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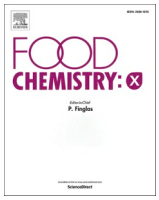

Effect of fat addition on the characteristics and interfacial behavior of chicken white soup emulsion from chicken skeleton

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ABSTRACT

The effects of varying fat additions (0 %, 1.0 %, 1.5 %, 2.0 %, and 2.5 %) on characteristics and interfacial properties of chicken white soup emulsion from stewing chicken skeleton were investigated. The results revealed that the chicken white soup emulsion obtained with the 2.0 % fat addition had smaller $D_{3,2}$ (1.889 μm), $D_{4,3}$ (2.944 μm), and higher absolute zeta potential value (23.32 mV). Viscosity values were higher for the 2.0 % fat addition compared to the other treatment groups. Techniques like scanning electron microscopy, laser confocal, and atomic force microscopy demonstrated that oil droplets and particles in the soup were smaller and more evenly dispersed with the 2.0 % fat addition. Moreover, the 2.0 % fat group exhibited higher interfacial protein concentration of 207.56 mg/m^2. Lastly, low field NMR images confirmed that the stability of the soup was enhanced with a 2.0 % fat addition. This research offers a foundational understanding for producing highly stable chicken white soup.

Introduction

Chicken skeletons are a major by-product of broiler processing, accounting for approximately 8 %-17 % of the total weight of broilers. They are inexpensive, nutritious, readily available, making them a promising raw material for food processing. Chicken skeletons contain 51 % moisture, 19 % collagen, 9 % fat, and 15 % ash (Dong et al., 2014), representing a valuable source of protein. Furthermore, compared to other animal bones, they have a higher content of soluble collagen, which more effectively promotes the body's absorption of calcium (Guo et al., 2015; Yue et al., 2017).

Soup is a crucial medium where substance exchange occurs during cooking and possesses better physiological functions and flavour than meat. For example, soup is conducive to the transport of nutrients, promotes digestion, and has the functions of supplementing energy and nourishing health. After extended cooking, the protein and fat in the chicken skeleton emulsify in the boiling state, producing a milky chicken white soup emulsion. It has been reported that gelatin, a key structural protein in chicken broth, affects the ability of chicken broth to emulsify oil droplets (Yan et al., 2024; Qi et al., 2023). Additionally, this emulsion is flavorful, nutritious, and beautifully colored, rich in free amino acids and volatile flavor compounds, making it popular among consumers. Previous research indicates that the colour of soup positively correlates with the content of proteins and lipids, with the milky white hue resulting from high concentrations of proteins and lipids (Zhang et al., 2013).

Protein and fat are the primary macromolecules in soup and can be driven by intermolecular forces to form a vast number of self-assembled spherical particles, ranging in size from nanoscale to macroscale (Wang et al., 2019). During the self-assembly process by intermolecular forces, protein adsorbs to the surface of oil droplets, playing a pivotal role in stabilizing oil-in-water emulsion systems (Qi et al., 2020). The role of fat in emulsion preparation is crucial. Smaller fat globules and ample solubilised protein to cover the fat surface (interfacial adsorption) are essential factors (Yang et al., 2021). The high dispersion and uniformity of the fat phase distribution positively impact the emulsion's viscosity, subsequently enhancing its stability. The Greenland halibut, a fatty fish species with over 10 % fat content, has this high fat content enhancing the emulsifying properties and flavour of its soup (He et al., 2022).

Additionally, research indicates that emulsion stability correlates with the mobility of the interfacial membranes (Urbina-Villalba, 2009). This alteration in the interfacial protein membrane can trigger a reduction in oil droplet particle size, thereby expanding the interfacial area of the oil phase. The quantity of oil droplets significantly impacts

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the emulsion. Sullivan and Kilpatrick (2002) also noted that the interfacial membrane augments emulsion stability by raising interfacial viscosity and reducing interfacial tension (IFT).

