Food Bioscience 59 (2024) 104166

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Effect of interaction between resveratrol and myofibrillar protein on the production of bound heterocyclic amines

Xiaoyue Yang [a,b,c,1], Dequan Zhang [a,b], Christophe Blecker [c], Chunjiang Zhang [a,b], Xiangxiang Sun [a,b], Zhenyu Wang [a,b,*]

[a] Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China
[b] Integrated Laboratory of Processing Technology for Chinese Meat and Dish Products, Ministry of Agriculture and Rural Affairs, Beijing, 100193, PR China
[c] Department of Food Science and Formulation, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, Gembloux, B-5030, Belgium

ARTICLE INFO

Keywords:
Bound heterocyclic amines
Resveratrol
Myofibrillar proteins
Structural characteristics
Interaction

ABSTRACT

To elucidate the pathways and mechanisms by which polyphenols inhibit protein-bound heterocyclic amines (HAs), the influence of resveratrol on bound/free HAs and the structure changes of myofibrillar proteins (MP) in the system of creatinine/MP/glucose model was studied. The results demonstrate that resveratrol significantly reduced bound and free HAs in the reaction systems ($P < 0.05$), and facilitates the transition from bound to free HAs. In addition, resveratrol altered the secondary structure of MP and had a static quenching effect on MP fluorescence. The strong interactions between resveratrol and MP are mainly hydrogen bonding and hydrophobic interactions. Furthermore, the results of molecular docking demonstrated that resveratrol exposed more hydrophobic amino acids (Ala, Lys and Pro) at the 556–578 amino acid sites of myosin. These results indicate that resveratrol controls the formation of bound HAs by reducing exposure to amino acids involved in the HAs reaction and promoting exposure to non-precursor amino acids.

1. Introduction

Heterocyclic amines (HAs) are easily produced when high-protein foods such as fish and meat are heated at high temperatures and have been widely concerned for their carcinogenic and mutagenic effects (Barzegar et al., 2019). Epidemiological studies suggest that always intake of heat-treated foods with HAs may increase the risk of several cancers (Gibis, 2016; Le Marchand et al., 2002; Nowell et al., 2002). Therefore, it is meaningful and necessary to study its mitigation strategies. In addition to the regulation of processing conditions (temperature, time, processing method), the addition of exogenous substances is a more effective means of inhibition (Dong et al., 2020; Lu et al., 2018). Numerous studies have shown that natural plant extracts, as a healthy and safe HAs inhibitor, are widely used in the meat industry and are inseparable from the polyphenol compounds they contain (Cheng et al., 2007; Oguri et al., 1998). At present, the mechanism of inhibiting HAs by polyphenols is mainly focused on scavenging and capturing intermediate free radicals and reactive carbonyls formed in the reaction (Vitaglione & Fogliano, 2004). The ability of some flavonoids to scavenge alkoxyl radicals was proportional to their ability to inhibit the formation of PhIP (IC50), indicating that scavenging of alkyl radicals is a pathway to inhibit PhIP generation (Yu et al., 2016). It was subsequently discovered that some of the polyphenols could trap or combine the intermediate active carbonyl compound phenylacetaldehyde produced by PhIP. Polyphenols indirectly inhibit the formation of PhIP by preventing further reactions between phenylacetaldehyde and creatinine. (Cheng et al., 2008, 2009).

The discovery of bound HAs has brought into view that proteins also play an important role in the formation of HA. Studies have demonstrated that HAs exist in two forms in food: free and bound. And the bound HAs will be released after gastrointestinal digestion in humans (Szterk, 2013; Xue et al., 2022). There are currently two pathways for the formation of bound HAs: (1) Firstly, free HAs are formed by the reaction of free amino acids, produced by protein degradation or originally present in the system, with other precursors. Then the free HAs bind to the protein to form protein-bound HAs; (2) Protein-bound HAs are directly formed by some groups in the protein peptide chain that react with glucose and creatinine (Chen et al., 2021). Induction of protein carbonylation in the presence of reducing sugars via the Maillard

* Corresponding author.
E-mail address: caasjgsmeat2021_6@126.com (Z. Wang).
[1] First Author: Xiaoyue Yang.

https://doi.org/10.1016/j.fbio.2024.104166
Received 12 March 2024; Received in revised form 18 April 2024; Accepted 20 April 2024
Available online 22 April 2024

Abbreviations

HAs	heterocyclic amines
Harman	1-methyl-9H-pyrido[*3,4-b*]-indole
IQx	2-amino-3-methyl-3H-imidazo[4,5-f]quinoxaline
MeAαC	2-amino-3-methyl-9H-pyrido[2,3-b]indole
MeIQx	2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
MP	myofibrillar proteins
Norharman	9H-pyrido[*3,4-b*]indole
PhIP	2-amino- 1-methyl-6-phenylimidazo[*4,5-b*]-pyridine
7,8-DiMeIQx	2-amino-3,4,7-3H-trimethyl-imidazo[4,5-f] quinoxaline.

reaction pathway, mainly through oxidative deamination of amino acid side chains in proteins, which may further involve in the formation of HAs (Villaverde & Estévez, 2013). The plant extracts piperine, sanshoamide and capsaicin have a significant impact on the inhibition of bound HAs (Xue et al., 2020). However, it is not clear what role polyphenols play in the formation of proteins involved in the bound HAs. And the mechanism by which polyphenols affect protein involvement in the formation of HAs is also unclear. Therefore, three hypotheses were proposed: (i) The binding of polyphenols and proteins affects the direct involvement of proteins in the Maillard reaction to form bound HAs; (ii) polyphenols reduce the formation of free HAs by inhibiting the breakdown of proteins into free amino acids; (iii) Polyphenols promote the formation of bound HAs between proteins and free HAs, reducing the content of free HAs.

Resveratrol, as a natural plant polyphenol mainly present in grapes and red wine, has been widely studied for its antioxidative effect. It can prevent and treat a variety of cancers, including breast cancer induced by PhIP (Dubuisson et al., 2002; Wu et al., 2022). In addition, it has been shown to have a significant inhibitory effect on HAs in a wide range of roasted meat and inhibits the formation of free HAs mainly by scavenging intermediate carbonyl compounds (Meurillon et al., 2020; Yang et al., 2023). Therefore, we chose resveratrol as a representative to explore the effect of polyphenols on the bound HAs and the mechanism was identified. The effects of resveratrol on MP participation in the HAs formation were investigated by establishing a Glucose/MP/creatinine model system and a direct reaction system of protein and HAs, and the intervention pathway was clarified. The mechanism of resveratrol's influence on the formation of bound HAs was further explored through its effects on the structure and physicochemical properties of MP. This study provides a reference and theoretical basis for supplementing and perfecting the mechanism of HAs formation inhibition by polyphenols and is of great significance for controlling the formation of HAs during meat processing.

2. Materials and methods

2.1. Materials

Resveratrol (98%) were from Yuanye (Shanghai, China). HAs standards (99.9%): Harman, Norharman, IQx, MeAαC, MeIQx, 7,8-DiMeIQx, PhIP, were purchased from TRC (Toronto, ONT, Canada). Ammonium acetate (99.9%), diethylene glycol (99%), sodium hydroxide, ethyl acetate (99.5%), creatinine and glucose, were purchased from Merck KGaA (Darmstadt, Hessian, Germany), Aladdin (Shanghai, China), and Macklin (Shanghai, China) and Solarbio (Beijing, China), respectively. Acetonitrile, acetic acid and methanol use chromatographic grades purchased from Thermo Fisher (Waltham, MA, USA). The analytical grade was used for all other reagents from Sinopharm Chemical Reagent (Shanghai, China). The *Longissimus dorsi* (LD) muscles were obtained from 8 months of age of small-tailed Han lambs, which were bought from Hebei Jinhong Halal Meat Company Limited and stored at −20 °C until use.

