



Emulsification properties of ovalbumin-fucoidan (OVA-FUC) binary complexes

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ABSTRACT

The poor thermal stability and emulsifying properties of ovalbumin (OVA) limit its functional performance, but these limitations may be overcome by forming binary complexes. We prepared binary complexes of OVA and fucoidan (FUC) through electrostatic self-assembly and investigated the emulsifying properties of the complex by measuring the particle size, interfacial membrane thickness, zeta potential, and stability of the emulsion prepared with camellia oil and the complex. The OVA-FUC emulsions have a thicker interfacial membrane, lower mobility, higher viscosity, and better stability compared with the OVA emulsions. The emulsion prepared with 1.5 % OVA-FUC remained stable and homogeneous during storage. They tended to become unstable with freeze-thaw, but the oil encapsulated did not leak after coalescence occurred. With the addition of Ca²⁺, the OVA-FUC emulsion will be converted into a gel state. These findings indicate that OVA-FUC binary complexes can be used to prepare high-performance emulsions with great potential for development.

1. Introduction

Ovalbumin (OVA) constitutes the majority of proteins in egg whites (54–60 %) and possesses diverse functional properties. However, its poor thermal stability and emulsification properties near the isoelectric point significantly limit its functionality (Li et al., 2020). To address this, food protein modification has been developed as a rational molecular design strategy to enhance emulsification performance (Liu, Ma, Gao, & McClements, 2017). Recent years have seen the application of various methods to improve the emulsification performance and stability of OVA emulsions, including phosphorylation and glycosylation reactions (Hu et al., 2020). Moreover, protein-polysaccharide complexes formed under the influence of electrostatic forces demonstrate superior performance in stabilizing emulsions, controlling structure, and safeguarding

the delivery of bioactive compounds compared to individual proteins or polysaccharides (Guo et al., 2020). In a previous study, we synthesized a novel binary complex (Duan et al., 2023) comprising OVA and Fucoidan (FUC) through electrostatic self-assembly. Our investigation into the formation mechanism of the binary complex confirmed its potential as a food-grade emulsifier for stabilizing emulsion systems, as demonstrated in interfacial tension studies, indicating promising development prospects.

Emulsions, as carriers, play a crucial role in transporting, protecting, and delivering bioactive substances or functional factors to enhance their bioavailability (McClements, 2007). They significantly impact the nutrition, functionality, sensory attributes, and shelf life of foods. The OVA-FUC binary complex has the potential to be a natural emulsifier for emulsions due to its high safety, nutritional value, and biocompatibility.

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External factors such as pH, ionic strength, and temperature changes can influence emulsions, making them susceptible to destabilization and breakage, thereby impacting their efficacy (Liao, Gharsallaoui, Dumas, & Elaissari, 2022). Recent research has extensively examined the interactions between proteins and polysaccharides (Xu et al., 2021). However, the internal interaction processes in protein-polysaccharide complex-stabilized emulsions are highly complex, and the impact of external environmental stresses remains unclear. For instance, the formation and thawing of ice crystals can destabilize the emulsion (Chen et al., 2019), while saline ions can promote the flocculation of oil droplets (Shao & Tang, 2014), among other factors. Further study is needed to determine whether stable emulsion systems exhibit good storage stability. Therefore, it is essential to investigate the performance of protein-polysaccharide complexes as emulsifiers and the effect of environmental factors on the stability of the constructed emulsion systems.

Ensuring the stability of emulsions during food processing and storage poses a significant challenge in emulsion research. Emulsions will suffer destabilization, often manifesting as flocculation, coalescence, sedimentation, creaming, phase inversion, and Ostwald ripening (McClements, 2012). Typically, the interfacial film of emulsion droplets preserves their original characteristics, with minimal changes in size and distribution (Gkinali, Matsakidou, & Paraskevopoulou, 2023). However, the rupture of the interfacial film leads to coalescence, causing an increase in droplet size. Coalescence is a non-reversible process that could cause the number of emulsion droplets to decrease and the emulsion to be destroyed, resulting in the separation of oil and water (Zhang et al., 2023). Due to the disparate densities of the oil and water phases, gravity can cause emulsions to delaminate, disrupting their homogeneity. The stability of emulsions was affected by factors including solid protein concentration, oil-to-water ratio, salt ions, oil equivalence, etc. For instance, both low and high-ionic-strength saline ions affect emulsion stability by influencing the emulsion surface potential (Delahaije, Wierenga, van Nieuwenhuijzen, Giuseppin, & Gruppen, 2013), protein distribution affects stability by influencing emulsion droplet size (Comas, Wagner, & Tomás, 2006), and the volume fraction of the oil phase affects emulsion particle size and stability (Tang & Liu, 2013). Emulsion systems prepared using OVA-FUC binary complexes as emulsion stabilizers have not been previously reported. Therefore, our study aimed to verify the potential of OVA-FUC binary complexes as emulsifiers and explore their efficient utilization. We investigated the emulsification properties of the OVA-FUC binary complex and examined the effects of different influencing factors on the stability of emulsion systems prepared using the OVA-FUC binary complex, including centrifugal stability, freeze-thaw stability, storage stability, and ion stability.

2. Materials and methods

2.1. Preparation of OVA-FUC emulsions

The OVA-FUC binary complexes were prepared following the method in our previous study (Duan et al., 2023). OVA (BR, 95 %, Shanghai Yuanye Bio-Technology Co., Ltd., China) and FUC (98 %, Shanghai Aladdin Biochemical Technology Co., Ltd., China) were dissolved in deionized water, gently stirred for 2 h at 25 °C until fully dissolved, and then refrigerated at 4 °C overnight to achieve full hydration. The solutions were diluted to the desired working concentration and mixed in a 1:1 ratio (v/v). The samples were thoroughly mixed with a magnetic stirrer for 30 min, then adjusted to pH 4.0 using 0.1 M hydrochloric acid or sodium hydroxide solution, mixed for 1 h, and refrigerated at 4 °C for 24 h. Each sample was centrifuged at 12000 × g for 20 min, and the supernatant was collected as the OVA-FUC binary complexes.

The OVA-FUC binary complex served as the water phase (W), while camellia oil (Yihai Kerry Arawana Holdings Co., Ltd., China) was used as the oil phase (O). A mixture with a total volume of 45 mL was prepared

using these two phases with ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 (O: W, v/v). The O/W emulsion was prepared using a two-step emulsification method. The O/W mixture was magnetically stirred for 5 min and then homogenized at 12,000 rpm for 3 min using a high-speed disperser (T25, IKA, Germany) to create the primary emulsion. The O/W emulsion was then prepared by subjecting the primary emulsion to ultrasonic treatment for 6 min at a power of 500 W (Li, Wang, Huang, & Geng, 2021).

2.2. Emulsion particle size determination

The particle size distribution of the emulsions was determined using the Malvern Mastersizer MS3000 laser particle sizer (MS-3000, Malvern, UK) (Huang et al., 2023). A suitable quantity of emulsion was carefully added drop by drop to the laser particle sizer cuvette to achieve 15–20 % shading. The refractive index of the dispersant was adjusted to 1.33, the refractive index of the emulsion particles was set to 1.46, the absorbance was set to 0.01, and the rotational speed was maintained at 2000 rpm to ensure uniform dispersion of the sample.

2.3. Zeta potential test

A volume of 620 µL of the emulsion sample was introduced into a designated cuvette and placed into the nanoparticle size potentiometer (Zetasizer Nano ZS90, Malvern Panalytical, UK) for Zeta potentiometric determination (Huang et al., 2021). The test temperature was 25 °C, and the samples were equilibrated for 120 s before measurement. Each sample was repeated three times, with 12 parallel values measured each time.

