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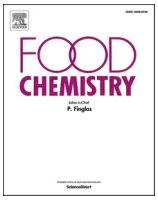

High-pressure microfluidization enhanced the stability of sodium caseinate-EGCG complex-stabilized fish oil emulsion

Wei Tang [a,b], Rui Wang [a,b], Minghui Li [a,b], Qingchun Zhang [a,b], Jianfei He [a,b], Dan Liu [c], Yuqi Feng [a,b], Weilin Liu [d,*], Jianhua Liu [a,b,*]

[a] College of Food Science and Technology, Zhejiang University of Technology, Hangzhou 310014, PR China
[b] Whole Grain Nutritious Food Processing Technology Research and Experimental Base of Ministry of Agriculture and Rural Affairs, Zhejiang University of Technology, Hangzhou 310014, PR China
[c] Key Laboratory of Pollution Exposure and Health Intervention of Zhejiang Province, College of Biology and Environmental Engineering, Zhejiang Shuren University, Hangzhou 310015, PR China
[d] School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310018, PR China

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ABSTRACT

Improving the emulsion-stabilizing effect of protein by chemical or physical modification has been paid much attention recently. Here, sodium caseinate (CS) was treated by high-pressure-microfluidization (HPM) under 0–100 MPa, and was further complexed with (-)-epigallocatechin-3-gallate (EGCG) to form an excellent emulsifier that stabilized fish oil emulsions. Results showed that HPM treatment (especially 80 MPa) significantly changed the secondary structure of CS, and 80 MPa-PCS-EGCG had the best emulsifying and antioxidant activities. In addition, after HPM treatment and EGCG bonding, CS formed a thicker interface layer on the surface of oil droplets, which could better protect the fish oil from the influence by oxygen, temperature and ion concentration. Moreover, the fish oil emulsion stabilized by PCS-EGCG complex significantly delayed the release of free fatty acids subjected to *in vitro* digestion. Conclusively, HPM-treated CS-EGCG complex could be a potential emulsifier to improve the stability of fish oil emulsions.

1. Introduction

Sodium caseinate (CS) is the sodium salt of casein, the main protein in cow's milk. It can be classified as an intrinsically unstructured protein aggregate consisting of four phosphoprotein fractions, namely α-$_{S1}$, α-$_{S2}$ casein, β-casein and κ-casein (El-Messery et al., 2020). CS has various bioactivities and remarkable emulsifying properties. (-)-Epigallocatechin-3-gallate (EGCG) is a prominent component of tea polyphenols, renowned for its exceptional antioxidant properties and various other bioactivities, particularly its preventing oxidative damage from reactive oxygen species (Li et al., 2023). At present, many studies have combined CS and EGCG to improve the performance of the digestion behaviors of emulsions (Li et al., 2021).

There are two main binding modes of CS and EGCG. These two compounds can be combined by non-covalent bonds such as hydrogen bonds, hydrophobic bonds and van der Waals forces, and can also be covalently combined by enzymatic and non-enzymatic methods (Liu et al., 2023). However, non-covalent binding is susceptible to environmental factors, and enzymatic covalent grafting process is complex (Quan, Benjakul, Sae-leaw, Balange, & Maqsood, 2019). Studies have shown that the emulsion stabilized by CS-EGCG covalent complexes can form a thick film on the droplet surface, preventing the oxidation of the droplet and improving the stability of the emulsion (Wei, Yang, Fan, Yuan, & Gao, 2015). Hence CS-EGCG covalent complexes can be used as an emulsifier to improve the stability of the oil-in-water emulsion.

Furthermore, the physical modification can alter the protein's structure and thus enhance its functions. Notably, HPM treatment can change the conformation of protein and further affect its function and interaction with polyphenols, during which the mechanical forces of HPM caused by shear stress, turbulence and cavitation play a key role (Ozturk & Turasan, 2022). For example, high-pressure could regulate the interaction between tea polyphenols and soybean isolate protein (SPI), thus reducing the surface hydrophobicity of SPI and making tea polyphenols more stable (Chen, Wang, Feng, Jiang, & Miao, 2019).

* Corresponding authors at: Whole Grain Nutritious Food Processing Technology Research and Experimental Base of Ministry of Agriculture and Rural Affairs, College of Food Science and Technology, Zhejiang University of Technology, Hangzhou 310014, PR China (J. Liu).
E-mail addresses: lwl512@zjgsu.edu.cn (W. Liu), jhliu@zjut.edu.cn (J. Liu).

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According to Zou, Xu, Zhao, Wang, and Liao (2019), HPM promoted the binding of proteins and anthocyanins, which improved the stability of anthocyanins. Nevertheless, there are few reports on the CS grafted with EGCG after HPM treatment, and the in-depth research has been hardly conducted on the emulsifying activity and antioxidant activities of HPM-treated CS-EGCG covalent complexes. Therefore, the study of the structure and function of CS treated under different pressures has important significance for further improving the performance of CS-EGCG covalent complexes.

Fish oil with high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has received widespread attention for their benefits in reducing the risk of heart disease and promoting brain health (Yesiltas et al., 2021). However, lipid oxidation, stratification, and low bioavailability in fish oil during processing and storage are a major concern, leading to the loss of stability and safety as well as sensory and nutritional quality (Liu et al., 2019). Protein-based emulsifiers for fish oil can effectively improve the stability of fish oils (Tang et al., 2023). It was found that CS could effectively improve the oxidative stability of fish oil, while EGCG with its strong antioxidant effect could enhance the effect of protein on fish oil against lipid oxidation (Peng & Tang, 2020), but the stabilizing effect of HPM-treated CS-EGCG complex on fish oil has been not yet known, and it is not clear how HPM-treated CS-EGCG further improves the stability of fish oil.

This study aims to explore the effect of HPM-treated CS-EGCG complex on the emulsifying and oxidation stability, and digestive properties of fish oil emulsion. Both CS-EGCG complexes with and without HPM treatment were investigated in detail on their structural characteristics, emulsifying activity, antioxidant activities, as well as their effect on fish oil by examining the environmental tolerance, storage stability and digestive behavior. This study provides a valuable reference for the stabilization of emulsions rich in ω-3 polyunsaturated fatty acids using novel protein–polyphenol complex emulsifiers.

2. Materials and methods

2.1. Materials

Sodium caseinate (CS, > 92 % purity) from bovine milk (C8654) was purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA), and (-)-epicatechin-3-gallate (EGCG, > 98 % purity) from green tea polyphenols were purchased from Rhawn Chemical Reagent Co., Ltd (Shanghai, China). All solutions in this experiment were prepared with Tris HCl buffer solution. Fish oil was provided by Zhoushan Xinnuojia Biological Engineering Co., Ltd. (Zhoushan, China).

2.2. Preparation of HPM-treated CS

CS (1 wt%) was prepared according to the method used in a previous study with minor changes (Tang et al., 2023). Briefly, CS powder was dispersed in 20 mmol/L Tris-HCl buffers set at pH 7.4. Then, the pH of CS was adjusted to 9, and the sample solution was prepared by continuous stirring for 2 h. The CS was subjected to a single treatment at 0, 20, 40, 60, 80 and 100 MPa by dynamic high-pressure microfluidization (Genizer, USA), and the CS treated by HPM was named PCS.

2.3. Complexation of EGCG with CS

The HPM-treated CS-EGCG complex was prepared by alkali-mediated grafting according to our previous study (Tang et al., 2023). Briefly, EGCG (0.05 %, 0.10 % and 0.15 % w/v) was slowly added to the CS dispersion system (Subsection 2.2) in a 1:1 ratio,and the pH of mixture was adjusted to 9 and then stirred continuously for 24 h in the presence of oxygen. The solutions were then dialyzed in visking tubes (MWCO 12–14 kDa) for 48 h to remove free EGCG molecules. The HPM-treated CS-EGCG samples were obtained after lyophilization.

The total phenolic content of HPM-treated CS-EGCG complex were assayed by Folin-Ciocalteu method (Wang et al., 2014). Briefly, 0.5 mL of adequately diluted sample was blended with 2.5 mL of freshly prepared Folin-Ciocalteu reagent at room temperature. After 5 min, 2 mL (7.5 % w/v) of sodium carbonate was added and the mixture was swirled and left in the dark at room temperature for 2 h. The absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Huyueming Scientific Instrument Co. Ltd., China).

2.4. Fourier transform-infrared (FTIR) spectroscopy

The infrared spectra (Thermo, USA) of HPM-treated CS (0–100 MPa) and CS-EGCG complex were performed using the potassium bromide pellet method (Yang et al., 2022), and the experimental results were processed by OMNIC 8 and PeakFit (v4.12) software.

2.5. Fluorescence spectroscopy

The fluorescence intensity of each sample (0.5 mg/mL, 20 mM PBS, pH 6.8) was analyzed using an F-6000 fluorometer (Shimadzu, Japan) with the method described in previous work (Xu et al., 2024). The excitation wavelength was set to 280 nm and the emission was recorded between 300 and 500 nm. The scanning speed was 12,000 nm/min and the slit widths were 3.3 and 2.0 nm.

2.6. Antioxidant activities

2.6.1. ABTS radical scavenging activity

The ABTS radical cation ($ABTS^{\bullet+}$) radical scavenging assay of the samples was assessed using the method described in a previous study (Zhang, Wang, He, Tang, & Liu, 2024). The 100 μL of diluted ABTS solution was mixed with 100 μL of sample (1 mg/mL) and left in the dark for 10 min. The absorbance of the resulting solution was measured at a wavelength of 734 nm.

2.6.2. DPPH radical scavenging activity

The DPPH radical scavenging activity was assessed using the method described by Zhang et al. (2024) with some modifications. In brief, 100 μL of each sample (0.5 mg/mL) was mixed with 100 μL DPPH solution (0.1 mM, ethanol) was mixed and kept in the dark at room temperature for 30 min. The absorbance of the remaining DPPH was determined at 517 nm.

2.6.3. Reducing power

The reducing power assay of HPM-treated CS (0–100 MPa) and HPM-treated CS-EGCG complex was determined according to a previously reported method (Zhang et al., 2024), and the results were covered as absorbance values. The absorbance values were measured at 700 nm after 10 min.