However, the fat content of chicken soup stewed with chicken bones is relatively low, in order to improve the emulsifying stability of the chicken soup, it is necessary to add a certain amount of fat. Lu et al. (2024) reported that emulsion with higher oil volume fraction has stronger resistance to delamination because of its high viscosity. Additionally, Wang, Zhu, Ji, and Chen (2021) also showed that the content of oil droplets has an impact on the lubrication and sensory properties of O/W emulsion systems. Usually, the direct addition of fat does not create emulsion films with evenly dispersed oil droplets, causing the oil to possibly migrate to the film's surface (Zhao, Ren, Shi, & Weng, 2023). Previous studies have shown that boiling acts as a form of self-emulsification, and adding fat during boiling process enhances emulsification. (Guan et al., 2023).

Despite some researches on the flavor of chicken broth have been reported, there are few studies on the stability of chicken white soup emulsion from chicken skeleton, especially the influence of added fat. Therefore, in this study, fat was added to chicken soup and boiled continuously for a certain amount of time to delve into the influence of fat addition on the stability and the interfacial membrane of chicken white soup, aiming to pinpoint the optimal fat addition and furnish a theoretical foundation for crafting stable chicken white soup.

Materials and methods

Materials

Chicken skeletons were sourced from Jinzhou vegetable market. Nile red was purchased from Beijing Baiao Laibo Technology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA) was obtained from Sigma Chemical Co., St. (Louis, MO, USA). Copper sulfate, potassium sulfate, sodium hydroxide, hydrochloric acid, concentrated sulfuric acid, and boric acid were purchased from Liaoning Quan Rui Reagent Co., Ltd. (Jinzhou, China). All reagents were analytical grade.

Extraction of chicken fat

The chicken suet was washed, drained, then placed in a hot pan (without water) and heated at 120 °C for 40 min. The liquid chicken fat was filtered through double gauze to remove oil residues and was subsequently solidified and stored at −20 °C. Before use, the chicken fat was placed in a 40 °C water bath for 30 mins.

Preparation of chicken white soup

The chicken skeleton was rinsed with warm water, removed the blood, tail fat gland, lungs, and trachea, and cut it into square pieces of 2 cm. Add cold water, blanch over high heat for 2 mins, skim off the foam, and pour off the water. The pre-treated chicken skeletons were placed into a stainless steel pot, maintaining a weight ratio of chicken skeletons to water of 1:2. After mixing, the chicken skeletons were boiled for 20 mins on an induction cooker at 1600 W power (100 ± 0.22 °C), then simmered for 100 mins at 800 W (98 ± 0.17 °C). Subsequently, the soup was divided equally into five portions and the fat content was measured. Then a specific amount of chicken fat was added to each portion to make the fat addition in the soup reached 0 %, 1.0 %, 1.5 %, 2.0 %, and 2.5 % respectively according to the report of Qi et al. (2023) and our pre-experimental findings. Continue to stew each soup for 1 h on an induction cooker at 600 W (95 ± 0.13 °C) to produce the chicken white soup emulsion. Throughout this process, check the water level every 30 min, adding boiling water as needed to maintain the pot's initial water level. Three independent repetitions were performed.

Chicken white soup characteristics

Multiple light scattering

The multiple light scattering of the chicken white soup emulsion was determined using a Turbiscan Lab Expert analyzer (Formulaction Inc., Toulouse, France) following a modified protocol described by Zhu et al. (2020a). The emulsion (20 mL) was placed in an instrument-specific glass test bottle and scanned every 2 mins continuously for 2 h. The stability of the emulsion was assessed using the Turbiscan stability index (TSI) via the Turbiscan software. The TSI was calculated as follows:

$$TSI = \sqrt{\frac{\sum_{i=1}^{n} (x_i - x_{BS})^2}{n - 1}}$$

where x_i is the average backscattering (each minute), x_{BS} is the average x_i, and n is the number of scans.

