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Research Article

Associated Changes in the Structural and Antioxidant Activity of Myofibrillar Proteins via Interaction of Polyphenolic Compounds and Protein Extracted from Lentil (*Lens culinaris*)

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This study evaluated the effects of different concentrations of green lentil acetone extract (GLA) (250, 500, 750, and 1000 μg/mL) and protein of green lentil (PGL) (1, 2, 3, and 4 g/100 g MP) on the functional attributes of myofibrillar protein (MP). GLA extract and PGL significantly affected the structure of MP by decreasing the carbonyl and sulfhydryl contents. Intrinsic fluorescence quenching studies showed that static quenching was involved in MP-GLA extract and MP-PGL complexes. Compared to the control (MP), the addition of GLA extract and PGL decreased the surface hydrophobicity, which correlated with the decrease in protein solubility. The MP-GLA and MP-PGL had lower cooking losses and slightly higher water-holding capacities ($P < 0.05$). FTIR spectroscopy demonstrated changes in MP secondary structure with the addition of GLA extract and PGL. GLA extract and PGL also decreased the thermal stability of MP and showed significant synergism in enhancing the radical scavenging activity of MP. Taken together, the results indicated that a high concentration of GLA extract (1000 μg/mL) and PGL (4 g/100 g MP) improved the functional properties of MP, and GLA extract was the most effective.

1. Introduction

Myofibrillar proteins (MP) form network structures and display a wide range of functional characteristics during the preparation of meat products [1]. Proteins are usually damaged by reactive oxygen species during processing and storage, and this is intensified in the presence of heme pigments, metal catalysts, and several other oxidizing chemicals. Protein oxidation causes several physicochemical modifications, including fragmentation, aggregation, crosslinking, thiol loss, and the production of carbonyl compounds [2]. It may lead to a significant reduction in the functionality of proteins, which results in changes to meat texture, a reduction in water-holding capacity, a loss of solubility, and cooking losses [3]. As a result, limiting protein oxidation in meat proteins is essential. Thus, the addition of natural compounds from plants, such as antioxidant polyphenols or proteins, can be one approach to solving this problem. For instance, Li et al. [4] reported that low concentrations (5–10 μmol/g protein) of tea polyphenols inhibited carbonyl group formation, reduced sulfhydryl groups, decreased surface hydrophobicity, and protected myofibrillar proteins from oxidation. It was suggested that hydroxyl groups in the ortho-phenolic structure of catechin might be responsible for the inhibition of MP oxidation. Similarly, mulberry polyphenols, including 3-O-glucoside,

cyaniding 3-O-rutinoside, caffeic acid, quercetin, and rutin, significantly improved the structure and functionality of myofibrillar protein in meat products [5].

Recently, various studies have shown that phenolic compounds and proteins can bind to other proteins through covalent and non-covalent bonds in protein-rich foods; this can alter the structural and functional attributes of proteins, such as their thermal stability, solubility, water-holding capacity, and antioxidant capacity [6–8]. Meng and Li [9] reported that WPI-phenolic compound (gallic acid, chlorogenic acid, and epigallocatechin gallate) complexes significantly reduced free sulfhydryl groups and the surface hydrophobicity of the protein. Moreover, polyphenols improved the functional properties of WPI, such as solubility, foaming, and emulsifying capacities. Wang et al. [10] explained that hydrophobic interactions performed the most crucial function in MP-heated soy protein isolate complexes, with hydrogen bonds stabilizing the MP-soy protein isolate gels. In another study, MP-soy protein isolates decreased water loss by trapping water molecules; hydrophobic interactions, hydrogen bonds, and disulfide bonds were crucial in the formation of the MP-soy protein isolate complex [11].

Lentil (*Lens culinaris* Medikus) is one of the most important legumes in the world due to its high nutritional quality, making it a complete food source for people with micronutrient malnutrition [12]. It contains high amounts of protein (20–30 g/100 g), beneficial fats (more than 2 g/100 g), carbohydrates (40–60 g/100 g), dietary fiber, and micronutrients. Phenolic compounds have demonstrated a variety of biological activity and beneficial properties, such as antioxidant, antiallergic, anti-inflammatory, anticancer, antimicrobial, and antiviral properties [13]. Various phenolic compounds, including catechins, procyanidins, quercetin, myricetin, luteolin, and apigenin, as well as dimers, trimers, and tetramers of proanthocyanidins, have been detected in lentils [14]. Fratianni et al. [15] reported that lentils had higher total polyphenolic contents than chickpeas and peas and strong antioxidant activity. Lentils are also a valuable source of protein with a high content of nonessential amino acids, such as arginine, aspartic acid, and glutamic acid, and a lower amount of essential amino acids, such as threonine, methionine, phenylalanine, tryptophan, histidine, valine, isoleucine, and leucine. Lentil proteins are considered a sustainable alternative to animal-derived proteins [16].

Considering their availability and high amounts of proteins and polyphenols (e.g., flavan-3-ol, proanthocyanidins, and some flavanols), the lentil has a strong potential for use in improving the functional and bioactive properties of meat products. To our knowledge, there is a dearth of information on the interaction of phenolic compounds and proteins of lentils with MP. The aim of this study was to evaluate the influence of various concentrations of phenolic extracts and lentil protein isolate on the configuration, functional, and physicochemical properties of MP. Carbonyl, sulfhydryl (SH), Fourier transform infrared spectroscopy (FTIR), surface hydrophobicity, and intrinsic tryptophan fluorescence were used to evaluate structural changes in MP, while solubility, cooking loss, water-holding capacity (WHC), thermal stability, and antioxidant activity were used to evaluate functional changes. This investigation was conducted to explore the potential of phenolic compounds and proteins in lentils as functional compounds for improving the structure and bioactive attributes of myofibrillar protein and meat products.

2. Materials and Methods

2.1. Materials. Green lentil seeds (*L. culinaris* ssp. *culinaris* Medikus) were ground into powder using a food blender and passed through a standard 40 mesh sieve (0.425 mm) to achieve a homogenous sample powder, which was stored in the dark at −18°C prior to extraction. Beef shoulder clod was obtained from a local market (Tehran, Iran). 2,4-Dinittrophenylhydrazine (DNPH), 5,5′-dithiobis (2nitrobenzoic acid) (DTNB), 1-anilino-8-naphtalene-sulphonic acid (ANS), 2,2-diphenyl-1-picrylhyrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazne-4′,4″-disulfonic acid (ferrozine) were obtained from Sigma-Aldrich (Vallensbaek Strand, Denmark). Other chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of Green Lentil Acetone Extract (GLA). Green lentil powder was extracted with a solvent mixture (acetone: water, 80 : 20 v/v) with a solid-to-solvent ratio of 1 : 40 (w/v) at 4°C for 24 h [17]. This extraction process was repeated twice, and the supernatants were combined. Thereafter, acetone was removed by a rotary evaporator, and the sample was freeze-dried (Labconco Corp., Kansas City, MO, USA). The resulting extracts were stored at −18°C until further analysis.

