disrupted the environment around tryptophan, thereby exposing the tryptophan residues in induced proteins, and consequently reducing the fluorescence of MP to cause a slight red shift of the tryptophan maximum fluorescence emission wavelength (λ_{max}). Huang et al. (2022) found similar results, with the increase of mulberry polyphenol concentration, the fluorescence intensity of MP in beef decreased, and the structure of MP was further expanded.

3.6 Electrophoresis analysis

Figure 2 shows the crosslinking and degradation of MP at various molecular weights after the addition of Eu at different concentrations under an oxidizing environment. As shown in Figure 2A, some macromolecular polymers were found on the top of the unoxidized MP sample stacking gel under nonreducing conditions. As described by Lu et al. (2017), these polymers might contain titin, actin, and their hydrolyzed fragments, and might also contain some polymers that were generated during MP extraction. Compared with the control group, the myosin heavy chain (MHC) bands in the oxidized MP samples were significantly reduced, while the macromolecular polymers at the top of the stacking gel and separating gel were significantly increased, indicating that these increased polymers were mainly formed during the polymerization of the myosin heavy chains. Under reducing conditions (with β-ME added, Figure 2B), most of the MHC bands were almost completely recovered, indicating that these polymers were primarily formed by disulfide cross-linking. In addition, in previous studies, the content of thiol groups have been decreasing, this result may be mirrored. Eu is an antioxidant that is oxidized to semiquinone or quinone after donating electrons and hydrogen atoms. These oxidizing substances promote the conversion of thiol groups to disulfide bonds or serve as cross-linking agents, thus the thiol-quinone derivatives are formed (Figure 3), at the same time, amino-quinone derivatives and other polymers are formed (Jongberg et al., 2015), which affect the cross-linking between proteins.

3.7 The gelation properties of MP

The properties of the MP gels; including gel strength, WHC, and whiteness; are shown in Table 2. The gel strength of

Table 2. Gelation properties of non-oxidized sample and samples under oxidative stress induced by adding different concentrations of Eu.

Samples	Gel strength (N)	Water holding capacity(%)	Whiteness
Control	57.78 ± 1.18^{cd}	85.86 ± 1.22^{b}	81.23 ± 2.05^{a}
Oxidized	58.97 ± 0.99^{bc}	89.75 ± 1.00^{a}	79.41 ± 1.69^{ab}
10 μM/g	62.77 ± 3.56^{ab}	90.23 ± 0.70^{a}	78.68 ± 1.57^{ab}
50 μM/g	67.28 ± 2.65^{a}	91.44 ± 0.96^{a}	78.35 ± 2.48^{ab}
100 μM/g	58.03 ± 1.65^{abc}	90.15 ± 1.44^{a}	77.40 ± 1.87^{ab}
200 μM/g	58.60 ± 0.85^{abc}	86.06 ± 2.56^{b}	77.06 ± 1.20^{b}
300 μM/g	53.80 ± 0.66^{d}	84.87 ± 0.79^{b}	76.93 ± 1.79^{b}

Note: Gelation properties of non-oxidized sample and samples under oxidative stress induced by adding different concentrations of Eu. Means with different letters (a–d) in each column differ significantly ($P < 0.05$).