2.4. Interfacial film thickness determination

The 1 % (v/v) solution of OVA-FUC, OVA, and FUC was prepared and then diluted with 10 mM citrate buffer (pH 3.5) to a series of concentrations ranging from 0.05–0.5 % (v/v), with the pH adjusted to 4.0. 2440 µL of OVA-FUC, OVA, and FUC solutions were individually combined with 60 µL of monodisperse polystyrene latex beads (2.5 % w/v). This reaction proceeded for 8 h to ensure adsorption equilibrium. The Zetasizer Nano ZS (ZS-90, Malvern, UK) was employed to measure the particle size of the latex beads. The thickness of the adsorbed layer was determined by calculating the difference between the initial and final particle sizes of the beads following the adsorption of the emulsion and the polystyrene latex beads:

$$dT = \frac{R_a - R_i}{2} \quad (1)$$

dT: the thickness of the adsorbed layer, Ra: the particle size of polystyrene latex beads adsorbed the emulsion, Ri: the particle size of polystyrene latex beads.

2.5. Determination of rheological properties

The rheological properties of the emulsions were assessed using a rotational rheometer (MCR 301, Anton Paar, Austria) with a dynamic frequency sweep, where the frequency range was set from 0.01 to 10 Hz, and the strain was maintained at 0.1 %. Subsequently, a power-law model (Ostwald-de Waele model) was applied and fitted according to the following:

$$\eta = K \cdot \dot{\gamma}^{n-1} \quad (2)$$

η: the apparent viscosity (Pa.s), γ: the shear rate (s⁻¹), K: the viscosity coefficient (Pa.sn), n: the fluid type index.

2.6. Apparent morphological observation

The differential interference contrast microscope (DM2500, Leica,

Germany) was utilized for observing the various treated emulsion samples. A 20 μL emulsion sample was placed at the center of the slide, covered with a coverslip to eliminate air bubbles and voids. Emulsion morphology was examined under the oil microscope at a magnification of 400 \times .

2.7. Storage stability test

The evaluation of emulsion storage stability was conducted following the approach outlined by Huang et al. (2023). Emulsion samples were prepared and transferred to transparent glass vials, then stored in a light-protected container at 25 $^{\circ}\text{C}$ for 15 d. At intervals of 24 h, samples were withdrawn for analysis, including measurement of particle size, zeta potential, and optical microscope examination. Subsequently, the creaming index (CI) was calculated to assess changes over time, utilizing the following formula:

$$CI = \frac{H}{H_0} \times 100\% \quad (3)$$

H: the height of the emulsion layer after several days of storage; H_0 : the total height of the emulsion

2.8. Freeze-thaw stability test

The freeze-thaw stability of the emulsions was assessed following the methodology outlined by Zhu et al. (2018). Triplicate samples of the emulsion were aliquoted into centrifuge tubes and subjected to freezing at -18°C for 24 h. After the freezing period, the samples were thawed at 25 $^{\circ}\text{C}$ until complete thawing occurred, and any alterations in the appearance of the emulsion were noted.

2.9. Centrifugal stability determination

The centrifugal stability of the emulsions was assessed following the method outlined by Liu, Wang, Sun, and Gao (2016) with certain modifications. A Dispersion Analyser (LUMisizer, LUM, Germany) was utilized to analyze the centrifugal stability of the emulsions. The test parameters were configured as follows: each test utilized a sample volume of 420 μL , the optical diameter of the sample tube was 2 mm, the NIR wavelength for irradiation was set at 880 nm, the centrifugation rate was 1308 $\times g$, the temperature was maintained at 25 $^{\circ}\text{C}$, and the emulsion samples were scanned at intervals of 10 s for data collection.

2.10. Ion stability test

To enhance the applicability of emulsifiers in food processing, they need to exhibit strong ion stability to navigate the intricate ionic milieu prevalent in food systems. Hence, it becomes imperative to assess the ion stability of emulsions derived from OVA-FUC binary complexes. O/W emulsions, formulated with 1.5 % OVA-FUC, were supplemented with varying quantities of NaCl /CaCl₂, resulting in final sodium ion concentrations of 100 mM, 200 mM, 300 mM, and 400 mM, or calcium ion concentrations of 5 mM, 10 mM, 20 mM, and 100 mM. Subsequently, the average particle size, zeta potential, and optical microscopic characteristics of the samples with added salt ions were measured following 30 min of magnetic stirring, serving as indicators to assess the stability of O/W emulsions under different ionic strengths (Huang et al., 2022).

2.11. Statistical analysis

The data were presented as the mean \pm standard deviation (SD) derived from a minimum of three independent experiments. Statistical analysis was conducted using one-way analysis of variance (ANOVA) via SPSS 25.0.0 software. Duncan's multiple tests were employed to assess average differences, with statistical significance determined at $P < 0.05$. Plots were generated using Origin 9.5 software.

3. Results and discussion

3.1. Effect of emulsifier and oil phase concentration on the emulsification properties of OVA-FUC

The spatial site resistance produced by OVA, FUC, and OVA-FUC emulsions was evaluated by measuring the thickness of the adsorbed hydration layer around the monodisperse polystyrene emulsion. From Fig. 1A, it is evident that with increasing OVA concentration, the thickness of the interfacial film of the prepared emulsion gradually decreases. Additionally, the influence of OVA on the emulsion interfacial film thickness does not appear to be as pronounced as that of FUC and OVA-FUC. This may be due to the relatively weak interaction forces between OVA molecules. Despite the ability of OVA to distribute more densely on the membrane surface at high concentrations, the proximity of the molecules, coupled with the increased ability of higher OVA concentrations to aggregate more tightly, results in a thinner interfacial film being formed. The concentration of FUC has the most significant impact on the thickness of the interfacial film of the prepared emulsion, as its concentration increases, the emulsion's interfacial film thickness significantly and rapidly increases. These observations indicate that FUC has a more substantial impact on the thickness of the emulsion's interfacial film, which may also explain why the trend of the OVA-FUC emulsion interfacial film thickness with varying OVA-FUC concentrations aligns with that of the FUC emulsion. The added FUC molecules reacted with OVA and formed the binary complex, which created a thicker interfacial film (Zhang et al., 2023), thus increasing the spatial site resistance and further improving the stability of the emulsion system.

The rheological properties of the emulsion samples are shown in Fig. 1B, and the corresponding viscosity indices (K , Pa.sⁿ) and fluid index types (n) of the emulsions as the concentration of the aqueous phase varies are listed in Table 1. The power-law model fits the experimental data well and has a high coefficient of determination ($R^2 > 0.999$). All emulsion samples showed shear thinning behavior ($0 < n < 1$) in the shear rate range of 0.01–100 s^{-1} , i.e., viscosity decreased with increasing shear rate (Hu et al., 2013), indicating that the tested emulsions were non-Newtonian fluids. In this case, the OVA-FUC binary complex emulsion, the viscosity increases with the increase in the concentration of the complex, which is in agreement with the findings of Wang et al. (2022), where an increase in the concentration of macromolecules in the aqueous phase leads to an increase in the viscosity of the emulsion system. The emulsion viscosity was found to be lower in the sample prepared with 1.5 % (w/v) OVA compared to that in the sample prepared with 1.5 % (w/v) OVA-FUC (i.e., containing 0.75 % (w/v) OVA), suggesting that the addition of FUC significantly enhanced the emulsion viscosity. Wu et al. (2022) highlighted that enhancing emulsion viscosity is advantageous for improving stability. Consequently, OVA-FUC binary complex emulsions exhibit greater stability compared to OVA emulsions.