2.3. Total Phenolic Content Determination. The total phenolic content of GLA extract was determined with the Folin–Ciocalteu reagent at 760 nm, as previously reported [17]. Total phenolic content was calculated as mg gallic acid equivalent per gram of sample dry matter.

2.4. Extraction of Protein of Green Lentil (PGL). Protein of green lentil was extracted with distilled water at a solid/solvent ratio of 1 : 20 (w/v) and pH 9 according to a previous method [18]. The extracted protein was then freeze dried, and the dry powder was stored at 4°C until further analysis.

2.5. Extraction of MP. MP was extracted following the procedure described in [19, 20]. Two batches of beef shoulder clod within 24 h postmortem (200 g) were added to 1 L of phosphate buffer (20 mM PBS, 150 mM NaCl, 25 mM KCl, 3 mM $MgCl_2$, and 4 mM EDTA at pH 6.5) and homogenized using the Ultra-Turrax homogenizer (IKA®, T18D, Staufen, Germany) at 10,000 rpm for 5 min. Thereafter, the suspension was centrifuged at $10,000 \times g$ for 15 min at 4°C (Sigma 3–30 K, Sigma Centrifuges GmbH, Osterode am Harz, Germany), and the supernatant was discarded. The pellet was collected, and the previous steps

were repeated. Next, the pellet was washed twice with 4 L of 0.1 M NaCl solution. The recovered protein was washed again with 4 L of 20 mM PBS (pH 6.5) and suspended in this solution until further analysis.

2.6. Preparation of MP-GLA Extract and MP-PGL Complexes. The MP-GLA extract and MP-PLG complexes were prepared by thoroughly mixing MP solutions (6.0 mg/mL, final protein concentration by 20 mM PBS containing 0.6 M NaCl, pH 7.0) with four final concentrations of GLA extract (250, 500, 750, and 1000 μg/mL) and PGL (1, 2, 3, and 4 g/100 g MP). The mixtures were stored in the dark at 25°C for 2 h.

2.7. Determination of Chemical and Structural Changes of MP

2.7.1. Total Carbonyl and Total Sulfhydryl Contents. The effect of different concentrations of GLA extract (250–1000 μg/mL) and PGL (1–4 g/100 g MP) on the total carbonyl content of MP was determined using DNPH following the method described by Zhang et al. [19]. The total carbonyl content of samples was calculated using a molar extinction coefficient of 22000 $M^{-1}\cdot cm^{-1}$.

Total sulfhydryl content of MP with different concentrations of GLA extract (250–1000 μg/mL) and PGL (1–4 g/100 g MP) was measured using DTNB as described by Meng and Li [9]. Total sulfhydryl content was calculated using a molar extinction coefficient of 13600 $M^{-1}\cdot cm^{-1}$.

2.7.2. Fluorescence Quenching Analysis. MP (6 mg/mL protein in 20 mM PBS buffer containing 0.6 mM NaCl pH 7) and different concentrations of GLA extract (250–1000 μg/mL) and PGL (1–4 g/100 g MP) solutions were mixed in the ratio of 1 : 1. Fluorescence spectra of the mixtures were examined using a PerkinElmer LS-45 spectrofluorometer (Waltham, MA, USA) after excitation at 280 nm, and the fluorescence emission spectra were recorded at 300 to 500 nm [21]. The type of quenching was determined using the Stern–Volmer equation

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{sv}. \tag{1}$$

F_0 is the fluorescence intensity of MP and F is the fluorescence intensity of MP in the presence of different concentrations of GLA extract and PGL. $[Q]$ is the concentration of GLA extract and PGL, K_q is the bimolecular quenching rate constant, and K_{sv} is the Stern–Volmer quenching constant. The mean lifetime (τ_0) is the biomolecule fluorophore in the absence of any quencher and equals to ~10^{-8} s [2].

2.7.3. Determination of Surface Hydrophobicity. Surface hydrophobicity of MP with different concentrations of GLA extract (250–1000 μg/mL) and PGL (1–4 g/100 g MP) was determined using ANS according to the method of Cao et al. [21]. Different concentrations of samples (4 mL) were mixed with ANS (40 μL of 8 mM) in phosphate buffer (0.1 M, pH 7.0). Surface hydrophobicity of the complexes was determined using linear analysis from the primary slope of plots of fluorescence intensity vs. GLA extract and PGL concentrations. The initial slope was referred to as SoANS.

2.7.4. Thermal Analysis by Differential Scanning Calorimetry. MP samples (final concentration of 60 mg/mL, 10–15 mg/mL) with 1000 μg/mL GLA extract and 4 g/100 g MP were hermetically sealed in aluminum pans. An empty pan was used as the control. Thermal scans were obtained using a differential scanning calorimeter (DSC) (TA, Q600, USA) at 30 to 120°C at a 5°C/min rate. The temperature maximum (T_{max}) for transition was determined [5].

2.7.5. FTIR Spectrum. All samples (2 mg) were dried, ground with KBr powder (100 mg), and pressed into a pellet for FTIR measurement (Thermo, AVATAR, USA) in the range of 4000–400 cm^{-1} [22].