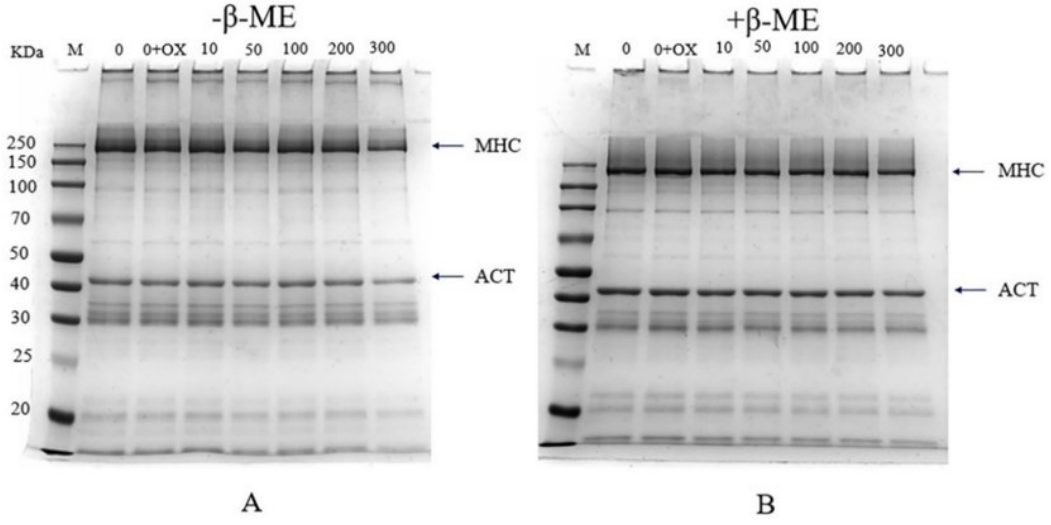

Figure 2. Images after SDS–PAGE of MP treated with different concentrations of Eu (10, 50, 100, 200 and 300 μM/g); 0: control, non-oxidized; 0+OX: oxidized; A, without β-ME and B, with β-ME.

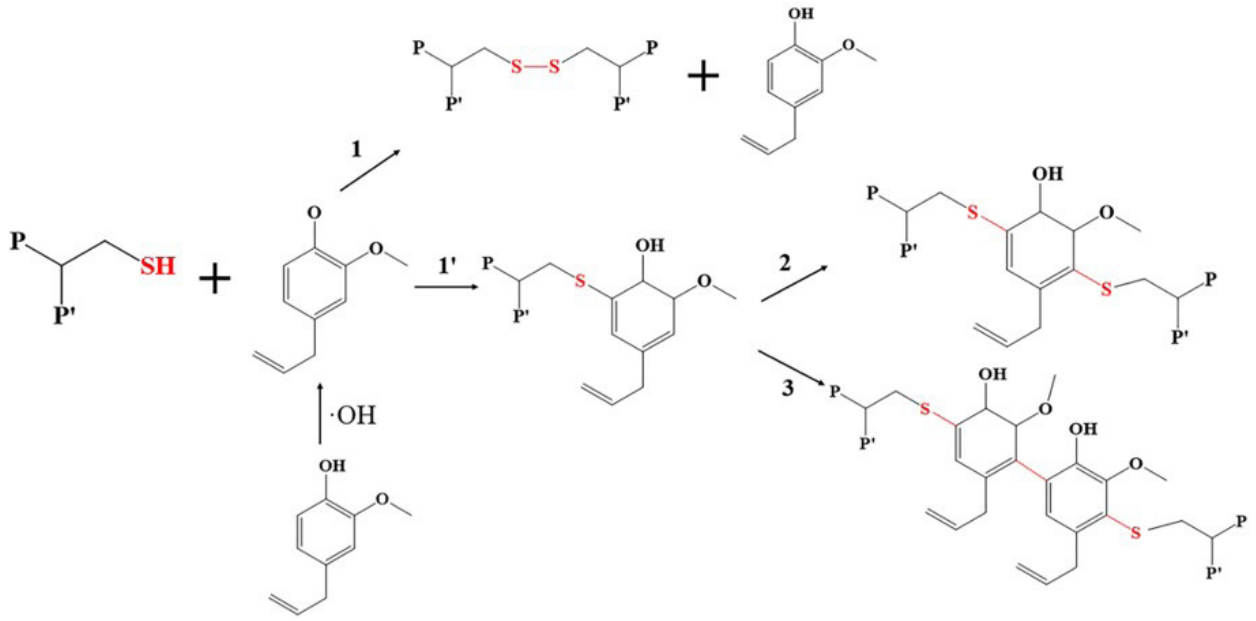

Figure 3. Mechanism of covalent connections between MP and Eu proposed, with emphasis on the significance of protein thiol groups. 1': adduction of quinone to -SH group in proteins; 2: development of cross-linking by another MP binding to the Eu derivative; 3: formation of cross-linking by quinone-MP adduct dimerization. The models were created using the findings of Jongberg et al. (2011) and Xiong & Guo (2020).

MP represents its capacity to form a gel. With the addition of 10 μM/g and 50 μM/g Eu, the gel strength rose by 6.05% and 12.35%, respectively, as compared with oxidized group ($P < 0.05$), suggesting the formation of a stronger gel network. However, the concentrations of 100, 200, and 300 μM/g Eu reduced the gel strength relative to the oxidized group; particularly at 300 μM/g, the network structure of gel was found to be significantly degraded. At moderate Eu treatment doses, the unfolding and cross-linking of MP may help in protein gelation by facilitating ordered protein aggregation. Because the synthesis of thiol–quinone and amine–quinone adducts may boost the cross-linking ability of Eu-MP, the Eu component might be oxidized to create quinones with better cross-linking capacity (Friesen et al., 2015).