Particle size and zeta potential

A BT-9300ST laser analyzer (Baite Co. Ltd., Dandong, China) was employed to measure the particle size and distribution of the emulsion at 25 °C. The mean surface-weighted diameter ($D_{3,2}$) and the mean volume-weighted diameter ($D_{4,3}$) of the droplets were determined. D_{10}, D_{50} and D_{90} indicate the particle sizes at 10 %, 50 %, and 90 % of the total particle volume, respectively.

The zeta potential of the chicken white soup emulsion were analyzed at room temperature (approximately 23 °C) using a Zeta potential analyzer (Malvern Instruments Ltd., Worcestershire, UK). For each measurement, 1 mL of the emulsion sample was injected into the folded capillary cell. Each measurement was conducted thrice.

Determination of surface protein coverage and centrifugal stability

Using the method described by Hebishy et al. (2015) with modifications, the concentration of interfacial protein adsorbed in the chicken white soup emulsion system was determined. The freshly prepared emulsion was centrifuged at 10,000 × *g* for 1 h at 25 °C. After centrifugation, the lower aqueous phase was gently aspirated with a syringe and filtered through a membrane (0.45 μm) to eliminate impurities. The protein content of the chicken white soup emulsion not adsorbed to the interface was determined by Lowry's method, with BSA as the standard protein. The amount of interfacial protein adsorbed was calculated as follows:

$$\Gamma = 1000 \times (Ct\text{-}Cs)/A$$

where Γ represents the interfacial protein adsorption (mg/m^2); A is the specific surface area of the droplet (m^2/g), determined by laser particle size distribution instrument; Ct is the total protein concentration in the soup (g/g); and Cs is the protein concentration in the aqueous phase (g/g).

Referring to the method of Duan et al. (2019) with minor adjustments, the light absorption A_0 of the sample was measured at 540 nm wavelength after diluting the chicken white soup emulsion 100 times. Subsequently, the light absorption value A_1 was measured at the same wave length after using a high-speed centrifuge at 2000 × *g* for 10 mins. The stability coefficient was calculated by A_1/A_0. A larger stability coefficient indicates better emulsifying stability of the resulting emulsion.

Low-field nuclear magnetic resonance (LF-NMR) imaging

LF-NMR imaging of the freshly prepared emulsion samples was conducted using an LF-NMR analyser (MesoMR23-040H-I, China). The method described by Cai, Du, Zhu, and Cao (2020) was adjusted and employed. A 6 g sample from the chicken white soup emulsion was transferred to a 10 mm diameter NMR glass tube and sealed with silver paper at the tube's opening. The emulsion imaging was conducted using

Spin echo (HSE) sequence, with specific parameters: FOV Read = 100 mm, FOV Phase = 100 mm, Averages = 2, TR = 4500 ms, TE = 10 ms, Slices = 1, Slices Width = 3 mm.

Microstructure observations

Confocal microscopic images of chicken white soup emulsions were acquired using an FV-1000 confocal laser scanning microscope (CLSM) (Leica Microsystems, Germany). Chicken white soup emulsions (1 mL) were mixed with 40 μL of staining solutions containing 0.02 % (w/v) Nile Red. The stained emulsions (10 μL) were placed on the concave confocal microscope slides and examined using Ar (488 nm) and He/Ne (633 nm) lasers.

Scanning electron microscopic images of the chicken white soup emulsion were captured using a scanning electron microscope (SEM) (S-3400 N, Hitachi, Japan) based on the method described by Guan et al. (2023). The middle layer of the chicken white soup emulsion was freeze-dried. A 10 mg portion of the chicken white soup emulsion powder was spread on special adhesive tape for SEM observation. The scanning electron microscopy was conducted at a 5 kV acceleration voltage, and images were obtained.