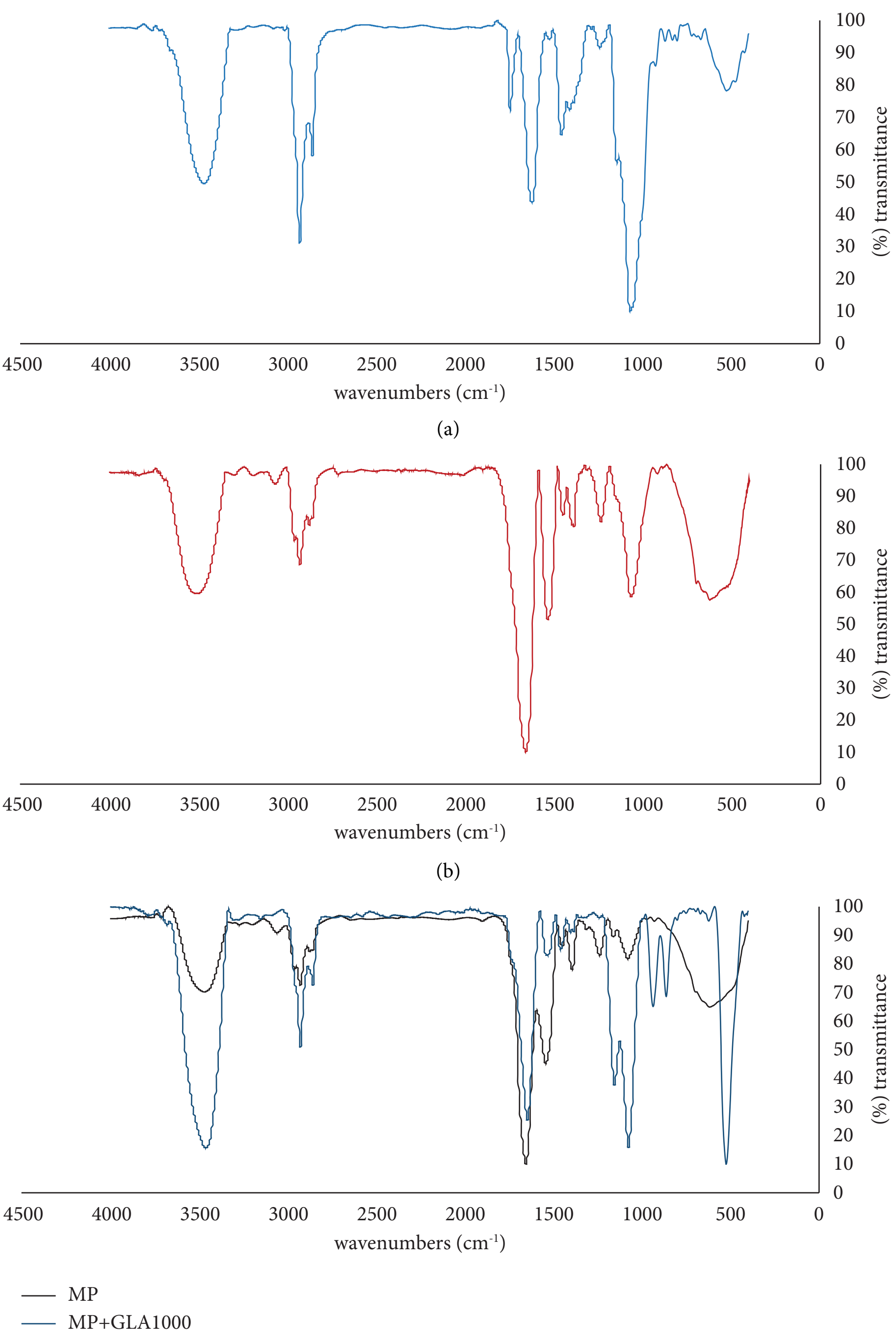
2.8. Solubility. The MP solution with various concentrations of GLA extract and PGL was centrifuged at 5000g for 20 min at 4°C. The protein concentration of the supernatant was determined by the Biuret method. The solubility (%) of the complexes was calculated as the proportion of protein content in the supernatant relative to the total protein content.

2.9. Cooking Loss and Water Holding Capacity. Cooking loss and water-holding capacity of the mixture of MP (final concentration of 60 mg/mL) with different concentrations of GLA extract and PGL were determined according to the methods of Wang et al. [23]. Gels (10 g, W_0) of the different solutions were moved into 16 mL polypropylene centrifuge tubes and heated in a water bath at 75°C for 20 min. After cooling, the formed gels were dried with a filter paper and weighed as W_1. Next, the weighed gels were stored in tubes and centrifuged at $10,000 \times$ g for 10 min at 4°C. The supernatant was removed, and the gels were dried with filter paper and weighed as W_2. Cooking loss and water-holding capacity were calculated using the following equations:

$$\begin{aligned} \text{cooking loss}\,(\%) &= \frac{w_0 - w_1}{w_0} \times 100, \\ \text{water holding capasity}\,(\%) &= \left(1 - \frac{w_0 - w_2}{w_0}\right) \times 100. \end{aligned} \tag{2}$$

2.10. Antioxidant Capacity

2.10.1. DPPH Radical Scavenging Activity Assay. The DPPH scavenging activity of MP with different concentrations of GLA extract and PGL was determined according to the method of Santos et al. [24]. DPPH radical scavenging activity (%) was calculated as $(A_0 - A_s) \times 100/A_0$, where A_0 is the absorbance of the control (methanol instead of the sample) at 517 nm and A_s is the absorbance of the sample.



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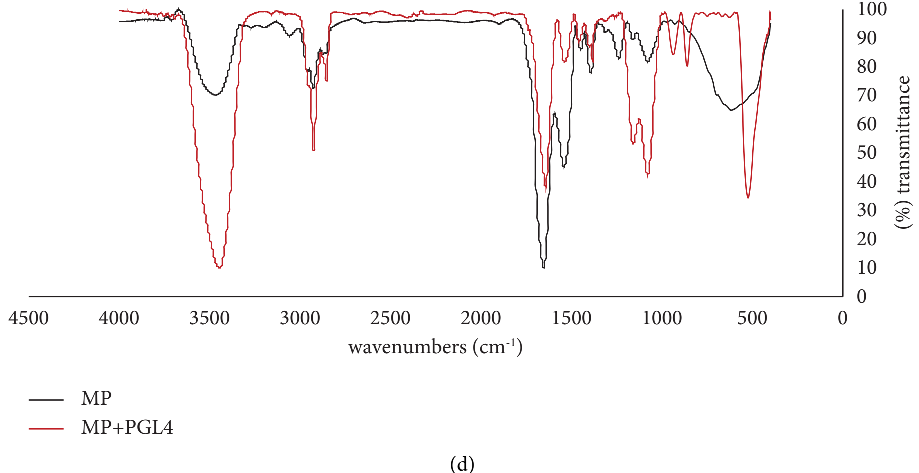


(d)

Figure 1: FTIR spectrum of green lentil acetone (GLA) (a), protein green lentil (PGL) (b), interaction between myofibrillar proteins (MP)-GLA complex (c), and MP-PGL complex (d).

2.10.2. ABTS Radical Scavenging Properties Assay. The 2,2′-azino-bis (3-ethybenzothiazoline-6-sulfonic acid) radical scavenging activity of MP with different concentrations of GLA extract and PGL was determined according to the method described by Almajano et al. [6]. ABTS radical scavenging activity (%) was calculated as $(A_0 - A_s) \times 100/A_0$, where A_0 is the absorbance of the control (distilled water was replaced with the sample) at 734 nm and A_s is the absorbance of the sample.

2.10.3. Fe^{2+} Chelating Activity Assay. The Fe^{2+} chelating activity of MP with different concentrations of GLA extract and PGL was determined as previously reported [25]. Fe^{2+} chelating activity (%) was calculated as $(A_0 - A_S) \times 100/A_{0,}$ where A_0 is the absorbance of the control (distilled water was replaced with the sample) at 562 nm and A_s is the absorbance of the sample.

2.11. Statistical Analysis. The data on antioxidant activity and chemical and structural changes were analyzed using the general linear model (GLM) procedure in SAS software (Ver. 9.2, SAS Institute Inc., Cary, NC, USA) applying one-way analysis of variance (ANOVA). The residual normality of the data was checked using the UNIVARIATE procedure. In ANOVA, different concentrations of GLA extracts (250, 500, 750, and 1000 μg/mL) and PGL concentrations (1, 2, 3, and 4 g/100 g MP) were assigned as fixed effects, and replication was assigned as a random effect. Three independent batches of MP suspensions with or without GLA extract and PGL were prepared for testing the changes in antioxidant activity, chemically and structurally. There were three cooking batches and three samples for each batch. Therefore, the statistical model for cooking loss included experimental and sampling errors. The data were expressed as the mean ± standard error (SE) of three replications (three different muscles). The means were compared using the Tukey test at the 0.05 level.