2.2. Preparation of MP and establishment of reaction systems

MP was prepared based on the method reported by Sun et al. (2023). Ground 60 g of meat with fat and connective tissue removed. One meat sample and 10 vol of (w/v) buffer A ($MgCl_2$, EGTA, 0.002 mol/L; KCl, 0.1 mol/L; K_2HPO_4, 0.02 mol/L; pH 6.8) were homogenized and centrifuged. The sediment was mixed in 8 vol (w/v) of buffer B ($MgCl_2$, EGTA, 0.002 mol/L; KCl, 0.1 mol/L; 10% Triton X-100; K_2HPO_4, 0.02 mol/L; pH 6.8) and centrifuged, the operation was repeated twice. Then the precipitate was dissolved in 8 vol (w/v) 0.1 mol/L KCl and centrifuged, repeated twice. All the conditions of centrifugation were 2000×*g* for 15 min at 4 °C (10000 rpm in an R10A3 rotor, CR21N High-Speed Refrigerated Centrifuge, Hitachi, Ltd., Tokyo, Japan). The final precipitate was myofibrillar proteins and the concentration was determined by a BCA kit.

The model reaction systems were constructed similarly to the previous research (Yang et al., 2023). (i) Glucose/MP/creatinine model system (MPMD + P): Myofibrillar protein (20 mg/mL,10 mL), glucose and resveratrol (both 0.2 mmol), creatinine (0.4 mmol) were mixture in closed reaction bottles and heated at 150 °C for 1 h (ii) MP/HAs reaction system (MP + HAs + P): Myofibrillar protein (20 mg/mL,10 mL), HAs mixture standard solution (5 mg/L,100 μL) and resveratrol (0.2 mmol) were mixture in closed reaction bottles and heated at 150 °C for 1 h. The systems without resveratrol were control groups (MPMD and MP + HAs). Moreover, the two groups with only HAs and HAs with resveratrol (HAs + P) were also heated at 150 °C for 1 h and served as controls.

2.3. Determination of free and bound HAs in reaction systems

The methods of extraction and determination of two types of HAs refer to previous methods (Yang et al., 2023). (i) Free HAs extraction: All simulated reaction solutions were homogeneously mixed with ethyl acetate (20 mL) and extracted for 30 min by ultrasonication, then centrifuged (3000 rpm, 10 min). Collected the supernatant ethyl acetate extraction phase and repeated the operation twice. The collected extract was pooled and nitrogen blasted to about 20 mL as a sample. Samples were separated, enriched and purified with a fully automated solid-phase extractor and MCX solid-phase extraction cartridge. (ii) Bound HAs extraction: The same volume of concentrated HCl was added to the lower liquid obtained by centrifugation in (i) to make the final concentration about 6 mol/L, and hydrolysis at 110 °C for a day. Before hydrolysis, ethyl acetate is completely volatilized. Filtered the hydrolysate and volume to 100 mL and 10 mL was taken for purification. The remaining steps were the same as for free HAs except that when activating the MCX column, 0.1 mol/L HCl (6 mL) was used instead of ethyl acetate (6 mL).

2.4. Determination of free amino acids

The sample solution was ultrasonicated for 10 min and centrifuged for 15 min at 5000 r/min, then the volume was determined to be 10 mL. Mixed 1 mL of the constant volume solution with an equal volume of sulfosalicylic acid and centrifuged (10000 r/min, 15 min). After centrifugation, dried 1 mL of supernatant with nitrogen, and 1 mL 0.02 mol/L HCl were added to redissolve. Then an L-8900 amino acid analyzer (Hitachi, Tokyo, Japan) was used for analysis.

2.5. Microstructure observation of scanning electron microscope (SEM)

The before and after heating reaction liquids of the Glucose/MP/creatinine model system in 2.2, MP, and MP with resveratrol (MP + P) were freeze-dried to obtain the samples for SEM and FTIR. The samples were observed by SEM-450 (FEI, Hillsboro, OR, USA) after spraying gold

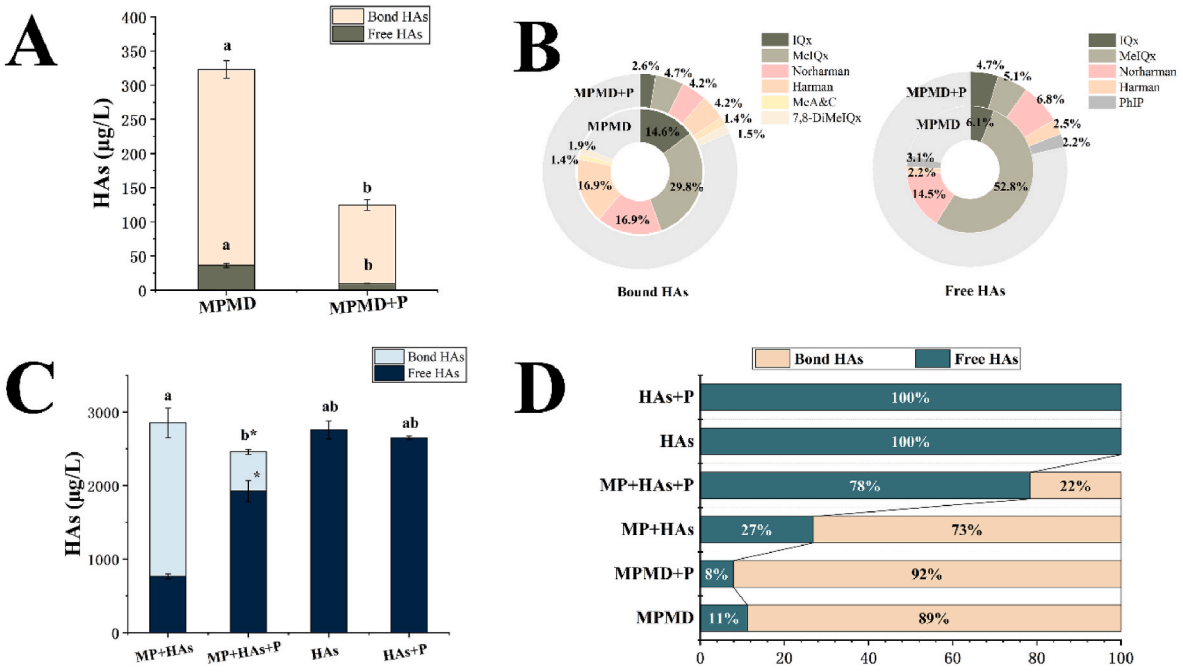

Fig. 1. Confirmation of resveratrol on the mechanism of MP involved in the formation of bound HAs. Different letters in the same index indicate significant differences ($P < 0.05$); * indicates a significant difference in the content of free and bound HAs as compared to no resveratrol added.

on the bonded plates.