Atomic force microscopy (AFM) images of the soups were obtained by using an XE-70 non-contact AFM (Park System Corp., Suwon, Korea) with the following instrument parameters: frequency 300 kHz; elasticity factor: 40 N/m; scan rate: 1 Hz. 10 μL of chicken white soup emulsion was dropped on the mica flakes and dried naturally at room temperature (25 °C) for 12 h. The three-dimensional structure was analyzed using Gwyddion analysis software.

Rheological properties

The steady-state flow viscoelasticity of the chicken white soup emulsion was assessed using a dynamic rheometer (AR-G2, TA Instruments, US). Measurements of steady shear flow were taken from 0.01 to 1000 s^{-1} for 120 s at 25 °C to gauge viscosity in relation to shear rate. The correlation among the shear rate and shear stress was modeled by applying the power law equation (Yan et al., 2024):

$$\tau = K\gamma^{n}$$

where τ represents shear stress (Pa); K represents consistency coefficient (Pa·s); γ represents shear rate (s^{-1}); and n represents flow behavior index.

Contact angle

The contact angle of the prepared emulsion was measured using a contact angle analyzer (OCA 25, Dataphiscis Instruments GmbH, Germany) following the sessile drop method. Briefly, 5 μL of deionized water was deposited onto the film's surface using a high-precision injector, after which the image was captured with a high-speed video camera. The profile of the droplet was then fitted using the LaPlace-Young equation (Zhao et al., 2023).

Characterization of the oil-in-water interface

Interfacial expansion rheology measurements

The dynamic interfacial properties of protein solution at the oil–water interface in chicken white soup emulsion were assessed using an optical contact angle meter (OCA25, Dataphysics Instruments GmbH, Germany). Before measurement, all solutions were placed in syringes and equilibrated for 30 mins to achieve a constant temperature (Wang et al., 2019). Following the methods of Zhao et al. (2023), a syringe needle containing chicken white soup emulsion was submerged in a glass cuvette filled with 3 mL of oil. Droplets containing the solution (5 μL) formed at the tip of the needle, and the droplet shape was captured by a camera linked to a computer.

Interfacial tension was monitored for 10800 s, recording data every 40 s. The oil-in-water interfacial pressure (π, mN/m) was calculated using $\pi = \gamma_0 - \gamma$, where γ_0 is the interfacial tensions of pure oil-DI water (37.88 mN/m) and γ is the interfacial tensions of chicken white soup emulsion (mN/m). The diffusion rate (K_{diff}) can be calculated using $\pi = 2C_0KT(D_t/3.14)^{1/2}$ (Zhao et al., 2023).where C_0 is the concentration in the continuous phase, K is the Boltzmann constant, T is the absolute temperature, and D_t is the diffusion coefficient of different adsorption time. If the graph of square root of time ($t^{1/2}$) dependence of surface pressure (π) is linear, and the slope in the graph is K_{diff}.

To further analyze the penetration and rearrangement rate of particles in the chicken white soup at the interface, a first-order equation was employed to fit the data (Xiong, Ren, Li, & Li, 2018).

$$\ln[(\pi_f - \pi_t)/(\pi_f - \pi_0)] = -k_i t$$

where π_f, π_0, and π_t are the interfacial pressure at the final, initial, and any given adsorption time, respectively. k_i is the first-order rate constant.

Dilated rheological properties of interfacial membranes

Surface dilatational results were obtained using periodical oscillations of a drop of liquid (10 μL) at a chosen amplitude (△A/A, 10 %) at a frequency of 0.1 Hz as per the method described by Zhu et al. (2020a), with slight adjustments. The droplets were incubated for 60 s before each experiment. Both active and blank runs consisted of 5 cycles during the experiments. The adsorption time was 10800 s, and the values of the surface dilatation modulus (E) and the dilatational elasticity (E_d) were calculated by the system software.

Statistical analysis

Origin 9.0 was used for plotting. Data were statistically analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). All measurements were performed in at least three independent experiments and results were expressed as mean ± standard deviation (SD).