3. Results and Discussion

3.1. Properties of GLA Extract and PGL. The chemical composition and properties of GLA extract and PGL were evaluated. The total phenolic content of the GLA extract was 166.79 ± 7.52 mg gallic acid/100 g dry sample, which indicates a high amount of phenolic compounds. According to Bubelova et al. [26], the total phenolic content of different species of lentils varied from 108 to 165 mg gallic acid/100 g dry samples. The protein content of PGL was 81.27 ± 1.07 g protein/100 g. Jarpa-Parra et al. [18] reported the protein content of lentil flour to be between 79.34 and 83.55 g of protein per 100 g of lentil flour, depending on extraction conditions. The FTIR spectra of GLA extract and PGL are shown in Figures 1(a) and 1(b). In both spectra, the absorbance band at 3400–3500 cm^{-1} was due to the stretching vibration of hydroxyl groups. The absorption peak at 2920 cm^{-1} is the C-H stretching vibration involving CH, CH_2, and CH_3 stretching and bonding vibrations. The C=O asymmetric stretching vibration was observed at 1700–1600 cm^{-1}. The absorption peak at around 1400 cm^{-1} indicates the presence of carboxyl groups [22], while the band observed at 1658 cm^{-1} in PGL indicates the presence of overlapped α-helices and random coil signals of the protein [18].

3.2. Protein Carbonyl Content and Total Sulfhydryl Groups. The carbonyl groups of proteins have been considered a measure of the degree of protein oxidation. The carbonyl compounds (α-aminoadipic semialdehyde and γ-glutamic semialdehyde) are produced from side-chain groups of several amino acids, such as threonine, proline, arginine, and lysine, in meat proteins [27]. Utrera et al. [28] showed that protein oxidation and the formation of carbonyl compounds can damage the essential amino acids, texture, and functionality of proteins.

The carbonyl content of control MP varied with different concentrations of GLA extract and PGL (Table 1). The carbonyl content of control MP was higher than that of MP with GLA extract, but there was no significant difference

Table 1: Effect of green lentil acetone extract (GLA) and protein green lentil (PGL) on carbonyl and sulfhydryl content, solubility, cooking loss, and water-holding capacity (WHC) of myofibrillar proteins (MP).

Concentration	Carbonyl content (nmol/mg pro)	Sulfhydryl content (nmol/mg pro)	Solubility (%)	Cooking loss (%)	WHC (%)
GLA (μg/mL)					
0	2.54 ± 0.25^{a}	49.62 ± 0.71^{a}	67.39 ± 1.27^{a}	31.50 ± 0.57^{a}	67.06 ± 0.79^{bc}
250	1.58 ± 0.01^{b}	31.81 ± 0.13^{b}	59.63 ± 0.76^{bc}	31.09 ± 0.36^{a}	66.87 ± 0.38^{c}
500	0.95 ± 0.09^{b}	28.61 ± 0.52^{c}	62.60 ± 0.49^{b}	28.88 ± 0.14^{b}	69.36 ± 0.27^{a}
750	1.19 ± 0.12^{b}	25.75 ± 0.55^{d}	56.61 ± 0.41^{cd}	28.47 ± 0.27^{b}	69.72 ± 0.58^{a}
1000	1.52 ± 0.11^{b}	23.72 ± 0.61^{d}	53.21 ± 0.51^{d}	27.09 ± 0.33^{b}	68.82 ± 1.01^{ab}
PGL (g/100 g MP)					
1	1.28 ± 0.02^{b}	38.35 ± 0.22^{b}	62.57 ± 1.13^{b}	28.79 ± 0.58^{b}	71.43 ± 0.71^{a}
2	1.04 ± 0.03^{b}	35.99 ± 0.33^{b}	63.96 ± 1.14^{ab}	27.50 ± 0.24^{b}	69.04 ± 0.37^{ab}
3	1.01 ± 0.04^{b}	30.75 ± 0.63^{c}	65.04 ± 0.87^{ab}	27.35 ± 0.63^{b}	68.81 ± 0.44^{b}
4	1.45 ± 0.07^{b}	26.69 ± 0.17^{d}	63.28 ± 1.20^{ab}	24.32 ± 0.17^{c}	69.25 ± 0.10^{ab}

Results are displayed as mean ± standard error. Values with different superscripted letters within the same row are significantly different ($n = 3$, $P < 0.05$).

