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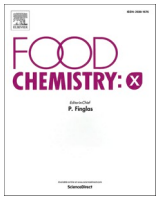

Understanding interactions between four main fishy compounds and grass carp myofibrillar proteins using the SPME-GC–MS, multiple spectroscopy, and molecular docking

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ABSTRACT

The interaction mechanism between four fishy compounds and myofibrillar proteins of grass crap was explored using solid phase microextraction-gas chromatography–mass spectrometry, multispectroscopy, and molecular docking. The result showed that the binding abilities of myofibrillar protein for the fishy compounds decreased in the order of decanal, octanal, hexanal, and 1-octen-3-ol. The interaction between myofibrillar proteins and four fishy compounds affected the aromatic amino acid residue microenvironment. The predominant binding force of myofibrillar proteins to the three aldehydes was hydrophobic, while those to 1-octen-3-ol were hydrogen bonds and van der Waals forces, and binding sites of these compounds occurred near tryptophan and tyrosine. A significant reduction in α-helical content and surface hydrophobicity in grass carp myofibrillar protein upon interaction with the four fishy compounds. Molecular docking confirmed that the different functional groups and chain lengths of the fishy components resulted in different binding sites and binding free energies with grass carp protein.

1. Introduction

Grass carp, the highest-producing freshwater species in the world, has provided consumers with cheap and high-quality protein, thus it is becoming increasingly popular (Tian et al., 2020). In China, the yield of grass carp reached 5.94 million tons in 2023, which accounts for about 21.44 % of China's total freshwater aquaculture production (FAO, 2024). Nevertheless, owing to the physiological impact and environmental influence, undesirable fishy odors occur in grass carp. These odors are regarded as unacceptable by a considerable proportion of consumers (Sun et al., 2023). Therefore, deodorization is of great importance in the current freshwater surimi industry.

Myofibrillar proteins, one of the main components of meat, account for between 55 % and 65 % of muscle proteins. Myofibrillar proteins exert a significant influence on the quality characteristics of meat products, including sensory and textural characteristics. Myofibrillar proteins bind or release volatile flavor compounds to influence the sensory characteristics of meat, thereby impacting the preferences of consumers (Zhang et al., 2024). Aldehydes and alcohols have been identified as the primary flavor compounds responsible for the undesirable fishy odor of freshwater fish (Xue et al., 2023). Proteins in fish with a large number of binding sites attract fishy compounds with reactive groups through different molecular interactions, and the number of binding sites as well as the type of molecular interaction will affect the release and retention of fishy compounds in the proteins (Nie et al., 2023).

Understanding the process by which fishy compounds interact with proteins in fish is crucial for effectively removing fishy odors from fish. Currently, the interactions between various proteins (e.g., soya proteins, whey proteins, bovine serum proteins, and lactoglobulins) and volatile flavor compounds have been wildly reported (Zhang et al., 2021). As for fish proteins, Xue et al. (2021) explore the interaction mechanism between myofibrillar protein in silver carp and the critical fishy compounds with fluorescence spectroscopy and molecular dynamics simulation. Similarly, Xue et al. (2023) use patial-temporal molecular dynamics simulation to reveal the dynamic interaction between silver

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carp myosin and fishy compounds at cold storage and oral temperatures. Chao et al. (2021) applied headspace solid-phase microextraction (HS-SPME) and gas chromatography (GC–MS) to investigate the ability of silver carp myofibrillar protein to adsorb fishy compounds under the impact of various environmental factors, including concentration, ionic strength, pH, temperature, and yeast glucan supplementation. Cao et al. (2018) used hydrogen peroxide to treat the grass carp skeletal muscle and explore the effect on the adsorption capacity of aldehyde and alcohol compounds. However, available information on the interaction between fish proteins and fishy compounds is still limited, especially grass carp, and few studies pay attention to the main binding interaction types, binding parameters, and protein conformational changes after proteins bind different fishy compounds.

The purpose of the present study was to reveal the interaction mechanism between the main protein (myofibrillar protein) and four fishy compounds (hexanal, octenal, decanal, and 1-octen-3-ol) responsible for the fishy odor in grass carp, which was investigated by the combination of SPME-GC–MS, ultraviolet-visible absorption spectroscopy, fluorescence spectroscopy, and Fourier infrared spectroscopy and molecular docking techniques. This study may provide some enlightenment for the effective removal of fishy odor compounds in grass carp and its products.

2. Material and method

2.1. Preparation of myofibrillar proteins from grass carp

Fifteen fresh grass carp were purchased from the supermarket, and each grass carp weighed 2.5 ± 0.2 kg. The myofibrillar protein from grass carp meat was extracted according to the method of Tan et al. (2024).

2.2. Preparation of stock solutions of fishy compounds

The stock solutions of fishy compounds were prepared according to the method of Gu et al. (2020). Different fishy compounds (e.g., hexanal, octanal, decanal, and 1-octen-3-ol) were added to Tris-HCl buffer (pH 7.0, 0.6 mol/L NaCl), and methanol was used as the solubilizer. The final concentration of each fishy compound was 1 g/L, and stored in a sealed brown bottle at 4 °C to prevent degradation.

2.3. Bind ability analysis

SPME-GC–MS was applied to determine the binding ability of grass carp myofibrillar protein to different fishy compounds (Wang et al., 2022). Grass carp myofibrillar protein samples and fishy compounds were put into a 20 mL brown headspace flask. The mass concentration of grass carp myofibrillar protein was fixed at 6 mg/mL, and mass concentrations of each fishy compound were set at 0.2, 0.5, 1, 2, 5, and 10 mg/L. The mixtures were thoroughly mixed, and incubated at 4 °C for 16 h to ensure the fishy compounds were adequately bound to grass carp myofibrillar proteins. The SPME needles were exposed to headspace vials and adsorbed at a constant temperature of 30 °C for 50 min to determine the content of the relevant fishy compounds. The GC program temperature rise conditions were set as follows: heating to 38 °C, holding for 6 min, increasing the temperature to 105 °C at 6 °C/min, then increasing the temperature to 220 °C at 15 °C/min, and finally holding for 5 min. The calculation of the binding ability of grass carp myofibrillar protein to fishy compounds was shown in Eq. 1:

$$\text{Binding rate}(\%) = \left(1 - \frac{S_{MP}}{S_{UMP}}\right) \times 100\% \quad (1)$$

S_{MP} is the peak area of fishy compounds after adding grass carp myofibrillar protein, and S_{UMP} is the peak area of fishy compounds without adding grass carp myofibrillar protein.

2.4. Ultraviolet-visible absorption spectroscopy analysis

The ultraviolet-visible absorption spectroscopy was applied to determine the grass carp myofibrillar proteins treated with various fishy compounds (Wang et al., 2022). Grass carp myofibrillar protein solution and fishy compounds of different concentrations were added into 5 mL brown test tubes. The mixtures were diluted to 5 mL with Tri-HCl buffer. The mass concentration of grass carp myofibrillar protein was fixed at 0.1 g/L, and the mass concentration of each fishy compound was set as 0, 0.2, 0.5, 1, 2, and 5 mg/L, respectively. The spectral scanning range of the samples was 190–500 nm.