The O/W emulsions were prepared using a two-step emulsification process, employing 0.5 % (v/v) OVA-FUC binary complex as the water phase and varying the oil phase proportion. The impact of oil phase concentration on the emulsification properties of O/W emulsions stabilized by OVA-FUC binary complexes is illustrated in Fig. 1(C–E). As depicted in Fig. 1C, in freshly prepared emulsions, an oil phase concentration exceeding 50 % (v/v) led to incomplete emulsification by the OVA-FUC binary complex, resulting in the inability to form a stable emulsion system and leading to phase separation. At a 50 % oil phase concentration, although a relatively stable emulsion system was achieved, there was slight delamination in the emulsion. After 24 h of storage, emulsions prepared from oil phases with concentrations ranging from 10 % to 50 % (v/v) exhibit a tendency for the emulsion layer to float in the higher concentration range. Specifically, when the oil phase concentration is between 30 % and 40 % (v/v), there is less water phase separation, indicating that at this concentration range, the

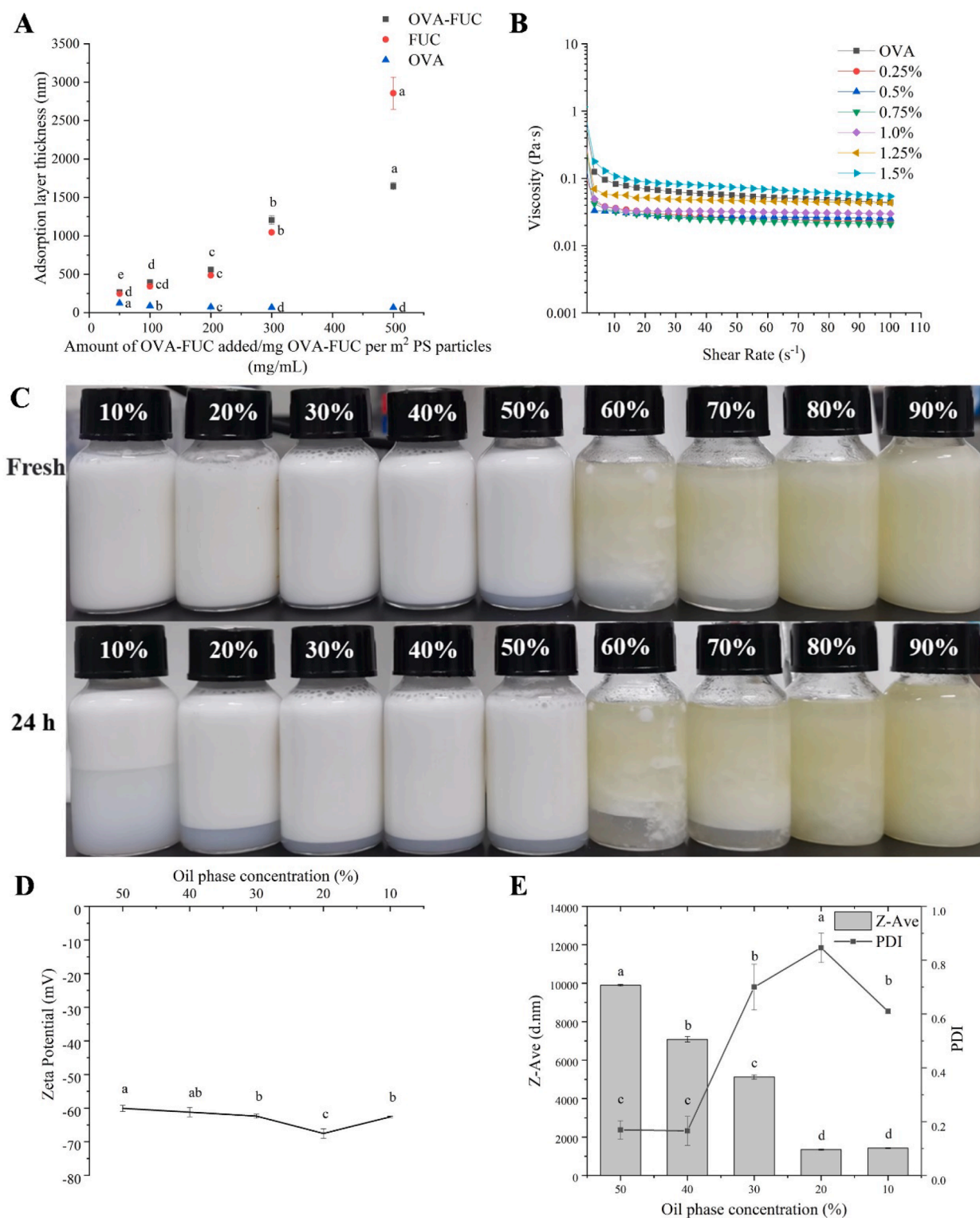


Fig. 1. Effect of emulsifier and oil phase concentration on the emulsification properties of OVA-FUC: (A) adsorption layer thickness; (B) rheological properties of the emulsions; (C) photographs of emulsions prepared with different oil concentrations; (D) Zeta potential diagram; (E) distribution of particle size and PDI. Different letters in the line graph and bar graphs indicate significant differences ($P < 0.05$).

complex can form a more homogeneous emulsion system with camellia oil. As depicted in Fig. 1D, the emulsions exhibited negative potentials across the range of tested oil phase concentrations. The absolute potentials decreased gradually as the oil phase concentrations increased within the 20–50 % (v/v) range, potentially attributed to the influence of the oil phase on the ionization of the proteins and polysaccharides

adsorbed on the oil droplet surfaces within the emulsion system. Furthermore, Fig. 1E demonstrates that as the oil phase concentration increased, the average particle size of the emulsion increased while the PDI gradually decreased. Notably, at an oil phase concentration of 30 %, the emulsion displayed relatively small particle size and PDI, indicating a relatively stable emulsion system at this concentration. Consequently,

Table 1

The fitting parameters of power law model for O/W emulsion prepared with different emulsifier concentration.

Emulsifier Concentration	K	n	R2
1.5 % OVA	0.19944	0.6603	0.99958
0.25 % OVA-FUC	0.05454	0.81108	0.99927
0.5 % OVA-FUC	0.06714	0.74574	0.98238
0.75 % OVA-FUC	0.08839	0.83460	0.99731
1.0 % OVA-FUC	0.10815	0.64723	0.99246
1.25 % OVA-FUC	0.12228	0.73651	0.99030
1.5 % OVA-FUC	0.25288	0.66968	0.99954

subsequent tests will be conducted at a 30 % oil phase concentration.

3.2. Storage stability of emulsions with different emulsifier concentrations

Storage stability serves as a crucial metric for assessing the characteristics of emulsion systems. Illustrated in Fig. 2A is the condition of O/W emulsion samples crafted with varied concentrations of the aqueous phase over a 15-day storage period. Within the graph, the onset of emulsion delamination is denoted by the red dashed line. Notably, the OVA emulsion began to delaminate on the eighth day of storage, signifying destabilization. Conversely, the 1.5 % (m/v) OVA-FUC emulsion remained free from delamination throughout the storage duration, showcasing a homogeneous and stable emulsion system. The Coalescence Index (CI) values provide insights into the extent of phase separation during emulsion storage (Kan et al., 2023). As depicted in Fig. 2B, the CI value for OVA emulsions reached 90.6 % after 15 d of storage, whereas the emulsion formulated with 1.5 % (w/v) OVA-FUC binary complexes exhibited no delamination, resulting in a CI value of 100 %, indicative of excellent storage stability. As the concentration increases, the interaction forces between OVA and FUC molecules strengthen, leading to greater repulsion between the molecules, which is advantageous for the stability of the emulsion. This trend aligns with the changes observed in the zeta potential.