2.6. Analysis of Fourier transform infrared spectroscopy (FTIR)

All operations were performed according to Sun et al. (2023), with slight modifications. KBr and the sample (100:1, w/w) were milled uniformly and pressed into thin pieces before measurements with the instrument (TENSOR 27, Bruker, Ettlingen, Germany). The spectra were recorded between 400 and 4000 cm^{-1}. Changes in the relative content of protein secondary structure of different samples were calculated by Peakfit v4.12 (Chicago, Illinois, USA).

2.7. Determination of chemical interaction

The chemical interactions were measured as protein solubility according to the previous research (Mi et al., 2022). Prepare the solution S1–S5 required for the reaction: S1, 0.05 mol/L NaCl; S2, 0.6 mol/L NaCl; S3, 0.6 mol/L NaCl +1.5 mol/L Urea; S4, 0.6 mol/L NaCl +8 mol/L Urea; S5, 0.6 mol/L NaCl +8 mol/L Urea +0.5 mol/L 2-β-mercaptoethanol. Ten milliliters of the MP and MP with resveratrol samples were homogenized with 10 mL of S1–S5 solutions at 4 °C, respectively. After centrifugation, the supernatant was obtained and the protein content was quantified by BCA kit. The chemical forces were calculated from the differences in solubilized proteins in these solutions, such as ionic bonds (S2–S1), hydrogen bonds (S3–S2), hydrophobic interactions (S4–S3), and disulfide bonds (S5–S4).

2.8. Fluorescence spectroscopy

MP and resveratrol were diluted with PBS solution to final concentrations of 0.4 mg/mL and 4–12 μg/mL, respectively, and mixed and left for 2 h. Fluorescence spectra were then determined with a Spark multifunctional microplate enzyme marker (Tecan, Männedorf, Switzerland). The test conditions of the enzyme-label instrument are excitation wavelength, 280 nm; emission spectra, 300–500 nm at 298 K; slit widths, 5 nm.

2.9. Molecular docking

MP as a mixture is not amenable to direct molecular docking. Therefore, we selected myosin, which accounts for 55–60% of myofibrillar components (Xia et al., 2019), for further analysis. The amino acid sequences of sheep myosin-1 (MYH-1, ID: W5PPG6) were acquired from UniProt (https://www.uniprot.org/). A suitable template for the construction of sheep MYH-1 fragment (XP_004012755.2) was found by NCBI BLAST analysis searching for sequence similarity. The three-dimensional structure of resveratrol (CID: 445154) was from the PubChem. The molecular docking studies refer to the methods of Morris et al. (2009) with the help of Autodock 4.2.6 and AutoDuck tools 1.5.7. The binding interaction of myosin-heavy chains with resveratrol was demonstrated using LigPlot^{+} software (Laskowski & Swindells, 2011). The visualization was displayed using PyMoL (Schrödinger & DeLano, 2020).

2.10. Statistical analysis

Each experiment was replicated at least in triplicate. Values were presented as mean ± SD. Analysis of ANOVA and Duncan's multiple range tests were performed by SPSS 20 (IBM Corp., New York, USA), where results of $P < 0.05$ were considered significant among samples.

3. Results and discussion

3.1. Inhibitory mechanism analysis of resveratrol on the participation of MP in HAs formation

In the model of the MP/creatinine/glucose system, the bound HAs produced by heating were much higher than free HAs. Resveratrol significantly reduced both free and bound HAs ($P < 0.05$), while increasing the proportion of bound HAs from 89% to 92% (Fig. 1A and D). A total of 5 free HAs (IQx, MeIQx, Norharman, Harman and PhIP) and 6 bound HAs (IQx, MeIQx, Norharman, Harman, MeA&C and 7,8-DiMeIQx) were detected in glucose/MP/creatinine model systems (Fig. 1B). Among them, PhIP mainly exists in the free state, MeA&C and 7,8-DiMeIQx are more inclined to exist in the combined state. In

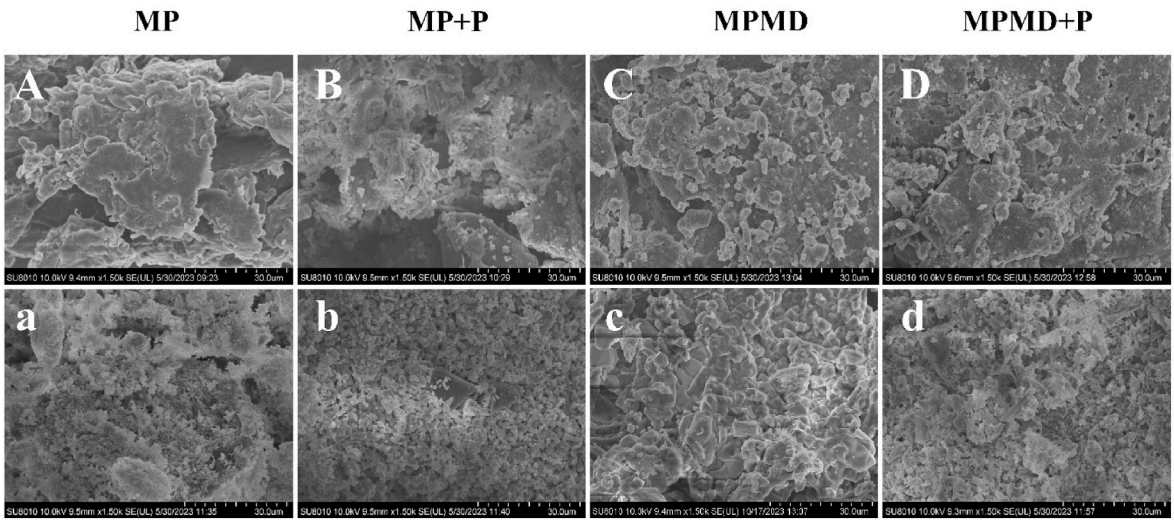

Fig. 2. Effect of resveratrol on the microstructure of MP before (A–D) and after (a–d) heating in the process of HAs formation.