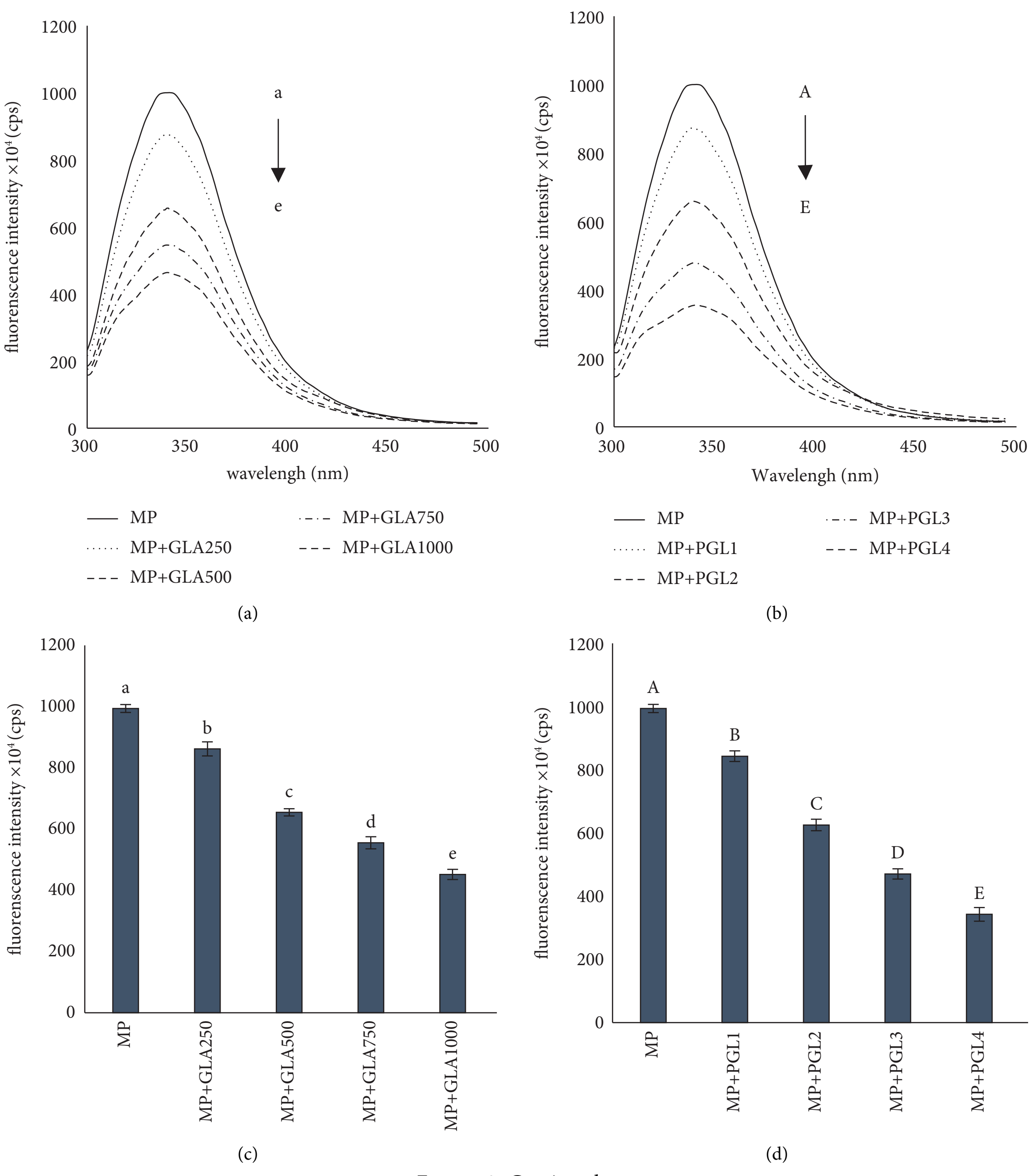
among the GLA extract concentrations. These results indicate that GLA extract might have interacted with carbonyl groups in MP or prevented the oxidation of MP. Zhang et al. [29] reported that phenolic compounds delay protein oxidation via prooxidant metal chelation and inhibition of free radicals. Furthermore, the covalent bonds formed by phenolic compounds with proteins, as well as the formation of accumulating protein-bound phenoxyl radicals with low reactivity, prevent the formation of carbonyl. Similarly, MP-PGL complexes displayed a decrease in carbonyl content compared with control MP. There was also no significant difference among the various concentrations of PGL. Metal chelating activity and radical scavenging activity of GLA extract and PGL could inhibit MP oxidation. Therefore, Schiff base adducts may be formed by carbonyls using NH_2 groups, leading to a decline in the concentration of carbonyls and likely the formation of cross-linkages between proteins [30].

MPs have sulfhydryl groups, which can easily oxidized from intraintermolecular disulfide bonds and, as a result, decrease the total sulfhydryl groups in MP [31]. Total sulfhydryl groups of MP decreased after the addition of various concentrations of GLA extract and PGL (Table 1). As shown in Table 1, the presence of phenolic extract in the complexes significantly decreased the SH content of MP. This effect might be associated with the covalent interaction between the sulfhydryl group and phenolic compounds extracted from lentil [5]. In general, thiol-quinone adducts can be formed by Michael addition due to interactions between the quinone of phenolic extracts and the sulfhydryl groups of MP [4]. Pan et al. [32] suggested that gallic acid reduced sulfhydryl groups through this mechanism. Also, Cao et al. [7] demonstrated that catechin, when exposed to •OH, can be converted to a quinone derivative and react with the sulfhydryl group or ε-NH_2 groups of lysine in MP. Moreover, the formation of covalent adducts caused a decrease in SH groups. Furthermore, mixing PGL with MP significantly decreased the SH content (Table 1). This might be due to the conversion of the SH group to disulfide (SS), resulting in protein-protein interaction between PGL and MP. Choudhary et al. [33] reported that polymerization and formation of covalent disulfide bonds after protein-protein interaction reduced total SH content.

3.3. Fluorescence Quenching Analysis. Changes in the tertiary structure of proteins were evaluated by tryptophan fluorescence. Partial or complete unfolding of proteins in a polar environment allows tryptophan to be exposed at the surface of the protein. Fluorescence quenching has been used to understand the interaction of proteins and polyphenols and the associated changes in the tertiary structure of proteins [2].

In this study, an increase in the concentration of GLA extract and PGL decreased significantly the fluorescence intensity of MP ($P < 0.05$). The maximum emission peak was observed at 340 nm for MP, and it did not shift for MP containing the samples (Figures 2(a) and 2(c)). The intrinsic fluorescence intensity of MP was reduced with increasing concentrations of GLA extract. The addition of GLA extract and PGL could unfold MP, thus exposing tryptophan residues to the surface of the protein and reducing fluorescence intensity [27]. Interaction between the phenolic extracts and MP quenched the tryptophan fluorescence of the protein, leading to a decrease in fluorescence intensity [34]. Hydroxyl groups in phenolic compounds might facilitate the non-covalent interaction with tryptophan in MP [2]. Similarly, the fluorescence intensity of the different concentrations of PGL with MP was lower than that of the control (Figures 2(b) and 2(d)). The results suggest that electron transfer from the electron-rich aromatic groups to electron-deficient side chains could be driving the interactions between MP and PGL [35]. An increase in the concentration of PGL decreased fluorescence intensities, suggesting that more PGL may have been incorporated into the MP. Furthermore, the hidden tryptophan residues of MP may be due to the formation of protein aggregates and increased hydrophobic interactions between the hydrophobic areas [8].

Figures 2(e) and 2(f) show the Stern–Volmer plots of the MP-GLA extract and MP-PGL complexes at different concentrations of GLA extract and PGL. The K_q for MP-GLA extract ($1.94 \times 10^{12} \pm 2.33 \times 10^{10}\,L{\cdot}mol^{-1}{\cdot}s^{-1}$) and MP-PGL ($1.64 \times 10^{12} \pm 1.06 \times 10^{10}\,L{\cdot}mol^{-1}{\cdot}s^{-1}$) were greater than the highest diffusion collision quenching constant, which is $2.0 \times 10^{10}\,(L{\cdot}mol^{-1}{\cdot}s^{-1})$ [36]. These results demonstrated that static quenching was involved in the interactions and formation of the complexes.