The fluctuations in the zeta potential of O/W emulsions, formulated with varying concentrations of the aqueous phase, throughout storage are depicted in Fig. 2C. Specifically, the zeta potential of the OVA emulsion measures at 36.9 mV, whereas the absolute zeta potential of the OVA-FUC emulsion notably surpassed that of the OVA emulsion ($P < 0.05$). Over time, the absolute zeta potential of the emulsions exhibited a gradual decline. By the conclusion of the 15-day storage period, the absolute zeta potential of the OVA-FUC emulsions peaked at 39.4 mV. This increase can be attributed to the thermodynamic instability of the emulsions, impacting the interaction of zeta proteins and polysaccharides during storage, consequently diminishing their absolute zeta potential values (Siddhuraju & Becker, 2003). Conversely, the absolute zeta potential of the OVA emulsion approximates 18.4 mV, resulting in protein flocculation and subsequent shielding of the protein's charge. Moreover, emulsions formulated with lower concentrations (0.25–0.75 % (w/v)) of OVA-FUC binary complexes exhibited gradual coalescence, accompanied by a significant increase in particle size during storage.

The stability of emulsions can be assessed by monitoring changes in droplet size and distribution, using volume-weighted average particle size and surface area-weighted average particle size as indicators (McClements, 2007). To provide a more objective assessment of emulsion stability during storage, we combined particle size distribution measurements with optical microscopy observations. The results revealed that freshly prepared OVA emulsions exhibited small and uniformly distributed particle sizes, with the particle size decreasing gradually with increasing concentration of the OVA-FUC binary complex. Prolonged storage led to a significant increase in particle size and evident coalescence in the OVA emulsion. In contrast, the 1.5 % (w/v) OVA-FUC emulsion demonstrated excellent emulsion stability with a uniform particle size distribution (Fig. S1). These findings indicate that despite the higher viscosity of emulsions prepared from 1.5 % (w/v) OVA (Fig. 1B), their lower spatial potential resistance (Fig. 1A) and reduced electrostatic repulsion (Fig. 2C) contribute to their propensity

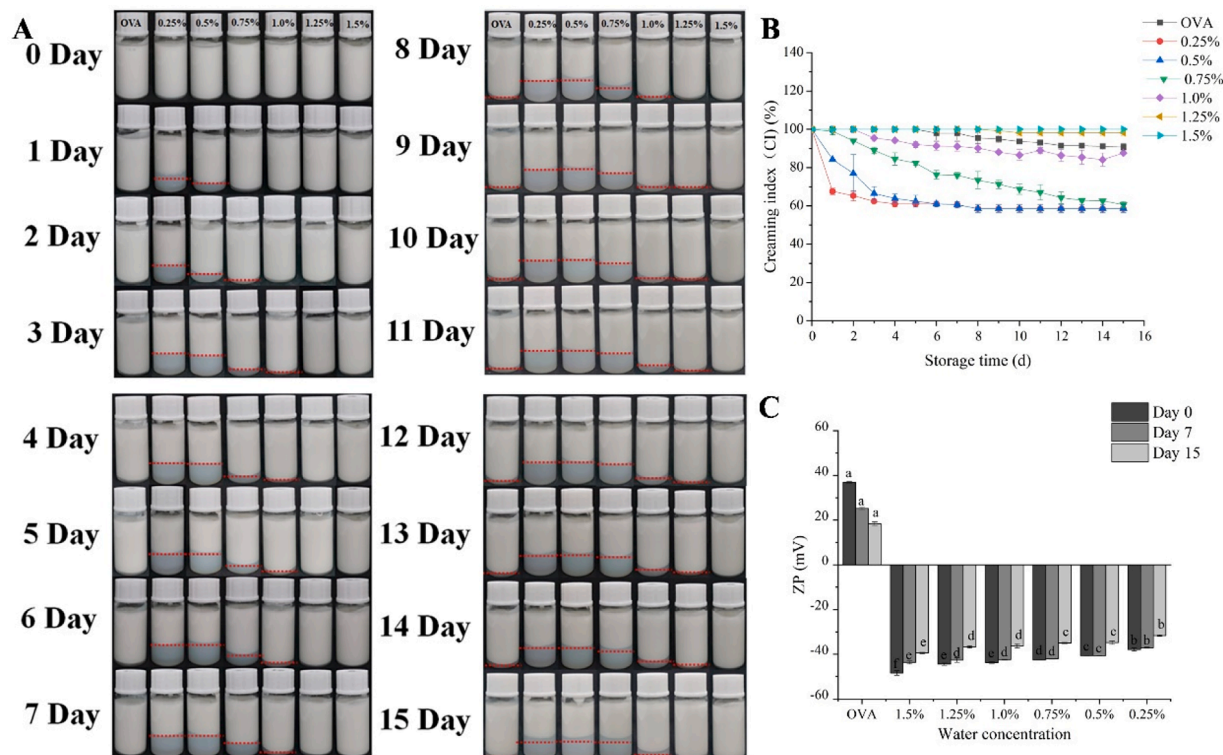


Fig. 2. Storage stability of emulsions with different emulsifier concentrations. (A) Plot of emulsions; the CI changes (B) and Zeta potential Changes (C) of emulsions with different emulsifier concentrations during storage. Different letters in the bar graphs indicate significant differences ($P < 0.05$).

for collision and coalescence during storage (Winuprasith et al., 2018). Generally, a higher absolute value of the zeta potential signifies stronger electrostatic repulsion and greater emulsion stability.

3.5. Freeze-thaw stability of emulsions with different emulsifier concentrations

The destabilization of emulsions due to freeze–thaw cycles is largely attributed to the formation of ice crystals during freezing, which disrupts hydrogen bonds and leads to the crystallization of the hydration layer/interfacial film (Wang et al., 2022). Protein-stabilized emulsions may experience a phenomenon where the emulsion layer floats upward during freeze–thaw cycling. Fig. 3 illustrates the stable appearance of the emulsions before freeze–thawing. However, after freeze–thawing, OVA and OVA-FUC emulsions of varying concentrations exhibited noticeable delamination. This observation suggests that the addition of FUC has only a limited effect on improving the freeze–thaw stability of the emulsion. As depicted in Fig. 3, the oil in the OVA emulsion and the 0.25 % OVA-FUC emulsion leaked out of the emulsion droplets following freeze–thawing. However, as the concentration of the OVA-FUC binary complex in the emulsions increased, the camellia oil in the droplets no longer leaked out. This phenomenon indicates that although the addition of FUC does not significantly enhance the freeze–thaw stability of the emulsion, it can prevent the camellia oil from leaking out during freeze–thaw cycling, demonstrating the continued protective effect of the encapsulated material (Chen et al., 2019). This indirectly underscores the crucial role of preparing OVA-FUC binary complexes in emulsion stability, particularly at higher concentrations of OVA-FUC.

3.6. Centrifugal stability of emulsions with different emulsifier concentrations

The impact of emulsifier concentration on the centrifugal stability of emulsions can be assessed by monitoring changes in transmission using the LUMISizer. As depicted in Fig. 4, the position at 130 mm corresponds to the bottom of the samples, while the position at approximately 105 mm represents the top. The first profile (red line) corresponds to the bottom, and the last profile (green line) represents the top. This

phenomenon is attributed to the tendency of emulsion droplets to move upward under centrifugal force due to the density disparity between the emulsion droplets and the water phase (Dammak & Sobral, 2021), where higher transmittance indicates lower emulsion droplet concentration. No leakage of camellia oil from the emulsions occurred in any of the samples tested, indicating high resistance to coalescence in the centrifugal field. The transmittance of the emulsion gradually decreased with increasing concentration of the complex (0.25–1.5 % (w/v)). The sample with the highest emulsifier concentration (1.5 % (w/v) OVA-FUC) exhibited minimal change in normalized transmittance at the bottom, suggesting little delamination in the emulsion (Liu et al., 2016). The emulsion prepared with 1.5 % (m/v) OVA showed the highest transmittance and the poorest centrifugal stability, indicating that the addition of FUC significantly enhanced the centrifugal stability of the emulsion. This improvement may be attributed to the thicker interfacial film of the emulsion prepared by the OVA-FUC complex compared to that of the emulsion prepared by OVA, resulting in greater resistance to coalescence or emulsion breakage and contributing to higher stability of the emulsion under high-speed centrifugation.