Results and discussion

Characterization of chicken white soup

Multiple light scattering

The static vertical scanning of the emulsion samples, based on the principle of multiple light scattering, provided insights into the sample's stability level and a qualitative analysis of the causes of its instability (settling, upwelling, particle size variation, etc.). The lower the slope and value of the TSI curve, the more stable the emulsion (Gavahian et al., 2018). Fig. 1A displayed the change in TSI of chicken white soup emulsion over 2 h. The TSI of all samples increased as the measurement time extended, suggesting a decline in stability (Zhu et al., 2020a. Concurrently, the TSI of the treated group with added fat was lower than that of the control group, suggesting that the addition of fat enhanced the emulsion stability of chicken white soup. However, no significant differences were observed between the treatment groups ($P > 0.05$).

Zeta-potential

As shown in the Fig. 1B, the zeta potential values of the chicken white soup emulsion were all negative, indicating a negative charge on the particle surface of the emulsified system. The absolute value of the potential for all samples was concentrated around 20–25 mV. Compared to the sample without fat, the addition of fat significantly increased the absolute value of the samples potential ($P < 0.05$), and the sample with 1.5 % fat had the largest absolute value. This observation suggests that the more fat added, the stronger the interaction or attraction between

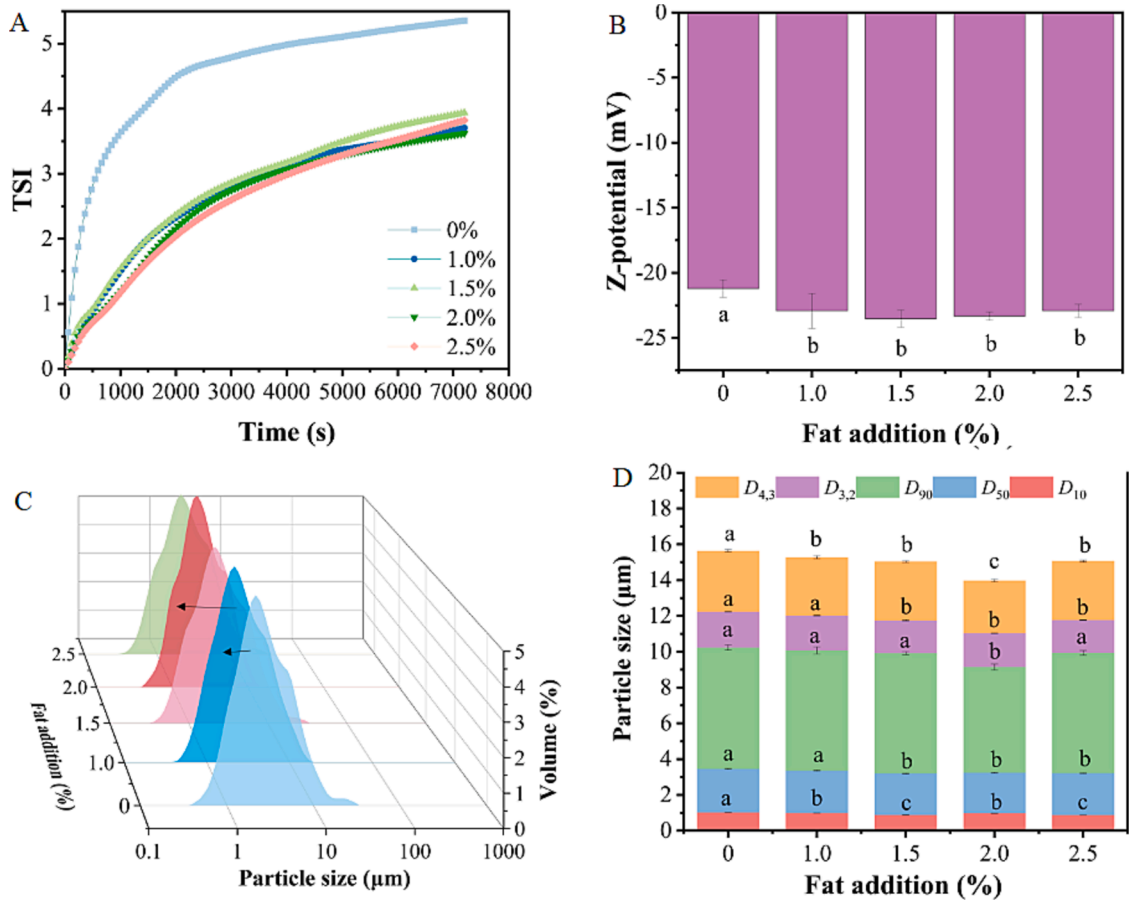

Fig. 1. Effect of different fat additions on multiple light scattering (A), zeta-potential (B), droplet size distribution (C), and particle size (D) of chicken white soup emulsion. Error bars represent standard errors obtained from triplicate sample analysis. Different letters in the same indexes indicate statistically significant differences ($P < 0.05$).

the particles in the emulsion, helping maintain emulsion stability and preventing large aggregation and flocculation. An increase in the absolute value of the zeta potential correlated with a decrease in the average particle size of the emulsion, possibly explaining the enhanced stability (Qi et al., 2023). However, when fat addition exceeded 1.5 %, the absolute value of the sample's potential showed a declining trend, suggesting that at higher fat concentrations, some phospholipids might precipitate, thereby impacting stability and causing a reduction in potential (Wang, Wang, Li, & Zhang, 2023). Notably, no significant difference was found between samples with 1.5 %, 2.0 %, and 2.5 % fat additions ($P > 0.05$). The results of Wang et al. (2023), who also found that as beef tallow content continuously increased, particle size grew, suggesting that the O/W emulsion contained more fat globule clusters, leading to reduced stability and potential.