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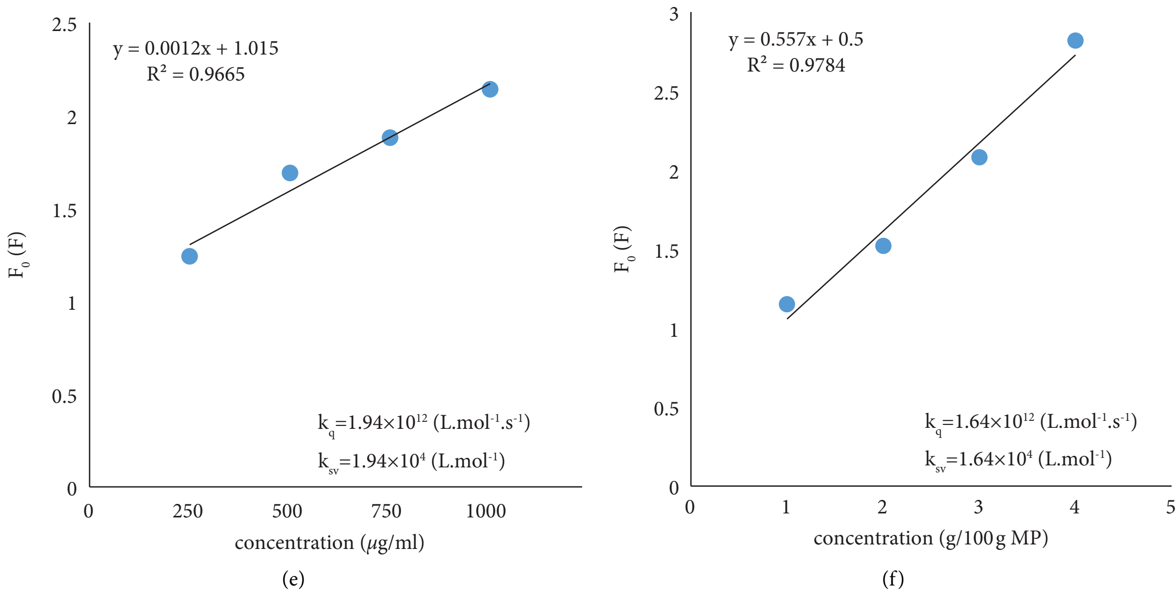

Figure 2: Fluorescence spectra (λ excitation: 280 nm) of MP suspensions with different concentrations of green lentil acetone extract (GLA) (a) and protein green lentil (PGL) (b). Fluorescence intensity (maximum intensity reached by the peak) corresponding to tryptophan residues measured with different concentrations of green lentil acetone extract (GLA) (c) and protein green lentil (PGL) (d) (mean ± standard error). Stern–Volmer plots of the interaction between myofibrillar proteins MP-GLA (e) and MP-PGL (f); a–e represent 0, 250, 500, 750, and 1000 μg/mL of GLA and A–E represent 0, 1, 2, 3, and 4 g/100 g MP of PGL.

3.4. Surface Hydrophobicity. Surface hydrophobicity is important in the determination of protein functional properties, such as emulsion formation and foam stability. An increase in surface hydrophobicity is associated with the oxidation, denaturation, and unfolding of MP [19]. As shown in Figures 3(a) and 3(b), the addition of GLA extract and PGL significantly decreased the surface hydrophobicity of MP ($P < 0.05$). The reduction in surface hydrophobicity occurred due to the aggregation of MP during the binding of the phenolic extracts. Indeed, binding of the polar groups (carboxyl and hydroxyl groups) of phenolic compounds to hydrophobic amino acid side chain groups (aromatic rings) on the protein surface reduces the binding sites of ANS [36]. Li et al. [4] studied the effect of tea phenolic compounds on the functional properties of MP. The results showed that the surface of MP treated with tea phenolic compounds became hydrophilic, which could be caused by the interaction between tea phenolic compounds and the hydrophobic amino acid residues of MP. Simultaneously, phenolic hydroxyl and carboxyl groups may provide a justification for the increase in surface hydrophobicity. Lv et al. [37] explained that the reduced concentrations of free amines and thiol groups after the addition of EGCG to proteins could decrease surface hydrophobicity. Similarly, results showed a significant reduction in surface hydrophobicity with the addition of PGL. The increase in the concentration of PGL can induce protein-protein aggregation phenomena due to the interaction of hydrophobic groups, resulting in decreased surface hydrophobicity. More-stable protein composites have been formed as the hydrophobic interaction between proteins has been strengthened [31, 38].

3.5. Thermal Stability of MP. A DSC analysis was conducted to study the thermal properties of MP. MP control displayed the usual thermal curve with two endotherm peaks at 42.28°C and 81.8°C, attributed to myosin heavy chain and actin, respectively. Myosin is essential for the formation of gels in meat products [38]. Therefore, only the results related to myosin were reported. The addition of GLA extract and PGL shifted the transition peak to lower temperatures (Table 2). MP-GLA extract and MP-PGL interactions might have damaged the structure of MP, resulting in the loss of thermal stability. Consequently, the transition enthalpies were reduced in MP-GLA extract and MP-PGL complexes, as previously reported, due to protein unfolding and cross linking [5].

3.6. FTIR Spectroscopy. Modification of protein secondary structure was evaluated using FTIR spectroscopy. Extension of the amide I band (1700–1600 cm^{-1}) and amide II band shift (1600–1500 cm^{-1}, N-H bending, and C-N stretching) explain the protein secondary structure; changes can be studied with spectral shift and changes in the intensity of these two bands [34]. The FTIR spectra of MP-GLA extract and MP-PGL were mostly similar to the FTIR spectrum of MP. This suggests that GLA extract and PGL may have interacted with MP through noncovalent interactions. Moreover, the shift of the peak at 3468 cm^{-1} (O-H) suggests that the GLA extract and PGL may have formed hydrogen bonds with MP [34]. The addition of GLA extract shifted amide I and amide II of MP from 1654.15 cm^{-1} to 1644.70 cm^{-1} and from 1542.16 cm^{-1} to 1539.55 cm^{-1}, respectively (Figure 1(c)). The addition of PGL moved the amide I and amide II bands of MP from 1654.15 cm^{-1} to

