Interaction and binding mechanism of lipid oxidation products to sturgeon myofibrillar protein in low temperature vacuum heating conditions: Multispectroscopic and molecular docking approaches

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Abstract

In this work, the binding mechanism of myofibrillar protein (MP) with malondialdehyde and 4-hydroxy-2-nonenal under low temperature vacuum heating was investigated via multispectroscopic and molecular docking. The results showed that binding interaction and increasing temperature caused significant changes in the conformations as well as a decrease in the value of protein intrinsic fluorescence, surface hydrophobicity, and fluorescence excitation-emission matrix spectra. Furthermore, the decrease in α-helix and β-turn, increase in β-sheet and a random coil of MP, imply the MP molecules to be more unfolded. Isothermal titration calorimetry and molecular docking results showed that main driving force for binding with MP was hydrogen bond, and the binding ability of malondialdehyde was superior to that of 4-hydroxy-2-nonenal. Moreover, increasing the heating temperature was beneficial to the binding reaction and intensified the conformational transition of MP. These results will provide a reference for further studies on the lipid and protein interaction of sturgeon.

1. Introduction

Russian sturgeon (Acipenser gueldenstaedti), as a typical source of highly digestible protein, is increasingly farmed due to its rich protein and polyunsaturated fatty acids, as well as its balanced amino acid composition (Liu et al., 2022), especially in China, which accounts for 85% of global production (Shen et al., 2020). At present, the processing of sturgeon fillets is still relatively basic, and an important reason is a loss caused by protein oxidation during processing. Protein oxidation changes the physicochemical properties of the protein in sturgeon fillets, which in turn affects the quality of sturgeon fillets and their processing properties (Zhang, Xiao, & Ahn, 2013). Regulation of protein oxidation during processing is a critical issue for improving sturgeon utilization.

Heat treatment is a common technical unit in the prefabrication or cooking process of fish meat. Most of the traditional heat treatment methods are air exposure conditions under normal pressure. The oxidative structure of protein is relatively serious, and it is easy to cause quality deterioration and economic losses due to excessive heat processing (Wan et al., 2021). Low temperature vacuum heating (LTVH) is a new heating technique, which can effectively improve the processing quality of sturgeon meat, form a good flavor (Liu et al., 2022), and prolong the shelf life (Shen et al., 2020). Compared to other vacuum heat treatment methods, LTVH was an easy-to-use processing method for processing large batch sizes of samples simultaneously. Therefore, LTVH is more suitable for industrial production. Based on this, it is necessary to study protein oxidation under LTVH processing conditions to expand the production and utilization of sturgeon fillets.

Protein and lipid oxidation reactions usually co-exist and mutually promote one another in the meat system (Wang, Zhao, Qiu, & Sun, 2018). The oxidation of both was generally initiated by reactive oxygen species (ROS), and there was a potential correlation between the two (Wang, He, Emara, Gan, & Li, 2019). Lipid oxidation products can cause
some damage to the protein. 4-Hydroxy-2-nonenal (HNE) altered the spatial structure of the protein and damaged the membrane of the protein, resulting in changes in membrane protein function, for example, Na+/K+ ion pump activity decreased (Fleuranceau-Morel, Barrier, Fauconneau, Piriou, & Huguet, 1999). Some studies have mainly focused on the oxidative damage of malondialdehyde (MDA) on myofibrillar protein (Wang et al., 2019) and the properties of myofibril gel (Wang, Zhang, Fang, & Bhandari, 2017), and some scholars have also paid attention to the effect of MDA on protein after adding antioxidants (Wang, He, Zhang, Chen, & Li, 2021). Previous studies did not apply external force and only relied on the interaction of MDA with proteins to promote protein oxidation. However, in the actual production process, factors such as heating, high pressure, and electric field will affect protein oxidation together with lipid oxidation products. Nevertheless, it is reasonable to speculate the LTVH could accelerate the reaction of lipid oxidation products and proteins, and the degree of both reactions increased with increasing temperature.

The purpose of this paper was to study the interaction and binding mechanism of different lipid oxidation products (MDA and HNE) on sturgeon myofibrillar proteins under low temperature vacuum heating (50, 60, 70 °C respectively). The multispectral methods including intrinsic fluorescence spectroscopy, Fourier transformation infrared (FTIR) spectroscopy, fluorescence excitation-emission matrix (EEM) spectra were used to analyze the structural changes in proteins. Meanwhile, the affinity of the interaction was confirmed by isothermal titration calorimetry (ITC). Besides, molecular docking technology was used to explore the binding mechanism of MDA and HNE to MP. The research results can provide new ideas for regulating protein oxidation during heating, and provide theoretical support for deep processing of sturgeon.