2.7. Emulsifying activity

The emulsifying activity were determined according to the method of Guo et al. (2021). Briefly, HPM-treated CS or CS-EGCG complex solution (15 mL, 1.0 mg/mL) was mixed with soybean oil (5 mL), and the mixture was homogenized (20,000 × *g*) for 3 min. An aliquot of 50 μL solution from the bottom of emulsion was mixed with an SDS solution (0.10 %, w/v), and the absorbance was measured at 500 nm at 0 min. The emulsifying activity index (EAI) was calculated as follows:

$$EAI = \frac{2 \times 2.303 \times A_0 \times dilutionfactor}{10000 \times \lambda \times \mathrm{C}} \quad (1)$$

Where the A_0 was the measured absorbance values for the diluted emulsion samples taken at 0 min.

2.8. Preparation of fish oil emulsion

The HPM-treated CS-EGCG complex (1 wt%) was dissolved in 20 mM Tris-HCl (pH 7.4), and stirred at 120 rpm for 2 h at constant speed to form an aqueous phase, and 10 % fish oil (w/w) was added to the above solution. The crude emulsion was then obtained by shearing with a homogenizer (Quanjian Electromechanical Co. Ltd., China) at 13,500 rpm for 1 min. The crude emulsion was finally processed into emulsion by passing it through HPM operating (Genizer, USA) under 60 MPa for three runs. The emulsion pH was adjusted to 7 and stirred for 1 h to obtain a stable emulsion. The prepared emulsion was stored at 4 °C.

2.9. Particle size and ζ-potential

Before testing, 100 μL of each emulsion was taken to dilute 1,000 times, and then the droplet size and potential value of samples were measured by a Malvern laser particle sizer (LSI3320, Beckman Coulter, USA) at 24 ± 1 °C.

2.10. Interfacial properties

The time-dependent interfacial pressure (π) and adsorption behavior of CS/CS-EGCG complex solutions adsorbed at the oil–water interface were measured at 25 °C by an optical contact angle meter (OCA35, DataPhysics Instruments Co. Ltd., Germany) as described by Wang et al. (2020) with some modifications.

2.11. Tolerance to environmental stresses

2.11.1. Thermal stability

The emulsion samples were subjected to heat treatment at 50 to 90 °C for 30 min and then immediately cooled to room temperature (Gu et al., 2017). After standing for 2 h, the average particle diameter of each nanoparticle dispersion was measured.

2.11.2. Salt stability

The salt stability of the emulsion was determined by adding different levels of sodium chloride. The original lotion sample was mixed with 4 volumes of sodium chloride solution (different moles) to make the final concentration of sodium chloride of the final emulsion reach 0, 30, 50, 100, 200 and 300 mM (Gu et al., 2017). After 2 h standing, the average particle diameter of each nanoparticle dispersion was measured.

2.12. Storage stability

The prepared emulsion was stored at 4 °C for 49 days, and samples were taken every 3 days to determine its particle size, light microscopy and oxidative stability. Among them, the determination method of particle size was according to Section 2.10. The emulsion was observed under a 40 × objective lens using a fluorescence inverted microscope (Olympus Co. Ltd., China). The POV and TBARS values of the samples were determined with a reference to the method of Kargar, Spyropoulos, and Norton (2011).

2.13. In vitro digestion

A three-step simulation of oral, gastric and small intestine was used to evaluate the potential gastrointestinal fate of different samples. Simulated oral phase [15.1 mM KCl, 3.7 mM KH_2PO_4, 13.6 mM $NaHCO_3$, 0.15 mM $MgCl_2(H_2O)_6$, 0.06 mM $(NH_4)_2CO_3$, 1.5 mM $CaCl_2(H_2O)_2$], simulated gastric phase [6.9 mM KCl, 0.9 mM KH_2PO_4, 25 M $NaHCO_3$, 47.2 mM NaCl, 0.1 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$, 0.15 mM $CaCl_2(H_2O)_2$] and simulated intestinal phase [6.8 mM KCl, 0.8 mM KH_2PO_4, 85 mM $NaHCO_3$, 38.4 mM NaCl, 0.33 mM $MgCl_2(H_2O)_6$, 0.6 mM $CaCl_2(H_2O)_2$, and 10 mM bile] were prepared using the method described by Liu et al. (2019) with some modifications.

2.13.1. Oral phase

The fish oil emulsion was mixed 1:1 with the above simulated saliva in 5.0 mL and finally stirred continuously for 5 min at 37 °C in a water bath, then 0.1 mL of water (instead of salivary amylase solution) was added to a final volume of 10.0 mL, and then stirred at a constant temperature of 37 °C for 2 min. At the end of the oral digestion, 0.5 mL of sample solution was taken immediately for analysis.

2.13.2. Gastric phase

The oral phase sample solution (9.5 mL) was mixed 1:1 with the above simulated gastric phase. The pH was then adjusted to 3.0 with 1 M HCl, and finally 12.7 mg of pepsin was added to a final concentration of 2,000 U/mL. The digestion was held in a water bath at 37 °C for 120 min. At the end of gastric digestion, 0.5 mL of sample solution was taken for analysis.

2.13.3. Intestinal phase

The gastric digestion sample solution (18.5 mL) was mixed with simulated intestinal fluid (simulated intestinal fluid hydrochloric acid adjusted to pH 7) in a 1:1 ratio. The pH was adjusted to 7.0 with 1.0 M NaOH, and 2 mL of porcine pancreatic enzyme stock solution was added to make a final concentration of 100 U/mL (making a final volume of 37 mL). The samples were then heated with stirring at 37 °C for 120 min and the pH in the intestinal phase was maintained at 7.0 with 0.05 M NaOH. The percentage of free fatty acids released was then calculated based on the volume of NaOH solution that neutralized the free fatty acids. At the end of intestinal digestion, 0.5 mL of sample solution was immediately taken for further analysis.

The particle size, ζ-potential and microstructure of the samples in three stages were measured. The determination method of particle size and ζ-potential is referred to Section 2.10. The microscopic image of the emulsion was observed using the method of Drapala, Auty, Mulvihill, and O'Mahony (2016). In short, the microstructure of the emulsion during gastrointestinal digestion was observed by Zeiss 800 laser confocal microscope (OLYMPUS BX41, China), and the magnification was 10–40 times. Nile red and fast green were used to label droplets and proteins, respectively.

2.14. Statistical analyses

All the measurements were carried out at least three times, and the accuracy between the data was represented by an error bar (Figs. 1, 3, 4 and 6) or an error band (Figs. 4 and 5). Statistical analyses were performed using the analysis of variance (ANOVA) procedure of the SPSS 21.0 statistical analysis program, and the differences between means of the trials were detected by a least significant difference test ($P \leq 0.05$).

3. Results and discussion

3.1. Amount of EGCG bound to CS

As shown in Fig. 1A, compared with the original CS-EGCG, the HPM treated CS-EGCG complex showed an increase in EGCG loading under 40 and 100 MPa, respectively, while it displayed a decrease in EGCG loading under 20, 60, and 80 MPa, respectively. This change suggested that HPM had huge effect on the grafting behavior between CS and EGCG molecules, which varied with the HPM pressures. In fact, different pressures can cause the change of protein conformation to a different extent (Ozturk & Turasan, 2022), resulting in different color reactions of Folin-Ciocalteu reagent (Wang et al., 2014), which might be responsible for the diversity of EGCG loading in CS-EGCG complexes.