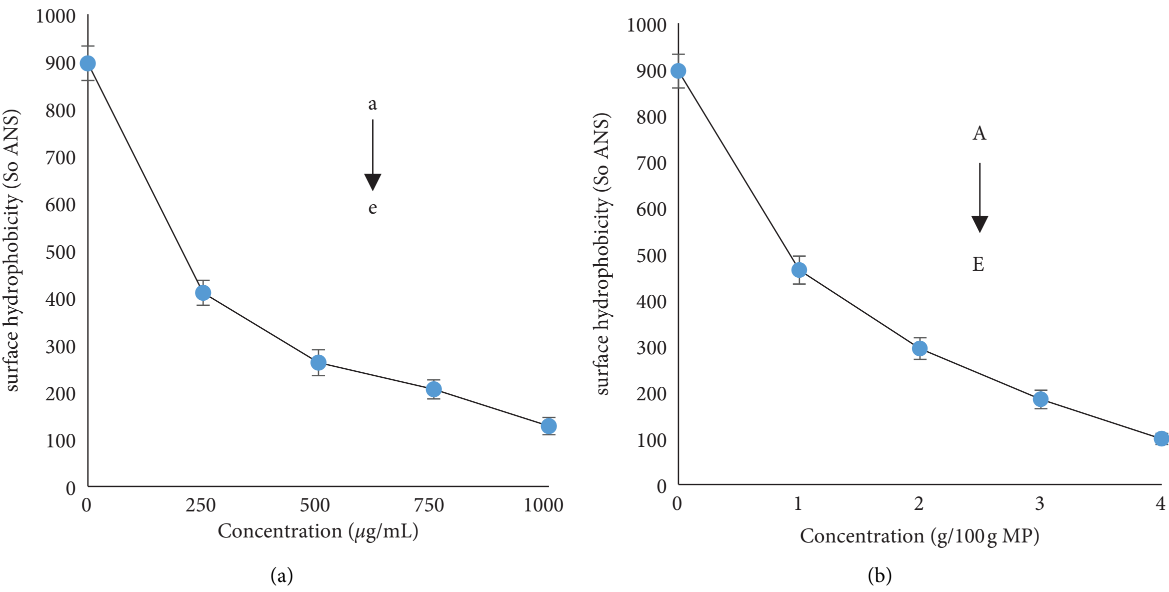

Figure 3: Surface hydrophobicity of myofibrillar proteins (MP) with different concentrations of green lentil acetone extract (a) and protein of green lentil (b) (mean ± standard error); a–e represent 0, 250, 500, 750, and 1000 μg/mL of GLA and A–E represent 0, 1, 2, 3, and 4 g/100 g MP of PGL.

Table 2: Temperature maximum for a transition (T_{max}) and total enthalpy of myofibrillar proteins (MP) with green lentil acetone extract (GLA) and protein green lentil (PGL).

Sample	Transition temperature (T_{max}) (°C)	ΔH (J/g)
MP	42.28 ± 0.07^{a}	1.70 ± 0.03^{a}
MP-GLA extract	40.33 ± 0.06^{b}	1.65 ± 0.04^{a}
MP-PGL	40.48 ± 0.07^{b}	1.57 ± 0.03^{b}

Results are displayed as mean ± standard error. Values with different superscripted letters within the same row are significantly different ($n = 3$, $P < 0.05$).

1640.75 cm^{-1} and from 1542.16 cm^{-1} to 1541.33 cm^{-1}, respectively (Figure 1(d)). The intensity of these bands decreased in MP-GLA extract and MP-PGL due to changes in myosin and N-H bending [8, 36].

3.7. Solubility. Protein solubility plays the most important role in determining the functional properties of proteins. Different intrinsic factors, such as ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds, affect the solubility of proteins [39]. The solubility of MP varied with the addition of different concentrations of GLA extract and PGL (Table 1). The protein solubility of the MP-GLA extract and MP-PGL complexes decreased compared to MP. This reduction in protein solubility may be attributed to the formation of hydrophobic interactions and disulfide bonds [31]. This was likely due to the unfolding of MP induced by GLA extract and PGL. According to Nie et al. [40], phenolic compounds of green tea and procyanidin of grapes reduced MP solubility. The decrease in protein solubility demonstrated that phenolic compounds included in green tea and grape seeds increased protein aggregation, predominantly via non-disulfide covalent bonds. The solubility of MP-PGL complexes improved with increasing concentrations of PGL, although these were not significantly different ($P > 0.05$). Niu et al. [11] confirmed that the presence of hydrogen bonding in complexes could improve protein solubility. Sha et al. [41] explained the modest reduction in MP solubility after adding soy isolate protein to the reduced solubility of soy isolate protein, resulting in MP dilution. They also identified a correlation between a reduction in surface hydrophobicity index and increased protein solubility.

3.8. Cooking Loss and Water Holding Capacity. The control MP had a higher cooking loss than the MP with added extract, except for GLA250 (Table 1). The reduction of cooking loss was decreased at a higher concentration of phenolic extract. However, the cooking loss of extracts higher than 250 μg/mL was not significantly different ($P < 0.05$). The cooking loss of the control MP was significantly higher than that of MP with different concentrations of PGL ($P < 0.05$). Among all samples, 4 g/100 g MP of PGL showed the highest reduction in cooking loss. The physical form of the protein matrix swells in contact with water and prevents the moisture and fat from escaping the system, consequently decreasing cooking loss [42]. Lee et al. [43] suggested that the combination of red bean protein isolate improved the cooking yield due to the stabilization of the protein structures and the promotion of the water-holding capacity through increased protein-protein interaction and protein-water interaction.

The WHC represents the capacity of designed protein gel networks to preserve water. The three-dimensional network formed in MP after heating and cooling causes water to be trapped in the protein network [2]. Results showed that