3.7. Effect of ions on the stability of OVA-FUC emulsions

Optical microscopy observations depicted an increase in droplet size alongside varying degrees of coalescence with escalating ionic concentration, as illustrated in Fig. S2. Beyond a Na^+ concentration of 300 mM, the emulsion transitioned into an inverted, non-flowing paste state. This occurrence likely stems from the electrostatic shielding effect of Na^+ . However, measurements of emulsion potentials, as shown in Fig. 5A, indicated no noteworthy alterations despite substantial Na^+ supplementation, suggesting that electrostatic shielding may not be the principal determinant of coalescence in emulsions (Pimentel-Moral, Ochando-Pulido, Segura-Carretero, & Martinez-Ferez, 2018). It is proposed that Na^+ could induce protein aggregation, forming gel-like network structures. Consequently, in our investigation, the formation of this paste-like substance may also be linked to the development of weak gel network structures of proteins at the interfacial membrane. This phenomenon results in diminished protein diffusion rates within the emulsion, fostering the creation of a gel network-like structure,

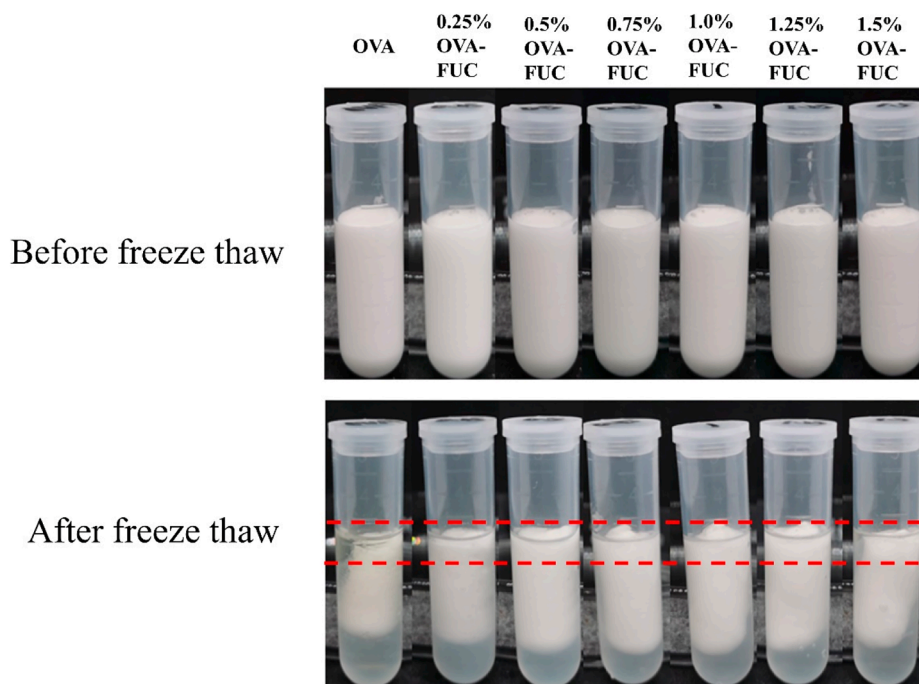


Fig. 3. Appearance of emulsion before and after freeze–thaw.

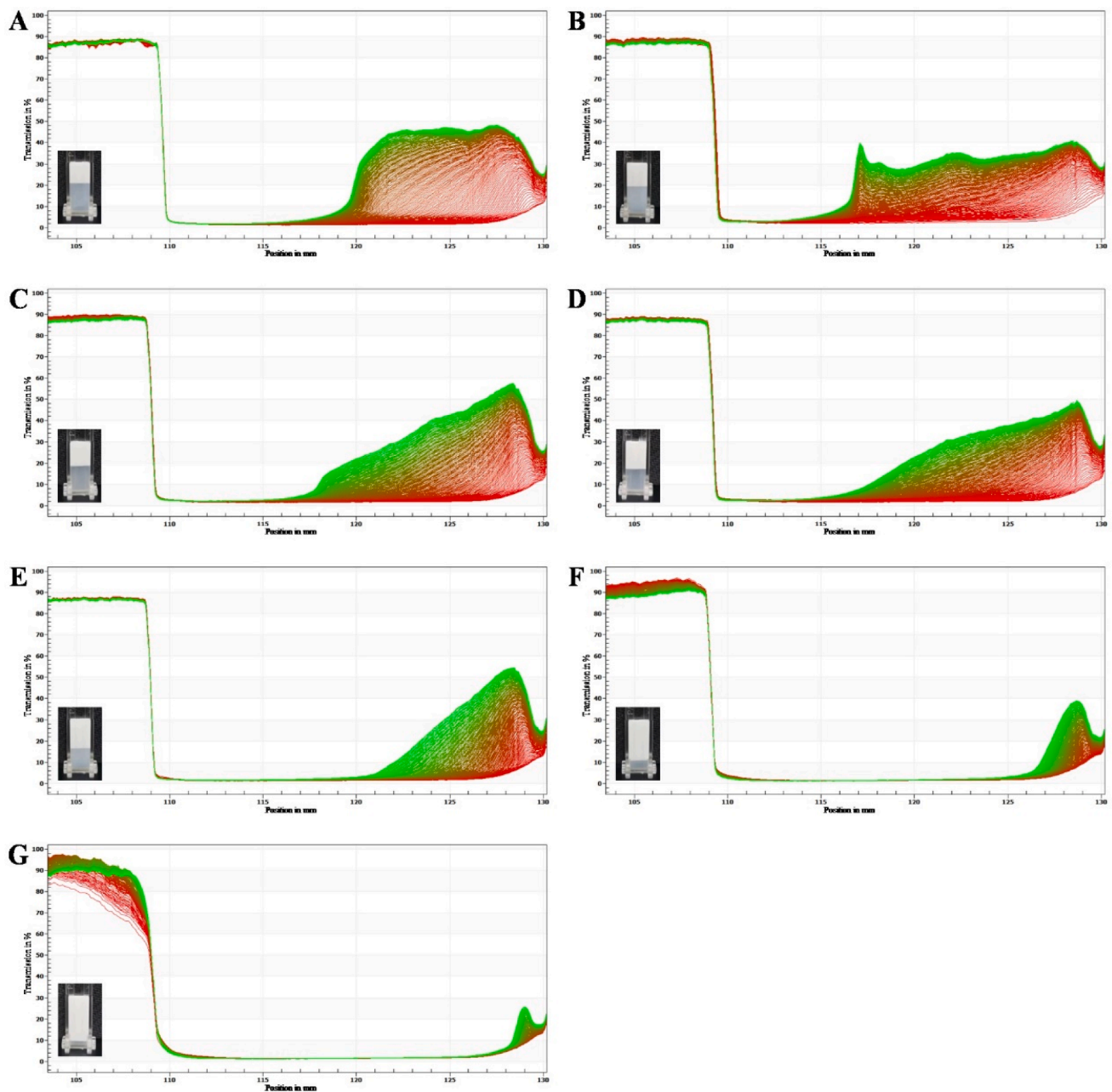


Fig. 4. LUMifuge analysis of O/W emulsions containing different emulsifier concentrations: (A) OVA; (B) 0.25 %; (C) 0.5 %; (D) 0.75 %; (E) 1.0 %; (F) 1.25 %; (G) 1.5 %.

ultimately impeding emulsion fluidity and leading to the formation of a paste-like consistency. The mechanical resilience of emulsions can be assessed by sweeping the modulus of elasticity (G') and modulus of loss (G'') of O/W emulsions across varying sodium ion concentrations. As illustrated in Fig. 5(B and C), the G' values of all emulsions significantly outweigh the G'' values across the frequency spectrum, indicating heightened viscosity with sodium ion supplementation.