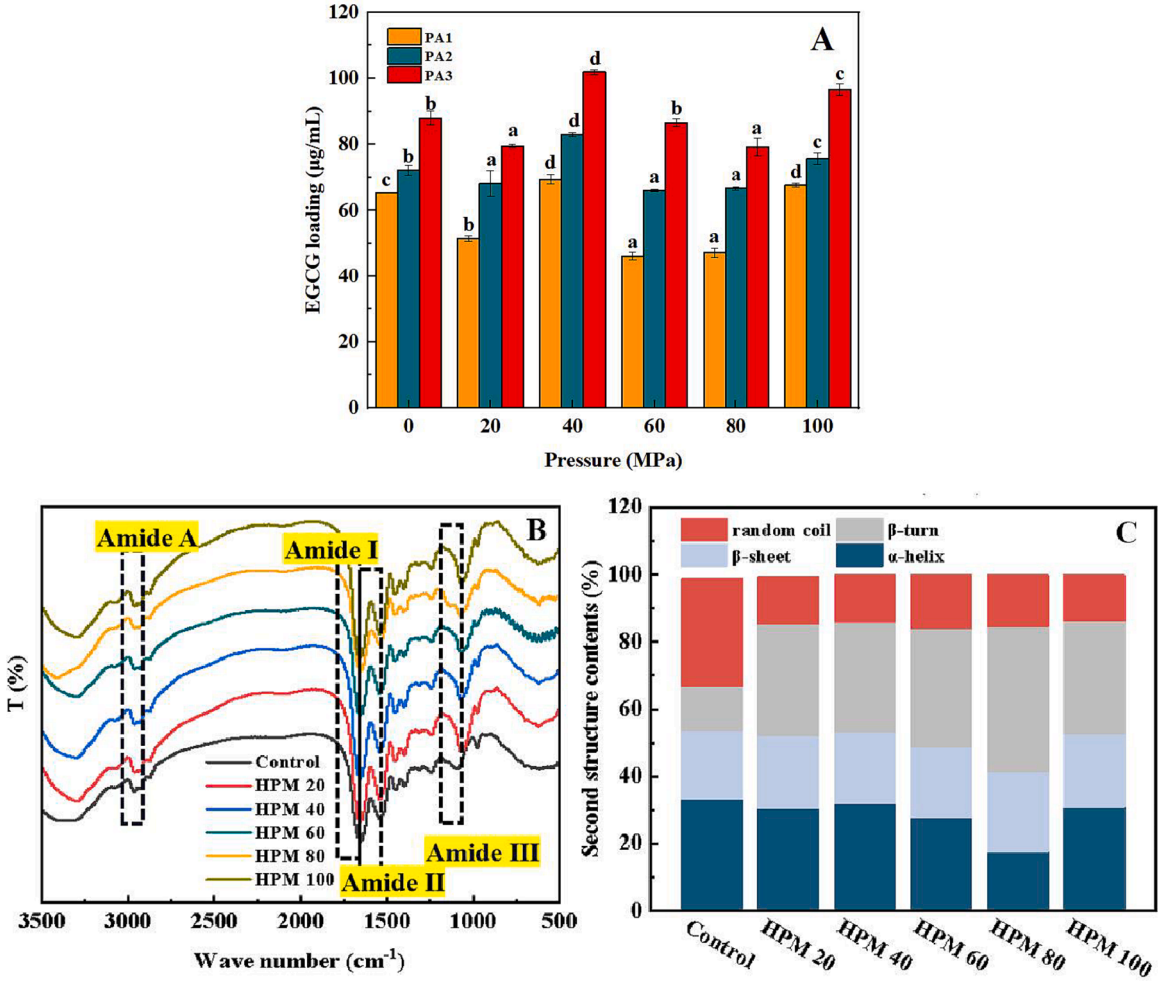

Fig. 1. Amount of EGCG (0.05 %, 0.10 %, 0.15 % w/v) bound to CS (**A**), FTIR of CS (**B**) and secondary structural elements of CS (**C**). The CS was treated with 0–100 MPa HPM, and the A1, A2 and A3 groups were CS-0.05 % EGCG, CS-0.10 % EGCG and CS-0.15 % EGCG, respectively. Different letters (a, b, c and d) indicated that there were significant differences between groups (the same sample was treated with different pressures) ($p < 0.05$).

3.2. Characterization of secondary structure

Fig. 1C shows the FTIR spectra of CS under 0–100 MPa pressure treatment. Firstly, the band fluctuation of original CS mainly occurs in the amide A (N—H stretching and hydrogen bonding vibration), amide I (C═O stretching, COO– and hydrogen bonding vibration), amide II (C—N stretching and N—H in-plane bending vibration) and amide III (C—N stretching vibration). The amide A, I, II and III band were located at 3404.76 cm^{-1}, 1654.48 cm^{-1}, 1545.51 cm^{-1}, and 1241.93 cm^{-1}, respectively. For the CS, the main changes occurred in the amide A and amide II bands, where the vibrational amplitude deepened and shifted mainly in the short-frequency direction (red-shift), and N—H stretching and hydrogen-bonded C—N stretching as well as bending vibrations in the N—H plane mainly occurred. The above phenomena indicated that HPM treatment affected the CS structure to different degrees.

Fig. 1D shows the content of original CS secondary structure treated at 0–100 MPa. It could be seen that after pressure treatment, the content of α-helix (33.30–17.31 %) and unordered coil (31.23–13.99 %) of original CS decreased, while the content of β-turn (13.30–43.20 %) and β-sheet structure (20.09–23.63 %) increased. Therefore, HPM treatment has huge impact on the secondary structure of protein.

The α-helix structure was related to the formation of intramolecular hydrogen bonds, and the decrease of α-helix structure indicated that HPM had a disruptive effect on the protein hydrogen bond structure, which increased the number of hydrogen bonds between molecules and enhanced intermolecular interactions (Chen et al., 2019). This suggested that HPM treatment not only changed the secondary structure but also made the protein–protein more tightly linked to each other. Moreover, the increase of β-sheet and β-turn content indicated that the aggregation of protein after HPM treatment, which changed the protein structure (Tang & Ma, 2009).

Overall, with the increase of HPM pressure from 0 to 80 MPa, the α-helix content decreased but significantly increased at 100 MPa, conversely the β-turn content showed an increasing trend with increasing pressure (0–80 MPa) and slightly decreased at 100 MPa. The same conclusion was obtained by Yang et al. (2022) when the soybean isolate was treated with 0–120 MPa. Notably, the least amount of α-helix content (17.31 %) and the most amount of β-sheet content (23.63 %) and β-turn content (43.20 %) were found at a pressure of 80 MPa. The effects of structural changes on functional properties (emulsifying activity and antioxidant activities) were discussed in the following subsections.

3.3. Fluorescence spectroscopy

Fig. 2A-F show the fluorescence emission spectra of CS under different pressure treatments in the presence of different concentrations of EGCG. As shown in Fig. 2A, CS had a strong fluorescence emission at 315 nm under 280 nm excitation. Among them, 100 MPa treated samples had the highest fluorescence emission, followed by 0, 80, 60, 40 and 20 MPa treated samples, indicating that 20–80 MPa treatment exposed

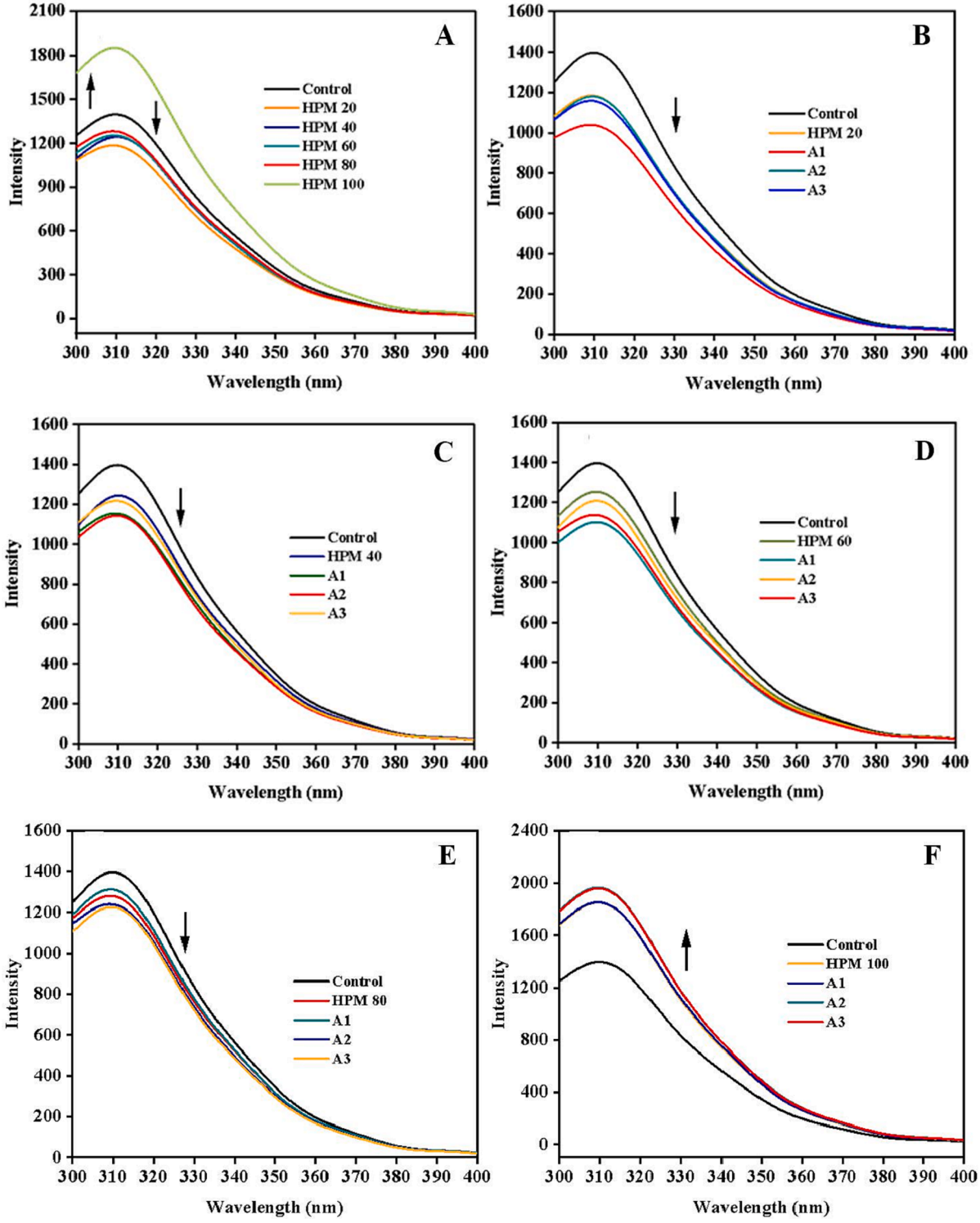

Fig. 2. Fluorescence spectra of CS (**A**) and CS-EGCG (**B-F**) at 0–100 MPa pressure. The CS was treated with 0–100 MPa HPM, and the A1, A2 and A3 groups were CS-0.05 % EGCG, CS-0.10 % EGCG and CS-0.15 % EGCG, respectively.

hydrophobic amino acids and decrease in the fluorescence intensity (Xu et al., 2024). After 100 MPa treatment, the degree of protein folding increased, resulting in tryptophan embedded in the protein and increased fluorescence intensity (Xu et al., 2024).