2. Materials and methods

2.1. Materials

The sturgeon was transported from Quzhou Sturgeon Aquatic Food Technology Development Co. Ltd. (Zhejiang, China) within 2 h at 4 °C. 4-hydroxy Nonenal (HNE) was purchased from GlibBio (Shanghai, China), sodium phosphate Dibasic dodecahydrate, 1,1,3,3-Tetramethoxypropane (TMP)sodium phosphate, monobasic dehydrate, sodium chloride, ethylenediaminetetraacetic acid (EDTA), brilliant blue G, sodium hydroxide, sodium 8-Anilino-1-naphthalenesulfonate (ANS-Na), 6-FITC were bought from Macklin biochemical Co. Ltd. (Shanghai, China). All chemicals were of analytical grade.

2.2. Preparation of lipid oxidation products

MDA solution was prepared according to Wang et al. (2019) with some modifications. Briefly, TMP (1.1 mL) was added with distilled water (38.9 mL) and 5 M HCl (10 mL). The reaction was magnetically stirred at 50 °C for 1 h in the dark. The color of the solution changed from colorless to light yellow. The pH of the mixture was adjusted to 7.4 using 6 M NaOH after the temperature was lowered to room temperature. Dilute the solution appropriately and measure the absorbance at 267 nm with a UV–Vis spectrophotometer (UV1900i, Shimadzu, Kyoto, Japan). The concentration of MDA was determined by the specific absorbance coefficient (31500 M$^{-1}$ cm$^{-1}$). The MDA solution was finally diluted in phosphate buffer B (25 mM, pH 7.0) containing 0.6 M NaCl to make 10 mM working solution. The HNE solution was diluted to a 10 mM solution in phosphate buffer B mentioned above.

2.3. Extraction of MP

The method for MP extraction was carried out in agreement with former reports (Shen et al., 2020). MP was stored at 4 °C and analyzed within 24 h. To determine the MP concentration, the volume of 20 μL MP solution was mixed with the 200 μL of Coomassie Brilliant Blue G-250 and the absorbance was measured at 595 nm.

2.3. In vitro oxidation of MP

The MP suspensions were adjusted to 1 mg mL$^{-1}$ using the phosphate buffer B. The MP suspension without any addition was the control group, the 10 mM MDA and HNE solution were added into MP as treated groups, respectively. The solution was immediately equally divided into three portions for low temperature vacuum heating (LTVH). The temperature of this period was set as 50, 60, 70 °C respectively. The control groups were named C50, C60, and C70. The MP solutions treated with MDA were M50, M60, and M70 while those treated with HNE were H50, H60, and H70. After heating for 15 min, the resulting MP solutions were dialyzed against 20 mM phosphate buffer A (pH 7.0) comprising 100 mM NaCl and 1 mM EDTA for 10 h. At the end of dialysis, the suspension was centrifuged at 10,000 g for 15 min at 4 °C, and the supernatants were discarded. The precipitate was subsequently lysed with phosphate buffer B and adjusted the MP solution to 1 mg mL$^{-1}$.

2.4. Intrinsic fluorescence spectroscopy

The fluorescence emission spectrum was obtained using the methods reported by Chizoba Ekezie, Cheng, & Sun (2018) with some modifications. The fluorescence spectra were recorded at wavelengths ranging from 300 to 400 nm with the excitation wavelength at 285 nm. The excitation slit width was set at 5 nm and the emission slit width was the same.

2.5. Fourier transformation infrared (FTIR) spectroscopy

The infrared spectra of the MP samples were obtained using a Fourier transform infrared spectrometer (NICOLET I55, Thermo Scientific, Germany). Samples were mixed with dry potassium bromide in a ratio of 1:100, ground, and pressed. The test was scanned in the range of 4000–400 cm$^{-1}$ and performed in transmissive mode with 32 scans (Liu et al., 2021).