Fig. S2 illustrates that upon the addition of varying concentrations of Ca^{2+} , the freshly prepared emulsions transformed into a paste-like consistency. This suggests that Ca^{2+} enhances the propensity of OVA-FUC emulsions to form network-like structures. This phenomenon can be attributed to several factors. Firstly, there is a potential reaction between Ca^{2+} and the free sulfate groups of Fucoidan, resulting in the formation of calcium bridges. These bridges facilitate the establishment of spatial network structures among the hydrated layers of the emulsion (Wang, Luo, Liu, Adhikari, & Chen, 2019). Secondly, akin to sodium

ions, calcium ions can induce protein gelation. The addition of Ca^{2+} leads to a substantial reduction in electrostatic repulsion within the emulsion, thereby increasing the likelihood of droplet collisions and facilitating the formation of gel networks (Tang, Chen, & Foegeding, 2011).

Fig. 5D illustrates the zeta potential distribution of O/W emulsions under varying strengths of calcium ions. The absolute value of the emulsion zeta potential exhibited a significant decrease upon the introduction of Ca^{2+} , plummeting from 67.2 mV to 23.5 mV. This reduction can be attributed to the bridging flocculation effect of calcium ions (Sok Line, Remondetto, & Subirade, 2005) which resulted in greater adsorption of OVA-FUC binary complexes at the interface. Additionally, salt ions can mitigate the impact of electrostatic repulsion on protein adsorption, leading to a diminished absolute value of the emulsion zeta potential (Liang, Leung Sok Line, Remondetto, & Subirade, 2010). Consequently, the interfacial film of OVA-FUC emulsions may thicken,

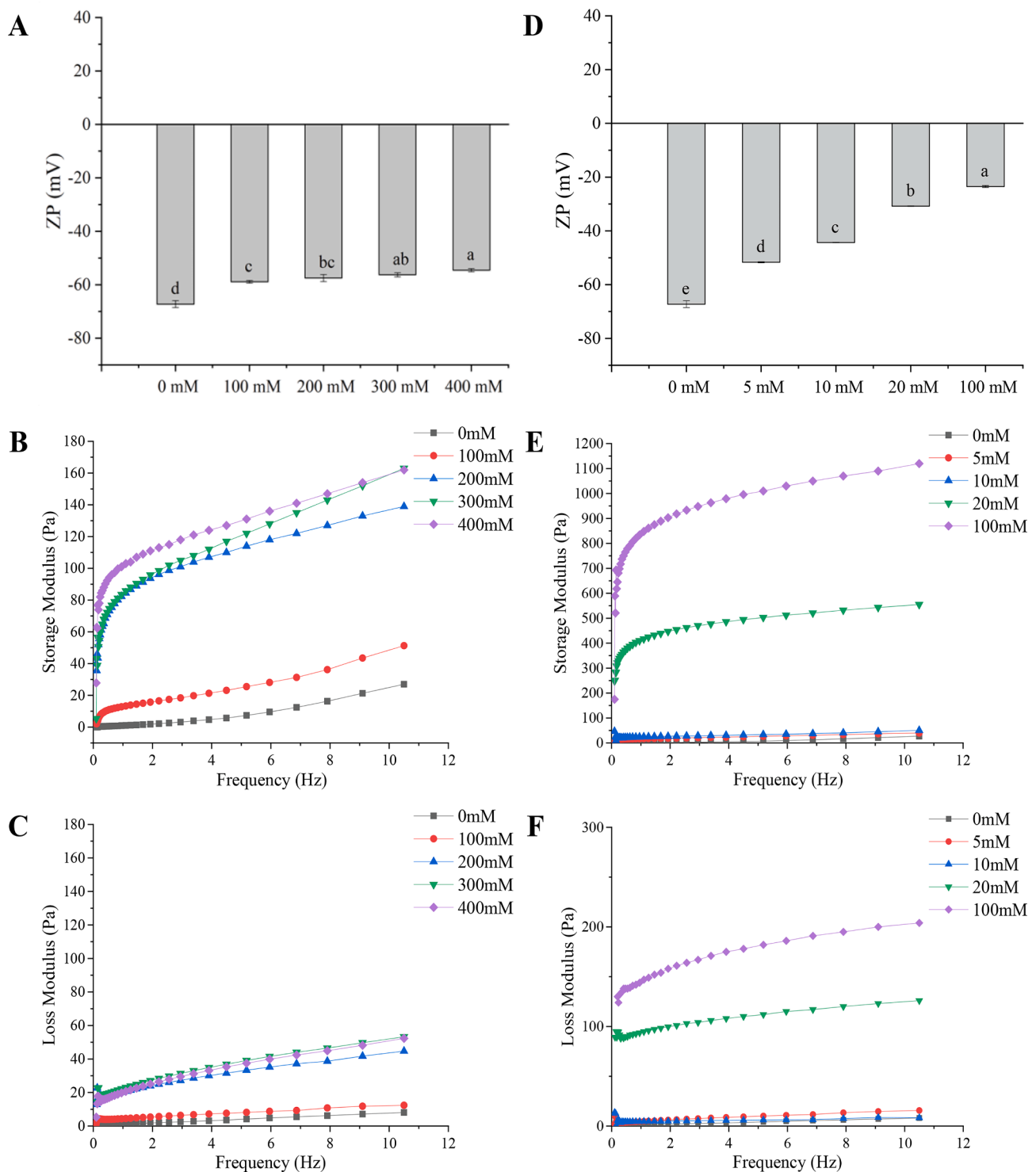


Fig. 5. Effect of ions on the stability of emulsion prepared with 70 % (v/v) of 1.5 % OVA-FUC. Zeta potential distribution of emulsions with different Na⁺ concentrations (A), and different Ca²⁺ concentrations (D); Rheology of emulsions with different Na⁺ concentrations: (B) Na⁺ storage modulus (G'), (C) Na⁺ loss modulus (G''); Rheology of emulsions with different concentrations of Ca²⁺: (E) Ca²⁺ storage modulus (G'), (F) Ca²⁺ loss modulus (G''). Different small letters in the bar graphs indicate significant differences ($P < 0.05$).

engendering robust repulsion between particles and impeding the coalescence of emulsion droplets. This dual effect suggests that even lower concentrations of Ca²⁺ can prompt the emulsions to assume a colloidal state like pastes (Liang et al., 2010).

Fig. S3 illustrates that after storing the emulsions in a dark box for 7 d, no deformation or oil was floating observed in the emulsions except for the sample with 5 mM of added Ca²⁺, suggesting excellent stability.

From Fig. 5(E and F), it is evident that the viscoelastic material possessed adequate mechanical strength, crucial for maintaining the layer-by-layer deposited structure and ensuring shape fidelity in the self-supporting phase (Shaddel et al., 2018). The G' values of the emulsions with added Ca²⁺ were consistently higher than the G'' values across the frequency range, indicating that the addition of Ca²⁺ conferred greater elastic-like properties to the emulsion system, forming a highly

interconnected network structure. Moreover, both G' and G'' values increased progressively with increasing Ca^{2+} concentration, reaching maximum values in the 100 mM emulsion gels, signifying the formation of a tighter and more robust intermolecular network between protein-protein and protein-polysaccharide molecules (Ma et al., 2022). These findings collectively suggest that Ca^{2+} enhances the mechanical strength and viscoelasticity of the gel. Consequently, it is apparent that even after the addition of FUC, the emulsion retains its high sensitivity to sodium and calcium ions. Nevertheless, the physical characteristics of the complex facilitate the formation of a solid-like emulsion gel under cationic stress, transforming the emulsion into a plastic-like solid. The properties of emulsion gels, such as adjustable morphology and microstructure, good physicochemical, mechanical, and functional properties, as well as good stability and controlled release, make them a promising gel with significant applications in the fields of processing, transporting, and targeted release of food additives, functional ingredients, and bioactivities, etc. (Abdullah, Liu, Javed, & Xiao, 2022), which is a critical insight into the use of OVA for the preparation of emulsion gels to enhance their utilization value. The results of ionic stabilization of OVA-FUC emulsions show that the interactions between macromolecules, especially the electrostatic repulsive force, play an important role in the stability of the emulsions. After forming a binary complex between FUC and OVA molecules through electrostatic self-assembly, the charge it carries strengthens the intermolecular force, which enhances the stability of the emulsions.