As shown in Fig. 2B-E, the grafting of EGCG further reduced the fluorescence intensity of the complex, indicating that the EGCG molecule could bind to the tryptophan residue in the protein and act as a quencher to quench the fluorescent substances (Cao et al., 2022). However, the different EGCG loading and HPM pressure had the irregular fluctuation effect on the fluorescence intensity. When the pressure increased to 100 MPa, the binding of EGCG caused severe distortion of the protein structure such as protein folding, which hence increased internal hydrophobic groups, and ultimately increased fluorescence intensity (Fig. 2F). In the meantime, it is worth noting that the change in fluorescence intensity has little correlation with EGCG loading, which may be attributed to the fact that the binding of polyphenols to proteins

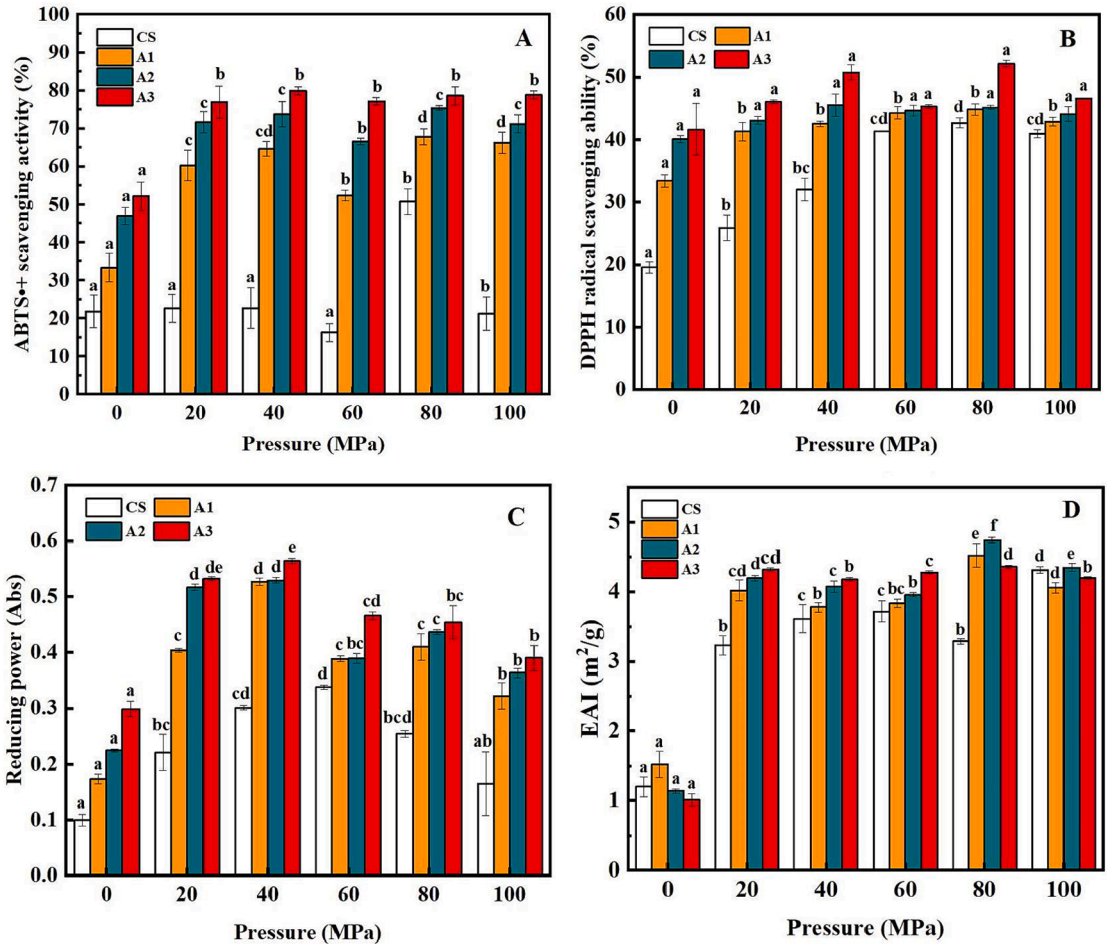

Fig. 3. ABTS radical scavenging activity (**A**), DPPH radical scavenging activity (**B**), reducing power (**C**) and emulsification activity index (**D**) of CS and CS-EGCG complex at 0–100 MPa pressure. The CS was treated with 0–100 MPa HPM, and the A1, A2 and A3 groups were CS-0.05 % EGCG, CS-0.10 % EGCG and CS-0.15 % EGCG, respectively. Different letters (a, b, c and d) indicated that there were significant differences between groups (the same sample was treated with different pressures) ($p < 0.05$).

is not limited to tryptophan. Similar results were also found in the study that polyphenols could bind to lysine and cystine (Parolia et al., 2022).

3.4. Antioxidant activities

As shown in Fig. 3A-C, the antioxidant capacity of CS changed after HPM (0–100 MPa) treatment. Overall, the 80 MPa treated CS had the highest free radical scavenging ability, especially the ABTS free radical scavenging ability (increasing 132.62 % comparing to original CS), though the 60 MPa treated CS had the best total reducing power, which might be due to the different antioxidant mechanisms of samples in different systems, such as hydrogen atom transfer and sphere electron transfer mechanisms as described by Tošović, Marković, Dimitrić Marković, Mojović, and Milenković (2017). In addition, Perreault et al. (2017) also proved that high pressure treatment could significantly enhance the antioxidant activity of proteins.

At the same time, the grafting of polyhydroxy EGCG could significantly enhance the antioxidant activities of the protein, which was consistent with our previous research (Tang et al., 2023) in which the complexes formed by covalent binding of CS with different concentrations of EGCG significantly improved the antioxidant activity of CS (increasing 52.73–2211.11 % comparing to original CS), which might be attributed to the presence of a large number of phenolic hydroxyl groups on the protein surface (Gu et al., 2017). It was worth noting that the antioxidant activities of CS-EGCG complex was further improved after HPM treatment. This indicated that HPM treatment and EGCG loading played a synergistic role in enhancing the oxidation resistance of CS, so that the oxidation stability of PCS-EGCG stabilized emulsion was the best (data in Section 3.9.3). As a consequence, the covalent complex formed by HPM-treated CS and EGCG had better antioxidant activities, and the main performance was that the antioxidant activities of PCS-EGCG enhanced by 52.73–266.15 %.

3.5. Emulsifying activity

As shown in Fig. 3D, compared with the original protein, the CS and CS-EGCG complex with HPM treatment had better emulsifying activity. For the original CS, the emulsifying activity index (EAI) of protein at 20 to 100 MPa showed a trend of first increase and later decrease, of which 80 MPa was the lowest. This might be attributed to that the degree of folding caused by HPM treatment affected its emulsifying properties (Guo et al., 2021). However, after polyphenols conjugation, the complex under 80 MPa had higher emulsifying activity, indicating that the exposed amino and sulfhydryl sites of proteins could exert better emulsifying activity after grafting with polyphenols under 80 MPa.

On the other hand, the HPM-treated CS-EGCG after 20–60 MPa HPM treatment showed an enlargement with the increase of EGCG concentration. However, for 80 and 100 MPa treated CS-EGCG complex, the EAI value varied with different concentrations of polyphenols. And it did not show a proportional growth trend with the change of concentration, even showing a downward trend at 100 MPa. This change occurred mainly because CS itself was a complex protein (α, β, κ-CS), and the

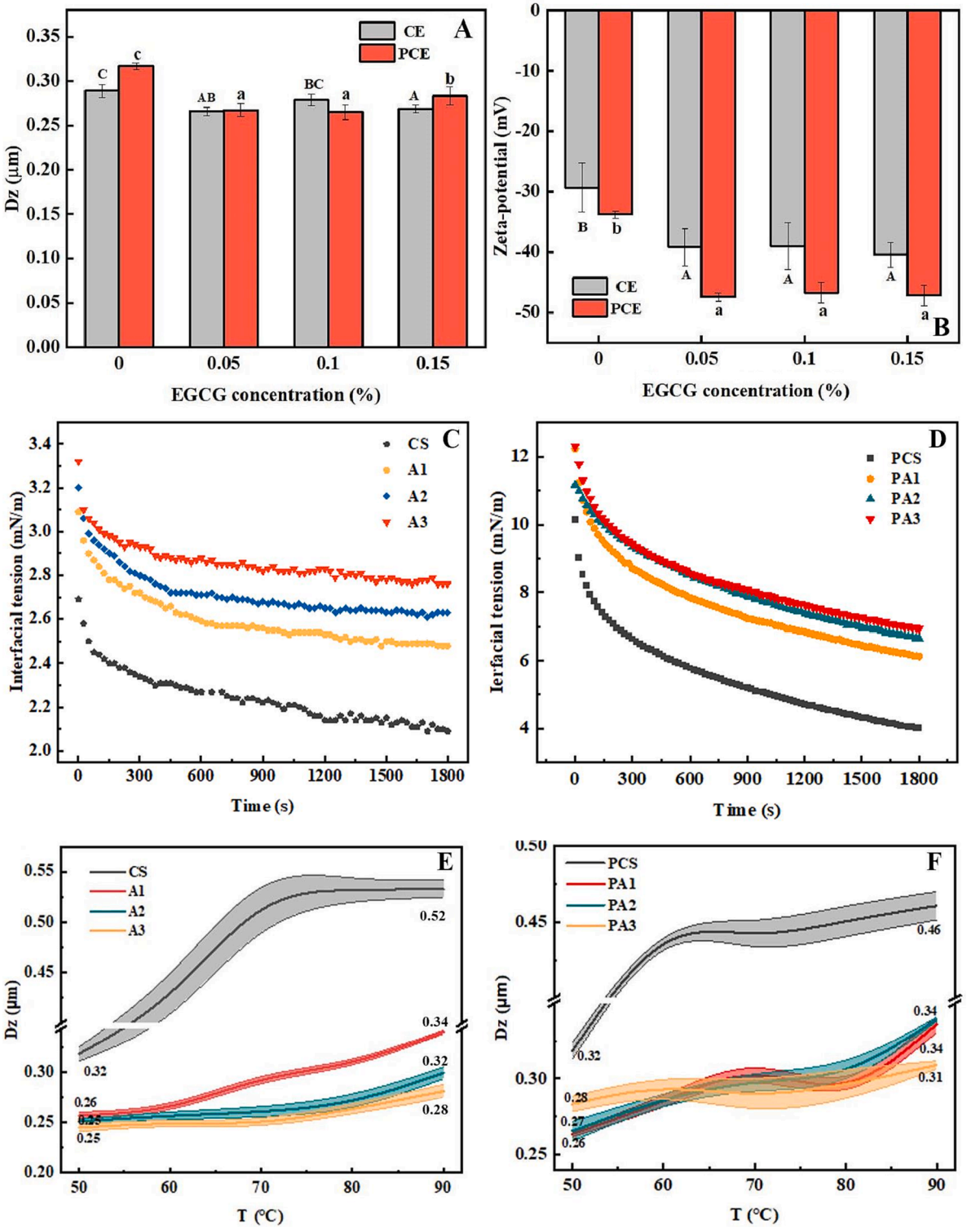

Fig. 4. Particle size (**A**), ζ-potential (**B**), surface tension (**C** and **D**) and tolerance to environmental stresses (**E**, **F**, **G** and **H**) of fish oil emulsions stabilized by CS/PCS and CS-EGCG/PCS-EGCG. The original group (CS) was treated with 0 MPa HPM, and the PCS was treated with 80 MPa. The A1/PA1, A2/PA2 and A3/PA3 groups were CS/PCS-0.05 % EGCG, CS/PCS-0.10 % EGCG and CS/PCS-0.15 % EGCG, respectively. Capital letters (A, B and C) indicated that there was a significant difference in CE group ($p < 0.05$), and lowercase letters (a, b and c) indicated that there was a significant difference in PCE group ($p < 0.05$).

covalent reaction with polyphenols was complex (Xue, Li, & Wang, 2021), so there might be proteins with different molecular weights and with different concentrations of EGCG have distinct affinity.