2.6. Surface hydrophobicity

MP samples (2 mL) was mixed with 20 μL of 1-anilino-8-naphthalenesulfonate (8 mM) and mixed vortex. After standing at room temperature for 3 min and being protected from light. The excitation and emission wavelengths were set at 390 and 470 nm, respectively (slit width 5 nm) (Guo, Jiang, & Xiong, 2021). The fluorescence spectrum for phosphate buffer B was subtracted from all sample spectra. The initial slope of the fluorescence intensity versus protein concentration (mg mL$^{-1}$) plot (calculated by linear regression analysis) was used as an index of surface hydrophobicity.

2.7. Fluorescence excitation-emission matrix (EEM) spectra

The three-fluorescence (3D) fluorescence emission spectra of MP were recorded using an RF-6000 fluorescence spectrophotometer (Shimadzu, Kyoto, Japan) as described by Lv et al. (2022) with modifications. The 3D map was collected with an excitation wavelength of 200–400 nm and an emission wavelength of 200–500 nm, with a 5 and 10 nm slit for excitation and emission, respectively.

2.8. Isothermal titration calorimetry (ITC)

The binding between lipid oxidation products (MDA and HNE) and MP was investigated using MicroCal iPEAQ-ITC (Malvern Pananalytical Limited, USA). MP was dissolved in a 6 M NaCl solution, 280 μL of MP solution was added to the sample cell and titrated with the MDA and HNE 19 times, respectively. The titrated samples were injected with a
2.9. Molecular docking

Molecular docking was performed to study the binding modes for the site of myosin with its ligands and obtain binding affinity (Xu et al., 2021, 2022). The molecular structure of MDA was obtained from the ZINC database (http://zinc.docking.org/substances/ZINC000005178380/). The 2D structures of HNE were obtained from the same website (http://zinc.docking.org/substances/ZINC000002020117/), which were then converted to PDB format via OpenBableGUI software. The crystal structure of myosin (PDB ID: 3QMA) was obtained from the RCSB Protein Data Bank (https://www.pdb.org/structure/3QMA). The crystal structure was prepared into receptors by removing water and substrate and adding polar hydrogen atoms by AutoDock software. At the same time, AutoDock Vina was used to conduct protein–ligand molecular docking studies. A grid box covered the entire binding site was centered at coordinates of X: 42.75, Y: 46.5, and Z: 47.25. The position with the highest Vina docking score was chosen, and its visual analysis was performed by PyMol software (https://www.pymol.org).

2.10. Statistical analysis

Data were expressed as means ± standard deviations for three independent experiments, each repeated four times. With the help of SPSS software (version 20.0), the significance between treatments was determined by using Multivariate Analysis of variance and least significant difference multiple comparison. The level for significance was defined as P < 0.05.

3. Results and discussion

3.1. Protein intrinsic fluorescence of the interaction between lipid oxidation products and MP

Intrinsic fluorescence quenching was used to study the potential interaction between MP and lipid oxidation products (MDA and HNE). There were two main forms of fluorescence quenching, one was protein–protein cross-linking resulting from protein oxidation and modification, and the other was the binding of covalent/non-covalent protein to lipid secondary oxidation ligands (such as MDA) (Zhang et al., 2022). Fig. 1 A showed the $\lambda_{\text{max}}$ values of MP in each group, which was around 330 nm and the peak value was tryptophan (Trp). An increase in the temperature led to a decrease in the fluorescence intensity of MP. It was consistent with Wan et al. (2021). As the temperature increased, the degree of protein aggregation increased, causing an increase in steric hindrance, the fluorescence intensity decreased as well. The aggregation degree of protein increases with increasing the temperature, which results in the increasing of the steric hindrance and lower fluorescence intensity (Wan et al., 2021). The fluorescence intensity will be significantly reduced when the protein containing a tryptophan residue exposed into the hydrophilic environment, and the decrease in fluorescence intensity stated that the MP was in a partially unfolded or fully unfolded state (Cao & Xiong, 2015).

Results are presented as the mean ± standard deviation. Different letters indicate significant difference (P < 0.05). The heating temperature of MP was set as 50, 60, 70 °C respectively. The control groups were named C50, C60, and C70. The MP solutions treated with MDA were M50, M60, M70 while those treated with HNE were H50, H60, H70.