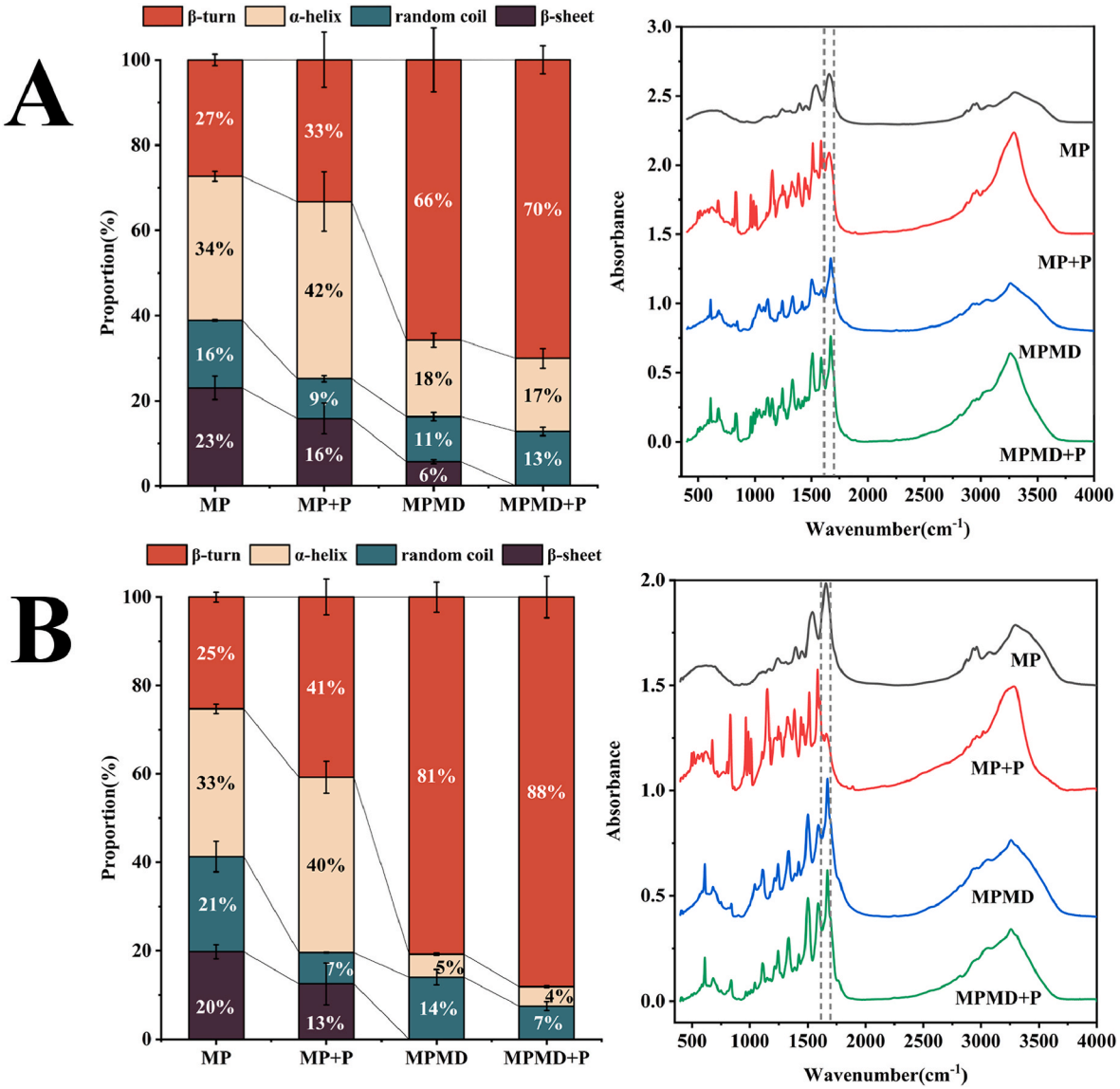

Fig. 3. Effects of resveratrol on the spectrum of FTIR and the relative content of secondary structure in MP before (A) and after (B) heating.

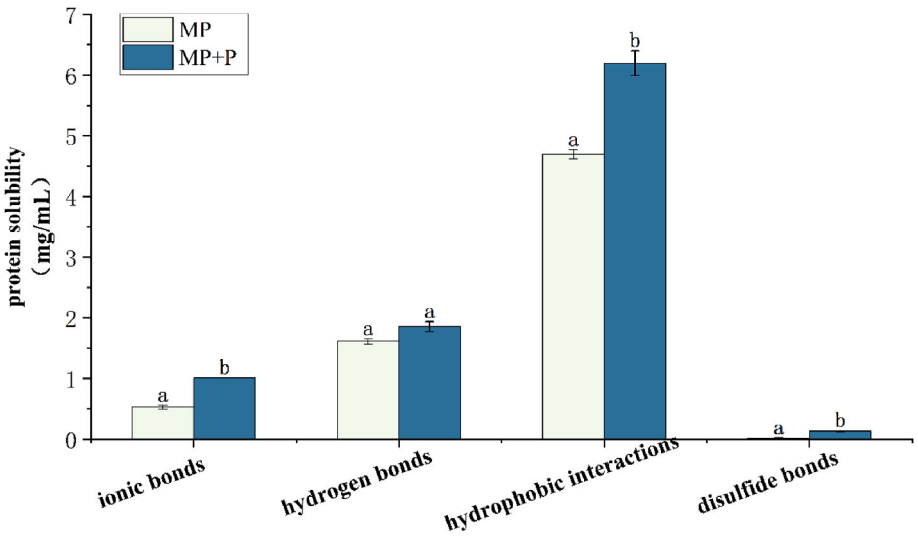

Fig. 4. Effects of resveratrol on the protein solubility of MP.

addition, MeIQx, Norharman, Harman and PhIP had higher percentages, similar to previous results in roasted meat (Yang et al., 2023), and the addition of resveratrol also mainly reduced the contents of these HAs. In addition, no free amino acids were detected in the MP solution before and after the addition of resveratrol, which suggests that MP does act as a precursor directly involved in the production of HAs and that polyphenols have an inhibitory effect on this process.

To further clarify whether polyphenols interact with muscle proteins to promote protein and HAs binding, the changes of two types of HAs in the direct response system of free HAs and MP were determined. The results showed that the bound HAs could be formed by the direct binding of MP and free HAs. In addition, resveratrol was effective in converting bound HAs to free HAs (Fig. 1C and D), indicating that the combination may be formed primarily by intermolecular interactions and is easily broken down. Xue et al. (2022) also showed that the addition of pepper, onion and apple, rich in polyphenols, promotes the transition from bound to free HAs during *in vitro* digestion. This further suggests that polyphenols reduce the production of bound HAs primarily by influencing MP to participate directly in the Maillard reaction.

3.2. Effect of resveratrol on morphologic changes in MP involved in HAs formation

To further clarify the structural changes of resveratrol on MP involved in the process of HAs production, the microstructure changes of proteins were observed by scanning electron microscopy with or without resveratrol in the model of MP/glucose/creatinine system before and after heating. It can be observed that heating breaks the orderly arrangement and tight union of the MP, the edges become rough and irregular clumps form (Fig. 2A and a). These changes can be due to heating-induced denaturation of MP (Liu et al., 2019). A similar result was shown in the addition of resveratrol, the surface of the protein became rough and formed dense spherical aggregates, indicating that the structure of the proteins changed with the addition of resveratrol (Fig. 2B, b,2D). Compared with the control group, MP becomes unfolded and loosely clustered in the glucose/MP/creatinine model system (Fig. 2C). It may be that excess glucose and amino acids may cover the MP to inhibit the interaction between MP molecules (Wang et al., 2023). Heating causes a large amount of aggregation and the surface becomes smooth, which may be caused by the formation of bound HAs (Fig. 2c). After the addition of resveratrol, the large aggregates disappear and return to a rough state to form more dense aggregation than the addition of resveratrol in MP alone (Fig. 2d). This may be due to the addition of resveratrol reducing the formation of bound HAs. These results indicated that resveratrol alters the structure of MP involved in the formation of bound HAs.