Particle size

Emulsion particle size is a crucial characteristic of emulsions, with droplet diameters in food emulsions typically ranging from 0.1 to 100 μm. As shown in Fig. 1C, the particle size distribution curve exhibited a single peak, and particle sizes were below 100 μm for all samples. Moreover, chicken white soup with added fat showed a leftward shift in distribution peaks towards smaller sizes compared to the control, and the soup with 2.0 % added fat had the most pronounced leftward shift. This indicates that the quantity of added fat influenced the particle size of chicken white soup, and at a 2.0 % fat addition, the fat interacted optimally with the protein to form smaller particles.

In emulsions, smaller droplet sizes generally result in greater stability. The mean diameters (D_{10}, D_{50} and D_{90}) of the oil droplets, volume-mean diameters ($D_{4,3}$) and volume-surface mean diameters ($D_{3,2}$) of different samples were listed in Fig. 1D. As fat addition increased, the value of $D_{4,3}$ first decreased, then increased, reaching its smallest value with the 2.0 % fat addition. This outcome is linked to the reduced interfacial tension of the chicken white soup (Fig. 5A). However, when fat addition reached 2.5 %, $D_{4,3}$ values rose, suggesting that with greater fat additions, there wasn't enough protein in the emulsion to coat the fat droplets. Consequently, some fat droplets re-aggregated into clusters more susceptible to aggregation and bridging, adversely impacting the emulsification system's stability.

Stability coefficient and surface protein coverage of chicken white soup

A larger stability coefficient signifies better emulsification performance and superior emulsion stability. As shown in Fig. 2, the emulsification stability of chicken white soup initially increased with added fat but later decreased. The stability coefficient for the 2.0 % fat addition was the highest, which may be attributed to the continuous agitation and cooking during the heating process promoting the emulsification of proteins and fat. At a fat addition of 2.5 %, the stability coefficient decreased, but not significantly when compared to the 2.0 % fat addition ($P > 0.05$). This suggests that higher amounts of fat are likely to induce emulsion aggregation and bridging, which in turn can increase the particle size of the emulsion (Zhou, Sun, Xu, Liu, & Shao, 2018).