In addition, according to the principal component analysis of CS secondary structure content (Fig. A1) under different HPM treatment conditions, the difference between P80 and P0 was greater than that under other pressure treatments, indicating that the expansion and overlap behavior of PCS structure under 80 MPa was significantly different from other treatment conditions, which was consistent with the conclusion obtained in section 3.2. At the same time, the correlation

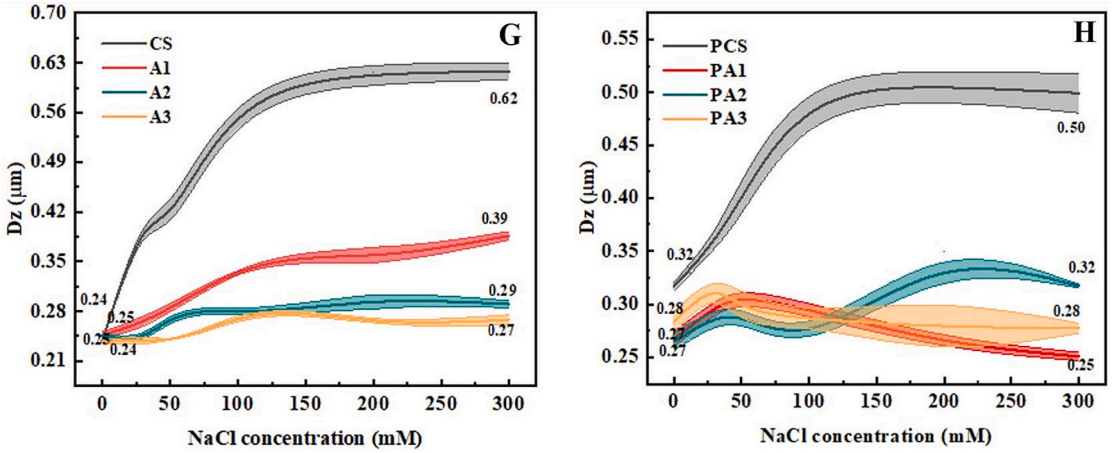

Fig. 4. (*continued*).

analysis of protein functional properties (Table A1) showed that the emulsifying activity and DPPH radical scavenging activity of the complex under 80 MPa treatment were the best among the main contribution values. Conclusively, 80 MPa treated CS-EGCG complex was selected to further investigate the effect of HPM treatment on stability of fish oil emulsion stabilized by this complex.

3.6. Particle size and ζ-potential

As shown in Fig. 4A-B, compared with the original CS-stabilized emulsion, the HPM treated CS-stabilized emulsion showed larger particle size and higher electrokinetic potential. This might be ascribed to the exposure of hydrophobic amino acids to the protein treated at 80 MPa, which caused the hydrophobic groups to re-aggregate or correlate (Xu et al., 2024). Moreover, the particle size of the complex-stabilized emulsion after grafting EGCG was significantly smaller than that of the individual protein-stabilized emulsion (Fig. 4A). This was mainly because CS/PCS and EGCG formed a complex under the covalent interaction, which could form an interface film on the oil–water surface in a more compact form, showing a lower particle size emulsion (Fan, Liu, Gao, Zhang, & Yi, 2018). Meanwhile, the negative charge of the emulsion stabilized by CS-EGCG/PCS-EGCG further increased (Fig. 4B). On the one hand, the covalent binding of CS/PCS to EGCG reduced the isoelectric point of the protein, thereby reducing its charge. On the other hand, the intervention of HPM and polyphenols makes the emulsion have stronger electrostatic interaction, which effectively prevented the aggregation of emulsion droplets, and further maintaining the stability of the emulsion (Zhang, Cheng, Luo, Hemar, & Yang, 2021).

3.7. Interfacial tension

In all cases, the water–oil interfacial tension decreased with the increase of adsorption time and increased with the increase of EGCG concentration (Fig. 4C-D). The former might be related to the adsorption capacity of CS/PCS or CS-EGCG/PCS-EGCG complexes at the interface (Pham, Wang, Zisu, & Adhikari, 2019), and the latter might be attributed to the additional loss of protein hydrophobicity caused by the hydrophilic hydroxyl groups provided by polyphenols (Wei et al., 2015). These observations are consistent with the reports on the complex of β-lactoglobulin and green tea polyphenols at the oil–water interface, and the interfacial activity of their interfacial proteins is adversely affected by the increase in polyphenol content (Von Staszewski, Pizones Ruiz-Henestrosa, & Pilosof, 2014). Furthermore, PCS and its complexes have higher interfacial tension than CS and CS-EGCG complexes without HPM treatment, indicating that HPM treatment decreased the interfacial activity of protein, which was mainly due to that HPM treatment changed the protein structure and increased the particle size of emulsion (Ho, Razzaghi, Ramachandran, & Mikkonen, 2022).

3.8. Thermal and salt stability

After high temperature treatment, the particle size of all samples increased, indicating that heating would flocculate the emulsion droplets (Fig. 4E-F). However, compared with CS and PCS, the temperature tolerance of CS-EGCG/PCS-EGCG complex-stabilized emulsion significantly enhanced, and a similar conclusion was also obtained in the study, in which the emulsion stabilized by catechin-egg white protein complex had better resistance to high temperature due to the stronger spatial repulsion between the two (Gu et al., 2017). In addition, PCS-stabilized emulsions showed better thermal stability than non-HPM-treated proteins, which might be attributed to that HPM treatment made the unfolding of proteins produced by high temperature change less, making them more resistant to heat (Tabilo-Munizaga et al., 2014). Although the temperature tolerance of the complex did not change significantly after HPM treatment, the thermal stability of the complex fluctuated under different pressures.

The salt stability of the emulsion stabilized by the protein and protein–polyphenol covalent complex before and after HPM treatment is shown in Fig. 4G-H. After grafting EGCG, the change of droplet size stabilized by all covalent complexes was much smaller than that of the original protein-embedded droplets, which might be ascribed to the increase of spatial repulsion on the droplet surface after EGCG bonding, thereby reducing the aggregation and flocculation between droplets (Gu et al., 2017). Moreover, after HPM treatment, the salt tolerance of emulsions stabilized by protein and protein–polyphenol complex significantly enhanced, indicating that HPM treatment could improve the salt ion stability of emulsions stabilized by protein and protein–polyphenol complex.

3.9. Storage stability

3.9.1. Light microscopy imaging

As can be seen from Fig. 5A, at day 0, the size of fish oil emulsions stabilized by all samples were evenly distributed and the particle dispersion was better. After 49 days of storage, fish oil emulsions stabilized by the sample without HPM treatment showed obvious enlargement and aggregation (Fig. 5A). Meanwhile, PCS-stabilized emulsion also showed aggregation, but its aggregation intensity greatly weakened compared with CS without HPM treatment. In addition, the stable emulsion droplets of HPM-treated CS-EGCG complexes did not change much after 49 days of storage (Fig. 5A). Therefore, the CS-EGCG complex treated by HPM has better ability to prevent emulsion