3.3. Effect of resveratrol on FT-IR of MP before and after heating

The amide I in the Raman bands with the range of 1600–1700 cm^{-1} reflects the changes in protein secondary structure (Herrero, 2008). Among them, 1615-1637 cm^{-1}, 1646-1664 cm^{-1}, 1637-1645 cm^{-1}, and 1664-1681 cm^{-1} are characteristic bands of β-sheet, α-helix, random coil and β-turn, respectively (Wu et al., 2023). Fig. 3 shows the percentage changes in the secondary structure of proteins in different treatments. After high-temperature heating, the proportion of random coil in MP increased while the β-turn and β-sheet decreased. The addition of resveratrol increased the α-helix in MP from 34% and 33% before and after heating to 42% and 40%, respectively. β-turn was also observed to increase from 27% and 25% to 33% and 41%, respectively, whereas the random coil was down from 16% and 21% to 9% and 7%, respectively. In general, the α-helix structure is maintained primarily by hydrogen bonding between the carbonyl and amino group in the polypeptide chain and is closely related to the stability of proteins (Kašička, 2013). The increase in its content suggests that resveratrol promotes tight binding and aggregation of proteins. In the Glucose/MP/creatinine model system, the decrease in α-helix and the increase in β-turn indicate that glucose and creatinine make MP more flexible and disordered. After heating, the generated HAs further converted the α-helix and β-sheet of MP to β-turn, indicating that the formation of bound HAs changes the structure of MP. In addition, the binding between HAs and MP may further weaken the intermolecular interaction of MP and reduce the number of hydrogen bonds.

3.4. Effect of resveratrol on intermolecular interaction forces of MP

The intermolecular forces were analyzed to further clarify how resveratrol affects MP participation in the formation of bound HAs. The difference in protein solubility of MP with and without resveratrol in different solutions reflects changes in specific chemical bonds, the results of which are shown in Fig. 4. It can be seen that the main interactions of MP are hydrophobic interaction and hydrogen bond, and the ionic and disulfide bonds are relatively less. The addition of resveratrol significantly increased hydrophobic interactions, ionic bonds, and disulfide bonds ($P < 0.05$), suggesting that the interaction between resveratrol and MP unfolds the protein, exposing more residues and increasing ionic and disulfide bonds (Yi et al., 2023).

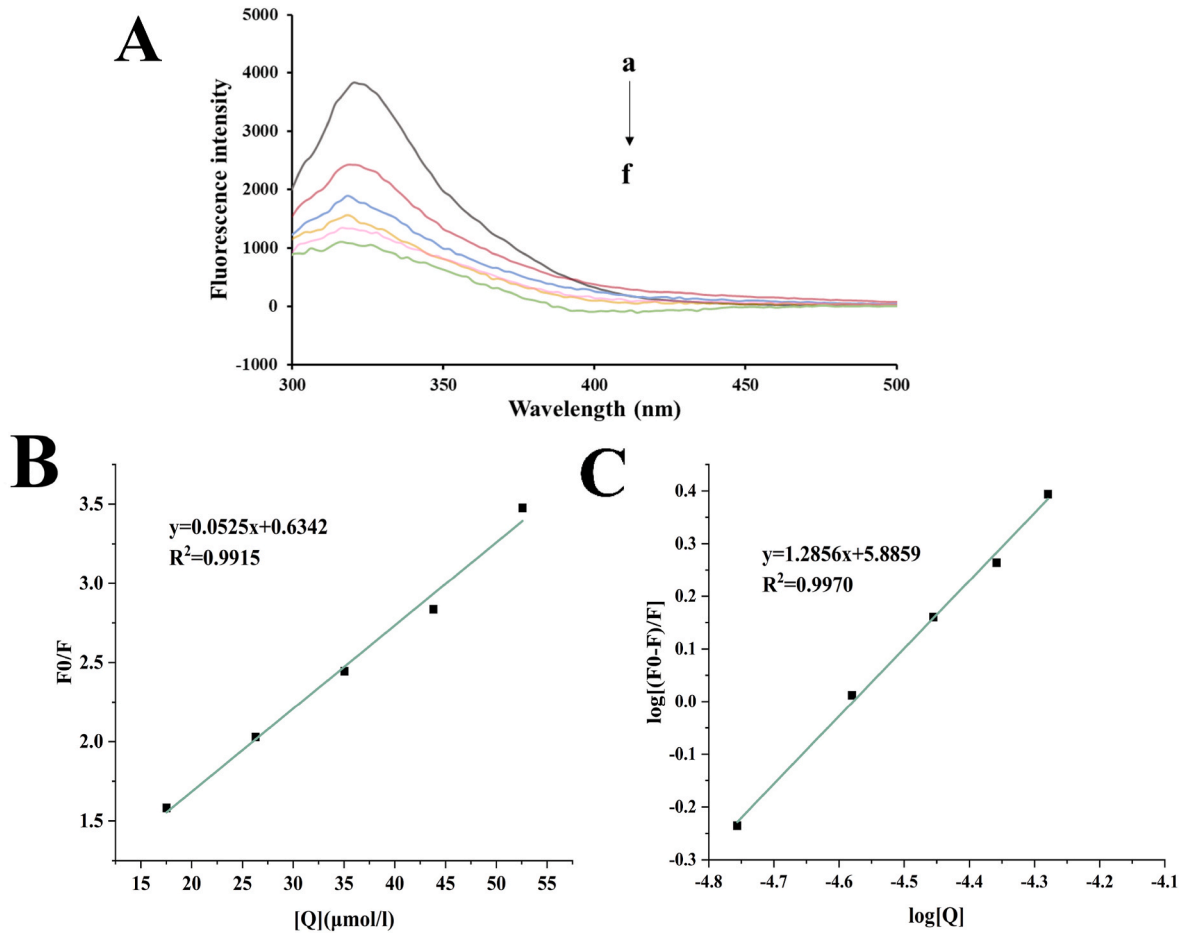

Fig. 5. Analysis of fluorescence quenching mechanism of MP by resveratrol. (A) Fluorescence emission spectrum (λex = 280 nm) of MP (0.5 mg/mL) with various concentrations of resveratrol (a–f: 0, 4, 6, 8, 10, 12 μM); (B) Stern-Volmer plots of MP with resveratrol added; (C) The plots for the static quenching of MP by resveratrol.

3.5. Fluorescence spectroscopy of resveratrol-MP systems

Proteins containing residues of aromatic amino acids (e.g. Tyr and Trp) will produce endogenous fluorescence under excitation light at 280 nm (Zhang et al., 2020). Fig. 5A shows the changes in MP fluorescence intensity with the addition of different concentrations of resveratrol. MP has a maximum fluorescence emission wavelength of 320 nm. With the concentration of resveratrol increased, the fluorescence intensity of MP decreased gradually and the maximum emission wavelength was slightly blue-shifted. The changes in fluorescence intensity and maximum emission wavelength indicate the changes in MP conformation and the surrounding environment of chromophore molecules (Klajnert & Bryszewska, 2002). Chromophore groups are stayed in a more hydrophobic environment with less exposure to solvents (Miriani et al., 2011). Tryptophan is also known to be a precursor for the formation of HAs, primarily Harman and Norharman. These results show that resveratrol may alter the structure of MP to reduce the exposure of residues directly involved in the production of HAs, such as tryptophan and phenylalanine, to inhibit their participation in the production of bound HAs (Fig. 7). The reduced fluorescence intensity is associated to the reduced exposure of Trp and Tyr residues, and may also be due to a shortening of the quenching distance of the quenching group, which leads to the increase in fluorescence quenching (Han et al., 2022). The mechanism of fluorescence quenching was further investigated.