Surface protein coverage can also serve as an indicator of the stability of the chicken white soup emulsion system. As depicted in Fig. 2, fat-treated groups had higher protein surface coverage than the control. This could be attributed to fat promoting protein adsorption onto droplet surfaces, thus elevating protein quantity at the interface. Research has shown that heating can denature proteins, exposing their internal non-polar groups at the molecular surface, which enhances

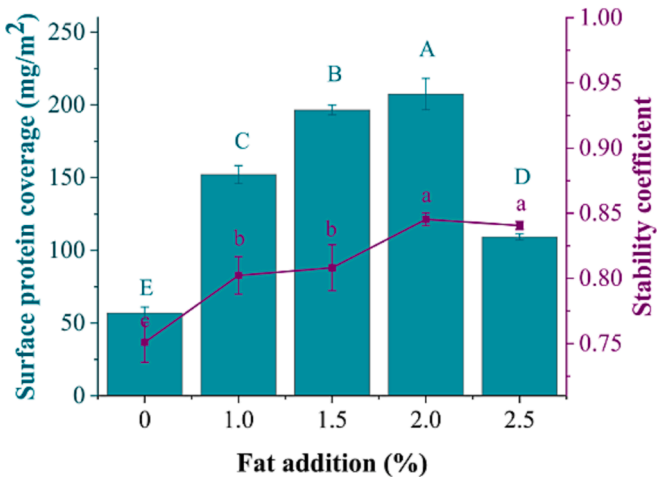

Fig. 2. Effect of different fat additions on surface protein coverage and centrifugal stability of chicken white soup emulsion. Error bars represent standard errors obtained from triplicate sample analysis. Different letters in the same indexes indicate statistically significant differences ($P < 0.05$).

protein water solubility as cooking progresses (Qi et al., 2018). The addition of fat can promote protein adsorption on the fat surface to some extent, resulting in a more uniform and stable emulsion system. However, when fat addition surpassed 2.0 %, surface protein coverage diminished. This suggests that optimal fat addition aids in the formation of a stable chicken white soup emulsion.

LF-NMR imaging

LF-NMR imaging is a simple method to study the distribution of moisture in an emulsion without destroying its structure. The denser the hydrogen protons (H_2O), the stronger the signal, Which means that when more moisture is present, it appears redder or appears (Tang, Gao, Zhang, & Tang, 2021). In pseudocolour images, higher moisture content colors are closer to red, while lower moisture content colors are closer to blue (Cai et al., 2020). In Fig. 3A, a clear dividing line exists between the blue and red parts of different treatment groups, indicating uneven water distribution in the prepared emulsion. However, the red color occupies more than half of the total image in the chicken white soup with 2.0 % fat. This is mainly because the addition of fat increases the stability of the chicken white soup emulsification system, enhancing its spatial and electrostatic repulsion, leading to a more homogeneous emulsion (Cai et al., 2020).