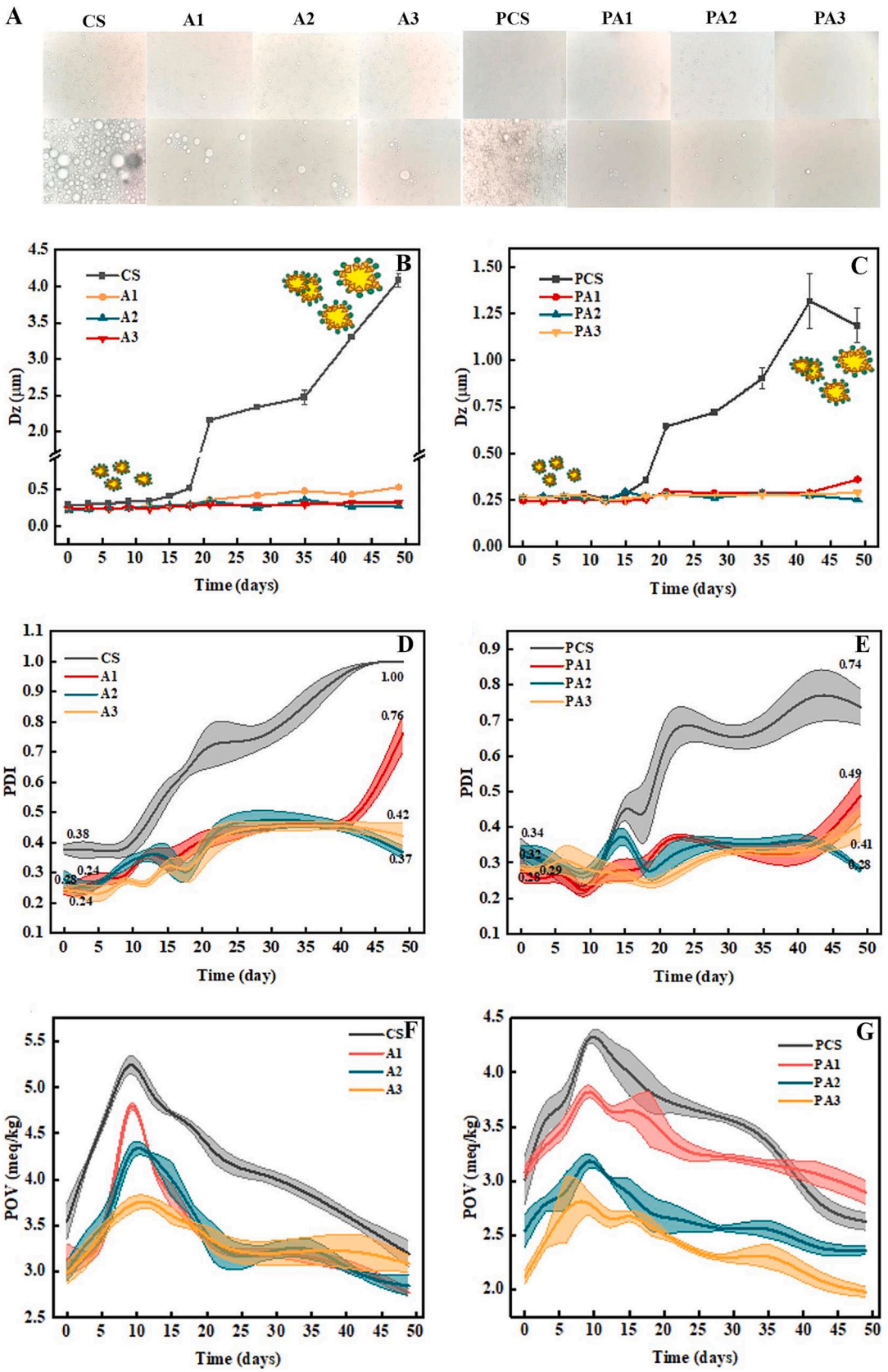

Fig. 5. Light microscope images (**A**), particle size (**B** and **C**), multiparticle dispersion (**D** and **E**), POV (**F** and **G**) and TBARS (**H** and **I**) of fish oil emulsions stabilized by CS/PCS and CS-EGCG/PCS-EGCG during storage. The original group (CS) was treated with 0 MPa HPM, and the PCS was treated with 80 MPa. The A1/PA1, A2/PA2 and A3/PA3 groups were CS/PCS-0.05 % EGCG, CS/PCS-0.10 % EGCG and CS/PCS-0.15 % EGCG, respectively.

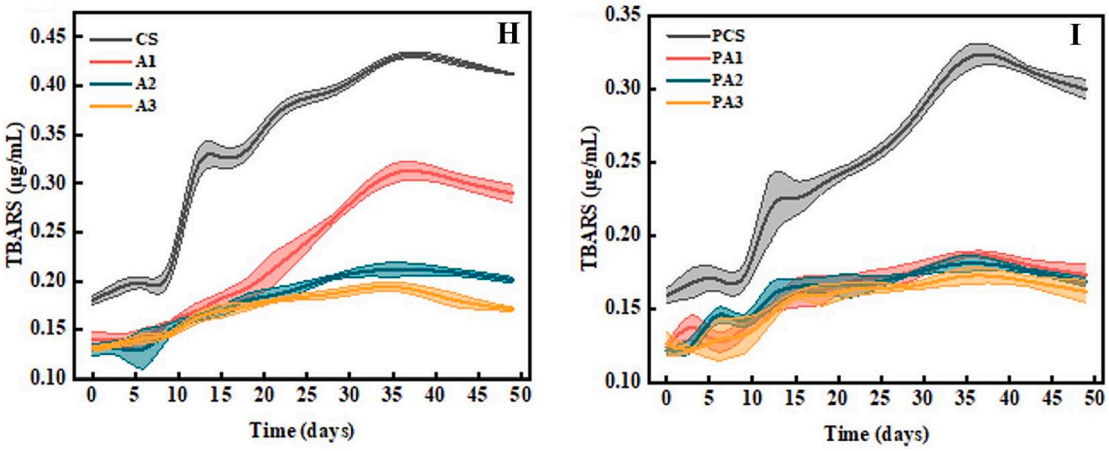

Fig. 5. (*continued*).

aggregation.

3.9.2. Particle size and multiparticle dispersion

Changes in particle size of the emulsion over a 49-day storage period (Fig. 5B-C) are similar to the results of light microscope. The particle size of CS-stabilized emulsion without HPM treatment varied greatly from 0.5 to 4.2 μm, while the particle size of PCS-stabilized emulsion varied from 0.4 to 1.2 μm. It showed that CS and PCS stabilized emulsions had flocculation and aggregation, but PCS stabilized emulsions showed good stability. Interestingly, the particle size of PCS-EGCG-stabilized emulsion showed no significant change during days 0–42, and the size of emulsion increased during days 42–49. It showed that the emulsion stabilized by PCS-EGCG complex had a strong electrostatic repulsion effect, and the droplets were not easy to aggregate. Particle dispersion (PDI) (Fig. 5D-E) showed a similar trend of change.

Microstructure, particle size and PDI are all important indicators for judging the stability of emulsions from the perspective of particles. The above results showed that the HPM-treated CS-EGCG complex had a better protective effect on lipid droplets than the original CS and CS-EGCG complex. The modified protein–polyphenol complex had a better effect on the ability to inhibit lipid droplet aggregation compared with CS and PCS. Among them, the emulsion stabilized by PCS-0.15 % EGCG complex showed the best storage stability.

3.9.3. Lipid oxidation stability

The POV value and TBARS value of the emulsion within 0–49 days of storage are shown in Fig. 5F-I. With the increase of storage time, the POV value and TBARS value of all emulsions increased first and then decreased. The overall increasing trend of POV value was CS > A1 > PCS > A2 > A3 > PA1 > PA2 > PA3, and the overall increasing trend of TBARS value was CS > PCS > A1 > A2 > A3 > PA1 > PA2 > PA3. This might be due to that HPM-treated proteins could form a stronger interface barrier at the water–oil interface (Perreault, Hénaux, Bazinet, & Doyen, 2017), and EGCG could provide the ability to scavenge free radicals for the emulsion system (Ge et al., 2022). This is the same as the above microscopic particle structure, particle size and PDI research results. The HPM modified protein–polyphenol covalent complex has the best inhibitory effect on the oxidation products of the emulsion. The reason is that HPM modification and EGCG loading provide CS with a more stable structure and stronger antioxidant activities as mentioned in Section 3.2 and 3.4, respectively, so that the fish oil emulsions have better storage stability.

3.10. Gastrointestinal fate

3.10.1. Zeta-potential and particle size

In the process of simulating gastrointestinal digestion, emulsions are subject to various disruptions, resulting in phenomena such as stratification and large changes in their droplet size and potential. After oral phase simulation, there was no significant difference among the particle size of all emulsions (Fig. 6A-B). This is because in the same pH buffer solution, dairy products do not contain starch and are not affected by simulated saliva (Chen, Yokoyama, Liang, & Zhong, 2020), and the charge characteristics of the emulsion have changed (Fig. 6C-D). The specific performance was as follows: after EGCG grafting, all complex-stabilized emulsions had more negative charges, and after oral phase simulation, each emulsion showed irregular changes. This might be attributed to the anionic carboxyl group carried by EGCG, which increased the absolute value of the potential (Liu, Liu, Liu, Li, & Liu, 2013).

In the gastric digestion stage, changes in low pH environment, ionic strength, and enzyme activity led to droplet aggregation (Ge et al., 2022), and the particle size of all emulsions increased significantly (Fig. 6A-B). However, compared with CS and PCS, the particle size of the emulsion stabilized by the complex increased little, indicating its stronger tolerance in a strong acidic environment (Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014). Additionally, when the emulsion entered the gastric phase, the negative charge values on the surface of all emulsions decreased (Fig. 6C-D). These changes were mainly due to the lower pH value in the gastric phase (Qiu, Zhao, Decker, & McClements, 2015).

During the small intestinal phase digestion, under the action of lipase, lipids are hydrolyzed to produce various particles, such as free fatty acids, bile salts and insoluble calcium soaps, and some unabsorbed oil droplets are further aggregated (Liu et al., 2013). However, the emulsion stabilized by PCS and PA1 (PCS-EGCG-0.05 %) were absorbed and utilized after decomposition, so the droplets were dispersed and the particle size was small (Fig. 6B). Furthermore, when the emulsion was exposed to the small intestinal phase, the negative charge of all systems increased (Fig. 6C-D), which might be due to the large amount of anionic free fatty acids produced by lipid digestion (Qiu et al., 2015). Notably, compared with the emulsion without HPM treatment, the particle size distribution range of PCS and PCS-EGCG stabilized emulsion droplets was small (Figs. A2-A3), indicating that HPM treatment was helpful to the uniform dispersion behavior of CS and CS-EGCG complex stabilized emulsions during gastrointestinal digestion.