However, when a considerable quantity of Eu is added, it disrupts the structure of MP to interfere with gelation. Several investigations have confirmed this concentration-dependent "dual effect" (Guo & Xiong, 2021). Cao & Xiong (2015) discovered that the concentrations of 6 and 30 μM/g Chlorogenic acid enhanced the quality of MP gels, whereas 150 μM/g deteriorated gelation due to severe protein aggregation and insolubility produced by the high concentration of chlorogenic acid. The dose-dependent effects of tea polyphenols were comparable (Li et al., 2020).

WHC is a key functional property of MP and is related to the creation of gel networks. Too loose of a gel network is not conducive to WHC. As shown in Table 1, compared with the oxidized group, the addition of 10 and 50 μM/g Eu each enhanced the WHC ($P > 0.05$). However, with Eu concentrations at 200 and 300 μM/g, WHC was reduced by 4.11% and 5.44%, respectively ($P < 0.05$). The current findings were in good agreement with those obtained for gel strength. This might be due to a poorer formation of the gel network, or even the disruption of the 3D network, resulting in a small amount of water retention in the gel matrix.

With Eu addition at the concentrations of 10, 50, and 100 μM/g, changes in whiteness were not evident. Conversely, the addition of Eu at the concentrations of 200 and 300 μM/g each significantly reduced the whiteness ($P > 0.05$). This negative relationship between Eu addition and gel whiteness may be attributable to the influence of the color of Eu itself, along with the additional effect of Fe^{3+} in the oxidation system (Tang et al., 2017).

3.8 SEM

The microstructures of MP gels were observed using SEM at 2500× magnification (Figure 4). In the control group, the pores of gel were more uniform. After oxidation, larger pores appeared in the microstructure of the gel. When 10 and 50 μM/g Eu were added, the MP gels displayed surface structures that were more flat, uniform, and compact. This may have been due to cross-linking between Eu and MP which promoted the formation of a protein gel. This result showed that the gel strength and water retention were also stronger. The network structure of the gel gradually became loose and rough with increasing concentrations of Eu; when it reached 300 μM/g, the microstructure of the gel became larger while the aggregation and distribution of protein micelles were not uniform. Excess Eu hindered the cross-linking between MP, which promoted the uneven aggregation and distribution of protein micelles, resulted in a poor protein network structure, and led to lower gel strength and WHC.

3.9 Dynamic rheological properties

The elastic properties of the gel system are described by the storage modulus (G'), whereby the larger the G' of the protein gel, the better its elasticity. During the gelation process, G′ was used to examine the rheological properties of MP samples. The G′