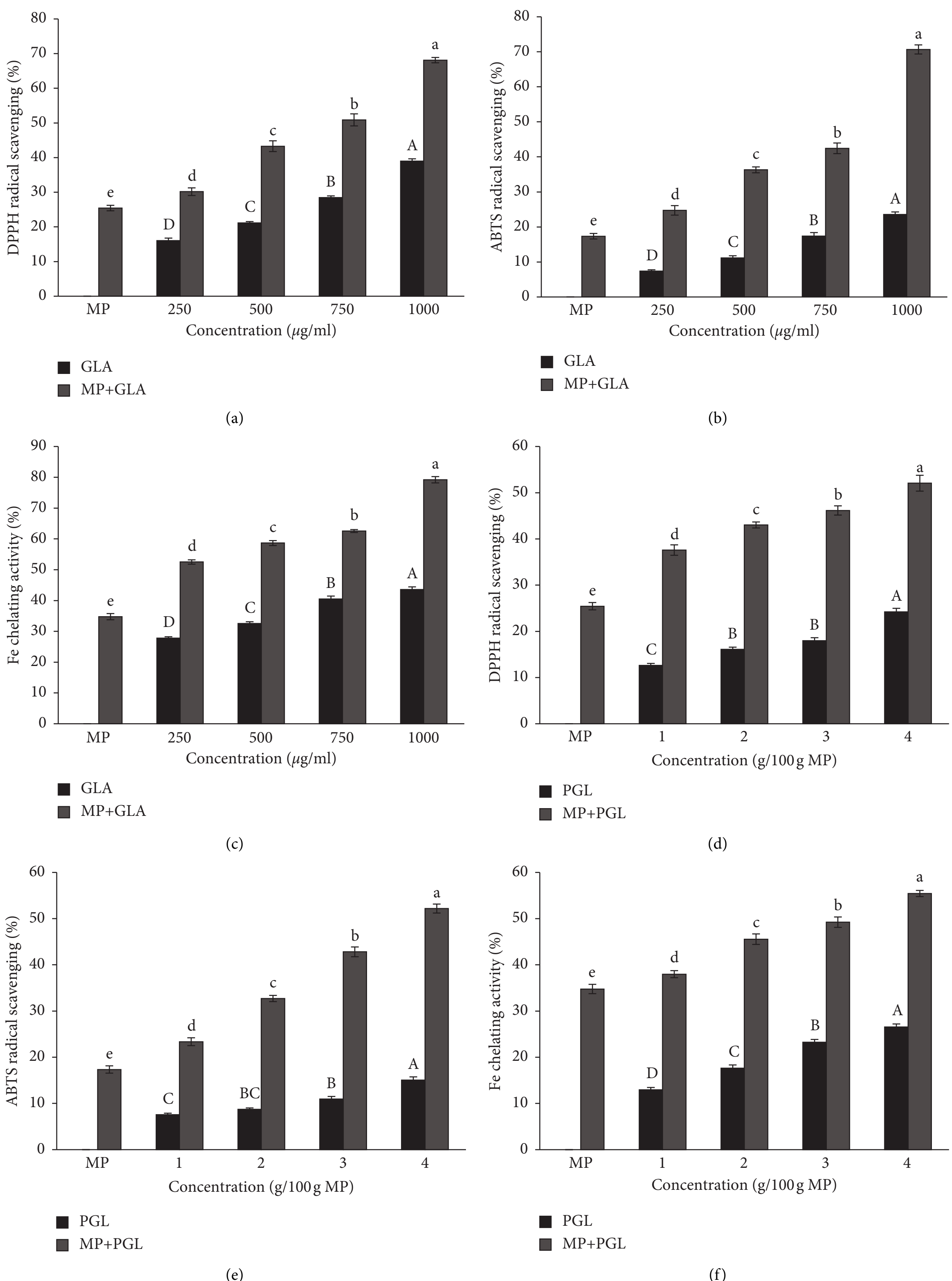

Figure 4: Effects of green lentil acetone extract (GLA) (a, b, c) and protein of green lentil (PGL) (d, e, f) on antioxidant activity of myofibrillar proteins (MP) (mean ± standard error). Different letters indicate significant differences among samples ($P < 0.05$). DPPH, DPPH radical scavenging; ABTS, $ABTS^{+}$ radical scavenging; Fe, Fe^{2+} chelating activity.

increasing the concentration of GLA extract and PGL slightly enhanced the WHC of MP ($P < 0.05$). However, there was no significant difference between the samples (Table 2). Niu et al. [11] reported that cohesive MP-soy protein isolate could promote reactive side chain groups and trap water molecules. Consequently, an increase in the hydrogen bonding within the protein gel improved the cooking loss and WHC.

3.9. Antioxidant Activity. Figure 4 shows the antioxidant activity of the MP-GLA extract and MP-PGL samples compared to the MP control. The results showed dose-dependent antioxidant activity for both GLA extract and PGL. The DPPH radical scavenging activity of MP was significantly enhanced from 25.44 ± 0.79% to 68.12 ± 0.77% with increasing concentrations of GLA extract between 0 and 1000 μg/mL (Figure 4(a)). As the concentration of PGL was increased, the DPPH scavenging activity of MP increased from 25.44 ± 0.46% to 52.06 ± 1.01% for 0 to 4 g/100 g MP (Figure 4(d)). $ABTS^+$ radical scavenging activity of GLA-MP complex also increased from 17.35 ± 1.10% to 70.68 ± 0.99% for 0 and 1000 μg/mL (Figure 4(b)). Similarly, $ABTS^+$ scavenging of MP significantly increased with the addition of PGL (0 and 4 g/100 g MP) from 17.35 ± 0.89% to 52.18 ± 0.71% (Figure 4(e)). Finally, the addition of 0–1000 μg/mL of GLA extract significantly increased the activity of MP in chelating Fe^{2+} ($P < 0.05$) from 34.77 ± 0.96% to 79.21 ± 0.62% (Figure 4(c)). The Fe^{2+} chelating activity of PGL-MP was enhanced from 34.77 ± 1.01% to 55.44 ± 0.66% for 0 and 4 g/100 g MP (Figure 4(f)).

The presence of hydroxyl groups in phenolic compounds contributes to electron or hydrogen atom transfer to free radicals, thus leading to antioxidant activity. Zhang et al. [14] reported that the phenolic compounds of lentils consisted of flavanols, flavonol glycosides, and procyanidin oligomers, and that the phenolic compounds (especially kaempferol, quercetin, catechin, and epicatechin) displayed strong antioxidant activity. Almajano et al. [6] found that the loss of aromaticity in one of the rings of EGCG on oxidation to quinone and binding to protein increased the antioxidant properties of the protein products. The scavenging activity of MP-GLA extract and MP-PGL complexes was higher than that of GLA extract and PGL alone. Thus, the MP-phenolic and protein-protein interactions produced adducts or complexes with higher antioxidant activities [5]. In contrast, phenolic compound-protein interactions had a masking effect on the antioxidant activity of phenolic compounds [44]. On the other hand, proteins are hydrogen atom donors and could react with free radicals to produce more stable compounds, thus exhibiting antioxidant activities [45]. Megías et al. [46] suggested that catechol groups are mostly responsible for the metal chelation capacity of phenolic extracts. Huyut et al. [47] attributed the chelating activity of phenolic compounds to the presence of multiple hydroxyl groups. Furthermore, the chelation of prooxidant metals using proteins could disrupt the conversion of hydroperoxide to volatile compounds and autoxidation. Protein, as an electron donor, can form complexes with metals, thus sterically hindering metal-peroxide interactions [48]. The high content of glutamic acid and aspartic acid could enhance the metal chelating activity of proteins [18]. Also, [49] reported that lentil proteins have a high antioxidant capacity due to their high content of amino acids with antioxidant activity (Val, Asp, Glu, Pro, Arg, His, Met, Leu, Ala, and Try). The presence of imidazole rings as a significant proton donor in his residues is credited with strong radical scavenging activity [50].

4. Conclusions

The addition of GLA extract and PGL influenced the physicochemical properties and functionality of MP. MP was modified by GLA extract and PGL, leading to decreased carbonyl content and total sulfhydryl groups at the highest concentrations of GLA extract (1000 μg/mL) and PGL (4 g/100 g MP), as well as changes in the tertiary structure and surface hydrophobicity. The interaction between the sulfhydryl groups of MP and phenolic compounds and lentil protein reduced the oxidation of MP. The noncovalent interaction of hydroxyl groups in GLA and protein-protein hydrophobic interaction decreased the tryptophan fluorescence intensity and surface hydrophobicity of MP. GLA extracted and PGL had a strong antioxidant activity, which may be due to the hydroxyl groups and amino acids of phenolic compounds and the protein of lentils. Taken together, the findings demonstrate that the interaction of phenolic extract and proteins from lentils with MP can be explored as a potential strategy for enhancing functional and bioactive properties in the production and processing of meat products.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Additional Points

The objective of this study was to evaluate the influence of various concentrations of phenolic extracts and lentil protein isolate on the configuration, functional, and physicochemical properties of myofibrillar protein. Carbonyl, sulfhydryl, Fourier transform infrared spectroscopy, surface hydrophobicity, and intrinsic tryptophan fluorescence were used to evaluate structural changes in the myofibrillar protein, while solubility, cooking loss, water-holding capacity, thermal stability, and antioxidant activity were used to evaluate functional changes. The findings demonstrated the potential of phenolic compounds and proteins in lentils as functional compounds for improving the structure and attributes of myofibrillar protein and meat products. The heat-induced gelation of myofibrillar proteins plays a vital role in the production and processing of meat products.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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