2.5. Fluorescence spectrometry assay

According to the method of Bijari et al. (2013), different concentrations of fishy compounds were added to 1 mL of grass carp myofibrillar protein solution (6 mg/L, *w*/w), and the volume was adjusted to 5 mL with Tris-HCl buffer. The final concentrations of fishy compounds were 0, 0.2, 0.5, 1, 2, and 5 mg/L. The samples were divided into three groups and kept in constant-temperature water baths at 298 K, 303 K, and 310 K for 30 min. Fluorescence quenching was applied to investigate the interaction between grass carp myofibrillar proteins and fishy compounds, and the result was analyzed using the Stern-Volmer equation (Eq. (2)). Thermodynamic binding parameters between grass carp myofibrillar protein and various fishy compounds were calculated using a variant of the following Stern-Volmer equation (Eq. (3))

$$\frac{F_0}{F} = 1 + K_{sv}[Q] \quad (2)$$

$$log\left(\frac{F_0 - \mathrm{F}}{F}\right) = logK_a + \mathrm{n}\log[Q] \quad (3)$$

F_0 and F represent fluorescence intensities using non-characteristic and fishy compounds as quenchers, respectively. K_{sv} and [Q] are the Stern-Volmer quenching constant and the concentration of the fishy compounds as the quencher, respectively. K_a is the corresponding binding constant (the binding affinity between the protein and the ligand), and n is the number of binding sites.

The values of Enthalpy change and entropy change could be calculated using the Van 't Hoff equation (Eq. (4) & (5))

$$ln\frac{k_2}{k_1} = \left(\frac{1}{T_1} - \frac{1}{T_2}\right) + \frac{\triangle H}{R} \quad (4)$$

$$G = -RTlnK = \triangle H - T\triangle S \quad (5)$$

where ΔH and ΔS are Enthalpy change and entropy change, respectively. R is gas state constant (8.314 J/(K·mol)).

2.6. Synchronous fluorescence spectroscopy analysis

The synchronous fluorescence spectra of grass carp myofibrillar protein with various fishy compounds were evaluated at a specific excitation wavelength (Xue et al., 2022). When the wavelength difference between the excitation wavelength and the emission wavelength (Δλ) was set as 15 nm, the microenvironment around the tyrosine (Tyr) residue could be reflected; when Δλ was set as 60 nm, the microenvironment around the tryptophan (Trp) residue could be reflected.

2.7. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (Nicolet is5, Thermo Fisher Scientific Inc., Germany) was used to determine the secondary structure of grass carp myofibrillar protein after binding with fishy compounds (Liu et al., 2022). The freeze-dried protein sample was placed on the sample stage. The spectrum was set to scan 64 times, with a resolution of

4 cm^{-1} and a wavelength scanning range of 400–4000 cm^{-1}.

2.8. Determination of surface hydrophobicity

The surface hydrophobicity of grass carp myofibrillar protein after adding different fishy compounds was measured using the ANS fluorescent probe method (Chelh et al., 2006). 50 μL of ANS (8 mmol/L) solution was added into the mixed solutions of grass carp myofibrillar protein (6 mg/L, *w*/w) and fishy compounds with mass concentrations (0, 1, and 5 mg/mL). Then the samples were kept away from light for 5 min and the fluorescence intensity of the protein sample was measured by a 970CR fluorescence spectrophotometer (Shanghai Precision Scientific Instrument Co., LTD, China).

2.9. Computational studies

The three-dimensional structure of grass carp myosin was generated through the homology modeling method. The homology modeling was carried out using the MODELLER 10.1, and the protein template was encoded as 6YSY. The PROCHECK program (http://services.mbi.ucla.edu/ SAVES/) was used to evaluate the quality of the built model. According to the Ramachandran Diagram, the model exceeding 90 % of residues in a reasonable region would be selected. The three-dimensional structures of hexanal (PubChem·CID:6184), octanal (PubChem·CID·: 454), decanal (PubChemCID:8175), and 1-octen-3-ol (PubChemCID:18827) were download from PubChem, and the geometry optimizations of these structures were performed using ORCA 6.0. Then these fishy compounds were docked into the grass carp myosin using Autodock vina 1.2. The result of molecular docking was visualized by using PyMol 2.4 and Discovery Studio 2016.

2.10. Statistical analysis

All assays in this study were repeated three times at least. The data were analyzed by one-way analysis of variance with SPSS 23.0. Statistically significant differences were calculated at $p < 0.05$ using multiple according to Duncan's method. The results were displayed as means ± standard deviations.

3. Results and discussions

3.1. Binding ability analysis

Proteins in food components can affect the existence state of volatile flavors in food components via the binding between proteins' molecular chains and volatile flavor (Martínez-Arellano et al., 2016). The diversity of primary structure and multiplicity of higher structures of proteins are the main factors affecting proteins and volatile flavor compounds in food components. In addition, the type and hydrophobicity of volatile flavor compounds, as well as binding partition coefficients in proteins, directly influence the ability of proteins to bind volatile flavor compounds (Weel et al., 2003). Fig. 1 showed the binding capacity of grass carp myofibrillar proteins to different concentrations of fishy compounds. With the increase of the concentration of fishy compounds, the binding ability of grass carp myofibrillar protein to each fishy compound increased first and then kept a stabilized trend, but the binding ability to different fishy compounds showed significant differences. Notably, at the same concentration, the binding abilities of each fishy compound to grass carp myofibrillar protein were found to decrease in the order as decanal, octanal, hexanal, and 1-octen-3-ol. The binding ability of grass carp myofibrillar protein to aldehydes was remarkedly greater than that to alcohols, which is similar to the results of Kühn et al. (2008) and Tan and Siebert (2008). Kühn et al. (2008) found that whey isolate proteins had a greatly higher binding capacity to aldehydes volatile compounds than alcohols and ketones volatile compounds. Tan and Siebert (2008) investigated the binding capacity of bovine serum albumin to four types

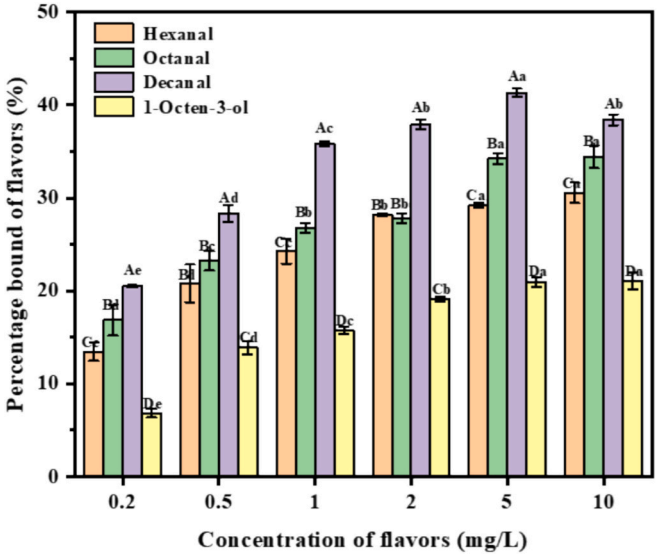

Fig. 1. Binding ability of grass carp myofibrillar protein to four fishy compounds at different concentrations.

of volatile compounds (alcohols, esters, aldehydes, and ketones), and found that bovine serum albumin had a greater binding capacity for aldehydes than for alcohols. The binding capacity for saturated aldehydes tends to increase gradually with the increase in the number of carbon atoms, which might be related to the enhanced hydrophobicity of aldehydes and the increased binding sites caused by the increased carbon atoms in the main chain of aldehydes (Weel et al., 2003).