4. Conclusion

In this investigation, the emulsification properties of the OVA-FUC binary complex were evaluated through an analysis of particle size, zeta potential, and changes in the emulsification index (CI) of emulsions formulated with OVA-FUC and camellia oil. Additionally, the study encompassed an assessment of storage stability, centrifugal stability, freezing and thawing stability, and ion stability of the emulsions. The findings revealed that in comparison to emulsions prepared from OVA alone, the OVA-FUC emulsion exhibited a thicker interfacial film, heightened viscosity, superior physical and storage stability, and the ability to produce a more uniform emulsion at oil phase concentrations ranging from 30 % to 40 %. Notably, the freeze-thaw stability test results indicated that the OVA-FUC binary complex emulsion did not demonstrate strong anti-freezing properties; however, FUC was effective in preventing camellia oil from seeping out of the emulsion droplets. Furthermore, the ion stability test revealed that the bridging flocculation induced by Ca^{2+} resulted in a gel-like state, suggesting the potential for the OVA-FUC binary complex emulsion to serve as an emulsion gel, an area of future research. The OVA-FUC binary complex displayed favorable emulsification properties, and the O/W emulsion systems constructed using this complex exhibited robust stability, offering enhanced protection for encapsulated bioactive compounds and promising prospects for further development. Nevertheless, the absence of a recognized effective emulsifier as a control in this study necessitates further research to ascertain the potential commercial utility of OVA-FUC as an emulsifier.

CRedit authorship contribution statement

Xiefei Li: Writing – review & editing, Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Yingmei Wu:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Conceptualization. **Wenshan Duan:** Software, Data curation. **Lei Chen:** Validation, Software. **Lujie Cheng:** Software, Resources. **Junmei Liu:** Validation, Software. **Yan Zhou:** Supervision, Formal analysis. **Chao Ai:** Supervision, Conceptualization. **Xin Li:** Supervision, Conceptualization. **Qun Huang:** Supervision, Resources, Conceptualization.

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.fochx.2024.101457>.

References

- Abdullah, Liu, L., Javed, H. U., & Xiao, J. (2022). Engineering emulsion gels as functional colloids emphasizing food applications: A review. *Frontiers Nutrition*, 9. <https://doi.org/10.3389/fnut.2022.890188>
- Chen, Y.-B., Zhu, X.-F., Liu, T.-X., Lin, W.-F., Tang, C.-H., & Liu, R. (2019). Improving freeze-thaw stability of soy nanoparticle-stabilized emulsions through increasing particle size and surface hydrophobicity. *Food Hydrocolloids*, 87, 404–412. <https://doi.org/10.1016/j.foodhyd.2018.08.020>
- Comas, D. I., Wagner, J. R., & Tomás, M. C. (2006). Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein–lecithin interaction. *Food Hydrocolloids*, 20(7), 990–996. <https://doi.org/10.1016/j.foodhyd.2005.11.006>
- Dammak, I., & Sobral, P. J. d. A. (2021). Curcumin nanoemulsions stabilized with natural plant-based emulsifiers. *Food Bioscience*, 43, Article 101335. <https://doi.org/10.1016/j.fbio.2021.101335>
- Delahajje, R. J. B. M., Wierenga, P. A., van Nieuwenhuijzen, N. H., Giuseppin, M. L. F., & Gruppen, H. (2013). Protein concentration and protein-exposed hydrophobicity as dominant parameters determining the flocculation of protein-stabilized oil-in-water emulsions. *Langmuir*, 29(37), 11567–11574. <https://doi.org/10.1021/la401314a>
- Duan, W., Chen, L., Liu, F., Li, X., Wu, Y., Cheng, L., Liu, J., Ai, C., Huang, Q., & Zhou, Y. (2023). The properties and formation mechanism of ovalbumin-fucoidan complex. *International Journal of Biological Macromolecules*, 241, Article 124644. <https://doi.org/10.1016/j.ijbiomac.2023.124644>
- Gkinali, A.-A., Matsakidou, A., & Paraskevopoulou, A. (2023). Assessing the emulsifying properties of *Tenebrio molitor* larvae protein preparations: Impact of storage, thermal, and freeze-thaw treatments on o/w emulsion stability. *International Journal of Biological Macromolecules*, 250, Article 126165. <https://doi.org/10.1016/j.ijbiomac.2023.126165>
- Guo, Q., Su, J., Shu, X., Yuan, F., Mao, L., Liu, J., & Gao, Y. (2020). Production and characterization of pea protein isolate-pectin complexes for delivery of curcumin: Effect of esterified degree of pectin. *Food Hydrocolloids*, 105, Article 105777. <https://doi.org/10.1016/j.foodhyd.2020.105777>
- Hu, B., Wang, K., Han, L., Zhou, B., Yang, J., & Li, S. (2020). Pomegranate seed oil stabilized with ovalbumin glycolated by inulin: Physicochemical stability and oxidative stability. *Food Hydrocolloids*, 102, Article 105602. <https://doi.org/10.1016/j.foodhyd.2019.105602>
- Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. *Food Hydrocolloids*, 30(2), 647–655. <https://doi.org/10.1016/j.foodhyd.2012.08.001>
- Huang, Q., Huang, X., Liu, L., Wang, G., Song, H., Geng, F., & Luo, P. (2021). Effect of nano eggshell calcium on the structure, physicochemical, and gel properties of threadfin bream (*Nemipterus virgatus*) actomyosin. *LWT*, 150, Article 112047. <https://doi.org/10.1016/j.lwt.2021.112047>
- Huang, X., Tu, R., Song, H., Dong, K., Geng, F., Chen, L., Huang, Q., & Wu, Y. (2022). Gelatin-EGCG-high methoxyl pectin ternary complex stabilized W1/O/W2 double emulsions loaded with vitamin C: Formation, structure, stability, in vitro gastrointestinal digestion. *International Journal of Biological Macromolecules*, 216, 891–905. <https://doi.org/10.1016/j.ijbiomac.2022.07.210>
- Huang, X., Tu, R., Song, H., Dong, K., Geng, F., Chen, L., Huang, Q., & Wu, Y. (2023). Fabrication and characterization of gelatin-EGCG–pectin ternary complex: Formation mechanism, emulsion stability, and structure. *Journal of the Science of Food and Agriculture*, 103(3), 1442–1453. <https://doi.org/10.1002/jsfa.12240>