There are two types of fluorescence quenching mechanisms, dynamic and static quenching, which are usually identified by Stern-Volmer quenching constants (Sung et al., 1992). The Stern-Volmer equation is expressed as follows:

$$F_0 / F = 1 + Ksv[Q] = 1 + K_q\tau_0[Q] \quad (1)$$

Ksv is the quenching constant; F is the maximum fluorescence intensity with the quencher added, while F_0 is without; [Q] is the quencher concentration; τ_0 is typically 10^{-8} s, which is the average lifetime of the fluorescent substance without of the quencher. Fig. 5B shows a Stern-Volmer plot of fluorescence quenching of MP by resveratrol. According to the equation and the linear relationship, the calculated quenching constant K_q is 5.25×10^{12} M^{-1} s^{-1}, which is much stronger than the maximum scattering collision quenching constant (2.0×10^{10} $M^{-1}s^{-1}$) between different quenchers and biopolymers (Lakowicz & Weber, 1973). These results demonstrate that resveratrol has a static quenching effect on MP, mainly due to the formation of non-luminous substances between quenchers and fluorescence molecules (Joye et al., 2015). Further calculation of the number of binding sites (*n*) and binding constant (*Ka*):

$$\log\left[(F_0 - F) / F\right] = \log K_a + n \log[Q] \quad (2)$$

The intercept and slope in the log (F_0 − F)/F with log [Q] plot are the values of *Ka* and *n*, respectively (Fig. 5C). In addition, the values of *Ka* (7.69×10^5 L/mol) and *n* (1.29) indicated that the binding strength of resveratrol to MP was stronger than that of some other polyphenols and myosin (Zhang et al., 2020). These results indicate that the MP conformation is significantly deformed when resveratrol interacts with MP.

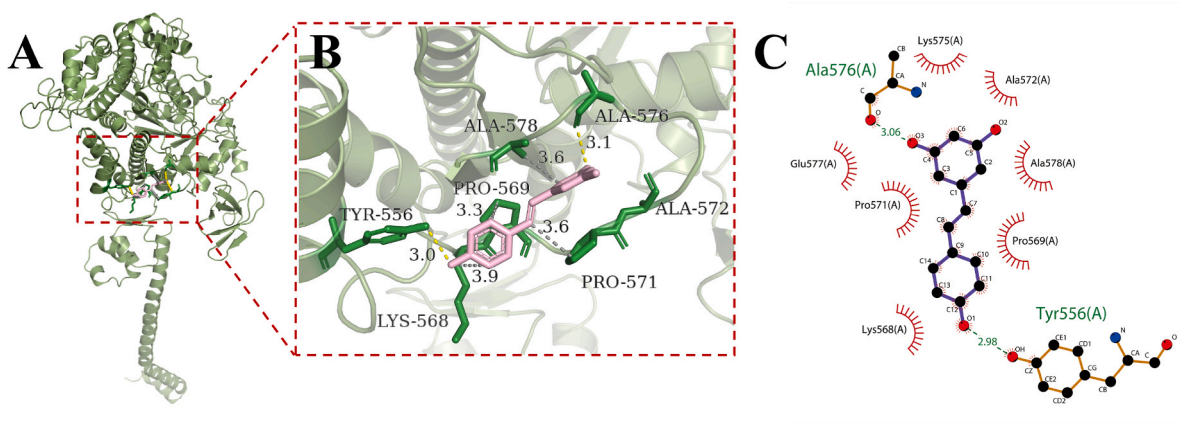

Fig. 6. Results of molecular docking between resveratrol and myosin. (A) Docking model of resveratrol and myosin with the lowest binding affinity (-5.18 kcal/mol); (B) The 3D binding mode of the resveratrol-myosin complex (The grey dashed line represents the hydrophobic interaction and the yellow dashed line represents the hydrogen bond); (C) 2D diagrams of resveratrol-myosin complex (The green dashed line represents hydrogen bonding and image of that eyebrows show hydrophobic interaction). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

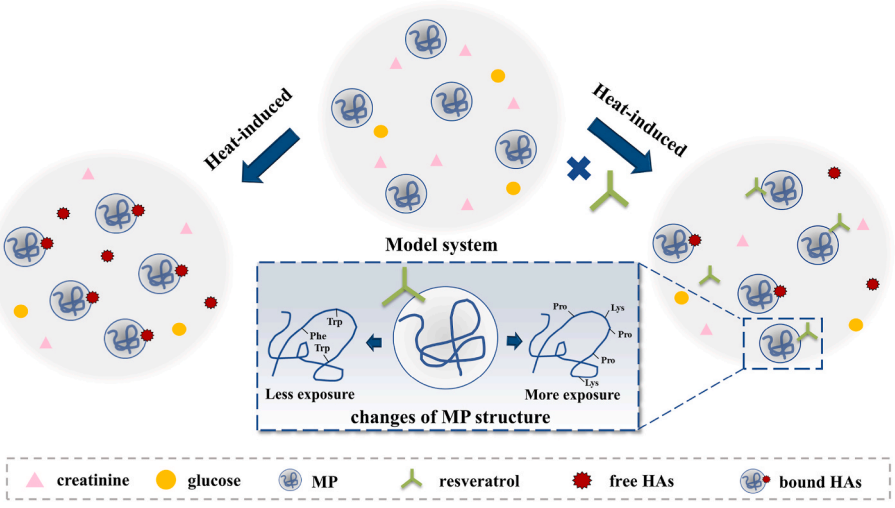

Fig. 7. Model for the inhibiting effect of resveratrol on MP participation in bound HAs production.

3.6. Molecular modeling

Binding sites were explored by molecular docking to further elucidate the details of the resveratrol's effect on the molecular structure of the protein. Of the 10 calculated results, the best-ranked one by energy is shown in Fig. 6A. The results showed that resveratrol and myosin could effectively bind and change the structure of the protein, and the binding between them was mainly through hydrophobic interaction and hydrogen bonding (Fig. 6B). The specific interaction between the key protein residue active site and resveratrol is shown in Fig. 6C. Resveratrol could interact with MP primarily via hydrophobic forces (Pro569, Ala578, Ala572, Lys575, Glu577, Pro571 and Lys568 residues). This phenomenon indicated that the binding points present in the 556–578 amino acids induced changes in the structure of the protein, and exposed many hydrophobic groups, resulting in higher hydrophobic interaction (Privalov & Gill, 1988). In addition, the Ala567 residue (O) and Tyr556 residue (OH) of myosin formed hydrogen bonds with O3 and O1 atoms of resveratrol, with lengths of 3.06 Å and 2.98 Å, respectively, which affected the stability of the complexes.

Similar to others' studies results, polyphenols can alter the structure of proteins by binding to them via noncovalent bonds (Quan et al., 2019). These properties are in agreement with the protein solubility results (Fig. 4) and the MP-resveratrol complex formed by this non-covalent binding may cause quenching of the intrinsic fluorescence in the protein. What's more, resveratrol increases exposure to hydrophobic amino acids in myosin, such as Ala, Lys and Pro. It has been shown that some non-precursor amino acids such as Pro and Lys inhibit the formation of HAs (Deng et al., 2022). Thus resveratrol may reduce the production of HAs by altering the structure of the protein to expose large amounts of non-precursor amino acids that inhibit the production of HAs (Fig. 7).