3.2. Ultraviolet-visible absorption spectroscopy analysis

Ultraviolet-visible absorption spectroscopy is a simple and effective method to determine the quenching mechanism of protein-small molecule complexes. The related study has shown that proteins and quenchers do not change the absorption spectrum of their fluorophores during the dynamic quenching, whereas the complexes formed by the fluorophore and the quencher will change the absorption spectra during the static quenching (Zhang et al., 2008). Fig. 2 showed the Ultraviolet-visible absorption spectroscopy analysis of grass carp myofibrillar protein after the treatment with different concentrations of fishy compounds. Obviously, the absorption spectrum of grass carp myofibrillar protein has two typical absorption peaks at 210 nm and 278 nm. The strong absorption peak at 210 nm originates from the peptide bond within the protein, which can reflect the peptide chain skeleton information of the protein. The weaker absorption peak at 278 nm is attributed to the aromatic amino acids (tryptophan, tyrosine, and phenylalanine residues) of the protein (Zhao et al., 2010). The absorption peak intensity of grass carp myofibrillar protein at 278 nm decreased and shifted slightly to the right after adding aldehyde and alcohol compounds. It indicated that the quenching type between grass carp myofibrillar protein and aldehyde and alcohol compounds is static quenching, and the addition of these volatiles will change the microenvironment of tryptophan and tyrosine residues in grass carp myofibrillar protein. In addition, the rightward shift of the maximum absorption peak indicated that the addition of aldehydes and alcohol volatiles might result in tryptophan and tyrosine residues of grass carp myofibrillar protein to be exposed to a less hydrophilic environment. Increased polarity in the microenvironment of both tryptophan and tyrosine residues will lead to a decrease in the hydrophobicity of grass carp myofibrillar proteins.

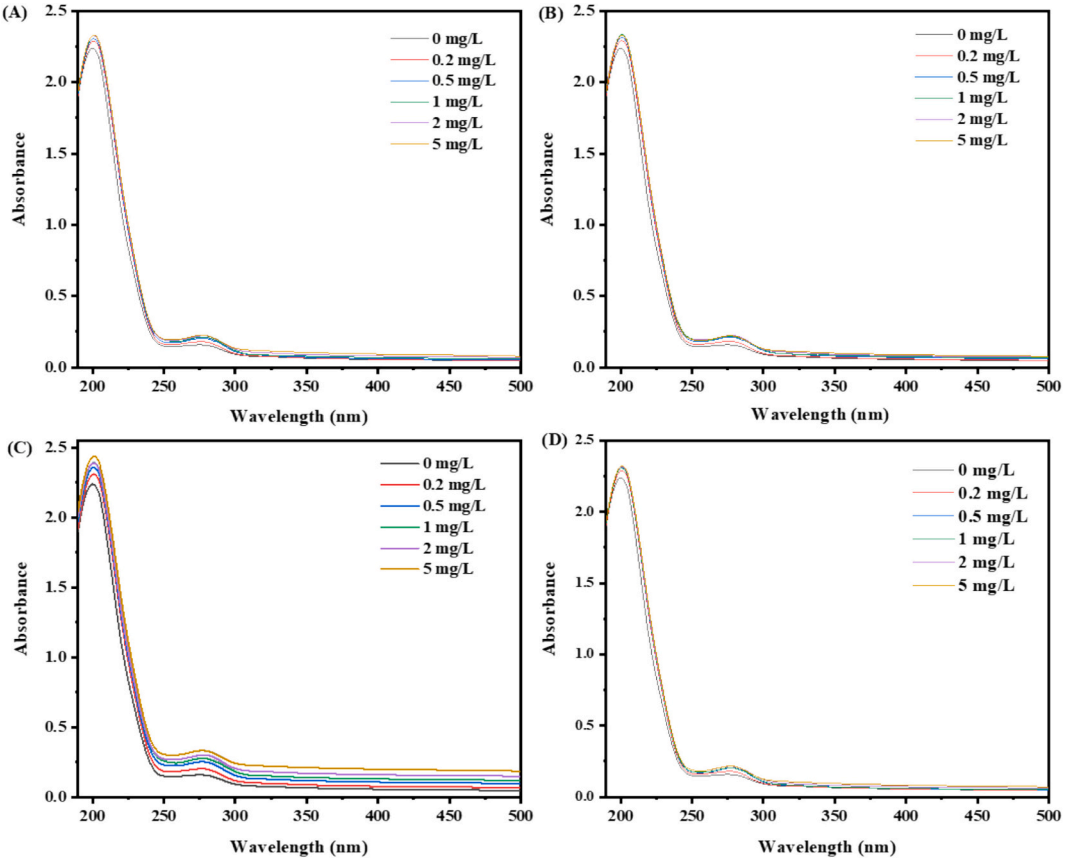

Fig. 2. Changes of ultraviolet-visible absorption spectra of myofibrillar protein in grass carp surimi after interaction with different concentrations of four fishy compounds.

3.3. Fluorescence spectroscopy mechanism analysis

Fluorescence spectroscopy is an effective method to explore the mechanism of fluorescence quenching of protein molecules by small molecules and their interaction. Since fluorescent groups are sensitive to the surrounding local polar environment, the intrinsic fluorescence of the protein will change through fluorescence quenching resulting from small molecules binding to proteins. Fluorescence quenching mechanisms can be divided into three types: static quenching mechanism, dynamic quenching mechanism, and the combination of the two mechanisms (Geng et al., 2020; Yu et al., 2020). The static quenching mechanism originates from the formation of a non-luminescent ground state complex between the quencher and the protein fluorophore, while the dynamic quenching mechanism originates from the collision between the quencher and the protein fluorophore in the excited state (Shen et al., 2019; Zhang et al., 2009). The effect of adding different fishy compounds on the fluorescence spectrum of grass carp myofibrillar protein was shown in Fig. 3. Grass carp myofibrillar protein has the largest fluorescence emission peak at a fluorescence wavelength of about 334 nm, which reflected the characteristic fluorescence peaks of tryptophan and tyrosine. With the increase of the concentration of aldehyde and alcohol compounds, the fluorescence intensity of grass carp myofibrillar protein showed a gradually decreasing trend, indicating that hexanal, octanal, decanal, and 1-octen-3-ol could quench the fluorescence of grass carp myofibrillar protein. Related studies have shown that tryptophan and tyrosine residues are usually enclosed in the water-gathering core of the protein, but these residues are exposed when the protein undergoes a process of denaturation or a change in its conformation following the binding of ligands. The fluorescence intensity of grass carp myofibrillar protein near 334 nm decreased, suggesting that the interaction between aldehyde and alcohol volatiles and grass carp myofibrillar protein might occur in the hydrophobic region near tryptophan and/or tyrosine residues.