3.10.2. Microstructure

Fig. 6E shows the microstructure of the emulsion during simulated

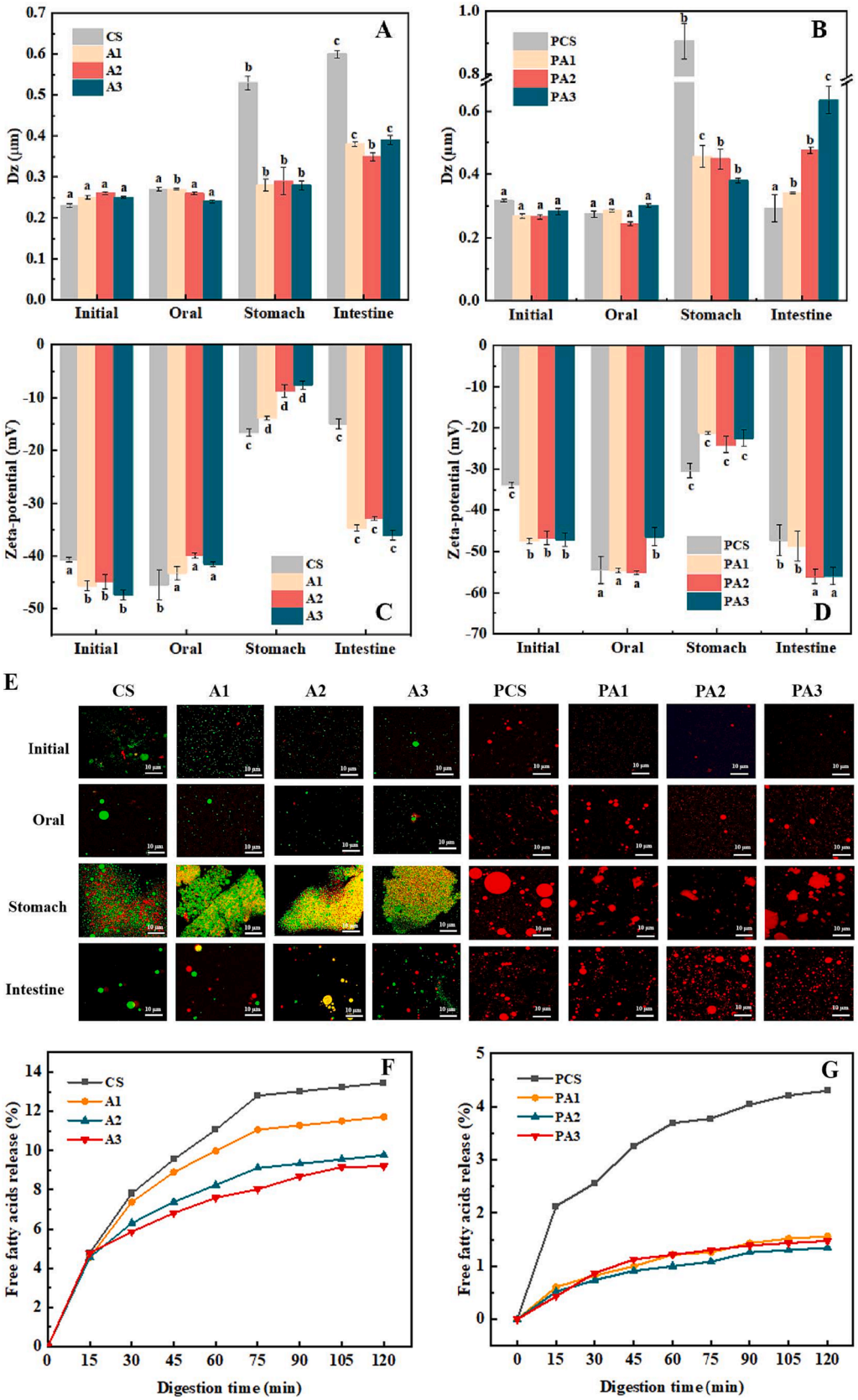

Fig. 6. Changes in particle size (**A** and **B**), zeta-potential (**C** and **D**), confocal micrographs (**E**) and release of free fatty acids (**F** and **G**) of fish oil emulsions stabilized by CS/PCS and CS-EGCG/PCS-EGCG during *in vitro* gastrointestinal tract (GIT) digestion. Fish oil emulsions were indicated as Initial, Oral, Stomach, and Intestine, respectively, before and in different phases of GIT digestion. The original group (CS) was treated with 0 MPa HPM, and the PCS was treated with 80 MPa. The A1/PA1, A2/PA2 and A3/PA3 groups were CS/PCS-0.05 % EGCG, CS/PCS-0.10 % EGCG and CS/PCS-0.15 % EGCG, respectively. The white bar indicates 10 μm. Different letters (a, b, c and d) indicated that there were significant differences between groups (the same sample at different digestion stages) ($p < 0.05$).

gastrointestinal digestion. In the initial and oral stages, all samples showed uniform oil droplets and small droplet sizes, but individual proteins still aggregated. Since the low pH, proteins in all samples aggregated and decomposed under the action of proteases during gastric digestion. In the small intestine stage, the droplet size returned to normal size under the action of lipase, and the protein-stabilized emulsions grafted with polyphenols (0.10 % and 0.15 %) contained more lipid droplets.

Compared with the microstructure of CS-stabilized emulsion during simulated gastrointestinal digestion, PCS-stabilized emulsion could resist the influence of droplets in the gastric environment to a greater extent. Both droplet aggregation and flocculation play a sustained-release role, benefiting for the digestion and absorption of fish oil emulsion in the small intestine. This further indicates that HPM treatment can enhance the ability of protein to stabilize droplets in the external environment, and its thick surface layer can enhance the stability of droplets during digestion. At the same time, the grafted EGCG improved the ability of each emulsion to resist the digestive environment, especially for the HPM-treated protein. Although the emulsion stabilized by PCS-EGCG complex also aggregated in the gastric phase, the degree of aggregation was smaller than the emulsions stabilized by CS, PCS and CS-EGCG. The PCS-EGCG complex enhanced the stability of the emulsion. Consequently, HPM treatment and EGCG grafting can increase the thickness of the surface layer of the emulsion, improve the ability of the emulsion to resist the gastric environment, making it safe to enter the small intestine, and further decompose for human absorption.

3.10.3. Free fatty acid release

Under the action of trypsin, the free fatty acid (FFA) release rate of the emulsion during intestinal digestion was measured by pH titration (Fig. 6F-G). In the first 15 min, the free fatty acids in CS and CS-EGCG stabilized emulsions increased sharply, indicating that lipase rapidly hydrolyzed fish oil triglycerides. However, the FFA of PCS and PCS-EGCG complex stabilized emulsions increased slowly, indicating that the rate of triglyceride decomposition by lipase was much lower than that of other emulsions. In addition, with the increase of digestion time, the FFA of PCS stabilized emulsion continued to increase significantly, while its complex stabilized emulsion only slightly increased the release rate of FFA. These effects were due to the formation of protein–polyphenol complex, which could reduce the sensitivity of protein to pepsin or pepsin catalysis (Lamothe et al., 2014). Therefore, HPM treatment and EGCG bonding can significantly delay the release of free fatty acids.

4. Conclusion

HPM treatment significantly changed the secondary structure of CS and its grafting with EGCG, which therefore affected the structure and physicochemical properties of PCS-EGCG complex. And the PCS-EGCG complex (80 MPa treated) was further studied on the stability for fish oil emulsion system due to its best emulsifying activity and antioxidant activities that increased 52.73–266.15 % comparing to original CS. It was found that the PCS-EGCG complex formed a thicker interface layer on the surface of oil droplets, which could better protect the oil layer from the influence of external environment such as oxygen, temperature and ion concentration. Notably, the PCS-EGCG complex significantly delayed the release of free fatty acids. However, the mechanism of HPM treatment and EGCG grafting affecting the internal structure and intermolecular behavior of CS still requires a further and harder study, which will be further focused on in our future work. This study may help us to better understand the covalent interaction between HPM-modified proteins and EGCG, and is of great significance for improving the physicochemical stability and nutritional delivery of fish oil emulsions.

CRediT authorship contribution statement

Wei Tang: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Rui Wang:** Formal analysis, Methodology, Writing – review & editing. **Minghui Li:** Formal analysis, Methodology, Writing – review & editing. **Qingchun Zhang:** Writing – review & editing. **Jianfei He:** Writing – review & editing. **Dan Liu:** Writing – review & editing. **Yuqi Feng:** Methodology. **Weilin Liu:** Writing – review & editing. **Jianhua Liu:** Funding acquisition, Investigation, Supervision, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138669.

References

Cao, J., Li, Y., Li, F., Liao, X., Hu, X., & Zhang, Y. (2022). Effect of high hydrostatic pressure on chlorophyll/soybean protein isolate interaction and the mixtures properties. *Food Hydrocolloids, 128*, Article 107555. https://doi.org/10.1016/j.foodhyd.2022.107555

Chen, G., Wang, S., Feng, B., Jiang, B., & Miao, M. (2019). Interaction between soybean protein and tea polyphenols under high pressure. *Food Chemistry, 277*, 632–638. https://doi.org/10.1016/j.foodchem.2018.11.024

Chen, L., Yokoyama, W., Liang, R., & Zhong, F. (2020). Enzymatic degradation and bioaccessibility of protein encapsulated β-carotene nano-emulsions during in vitro gastro-intestinal digestion. *Food Hydrocolloids, 100*, Article 105177. https://doi.org/10.1016/j.foodhyd.2019.105177

Drapala, K. P., Auty, M. A. E., Mulvihill, D. M., & O'Mahony, J. A. (2016). Performance of whey protein hydrolysate–maltodextrin conjugates as emulsifiers in model infant formula emulsions. *International Dairy Journal, 62*, 76–83. https://doi.org/10.1016/j.idairyj.2016.03.006

El-Messery, T. M., Mwafy, E. A., Mostafa, A. M., El-Din, H. M. F., Mwafy, A., Amarowicz, R., & Ozçelik, B. (2020). Spectroscopic studies of the interaction between isolated polyphenols from coffee and the milk proteins. *Surfaces and Interfaces, 20*, Article 100558. https://doi.org/10.1016/j.surfin.2020.100558

Fan, Y., Liu, Y., Gao, L., Zhang, Y., & Yi, J. (2018). Oxidative stability and in vitro digestion of menhaden oil emulsions with whey protein: Effects of EGCG conjugation and interfacial cross-linking. *Food Chemistry, 265*, 200–207. https://doi.org/10.1016/j.foodchem.2018.05.098

Ge, S., Jia, R., Liu, W., Xie, J., Liu, M., Cai, D., … Liu, J. (2022). Lipid oxidation and in vitro digestion of pickering emulsion based on zein-adzuki bean seed coat polyphenol covalent crosslinking nanoparticles. *Food Chemistry, 386*, Article 132513. https://doi.org/10.1016/j.foodchem.2022.132513

Gu, L., Su, Y., Zhang, M., Chang, C., Li, J., McClements, D. J., & Yang, Y. (2017). Protection of β-carotene from chemical degradation in emulsion-based delivery systems using antioxidant interfacial complexes: Catechin-egg white protein conjugates. *Food Research International, 96*, 84–93. https://doi.org/10.1016/j.foodres.2017.03.015