Fig. 1B showed the secondary structure of the interaction between lipid oxidation products and MP subjected to the different heating temperatures. The secondary structure composition was determined by FTIR by measuring absorbance within the amide I region (1600–1701 cm$^{-1}$) (Shi et al., 2020), and the results were shown in Table 1. It was clear that the ratio of $\alpha$-helix significantly decreased and $\beta$-sheet and random increased concomitantly with the temperature increasing (P < 0.05). It was conceptualized that intramolecular hydrogen bond networks played a critical role in the stability of $\alpha$-helix, while heating weakened or even broke the hydrogen bond between the amino hydrogen and the carbonyl oxygen, thereby unwinding the $\alpha$-helical structure of MP. The ratio of $\beta$-turn decreased while the ratio of random increased with increasing the temperature.

![Figure 1](image.png)

**Fig. 1.** Changes in intrinsic fluorescence (A) and secondary structure (B) of MP extracted from sturgeon fillets. The heating temperature of MP was set as 50, 60, 70 °C respectively. The control groups were named C50, C60, and C70. The MP solutions treated with MDA were M50, M60, M70 while those treated with HNE were H50, H60, H70.

### Table 1

| MP Treatment | $\alpha$-helix/% | $\beta$-sheet/% | $\beta$-turn/% | Random/% |
|--------------|-----------------|----------------|---------------|----------|
| C50          | 13.60 ± 0.82$^a$ | 38.16 ± 0.10$^a$ | 34.78 ± 2.13$^a$ | 12.93 ± 0.71$^a$ |
| C60          | 12.44 ± 0.27$^a$ | 39.89 ± 0.64$^c$ | 35.36 ± 0.50$^b$ | 12.28 ± 0.13$^a$ |
| C70          | 11.71 ± 0.55$^b$ | 41.90 ± 1.49$^c$ | 32.19 ± 1.10$^a$ | 17.60 ± 0.94$^a$ |
| M50          | 12.83 ± 1.06$^b$ | 39.63 ± 0.73$^b$ | 29.55 ± 2.52$^b$ | 15.98 ± 0.70$^b$ |
| M60          | 12.46 ± 0.67$^b$ | 42.47 ± 1.77$^b$ | 35.07 ± 0.42$^c$ | 16.97 ± 1.35$^c$ |
| M70          | 11.69 ± 0.38$^b$ | 45.49 ± 0.91$^a$ | 33.56 ± 1.81$^c$ | 17.64 ± 0.07$^c$ |
| H50          | 13.19 ± 0.35$^b$ | 38.08 ± 0.15$^a$ | 32.93 ± 2.60$^a$ | 15.28 ± 1.06$^a$ |
| H60          | 11.81 ± 0.47$^c$ | 39.98 ± 0.11$^c$ | 34.32 ± 0.57$^a$ | 16.37 ± 0.69$^c$ |
| H70          | 10.98 ± 0.84$^c$ | 43.99 ± 0.38$^c$ | 34.42 ± 1.92$^a$ | 17.09 ± 1.15$^a$ |

Results are presented as the mean ± standard deviation. Different letters indicate significant difference (P < 0.05). The heating temperature of MP was set as 50, 60, 70 °C respectively. The control groups were named C50, C60, and C70. The MP solutions treated with MDA were M50, M60, M70 while those treated with HNE were H50, H60, H70.
structure of the protein (Yin, Zhou, Pereira, Zhang, & Zhang, 2020). Generally, the decline of the α-helix and the increase of the β-sheet usually indicated the unfolding of MP molecules with further aggregation and less structural order (Jiang et al., 2017; Zhang et al., 2022).

For the interaction of different lipid oxidation products and MP, the effect of MDA on MP was more obvious. The interaction between HNE and MP was beneficial to the unfolding of the α-helix segment in MP, which can expose HIS residues buried inside the molecule, such as HIS24 (Friend & Gurd, 1979), followed by further binding of HNE (Viana et al., 2020), making the MP helical structure slightly recycled, so compared with the control groups differences were not significant (P > 0.05). As for MDA, it reacted readily with ε-NH2 groups of MP and reported that MDA can modify proteins by reacting with 50–60% of ε-NH2 (Buttkus, 1967; Wang et al., 2017). Wang et al. (Wang et al., 2021) verified that MDA can reduce in α-helix and increase in the random coil of MP.