Given that the four fishy compounds (hexanal, octanal, decanal, and 1-octen-3-ol) could quench the intrinsic fluorescence of grass carp myofibrillar protein, the quenching mechanism of the above four fishy compounds and grass carp myofibrillar protein was further explored. In general, the different temperature dependence of the fluorescence burst behavior can be used to distinguish between dynamic and static quenching (Liu et al., 2024). According to the Stern-Volmer equation plots (Fig. 4), it could be found the F_0/F value gradually increased with the increased concentration of the fishy compound, proving that the maximum fluorescence value of grass carp myofibrillar proteins gradually decreased with the increased concentration of the fishy compound, and indicated hexanal, octanal, decanal, and 1-octen-3-ol could be used as fluorescence bursting agents to grass carp myofibrillar proteins. Bursting parameters of grass carp myofibrillar proteins with different fishy compounds under different temperature conditions were shown in Table 1. The burst rate constants (K_{sv}) of hexanal, octanal, and decanal in the interaction system with grass carp myofibrillar proteins increased with the increase in temperature, indicating that the type of fluorescence quenching was dynamic quenching. However, the molecular quenching constant (K_q) of saturated aldehyde volatiles was greatly higher than the diffusion rate constant of biomolecules (2×10^{10} M^{-1} s^{-1}), indicating that static quenching was also involved in the interaction of saturated aldehyde volatiles with grass carp myofibrillar protein. Therefore, the quenching type of grass carp myofibrillar protein with hexanal, octanal, and decanal was a combination of static and dynamic quenching. The K_{sv} of the interaction between 1-octen-3-ol and grass carp myofibrillar protein decreased with increasing temperature, and the molecular quenching constant (K_q) was greatly higher than the diffusion rate constant of biological molecules, indicating that the quenching type of

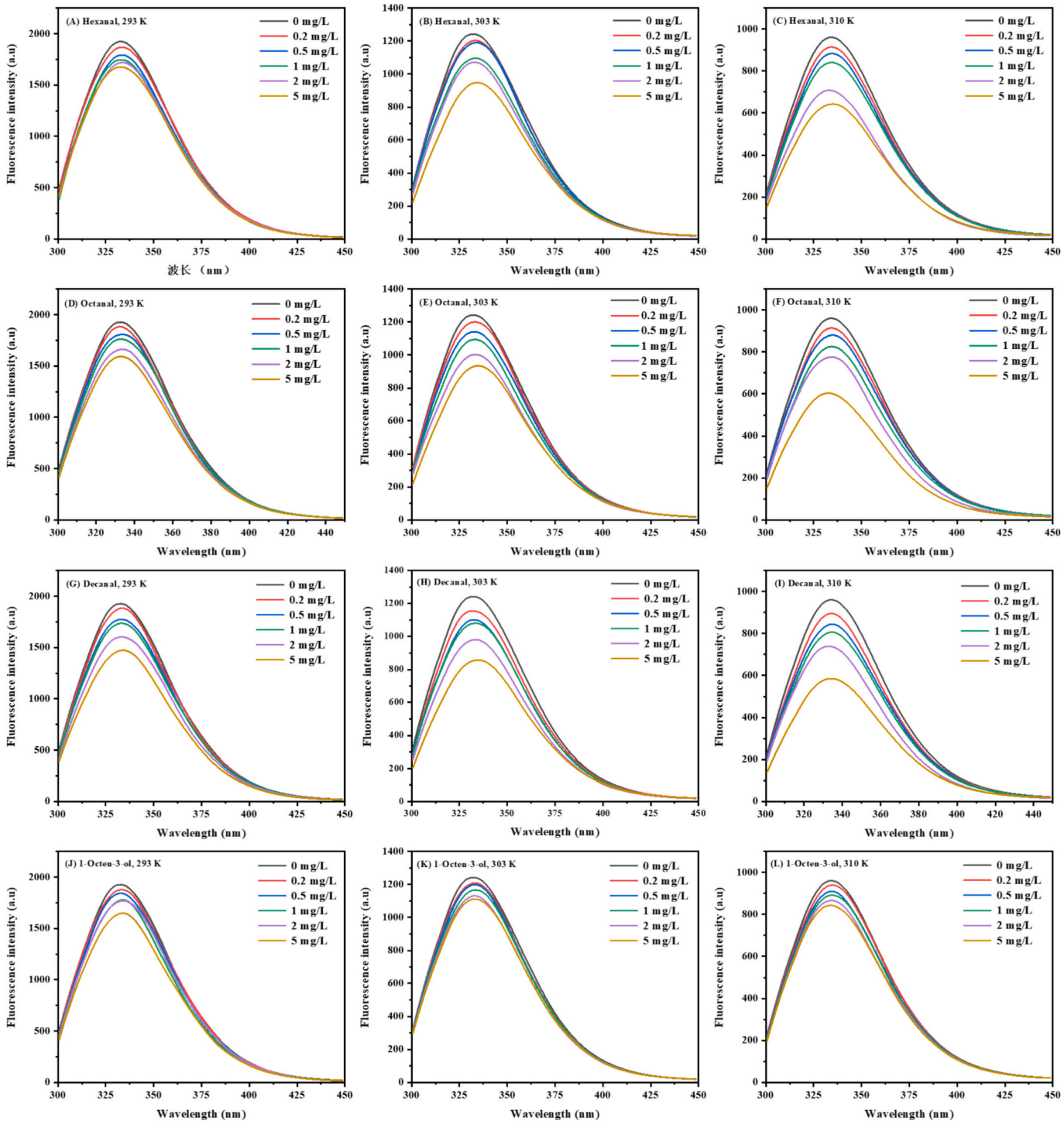

Fig. 3. Changes of fluorescence spectra of myofibrillar protein in Grass carp after interaction with different concentrations of four fishy compounds.

1-octen-3-ol on grass carp myofibrillar protein was static quenching.

To gain a deeper understanding of the binding mechanism between grass carp myofibrillar protein and hexanal, octanal, decanal and 1-octen-3-ol, the modified Stern-Volmer equation was used to calculate the thermodynamic binding parameters between grass carp myofibrillar protein and each fishy compound (Fig. 4). For systems of saturated aldehyde quenching grass carp myofibrillar protein, their K_a and n increased with increasing temperature (Table 1), indicating that the increase in temperature may be conducive to the binding of grass carp myofibrillar protein with saturated aldehyde compounds and the stability of the complexes. However, for the system of quenching grass carp myofibrillar protein with 1-octen-3-ol, K_a and n decreased with increasing temperature, indicating that the increased temperature might have a negative effect on the binding of grass carp myofibrillar protein with 1-octen-3-ol and the stability of the complex. In addition, the number of binding sites of the four fishy compounds with grass carp myofibrillar protein decreased in the following order: decanal, octanal, hexanal, 1-octen-3-ol. It was consistent with the result of the binding ability between various fishy compounds and grass carp myofibrillar protein.