Guo, Z., Huang, Z., Guo, Y., Li, B., Yu, W., Zhou, L., … Wang, Z. (2021). Effects of high-pressure homogenization on structural and emulsifying properties of thermally soluble aggregated kidney bean (Phaseolus vulgaris L.) proteins. *Food Hydrocolloids, 119*, Article 106835. https://doi.org/10.1016/j.foodhyd.2021.106835

Ho, T. M., Razzaghi, A., Ramachandran, A., & Mikkonen, K. S. (2022). Emulsion characterization via microfluidic devices: A review on interfacial tension and stability to coalescence. *Advances in Colloid and Interface Science, 299*, Article 102541. https://doi.org/10.1016/j.cis.2021.102541

Kargar, M., Spyropoulos, F., & Norton, I. T. (2011). The effect of interfacial microstructure on the lipid oxidation stability of oil-in-water emulsions. *Journal of Colloid and Interface Science, 357*(2), 527–533. https://doi.org/10.1016/j.jcis.2011.02.019

Lamothe, S., Azimy, N., Bazinet, L., Couillard, C., & Britten, M. (2014). Interaction of green tea polyphenols with dairy matrices in a simulated gastrointestinal environment. *Food & Function, 5*(10), 2621–2631. https://doi.org/10.1039/C4FO00203B

Li, M., Ritzoulis, C., Du, Q., Liu, Y., Ding, Y., Liu, W., & Liu, J. (2021). Recent progress on protein-polyphenol complexes: Effect on stability and nutrients delivery of oil-in-water emulsion system. *Frontiers in Nutrition, 8*, Article 765589. https://doi.org/10.3389/fnut.2021.765589

Li, Y., Liu, X., Yin, F., Li, D., Jiang, P., Song, L., … Zhou, D. (2023). Characterization of oyster water-soluble protein-EGCG conjugate and its antioxidant effects on linolic acid in emulsion system. *Food Bioscience, 51*, Article 102215. https://doi.org/10.1016/j.fbio.2022.102215

Liu, J., Liu, W., Salt, L. J., Ridout, M. J., Ding, Y., & Wilde, P. J. (2019). Fish Oil emulsions stabilized with caseinate glycated by dextran: Physicochemical stability and gastrointestinal fate. *Journal of Agricultural and Food Chemistry, 67*(1), 452–462. https://doi.org/10.1021/acs.jafc.8b04190

Liu, J., Song, G., Zhou, L., Yuan, Y., Wang, D., Yuan, T., … Gong, J. (2023). Recent advances in the effect of ultrasound on the binding of protein−polyphenol complexes in foodstuff. *Food Frontiers, 4*(2), 721–732. https://doi.org/10.1002/fft2.221

Liu, W., Liu, J., Liu, W., Li, T., & Liu, C. (2013). Improved physical and in vitro digestion stability of a polyelectrolyte delivery system based on layer-by-layer self-assembly alginate–chitosan-coated nanoliposomes. *Journal of Agricultural and Food Chemistry, 61*(17), 4133–4144. https://doi.org/10.1021/jf305329n

Ozturk, O. K., & Turasan, H. (2022). Latest developments in the applications of microfluidization to modify the structure of macromolecules leading to improved physicochemical and functional properties. *Critical Reviews in Food Science and Nutrition, 62*(16), 4481–4503. https://doi.org/10.1080/10408398.2021.1875981

Parolia, S., Maley, J., Sammynaiken, R., Green, R., Nickerson, M., & Ghosh, S. (2022). Structure – Functionality of lentil protein-polyphenol conjugates. *Food Chemistry, 367*, Article 130603. https://doi.org/10.1016/j.foodchem.2021.130603

Peng, L.-P., & Tang, C.-H. (2020). Outstanding antioxidant pickering high internal phase emulsions by co-assembled polyphenol-soy β-conglycinin nanoparticles. *Food Research International, 136*, Article 109509. https://doi.org/10.1016/j.foodres.2020.109509

Perreault, V., Hénaux, L., Bazinet, L., & Doyen, A. (2017). Pretreatment of flaxseed protein isolate by high hydrostatic pressure: Impacts on protein structure, enzymatic hydrolysis and final hydrolysate antioxidant capacities. *Food Chemistry, 221*, 1805–1812. https://doi.org/10.1016/j.foodchem.2016.10.100

Pham, L. B., Wang, B., Zisu, B., & Adhikari, B. (2019). Complexation between flaxseed protein isolate and phenolic compounds: Effects on interfacial, emulsifying and antioxidant properties of emulsions. *Food Hydrocolloids, 94*, 20–29. https://doi.org/10.1016/j.foodhyd.2019.03.007

Qiu, C., Zhao, M., Decker, E. A., & McClements, D. J. (2015). Influence of protein type on oxidation and digestibility of fish oil-in-water emulsions: Gliadin, caseinate, and whey protein. *Food Chemistry, 175*, 249–257. https://doi.org/10.1016/j.foodchem.2014.11.112

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

Tabilo-Munizaga, G., Gordon, T. A., Villalobos-Carvajal, R., Moreno-Osorio, L., Salazar, F. N., Pérez-Won, M., & Acuña, S. (2014). Effects of high hydrostatic pressure (HHP) on the protein structure and thermal stability of Sauvignon blanc wine. *Food Chemistry, 155*, 214–220. https://doi.org/10.1016/j.foodchem.2014.01.051

Tang, C., & Ma, C. (2009). Effect of high pressure treatment on aggregation and structural properties of soy protein isolate. *LWT - Food Science and Technology, 42*(2), 606–611. https://doi.org/10.1016/j.lwt.2008.07.012

Tang, W., Li, M., Ritzoulis, C., Liu, Y., Ding, Y., Liu, W., & Liu, J. (2023). Chemical and thermodynamic characterization of antioxidant emulsifiers: The case of complex of sodium caseinate with EGCG. *Food Frontiers, 4*(3), 1382–1394. https://doi.org/10.1002/fft2.237

Tang, W., Zhang, Q., Ritzoulis, C., Walayat, N., Ding, Y., & Liu, J. (2023). Food protein glycation: A review focusing on stability and in vitro digestive characteristics of oil/water emulsions. *Comprehensive Reviews in Food Science and Food Safety, 22*(3), 1986–2016. https://doi.org/10.1111/1541-4337.13138

Tošović, J., Marković, S., Dimitrić Marković, J. M., Mojović, M., & Milenković, D. (2017). Antioxidative mechanisms in chlorogenic acid. *Food Chemistry, 237*, 390–398. https://doi.org/10.1016/j.foodchem.2017.05.080

Von Staszewski, M., Pizones Ruiz-Henestrosa, V. M., & Pilosof, A. M. R. (2014). Green tea polyphenols-β-lactoglobulin nanocomplexes: Interfacial behavior, emulsification and oxidation stability of fish oil. *Food Hydrocolloids, 35*, 505–511. https://doi.org/10.1016/j.foodhyd.2013.07.008

Wang, S., Yang, J., Shao, G., Qu, D., Zhao, H., Yang, L., … Zhu, D. (2020). Soy protein isolated-soy hull polysaccharides stabilized O/W emulsion: Effect of polysaccharides concentration on the storage stability and interfacial rheological properties. *Food Hydrocolloids, 101*, Article 105490. https://doi.org/10.1016/j.foodhyd.2019.105490

Wang, X., Zhang, J., Lei, F., Liang, C., Yuan, F., & Gao, Y. (2014). Covalent complexation and functional evaluation of (−)-epigallocatechin gallate and α-lactalbumin. *Food Chemistry, 150*, 341–347. https://doi.org/10.1016/j.foodchem.2013.09.127

Wei, Z., Yang, W., Fan, R., Yuan, F., & Gao, Y. (2015). Evaluation of structural and functional properties of protein–EGCG complexes and their ability of stabilizing a model β-carotene emulsion. *Food Hydrocolloids, 45*, 337–350. https://doi.org/10.1016/j.foodhyd.2014.12.008

Xu, M., Ni, X., Liu, Q., Chen, C., Deng, X., Wang, X., & Yu, R. (2024). Ultra-high pressure improved gelation and digestive properties of Tai Lake whitebait myofibrillar protein. *Food Chemistry: X, 21*, Article 101061. https://doi.org/10.1016/j.fochx.2023.101061

Xue, F., Li, C., & Wang, S. (2021). Effects of high-intensity ultrasound treatment on physiochemical properties of caseins-cyanidin-3-galactoside conjugates. *International Journal of Food Science & Technology, 56*(7), 3378–3387. https://doi.org/10.1111/ijfs.14961

Yang, H., Li, L., Xie, C., He, M., Guo, Z., Zhao, S., … Li, Y. (2022). Characteristics and structure of a soy protein isolate–lutein nanocomplex produced via high-pressure homogenization. *Journal of the Science of Food and Agriculture, 102*(12), 5411–5421. https://doi.org/10.1002/jsfa.11894

Yesiltas, B., García-Moreno, P. J., Sørensen, A.-D.-M., Soria Caindec, A. M., Hyldig, G., Anankanbil, S., … Jacobsen, C. (2021). Enrichment of mayonnaise with a high fat fish oil-in-water emulsion stabilized with modified DATEM C14 enhances oxidative stability. *Food Chemistry, 341*, Article 128141. https://doi.org/10.1016/j.foodchem.2020.128141

Zhang, Q., Wang, R., He, J., Tang, W., & Liu, J. (2024). Innovative multistep modifications of β-Lactoglobulin for enhanced emulsifying and antioxidant activities. *Food Hydrocolloids, 148*, Article 109465. https://doi.org/10.1016/j.foodhyd.2023.109465

Zhang, R., Cheng, L., Luo, L., Hemar, Y., & Yang, Z. (2021). Formation and characterisation of high-internal-phase emulsions stabilised by high-pressure homogenised quinoa protein isolate. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 631*, Article 127688. https://doi.org/10.1016/j.colsurfa.2021.127688

Zou, H., Xu, Z., Zhao, L., Wang, Y., & Liao, X. (2019). Effects of high pressure processing on the interaction of α-lactalbumin and pelargonidin-3-glucoside. *Food Chemistry, 285*, 22–30. https://doi.org/10.1016/j.foodchem.2019.01.129