3.3. Tertiary structure of the interaction between lipid oxidation products and MP

ANS was a fluorescent probe with enhanced fluorescence after binding to a hydrophobic surface, which can be used to observe the conformational changes of proteins, mainly the exposure of hydrophobic regions (Fu et al., 2020). As depicted in Fig. 2, the effect of temperature on the surface hydrophobicity was very significant, the temperature increased, the surface hydrophobicity increased significantly (P < 0.05). The results were supported by Traore et al. (2012) who reported that increasing the heating temperature increased the surface hydrophobicity of pork MPs. After heating, part of non-polar amino acids was exposed to the hydrophobic clusters, leading to unfolding and rearrangement of protein molecules (Fu et al., 2020; Kijroongrojana, 2009), increasing surface hydrophobicity, which was consistent with the result of Fourier transformation infrared spectroscopy. In addition, it was clear that the addition of MDA and HNE significantly increased the surface hydrophobicity (P < 0.05). Except that the surface hydrophobicity of the added MDA was lower than that of HNE in the 60 °C treatment group, the other two groups had the highest MDA. This indicated that due to the action of MDA, electrostatic interactions or hydrogen bonds between proteins are disrupted, resulting in the unfolding of proteins and exposing non-polar amino acids to the protein surface, thereby increasing the surface hydrophobicity (Wang et al., 2017; Wang et al., 2021).

Fluorescence excitation-emission matrix (EEM) spectra can simultaneously obtain and characterize the spectral peak information of different fluorophores of proteins, which contributed to the confidence of conformational studies (Fu et al., 2020). Fig. 3 showed the spectra of the interaction between lipid oxidation products and MP. Generally, Peak 1 (λex = 280.0 nm, λem = 330.0 nm) characterized the spectral properties of Trp and Tyr residues, and peak 2 (λex = 230.0 nm, λem = 330.0 nm) can represent the fluorescence spectral changes of the polypeptide backbone structure (Jin, Yang, Yang, & Feng, 2021; Lv et al., 2022). The fluorescence intensity in Fig. 3 showed decreasing trends as temperature increased, for both two peaks. Meanwhile, it can be observed that the peak 1 fluorescence intensity of MP decreased after adding MDA and HNE in heating conditions. Besides, the fluorescence intensity of peak 2 also decreased with the addition of MDA and HNE. The chromophores of peak1 were mainly tryptophan (Trp) and tyrosine (Tyr), which were usually located in the hydrophobic region of the protein. The changes in fluorescence intensity were usually due to swelling of proteins exposing them to a hydrophilic environment (Jin et al., 2021). The interaction of lipid oxidation products with MP altered the polarity of the microenvironment of Trp and Tyr residues, resulting in reduced MP polarity and enhanced hydrophobicity, and a tendency for the protein to fold (Lv et al., 2022), it was validated the conclusion of surface hydrophobicity and intrinsic fluorescence spectroscopy.

3.4. Affinity of lipid oxidation products to MP

It was reported that ITC was an important method to explain the mechanisms of interaction (Demers & Mittermaier, 2009). Dissociation constants (Kd), enthalpy change (ΔH), Gibbs free energy change (ΔG), and entropy change (ΔS) were crucial thermodynamic parameters for the investigation of interaction mechanism (Fu et al., 2020). A summary of the thermodynamic parameters from the ITC assay were presented in Table 2. The Kd value of MDA and MP was smaller than HNE (P < 0.05), suggesting that the binding capacity of MP with MDA was stronger than that with HNE. The negative values of ΔG indicated the feasible and spontaneous nature of reaction (Fu et al., 2020). Besides, according to the size of thermodynamic enthalpy and entropy change, the main types of ligand–protein binding can be obtained (Frazier, Papadopoulou, & Green, 2006). Through the symbols of some thermodynamic constants, the type of molecular bonds can be judged: When ΔH > 0 and ΔS > 0, the reaction was a typical hydrophobic interaction; when ΔH < 0 and ΔS < 0 represented van der Waals forces and/or hydrogen bonds; when ΔH ≈ 0 or less, ΔS > 0 was an electrostatic interaction (Ross & Subramanian, 1981). It can be seen from Table 2 that both ΔH and ΔS of interaction lipid oxidation products and MP were negative, indicating that the major interaction forces in MP-MDA or MP-HNE complexation were hydrogen bonding and/or van der Waals forces. Besides, the increase in temperature made the Kd value decrease, indicating that increasing the temperature was beneficial to the reaction.