The binding interaction between small molecule ligand compounds and biological macromolecules mainly includes hydrogen bonding,

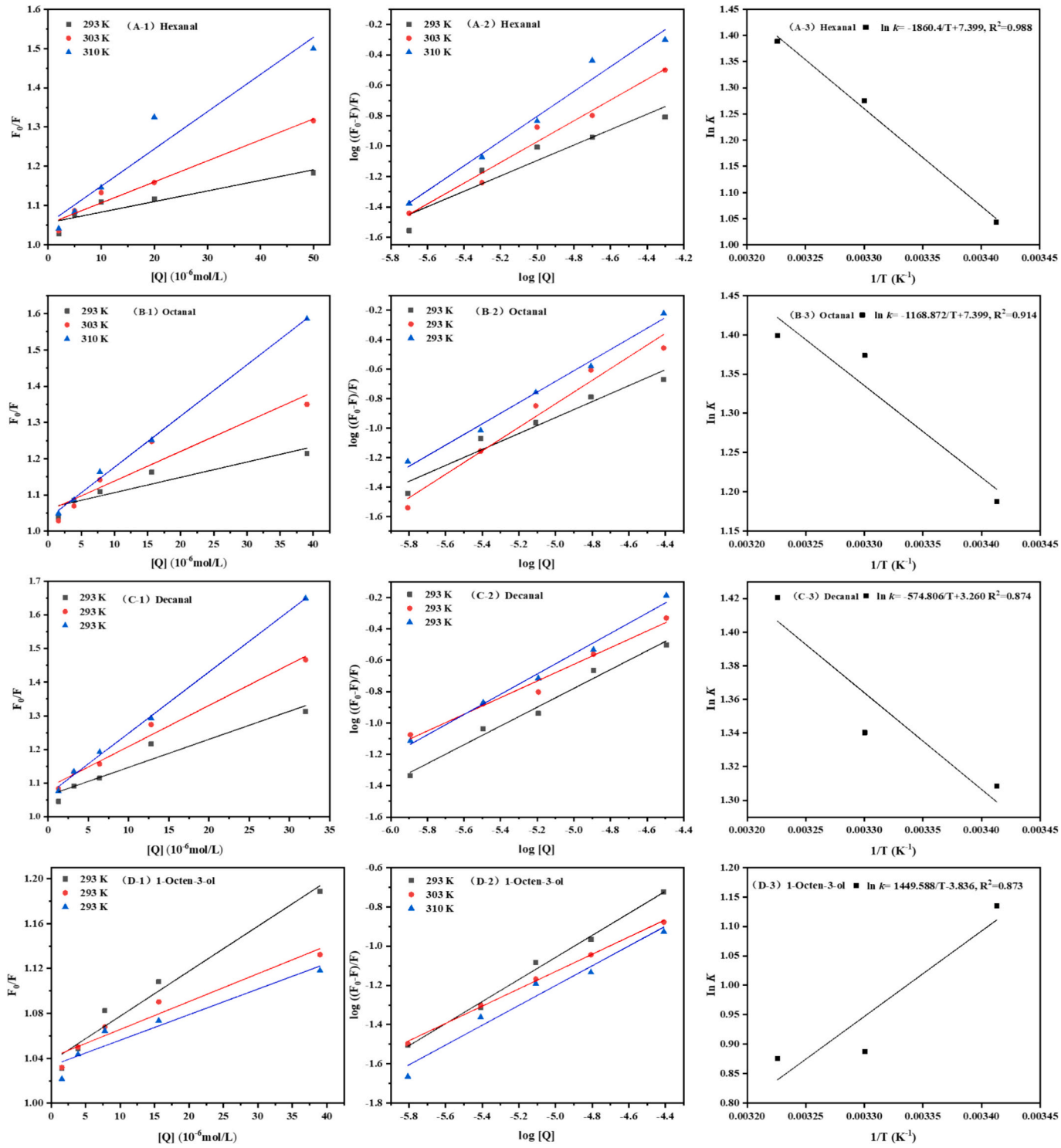

Fig. 4. Changes of synchronous fluorescence spectra of myofibrillar protein in Grass carp after interaction with different concentrations of four fishy compounds.

electrostatic interaction, hydrophobic force, and van der Waals force (Paiva et al., 2020; Ross & Subramanian, 1981). The main interaction can be identified according to the thermodynamic parameters. When $\Delta H < 0$ and $\Delta S > 0$, the main interaction is electrostatic interaction; when $\Delta H < 0$ and $\Delta S < 0$, it is van der Waals force and hydrogen bond interaction; when $\Delta H > 0$ and $\Delta S > 0$, it is hydrophobic interaction (Wu et al., 2011). Thermodynamic parameters for the binding of grass carp myofibrillar proteins with the four fishy compounds were shown in Table 1. Free energy variation values of hexanal, octanal, decanal, and 1-octen-3-ol in the interaction system with grass carp myofibrillar proteins were less than 0, which indicated that the four fishy compounds could spontaneously bind to the grass carp myofibrillar proteins. For hexanal, octanal and decanal, ΔH and ΔS values were all greater than 0, indicating that the main interaction force between saturated aldehydes and grass carp myofibrillar proteins was hydrophobic interaction force. For 1-octen-3-ol, ΔH and ΔS values were all smaller than 0, indicating that the main interaction force between 1-octen-3-ol and grass carp myofibrillar protein was hydrogen bonding or van der Waals force.

Table 1
The quenching rate constant (K_{sv}), bimolecular quenching constant (K_q), binding constant (K_a), binding site number (n), and thermodynamic parameters (ΔH, ΔS, and ΔG) for the interaction of myofibrillar proteins of Grass carp with four fishy compounds.

Compounds	*T* (K)	K_{sv} (10^3 M^{-1})	Kq (10^{11} M^{-1} S^{-1})	K_a (10^3 M^{-1})	n	ΔH (KJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
	293	2.70	2.70	2.84	0.612			−2.56
Hexanal	303	5.35	5.35	3.58	0.741	15.47	61.52	−3.17
	310	9.49	9.49	4.01	0.845			−3.60
	293	4.22	4.22	3.28	0.667			−2.93
Octanal	303	8.18	8.18	3.95	0.814	9.717	43.17	−3.36
	310	14.11	14.11	4.05	0.883			−3.67
	293	8.33	8.33	3.70	0.784			−3.16
Decanal	303	12.18	12.18	3.82	0.942	4.78	27.10	−3.43
	310	18.20	18.20	4.14	1.107			−3.62
	293	4.01	4.01	3.11	0.693			−2.71
1-Octen-3-ol	303	2.48	2.48	2.43	0.431	−12.05	−31.89	−2.39
	310	2.28	2.28	2.40	0.409			−2.16

3.4. Synchronous fluorescence spectroscopy analysis

The information provided by synchronous fluorescence spectroscopy can reflect the polarity changes of the microenvironment surrounding the fluorophore (Xu et al., 2015). When the difference between the excitation wavelength and the emission wavelength (Δλ) is 15 or 60 nm, the synchronous fluorescence spectrum can reflect the characteristic information of Tyr or Trp residues (Wang et al., 2008). In addition, the change of the maximum emission wavelength in synchronous fluorescence spectroscopy can reflect the information of protein conformation. The red shift of the maximum absorption peak indicates the increased polarity of the microenvironment in which the fluorophore is located and the hydrophobicity is weakened, while the blue shift indicates that the hydrophobicity of the microenvironment is enhanced and the polarity is weakened (Meng et al., 2012). The changes in synchronous fluorescence spectra of grass carp myofibrillar protein after interaction with fishy compounds of different concentrations was shown in Fig. 5. The addition of hexanal, octanal, decanal, and 1-octen-3-ol led to different degrees of reduction in fluorescence intensity related to Tyr and Trp residues in grass carp myofibrillar protein, indicating that the four fishy compounds could interact with grass carp myofibrillar protein, resulting in fluorescence quenching and conformational changes. In addition, with the increase of volatile concentration, the maximum absorption peaks of Tyr and Trp residues gradually red-shifted, indicating that the polarity of the microenvironment around the tryptophan and tyrosine residues increased and the hydrophobicity decreased, which is consistent with the results of Ultraviolet-visible absorption spectroscopy analysis. In addition, the changes in the microenvironment of Trp and Tyr residues further proved that the sites of interaction with aldehyde and alcohol compounds in grass carp myofibrillar protein might occur near Tyr and Trp residues.