- Kan, G., Zi, Y., Li, L., Gong, H., Peng, J., Wang, X., & Zhong, J. (2023). Curcumin-encapsulated hydrophilic gelatin nanoparticle to stabilize fish oil-loaded Pickering emulsion. *Food Chemistry: X*, 17, Article 100590. <https://doi.org/10.1016/j.fochx.2023.100590>
- Li, Z., Kuang, H., Yang, J., Hu, J., Ding, B., Sun, W., & Luo, Y. (2020). Improving emulsion stability based on ovalbumin-carboxymethyl cellulose complexes with thermal treatment near ovalbumin isoelectric point. *Scientific Reports*, 10(1), 3456. <https://doi.org/10.1038/s41598-020-60455-y>
- Li, S., Wang, K., Huang, Q., & Geng, F. (2021). Microwave pretreatment enhanced the properties of ovalbumin-inulin-oil emulsion gels and improved the storage stability of pomegranate seed oil. *Food Hydrocolloids*, 113, Article 106548. <https://doi.org/10.1016/j.foodhyd.2020.106548>
- Liang, L., Leung Sok Line, V., Remondetto, G. E., & Subirade, M. (2010). In vitro release of α -tocopherol from emulsion-loaded β -lactoglobulin gels. *International Dairy Journal*, 20(3), 176–181. <https://doi.org/10.1016/j.idairyj.2009.09.008>
- Liao, W., Gharsallaoui, A., Dumas, E., & Elaissari, A. (2022). Understanding of the key factors influencing the properties of emulsions stabilized by sodium caseinate. *Comprehensive Reviews in Food Science and Food Safety*, 21(6), 5291–5317. <https://doi.org/10.1111/1541-4337.13062>
- Liu, F., Ma, C., Gao, Y., & McClements, D. J. (2017). Food-grade covalent complexes and their application as nutraceutical delivery systems: A review. *Comprehensive Reviews in Food Science and Food Safety*, 16(1), 76–95. <https://doi.org/10.1111/1541-4337.12229>
- Liu, F., Wang, D., Sun, C., & Gao, Y. (2016). Influence of polysaccharides on the physicochemical properties of lactoferrin–polyphenol conjugates coated β -carotene emulsions. *Food Hydrocolloids*, 52, 661–669. <https://doi.org/10.1016/j.foodhyd.2015.08.007>
- Ma, T., Cui, R., Lu, S., Hu, X., Xu, B., Song, Y., & Hu, X. (2022). High internal phase Pickering emulsions stabilized by cellulose nanocrystals for 3D printing. *Food Hydrocolloids*, 125, Article 107418. <https://doi.org/10.1016/j.foodhyd.2021.107418>
- McClements, D. J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition*, 47(7), 611–649. <https://doi.org/10.1080/10408390701289292>
- McClements, D. J. (2012). Advances in fabrication of emulsions with enhanced functionality using structural design principles. *Current Opinion in Colloid & Interface Science*, 17(5), 235–245. <https://doi.org/10.1016/j.cocis.2012.06.002>
- McClements, D. J., Decker, E. a., & Weiss, J. (2007). Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. *Journal of Food Science*, 72(8), R109–R124. <https://doi.org/10.1111/j.1750-3841.2007.00507.x>
- Pimentel-Moral, S., Ochando-Pulido, J. M., Segura-Carretero, A., & Martinez-Ferez, A. (2018). Stabilization of W/O/W multiple emulsion loaded with Hibiscus sabdariffa extract through protein-polysaccharide complexes. *LWT*, 90, 389–395. <https://doi.org/10.1016/j.lwt.2017.12.054>
- Shaddel, R., Hesari, J., Azadmard-Damirchi, S., Hamishehkar, H., Fathi-Achachlouei, B., & Huang, Q. (2018). Double emulsion followed by complex coacervation as a promising method for protection of black raspberry anthocyanins. *Food Hydrocolloids*, 77, 803–816. <https://doi.org/10.1016/j.foodhyd.2017.11.024>
- Shao, Y., & Tang, C.-H. (2014). Characteristics and oxidative stability of soy protein-stabilized oil-in-water emulsions: Influence of ionic strength and heat pretreatment. *Food Hydrocolloids*, 37, 149–158. <https://doi.org/10.1016/j.foodhyd.2013.10.030>
- Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry*, 51(8), 2144–2155. <https://doi.org/10.1021/jf020444+>
- Sok Line, V. L., Remondetto, G. E., & Subirade, M. (2005). Cold gelation of β -lactoglobulin oil-in-water emulsions. *Food Hydrocolloids*, 19(2), 269–278. <https://doi.org/10.1016/j.foodhyd.2004.06.004>
- Tang, C.-H., Chen, L., & Foegeding, E. A. (2011). Mechanical and water-holding properties and microstructures of soy protein isolate emulsion gels induced by CaCl₂, glucono- δ -lactone (GDL), and transglutaminase: Influence of thermal treatments before and/or after emulsification. *Journal of Agricultural and Food Chemistry*, 59(8), 4071–4077. <https://doi.org/10.1021/jf104834m>
- Tang, C.-H., & Liu, F. (2013). Cold, gel-like soy protein emulsions by microfluidization: Emulsion characteristics, rheological and microstructural properties, and gelling mechanism. *Food Hydrocolloids*, 30(1), 61–72. <https://doi.org/10.1016/j.foodhyd.2012.05.008>
- Wang, Y., Lin, R., Song, Z., Zhang, S., Zhao, X., Jiang, J., & Liu, Y. (2022). Freeze-thaw stability and oil crystallization behavior of phospholipids/whey protein-costabilized acidic emulsions with four oil types. *Food Hydrocolloids*, 125, Article 107385. <https://doi.org/10.1016/j.foodhyd.2021.107385>
- Wang, X., Luo, K., Liu, S., Adhikari, B., & Chen, J. (2019). Improvement of gelation properties of soy protein isolate emulsion induced by calcium cooperated with magnesium. *Journal of Food Engineering*, 244, 32–39. <https://doi.org/10.1016/j.jfoodeng.2018.09.025>
- Wang, T., Wang, N., Li, N., Ji, X., Zhang, H., Yu, D., & Wang, L. (2022). Effect of high-intensity ultrasound on the physicochemical properties, microstructure, and stability of soy protein isolate-pectin emulsion. *Ultrasonics Sonochemistry*, 82, Article 105871. <https://doi.org/10.1016/j.ultsonch.2021.105871>
- Winuprasith, T., Khomein, P., Mitbumrung, W., Suphantharika, M., Nitithamyong, A., & McClements, D. J. (2018). Encapsulation of vitamin D3 in pickering emulsions stabilized by nanofibrillated mangosteen cellulose: Impact on in vitro digestion and bioaccessibility. *Food Hydrocolloids*, 83, 153–164. <https://doi.org/10.1016/j.foodhyd.2018.04.047>
- Wu, Y., Lei, C., Li, J., Chen, Y., Liang, H., Li, Y., Li, B., Luo, X., Pei, Y., & Liu, S. (2022). Improvement of O/W emulsion performance by adjusting the interaction between gelatin and bacterial cellulose nanofibrils. *Carbohydrate Polymers*, 276, Article 118806. <https://doi.org/10.1016/j.carbpol.2021.118806>
- Xu, Q., Qi, B., Han, L., Wang, D., Zhang, S., Jiang, L., Xie, F., & Li, Y. (2021). Study on the gel properties, interactions, and pH stability of pea protein isolate emulsion gels as influenced by inulin. *LWT*, 137, Article 110421. <https://doi.org/10.1016/j.lwt.2020.110421>
- Zhang, Y., Guo, Y., Liu, F., & Luo, Y. (2023). Recent development of egg protein fractions and individual proteins as encapsulant materials for delivery of bioactives. *Food Chemistry*, 403, Article 134353. <https://doi.org/10.1016/j.foodchem.2022.134353>
- Zhang, X., Wang, Q., Liu, Z., Zhi, L., Jiao, B., Hu, H., Ma, X., Agyei, D., & Shi, A. (2023). Plant protein-based emulsifiers: Mechanisms, techniques for emulsification enhancement and applications. *Food Hydrocolloids*, 144, Article 109008. <https://doi.org/10.1016/j.foodhyd.2023.109008>
- Zhu, X.-F., Zheng, J., Liu, F., Qiu, C.-Y., Lin, W.-F., & Tang, C.-H. (2018). Freeze-thaw stability of Pickering emulsions stabilized by soy protein nanoparticles. Influence of ionic strength before or after emulsification. *Food Hydrocolloids*, 74, 37–45. <https://doi.org/10.1016/j.foodhyd.2017.07.017>