3.5. Molecular docking of the interaction between lipid oxidation products and MP

Molecular docking modeling was used to further validate the binding ability of lipid oxidation products and MP (Fig. 4). The binding energy of MP against MDA and HNE were −5.5 and −4.1 kcal/mol and indicated that the binding ability of MP to MDA was stronger than HNE. The results were consistent with the findings of the results of ITC. The HNE and MP complex was formed three conventional hydrogen bonds (O-H-X), in which the hydroxyl oxygen of HNE and the residues of LYS-67 formed two hydrogen bonds with the length of 3.0 Å and 3.2 Å, respectively (Fig. 4A). MDA was in close contact with HIS-78, LEU-71, and LEU-72, and formed six hydrogen bonds with the bond length of 3.5 Å, 3.0 Å, 2.8 Å, 2.1 Å, 1.8 Å and, 3.5 Å, respectively (Fig. 4B). The results showed that the lipid oxidation products interacted with MP through hydrogen bonds and further confirmation of the results of ITC. The weak interactions suggested that the binding between lipid oxidation products and MP was reversible. It was reported that amino acid residues in proteins provide a more favorable environment for the reaction of MDA (Esterbauer,
In general, His only reacted with the corresponding allyl group on the α-amino group, since MDA had an amino-imino-propene structure, it was easier to react with amino acids such as histidine and lysine. And the methyl-esters of His also reacted with MDA to enaminals of the general structure RNH–CH–CH–CHO (Pietrzyk & Stodola, 1981).

### Table 2

| T (°C) | K0 (M, 10⁻⁵) | ΔH (kcal/mol) | ΔG (kcal/mol) | ΔS (kcal/mol) |
|--------|--------------|--------------|--------------|---------------|
| MDA 50 | 12.1 ± 0.5a  | -1.5 ± 0.2a  | -29.8        | 28.9          |
| 60     | 7.0 ± 1.1c   | -1.3 ± 0.3c  | -25.7        | 27.3          |
| 70     | 2.9 ± 0.9e   | -0.8 ± 0.1e  | -25.8        | 23.8          |
| HNE 50 | 36.3 ± 1.4a  | -3.3 ± 0.1a  | -78.9        | 261           |
| 60     | 22.6 ± 0.3b  | -2.1 ± 0.2b  | -74.3        | 138           |
| 70     | 10.5 ± 2.0c  | -1.6 ± 0.1c  | -76.6        | 123           |

Different letters indicate significant difference (P < 0.05).

In conclusion, the interactions of different lipid oxidation products (MDA and HNE) with MP in LTVH treatment and the corresponding effects on the structure at the molecular level were investigated using multispectroscopic and molecular docking. The addition of MDA and HNE altered the spatial structure of MP; however, this change was detrimental to the MP. And increasing the heating temperature can increase the damage degree of protein structure and make the fluorescence spectrum change more significant. The result of ITC showed that the lipid oxidation products interact spontaneously with MP and by hydrogen bonding, and molecular docking further confirmed this. However, the ability of MDA to bind to MP was stronger than that of HNE. The amino acid residues related to binding of MDA to MP were identified, and HIS-78, LEU-71, and LEU-72 was confirmed as the key amino acid residue. As for HNE, LYS-67 was the key amino acid residue. The results can provide a theoretical basis for the industrial application of modified proteins induced by lipid oxidation products. At present, we only study the interaction between lipid oxidation products and protein and the changes to the spatial structure of protein, but lack of research.

![Fig. 3. Changes in Fluorescence excitation-emission matrix spectra of MP extracted from sturgeon fillets. A- C represented the control groups, D-F represented the MDA groups, G-I represented the HNE groups, from left to right, these were 50 °C, 60 °C, 70 °C.](image-url)
Fig. 4. The best docked conformations for the complex of MP with HNE (A) and MDA (B). The red stick structure was used to represent the lipid oxidation products, while the blue stick showed the MP. The dashed yellow line represents hydrogen-bonding.

and specific application of specific characteristics of protein. Further studies are needed to focus on the study of the emulsifying properties of the lipid oxidation products for protein and the loading capacity of the emulsion.

CRediT authorship contribution statement

Shi-ke Shen: Writing – original draft, Conceptualization. Qian-yun Bu: Methodology. Wen-tao Yu: Validation. Yue-wen Chen: Writing – review & editing, Validation. Fei-jian Liu: Writing – original draft, Conceptualization, Supervision. Zhi-wen Ding: Supervision. Jun-long Mao: Methodology.

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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