3.5. Fourier transform infrared spectroscopy analysis

Fourier transform infrared spectroscopy can be used to investigate changes in secondary structure in proteins. According to infrared spectroscopy analysis, information on changes in the microenvironment of some active groups (e.g., hydrogen-bonded amino and carboxyl groups) and amino acid residue side chains in relevant proteins can be obtained. The changes in the infrared spectrum of grass carp myofibrillar protein after adding four fishy compounds were shown in Fig. 6A. The peak at 1600–1700 cm^{-1} is caused by the stretching vibration of C═O, representing the amide I band (Canon et al., 2009); the peak at 1500–1600 cm^{-1} is caused by the N—H bending vibration and the C—N stretching vibration, representing the amide II band (McClements, 2010). The peak shapes and wave numbers at the amide bands I and II of grass carp myofibrillar protein changed significantly by adding aldehyde and alcohol volatile compounds, indicating that grass carp myofibrillar protein may interact with aldehyde and alcohol compounds and lead to changes in its secondary structure. The changes in the relative content of the secondary structure of grass carp myofibrillar protein after interaction with the four fishy compounds were shown in Table 2. After adding fishy compounds, the content of α-helix structure in grass carp myofibrillar protein was significantly reduced, and the content of β-sheet and β-turn structure was significantly increased, indicating that the addition of aldehydes and alcohols could lead to the expansion of grass carp myofibrillar protein structure and transform its α-helix structure into β-sheet and β-turn structure. Compared with grass carp myofibrillar protein without the treatment of fishy compounds, the content of α-helix structure in grass carp myofibrillar protein decreased by 9.05 %, 9.65 %, 11.58 %, and 5.41 % respectively after adding hexanal, octanal, decanal and 1-octen-3-ol.

3.6. Surface hydrophobicity of myofibrillar proteins analysis

The surface hydrophobicity of the protein represents the number of hydrophobic groups on the surface of its molecule, which has a great impact on the functional properties of the protein (Chandrapala et al., 2011). The changes in surface hydrophobicity of grass carp myofibrillar protein after interaction with different fishy compounds were shown in Fig. 6B. The surface hydrophobicity of grass carp myofibrillar proteins displayed varying degrees of decrease with the addition of all four fishy compounds. To conveniently observe, the surface hydrophobicity of grass carp myofibrillar proteins without the addition of fishy compounds was converted to 100 %. The surface hydrophobicity of grass carp myofibrillar proteins decreased by 2.62 %, 5.28 %, 8.56 %, 9.79 %, and 1.73 %, respectively, with the addition of 1 mg/mL of hexanal, octanal, decanal and 1-octen-3-ol, and further decreased by 5.76 %, 6.89 %, 10.82 % and 3.21 %, respectively, with the addition of 5 mg/mL of hexanal, octanal, decanal and 1-octen-3-ol. The results of surface hydrophobicity can reflect the changes in the secondary structure of proteins, indicating that interactions could occur between grass carp myofibrillar protein and the four fishy compounds. According to the results of thermodynamic parameters, the free energy change values of the interaction systems of hexanal, octanal, decanal, and 1-octen-3-ol with grass carp myofibrillar protein were all less than 0, indicating that interaction between the grass carp myofibrillar protein and the above four fishy compounds occurred spontaneously. The hydrophobic structure of the fishy compounds interacted with the hydrophobic groups on the protein surface, resulting in a decrease in the measurable surface hydrophobicity. The decrease in the surface hydrophobicity of grass carp myofibrillar protein caused by the addition of hexanal, octanal, and decanal gave evidence to support the thermodynamic parameter results, which reflected that a hydrophobic interactions occur between hexanal, octanal, and decanal and grass carp myofibrillar protein. At the same concentration, with the increase of carbon atom number, the degree of decrease in surface hydrophobicity of grass carp myofibrillar protein caused by saturated aldehydes gradually increased,