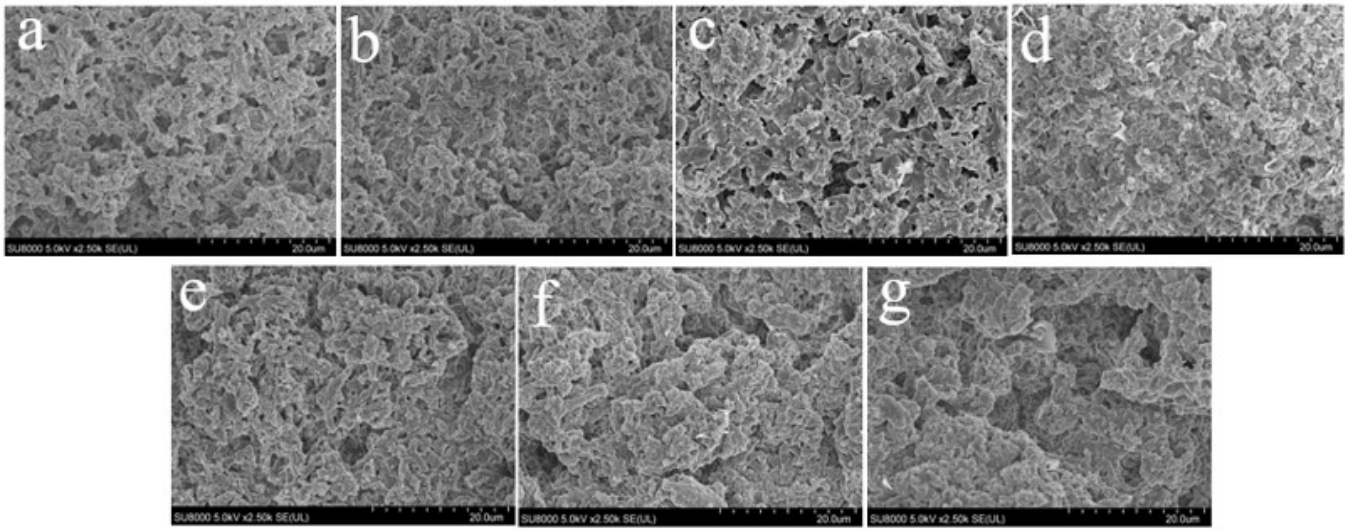

Figure 4. SEM images of MP samples under oxidative stress added with Eu at different concentrations (magnification: 2500×): (a) control; (b) oxidized; (c) 10 μM/g Eu; (d) 50 μM/g Eu; (e) 100 μM/g Eu; (f) 200 μM/g Eu; and (g) g 300 μM/g Eu.

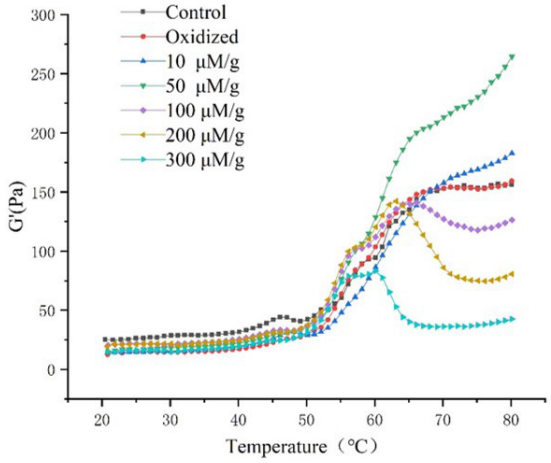

Figure 5. Storage modulus (G′) development of MP during thermal gelation with different.

of the samples is shown in Figure 5. The control group without Eu and the oxidized group showed a typical G' curve with two transition peaks at 45 °C and 50 °C. The initial peak primarily represents the unfolding of the myosin head and suggests an improvement in gel quality. Low concentrations of Eu (10 and 50 μM/g) significantly contributed to the final elastic modulus, suggesting that the addition of low-to-medium concentrations of Eu facilitated protein intermolecular interactions and molecular cross-linking aggregation under slightly oxidizing conditions. This is because, under this condition, the presence of Eu facilitates the unfolding of the protein structure, exposure of more amino acid residues, and no significant reduction in functional groups (thiol and free amino groups). All these changes are beneficial for protein intermolecular interactions and cross-linking. However, high concentrations of Eu (100, 200, and 300 μM/g) completely changed the gel properties of MP. The two typical transition peaks completely disappeared, a new transition peak appeared at approximately 60 °C, and the final G′ value decreased sharply, suggesting that the performance of the MP gel was severely damaged, especially at a concentration of 300 μM/g Eu.

Therefore, combined with the changes of MP structure and gel properties, we simulated the changes of MP structure under different concentrations of Eu (Figure 6). MP treated with low doses of Eu (10, 50 μM/g) had good rheological properties and generated good cross-linking with protein, which played a positive role in the construction of three-dimensional network structure of gel. Excessive cross-linking between high doses of Eu (100, 200, 300 μM/g) and MP, such as the formation of thiol-quinone derivatives, amino-quinone derivatives and other polymers, damaged the stability of the gel, especially the disulfide bond, which had an important impact on the gel formation process.