4. Conclusions

In conclusion, resveratrol inhibits the formation of bound HAs mainly by influencing the direct involvement of proteins in the Maillard reaction. The structural changes of proteins are key to their involvement in the formation of HAs and also the key to making resveratrol exert its inhibitory effect. Resveratrol changes the structure of MP mainly through hydrophobic interactions and hydrogen bonds. On the one hand, changes in protein structure reduce the exposure of some amino acid residues that are directly involved in the production of HAs, reducing the production of bound HAs. On the other hand, resveratrol promotes the exposure of certain hydrophobic amino acids in MP. These amino acids are also non-precursor amino acids that inhibit the formation of HAs. Therefore, this study provides a new perspective to

understand the mechanism of polyphenol inhibition of HAs formation, as well as a basis for screening more effective HAs inhibitors and developing safer high-protein thermally processed foods.

Notes

The authors declare no competing financial interest.

CRediT authorship contribution statement

Xiaoyue Yang: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Dequan Zhang:** Writing – review & editing. **Christophe Blecker:** Writing – review & editing. **Chunjiang Zhang:** Writing – review & editing. **Xiangxiang Sun:** Visualization. **Zhenyu Wang:** Writing – review & editing, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

This work was financially supported by China Agriculture Research System of MOF and MARA (CARS-38), Beijing Innovation Consortium of Agriculture Research System (BAIC06-2023-BJJQ-G12), and Agricultural Science and Technology Innovation Program, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2024-IFST).

References

Barzegar, F., Kamankesh, M., & Mohammadi, A. (2019). Heterocyclic aromatic amines in cooked food: A review on formation, health risk-toxicology and their analytical techniques. *Food Chemistry, 280*, 240–254. https://doi.org/10.1016/j.foodchem.2018.12.058

Chen, Q., Xue, C., He, Z., Wang, Z., Qin, F., Chen, J., & Zeng, M. (2021). Generation of sarcoplasmic and myofibrillar protein-bound heterocyclic amines in chemical model systems under different heating temperatures and durations. *Journal of Agricultural and Food Chemistry, 69*(10), 3232–3246. https://doi.org/10.1021/acs.jafc.0c06885

Cheng, K. W., Chen, F., & Wang, M. (2007). Inhibitory activities of dietary phenolic compounds on heterocyclic amine formation in both chemical model system and beef patties. *Molecular Nutrition & Food Research, 51*(8), 969–976. https://doi.org/10.1002/mnfr.200700032

Cheng, K. W., Wong, C. C., Chao, J., Lo, C., Chen, F., Chu, I. K., Che, C. M., Ho, C. T., & Wang, M. (2009). Inhibition of mutagenic PhIP formation by epigallocatechin gallate via scavenging of phenylacetaldehyde. *Molecular Nutrition & Food Research, 53*(6), 716–725. https://doi.org/10.1002/mnfr.200800206

Cheng, K. W., Wong, C. C., Cho, C. K., Chu, I. K., Sze, K. H., Lo, C., Chen, F., & Wang, M. (2008). Trapping of phenylacetaldehyde as a key mechanism responsible for naringenin's inhibitory activity in mutagenic 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine formation. *Chemical Research in Toxicology, 21*(10), 2026–2034. https://doi.org/10.1021/tx800220h

Deng, P., Xue, C., He, Z., Wang, Z., Qin, F., Oz, E., Chen, J., El Sheikha, A. F., Proestos, C., Oz, F., & Zeng, M. (2022). Synergistic inhibitory effects of selected amino acids on the formation of 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in both benzaldehyde- and phenylacetaldehyde-creatinine model systems. *Journal of Agricultural and Food Chemistry, 70*(35), 10858–10871. https://doi.org/10.1021/acs.jafc.2c03122

Dong, H., Xian, Y., Li, H., Bai, W., & Zeng, X. (2020). Potential carcinogenic heterocyclic aromatic amines (HAAs) in foodstuffs: Formation, extraction, analytical methods, and mitigation strategies. *Comprehensive Reviews in Food Science and Food Safety, 19*(2), 365–404. https://doi.org/10.1111/1541-4337.12527

Dubuisson, J. G., Dyess, D. L., & Gaubatz, J. W. (2002). Resveratrol modulates human mammary epithelial cell O-acetyltransferase, sulfotransferase, and kinase activation of the heterocyclic amine carcinogen N-hydroxy-PhIP. *Cancer Letters, 182*(1), 27–32. https://doi.org/10.1016/s0304-3835(02)00061-7

Gibis, M. (2016). Heterocyclic aromatic amines in cooked meat products: Causes, formation, occurrence, and risk assessment. *Comprehensive Reviews in Food Science and Food Safety, 15*(2), 269–302. https://doi.org/10.1111/1541-4337.12186

Han, P., An, N., Yang, L., Ren, X., Lu, S., Ji, H., & Dong, J. (2022). Molecular dynamics simulation of the interactions between sesamol and myosin combined with spectroscopy and molecular docking studies. *Food Hydrocolloids, 131*, Article 107801. https://doi.org/10.1016/j.foodhyd.2022.107801

Herrero, A. M. (2008). Raman spectroscopy for monitoring protein structure in muscle food systems. *Critical Reviews in Food Science and Nutrition, 48*(6), 512–523. https://doi.org/10.1080/10408390701537385

Joye, I. J., Davidov-Pardo, G., Ludescher, R. D., & McClements, D. J. (2015). Fluorescence quenching study of resveratrol binding to zein and gliadin: Towards a more rational approach to resveratrol encapsulation using water-insoluble proteins. *Food Chemistry, 185*, 261–267. https://doi.org/10.1016/j.foodchem.2015.03.128

Kašička, V. (2013). Amino acids, peptides and proteins. *Electrophoresis, 34*(18), 2603. https://doi.org/10.1002/elps.201370174

Klajnert, B., & Bryszewska, M. (2002). Fluorescence studies on PAMAM dendrimers interactions with bovine serum albumin. *Bioelectrochemistry, 55*, 33–35. https://doi.org/10.1016/S1567-5394(01)00170-0

Lakowicz, J. R., & Weber, G. (1973). Quenching of protein fluorescence by oxygen. Detection of structural fluctuations in proteins on the nanosecond time scale. *Biochemistry, 12*, 4171–4179. https://doi.org/10.1021/bi00745a021

Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling, 51*(10), 2778–2786. https://doi.org/10.1021/ci200227u

Le Marchand, L., Hankin, J. H., Pierce, L. M., Sinha, R., Nerurkar, P. V., Franke, A. A., Wilkens, L. R., Kolonel, L. N., Donlon, T., Seifried, A., Custer, L. J., Lum-Jones, A., & Chang, W. (2002). Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. *Mutation Research, 506–507*, 205–214. https://doi.org/10.1016/s0027-5107(02)00167-7

Liu, H., Wang, Z., Suleman, R., Shen, Q., & Zhang, D. (2019). Effect of protein thermal stability and protein secondary structure on the roasted mutton texture and colour from different cuts. *Meat Science, 156*, 52–58. https://doi.org/10.1016/j.meatsci.2019.05.014

Lu, F., Kuhnle, G. K., & Cheng, Q. (2018). The effect of common spices and meat type on the formation of heterocyclic amines and polycyclic aromatic hydrocarbons in deepfried meatballs. *Food Control, 92*, 399–411. https://doi.org/10.1016/j.foodcont.2018.05.018

Meurillon, M., Angénieux, M., Mercier, F., Blinet, P., Chaloin, L., Chevolleau, S., Debrauwer, L., & Engel, E. (2020). Mitigation of heterocyclic aromatic amines in cooked meat. Part I: Informed selection of antioxidants based on molecular modeling. *Food Chemistry, 331*, Article 127264. https://doi.org/10.1016/j.foodchem.2020.127264