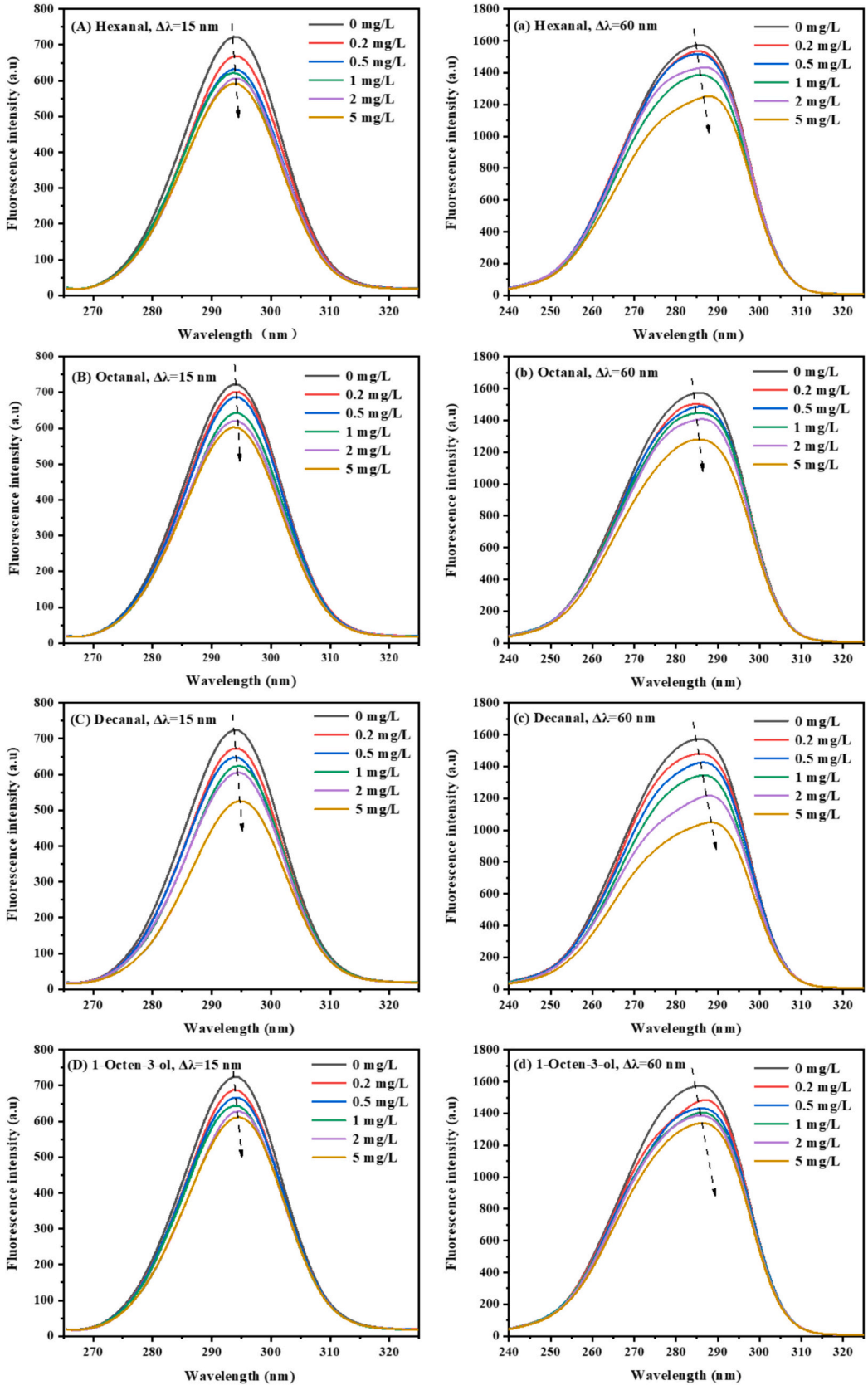

Fig. 5. Stern-Volmer plot, modified Stern-Volmer plots and Van't Hoff plot for myofibrillar proteins of Grass carp with four fishy compounds.

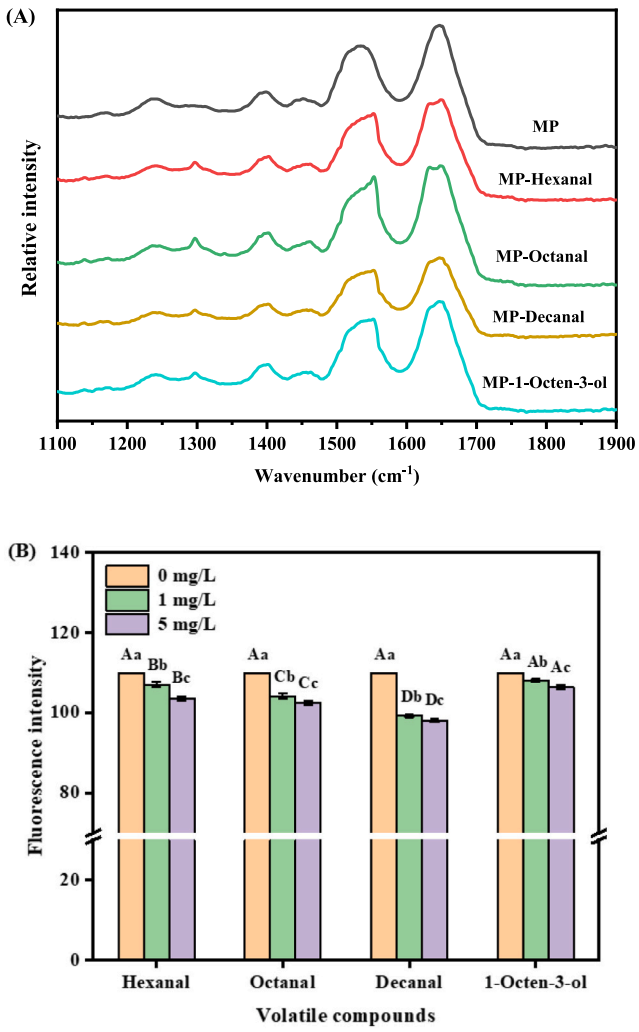

Fig. 6. Changes of Fourier infrared spectrum and surface hydrophobicity of grass carp myofibrillar protein after interaction with four fishy compounds.

Table 2
Changes of Secondary structure of myofibrillar proteins in Grass carp after interaction with four fishy odor compounds.

Sample	α-Helix	β-Sheet	β-Turn	Random coil
MP	43.44 ± 0.26^{a}	9.17 ± 0.13^{b}	19.41 ± 0.31^{c}	27.98 ± 0.44^{a}
MP-Hexanal	39.51 ± 0.39^{c}	11.74 ± 0.16^{a}	20.49 ± 0.21^{b}	28.26 ± 0.28^{a}
MP-Octanal	39.25 ± 0.15^{c}	10.97 ± 0.41^{a}	22.01 ± 0.73^{a}	27.77 ± 1.78^{a}
MP-Decanal	38.41 ± 0.42^{d}	11.23 ± 0.51^{a}	21.99 ± 0.48^{a}	28.37 ± 0.70^{a}
MP-1-Octen-3-ol	41.09 ± 0.75^{b}	11.44 ± 0.38^{a}	19.77 ± 0.39^{c}	27.69 ± 0.47^{a}

which also indirectly proved that with the increase of carbon atom number, the binding strength between saturated aldehydes and grass carp myofibrillar protein gradually increased. Notably, 1-octen-3-ol caused a downward trend in the change of the surface hydrophobicity of grass carp myofibrillar protein, which might be due to the existence of secondary hydrophobic interaction besides hydrogen bond or van der Waals force as the main interaction mode.

3.7. Homology modeling and molecular docking

Myofibrillar protein is a mixed system, which is mainly composed of myosin (47.83 %) and actin (21.73 %) (Wu et al., 2023). Related studies have confirmed that myosin is the main binding receptor for flavor compounds in myofibrillar protein (Gu et al., 2020), while actin cannot bind to fishy compounds (Pérez-Juan et al., 2007). Therefore, to analyze the interaction between grass carp myofibrillar protein and fishy compounds (hexanal, octanal, decanal, and 1-octen-3-ol), grass carp myosin was used as the receptor protein, and the molecular docking simulation between the myosin and the four fishy compounds was carried out.

The three-dimensional structure of grass carp myosin was built using the homologous modeling method with MODELLER 10.1, and the three-dimensional model of grass carp myosin was obtained (Fig. 7A). The Ramachandran plot of the obtained model was generated with PROCHECK program to evaluate its quality. As shown in Fig. 7B, black (or red) squares represent amino acids, red areas represent most favored areas, bright yellow areas represent additional allowed areas, light yellow areas represent generally allowed areas, and white areas represent impermissible areas. The number of amino acids in the optimal region reached 90.8 %, the number of amino acids in the impermissible region only accounted for 0.3 %, and 99.7 % of the amino acids in the model were within the reasonable region, suggesting the structure of grass carp myosin obtained by homologous modeling was reasonable.

The four fishy compounds (hexanal, octanal, decanal, and 1-octen-3-ol) were further docked to the grass carp myosin. As shown in Table 3, the absolute values of the binding energy between grass carp myosin and four fishy compounds were found to decrease in the following order: decanal > octanal > hexanal > 1-octen-3-ol, which was consistent with the K_a comparison results of each fishy compound in fluorescence quenching analysis. The binding ability was enhanced with increased carbon chain length of aldehyde compounds, and the binding ability of aldehyde volatile compounds is greatly stronger than that of alcohol volatile compounds.