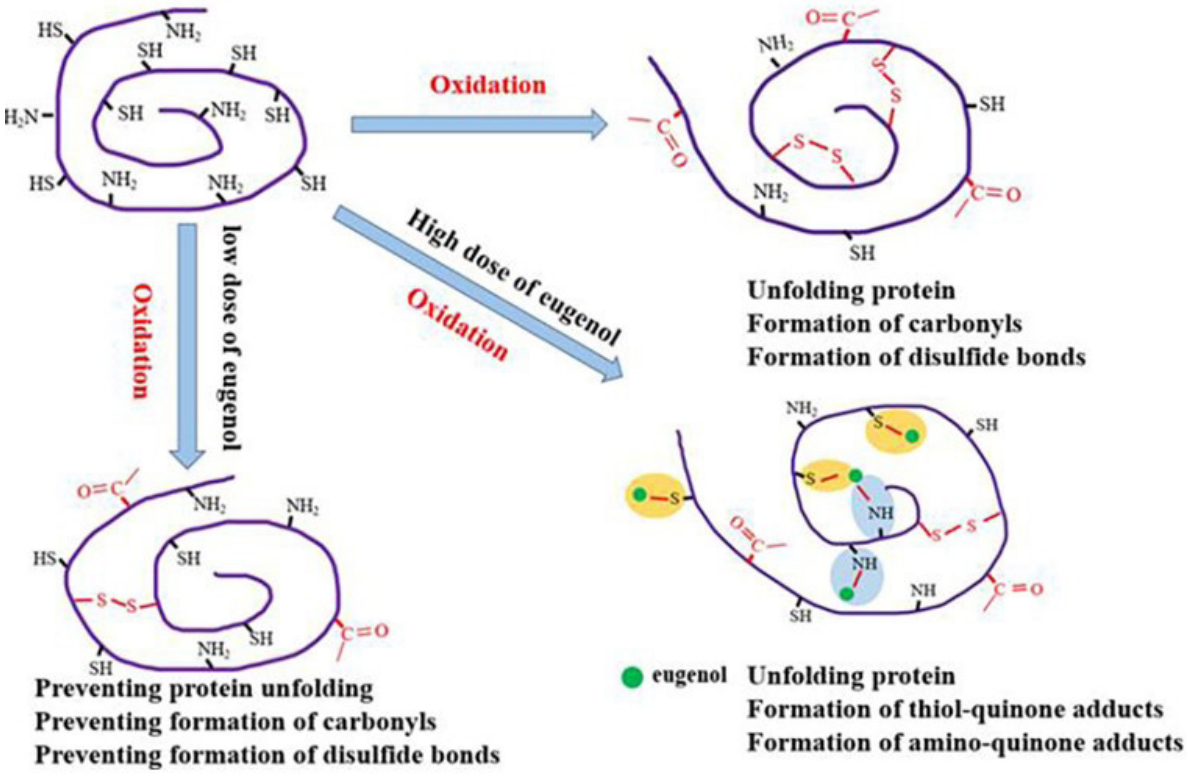

Figure 6. Schematic representation of the proposed reactions between MP and Eu.

4 Conclusions

The effects of various concentrations of Eu on the structural and gel properties of MP under oxidative stress were investigated. With the addition of Eu, the carbonyl content decreased significantly due to the antioxidant effect of Eu. However, Eu did not inhibit the decrease of the total thiol content, the unfolding of the structure, or the cross-linking of MP. With the addition of eugenol, the content of α-helices gradually decreased, thus altering the secondary structure of the protein. With the gradual decrease of the fluorescence intensity of intrinsic tryptophan, the tertiary structure and surface hydrophobicity correspondingly changed. In terms of gelation, Eu affected the gel properties of proteins by inducing structural changes. When the concentration of Eu was 10 and 50 μM/g, it exerted positive effects on gel formation. But Eu was not conducive to the gel formation of proteins at concentrations above 100 μM/g, especially at 300 μM/g. In summation, both low and moderate concentrations of Eu were observed to improve the quality of MP gels, possibly due to interactions between MP and MP; however, when high concentrations of Eu were present, excessive MP-MP interactions and Eu-MP interactions decreased the quality of MP gel properties.

Conflict of interest

The authors declare that they do not have any known competing financial interests or personal ties that may seem to have influenced the work reported in this study.

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Author contributions

Ning An: Conceptualization, Methodology, Validation, Writing - Original Draft. Mou Zhao: Methodology, Software. Juan Dong: Conceptualization, Writing-Review & Editing, Supervision, Funding acquisition. Ping Han: Investigation. Jiamei Li: Investigation. Shuyao Zhang: Investigation. Qingling Wang: Supervision, Resources. Shiling Lu: Supervision, Validation. Hua Ji: Supervision.

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