Mi, J., Ni, W., Huang, P., Hong, J., Jia, R., Deng, S., Yu, X., Wei, H., & Yang, W. (2022). Effect of acetylated distarch adipate on the physicochemical characteristics and structure of shrimp (Penaeus vannamei) myofibrillar protein. *Food Chemistry, 373*, Article 131530. https://doi.org/10.1016/j.foodchem.2021.131530

Miriani, M., Keerati-u-rai, M., Corredig, M., Iametti, S., & Bonomi, F. (2011). Denaturation of soy proteins in solution and at the oilewater interface: A fluorescence study. *Food Hydrocolloids, 25*, 620–626. https://doi.org/10.1016/j.foodhyd.2010.07.020

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry, 30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Nowell, S., Coles, B., Sinha, R., MacLeod, S., Luke Ratnasinghe, D., Stotts, C., Kadlubar, F. F., Ambrosone, C. B., & Lang, N. P. (2002). Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: Contribution of metabolic variation to risk. *Mutation Research, 506–507*, 175–185. https://doi.org/10.1016/s0027-5107(02)00164-1

Oguri, A., Suda, M., Totsuka, Y., Sugimura, T., & Wakabayashi, K. (1998). Inhibitory effects of antioxidants on formation of heterocyclic amines. *Mutation Research, 402*(1–2), 237–245. https://doi.org/10.1016/s0027-5107(97)00303-5

Privalov, P. L., & Gill, S. J. (1988). Stability of protein structure and hydrophobic interaction. *Advances in Protein Chemistry, 39*, 191–234. https://doi.org/10.1016/s0065-3233(08)60377-0

Quan, T. H., Benjakul, S., Sae-leaw, T., Balange, A. K., & Maqsood, S. (2019). Protein–polyphenol conjugates: Antioxidant property, functionalities and their applications. *Trends in Food Science & Technology, 91*, 507–517. https://doi.org/10.1016/j.tifs.2019.07.049

Schrödinger, L., & DeLano, W. (2020). *PyMOL*. Retrieved from http://www.pymol.org/pymol.

Sun, X., Yu, Y., Saleh, A. S. M., Yang, X., Ma, J., Li, W., Zhang, D., & Wang, Z. (2023). Understanding interactions among flavor compounds from spices and myofibrillar proteins by multi-spectroscopy and molecular docking simulation. *International Journal of Biological Macromolecules, 229*, 188–198. https://doi.org/10.1016/j.ijbiomac.2022.12.312

Sung, J., Shin, K. J., & Lee, S. (1992). Theory of diffusion-influenced fluorescence quenching. Effects of static quenching on the Stern-Volmer curve. *Chemical Physics, 167*, 17–36. https://doi.org/10.1016/0301-0104(92)80020-V

Szterk, A. (2013). Chemical state of heterocyclic aromatic amines in grilled beef: Evaluation by *in vitro* digestion model and comparison of alkaline hydrolysis and organic solvent for extraction. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 62*, 653–660. https://doi.org/10.1016/j.fct.2013.09.036

Villaverde, A., & Estévez, M. (2013). Carbonylation of myofibrillar proteins through the maillard pathway: Effect of reducing sugars and reaction temperature. *Journal of Agricultural and Food Chemistry, 61*(12), 3140–3147. https://doi.org/10.1021/jf305451p

Vitaglione, P., & Fogliano, V. (2004). Use of antioxidants to minimize the human health risk associated to mutagenic/carcinogenic heterocyclic amines in food. *Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences, 802*(1), 189–199. https://doi.org/10.1016/j.jchromb.2003.09.029

Wang, M., Kang, J., Chen, L., He, G., Liu, Y., Fan, X., Lv, X., Xu, X., Zhou, G., & Feng, X. (2023). Suppression mechanism of L-lysine on the Epigallocatechin-3-gallate-induced loss of myofibrillar protein gelling potential. *Food Research International, 169*, Article 112928. https://doi.org/10.1016/j.foodres.2023.112928

Wu, D., Wang, H., Guo, X., Zhang, Z., Gao, Z., Gao, S., Liu, Z., Rao, S., & Meng, X. (2023). Insight into the mechanism of enhancing myofibrillar protein gel hardness by ultrasonic treatment combined with insoluble dietary fiber from oat. *LWT-food Science & Technology, 178*, Article 114539. https://doi.org/10.1016/j.lwt.2023.114539

Wu, S. X., Xiong, R. G., Huang, S. Y., Zhou, D. D., Saimaiti, A., Zhao, C. N., Shang, A., Zhang, Y. J., Gan, R. Y., & Li, H. B. (2022). Effects and mechanisms of resveratrol for prevention and management of cancers: An updated review. *Critical Reviews in Food Science and Nutrition, 63*(33), 12422–12440. https://doi.org/10.1080/10408398.2022.2101428

Xia, M., Chen, Y., Guo, J., Huang, H., Wang, L., Wu, W., … Sun, W. (2019). Water distribution and textual properties of heat-induced pork myofibrillar protein gel as affected by sarcoplasmic protein. *LWT-food Science & Technology, 103*, 308–315. https://doi.org/10.1016/j.lwt.2019.01.009

Xue, C., Chen, Q., He, Z., Qin, F., Wang, Z., Chen, J., & Zeng, M. (2022). Release profiles of beef myofibril protein-bound heterocyclic amines and effects of dietary components on *in vitro* digestion. *Food Research International, 155*, Article 111006. https://doi.org/10.1016/j.foodres.2022.111006

Xue, C., He, Z., Qin, F., Chen, J., & Zeng, M. (2020). Effects of amides from pungent spices on the free and protein-bound heterocyclic amine profiles of roast beef patties by UPLC-MS/MS and multivariate statistical analysis. *Food Research International, 135*, Article 109299. https://doi.org/10.1016/j.foodres.2020.109299

Yang, X. Y., Blecker, C., Liu, H., Zhang, D. Q., & Wang, Z. Y. (2023). Effect of plant polyphenols with different m-hydroxy and o-hydroxy groups on the inhibition of heterocyclic amines formation in roasted meat. *Food Control, 153*, Article 109960. https://doi.org/10.1016/j.foodcont.2023.109960

Yi, X., Pei, Z., Xia, G., Liu, Z., Shi, H., & Shen, X. (2023). Interaction between liposome and myofibrillar protein in surimi: Effect on gel structure and digestive characteristics. *International Journal of Biological Macromolecules, 253*, Article 126731. https://doi.org/10.1016/j.ijbiomac.2023.126731

Yu, C., Shao, Z., Liu, B., Zhang, Y., & Wang, S. (2016). Inhibition of 2-Amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) formation by alkoxy radical scavenging of flavonoids and their quantitative structure-activity relationship in a model system. *Journal of Food Science, 81*(8), C1908–C1913. https://doi.org/10.1111/1750-3841.13381

Zhang, L., Wang, P., Yang, Z., Du, F., Li, Z., Wu, C., & Zhou, G. (2020). Molecular dynamics simulation exploration of the interaction between curcumin and myosin combined with the results of spectroscopy techniques. *Food Hydrocolloids, 101*, Article 105455. https://doi.org/10.1016/j.foodhyd.2019.105455