Two-dimensional and three-dimensional visualization results of the conformation of grass carp myosin docked with four fishy compounds were shown in Fig. 7C. Hexanal bond to grass carp myosin by its hydrophobic interactions with Tyr269 and Leu659 and van der Waals forces with Arg246, Phe247, Gly248, Leu270, Ile465, Ala466, Ile481, Thr484 and Leu662. Similarly, octanal bond to grass carp myosin by its hydrophobic interactions with Tyr130 and van der Waals forces with Ile116, Asn128, Pro129, Lys131, Trp132, Tyr136, and Val188. As for decanal, it bond to the myosin by its hydrophobic interactions with Tyr 130, van der Waals forces with Ile116, Asn128, Pro 129, Tyr130, Lys131, Gly185, Val188, and Leu675, and hydrogen-bonding forces with Tyr 136 and Asn 189, while 1-octen-3-ol bond to the myosin with its hydrophobic interactions with Ile116, Pro129 and Leu675, van der Waals forces with Tyr117, Thr126, Val127, Asn128, Tyr130, Lys131, Gly185, and Val188, and hydrogen-bonding forces with Tyr136 and Asn189. This result suggested that different fishy compounds displayed diverse binding sites. Besides, the main interaction forces between grass carp myosin and fishy compounds with different functional groups exhibited significant differences, which is consistent with the results reported by Zhao et al. (2024) and Xue et al. (2022).

4. Conclusions

In the present study, the interaction between the myofibrillar protein in grass carp and four fishy compounds (hexanal, octanal, decanal and 1-octen-3-ol) was investigated. The binding abilities of myofibrillar protein for the fishy compounds were ranked in descending order as follows: decanal, octanal, hexanal, and 1-octen-3-ol. The aromatic amino acid residue microenvironment was affected by the interaction between grass carp myofibrillar proteins and these flavor fishy compounds. The quenching mechanism of aldehyde volatiles with grass carp myofibrillar proteins was a combination of static and dynamic quenches, whereas

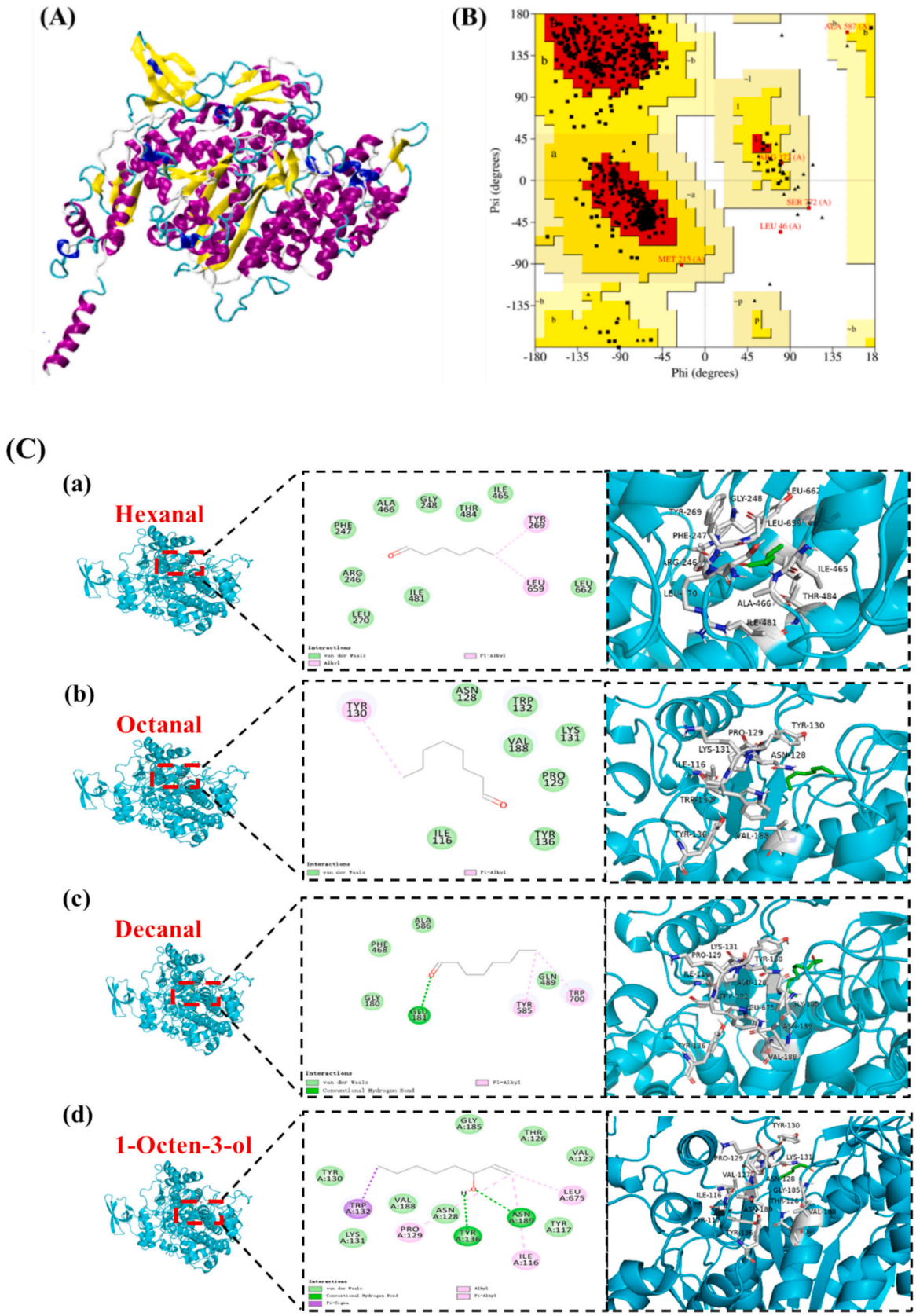

Fig. 7. Homology modeling of myosin in grass carp and molecular docking between grass carp myosin and the four fishy compounds. (A) and (B) represent the three-dimensional structure and Ramachandran plot of myosin, respectively. (C) represent two-dimensional and three-dimensional diagrams of the molecular docking of grass carp myosin and (a) hexanal, (b) octanal, (c) decanal and (d) 1-Octen −3-ol, respectively.

Table 3
Docking summary of four fishy odor compounds with grass carp myosin.

Compounds	E_b/(kcal/mol)	Residual number of interacting receptors
Hexanal	−3.091	11
Octanal	−3.627	8
Decanal	−4.419	11
1-Octen-3-ol	−2.114	14

that of 1-octen-3-ol was a static quench. The main binding interaction of myofibrillar proteins to hexanal, octanal, and decanal was found to be hydrophobic, while those to 1-octen-3-ol were characterized by hydrogen bonding and van der Waals forces, and binding sites with these fishy compounds in grass carp myofibrillar proteins might occur in the vicinity of Tyr and Trp residues. The further structure analysis showed decreased the α-helical structure content, increased the β-sheet and β-turning structure contents, and a decrease in surface hydrophobicity occurred in grass carp myofibrillar protein through interaction with the four fishy compounds. Molecular docking exhibited the absolute value of the binding energy between grass carp myosin and four fishy compounds was decreased in the order as decanal, octanal, hexanal, and 1-octen-3-ol, and different fishy compounds might format various biding sites and interactions. The study may provide a theoretical basis for eliminating fishy odor in freshwater fish.

CRediT authorship contribution statement

Naiyong Xiao: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation. **Yutao Pang:** Investigation. **Sirui Chen:** Writing – review & editing, Conceptualization. **Lilang Li:** Writing – review & editing, Writing – original draft. **Yantao Yin:** Writing – review & editing, Methodology. **Wen Xia:** Formal analysis. **Qinxiu Sun:** Writing – review & editing. **Shucheng Liu:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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