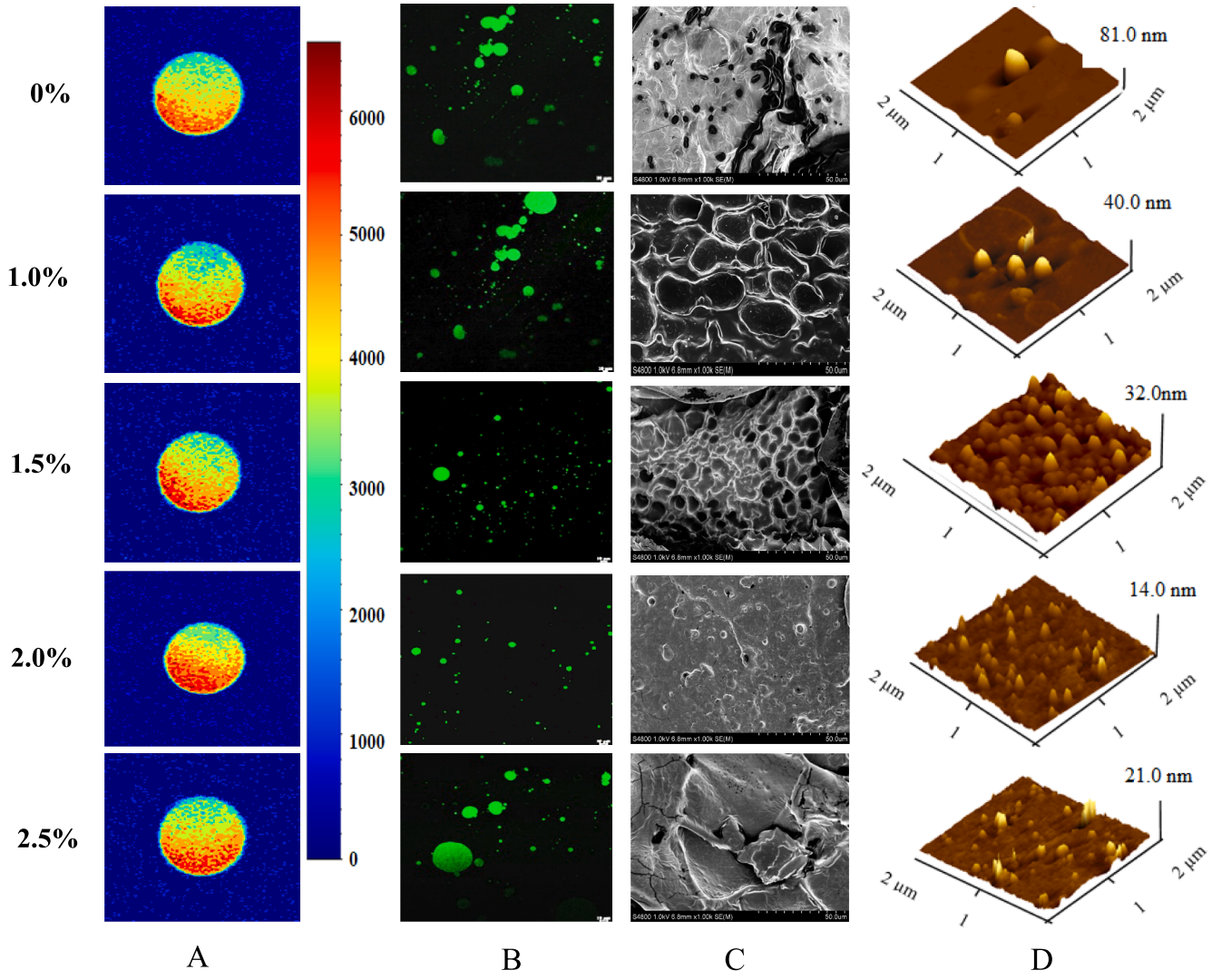

Fig. 3. LF-NMR images (A), CLSM images (B), SEM images (C), and AFM images (D) of chicken white soup emulsion obtained under different fat additions.

Microstructure analysis

Changes of oil droplets in chicken white soup emulsion at different fat additions were observed under CLSM. Nile red, which has a high affinity for fat, labels oil droplets adsorbed on the interface with a green color. In Fig. 3B, as fat addition increased, oil droplets shrank. With 2.0 % fat addition, distribution was most uniform. This can be attributed to the formation of a dense network based on complex particles at the oil–water interface, preventing flocculation and coalescence of emulsions, aligning with the findings of Liu, Shen, Yang, and Lin (2021). When fat addition reached 2.5 %, oil droplets varied in size and were unevenly distributed.

Scanning electron micrographs of chicken white soup samples at different fat additions were shown in Fig. 3C. As fat addition increased, fat filled the chicken white soup emulsion gaps, leading to a uniform and stable emulsion system. This may be due to protein-lipid interaction in the emulsion, with oil droplets evenly distributed in the protein-lipid complex network. Prior research indicated that phospholipids access the globule interface through small gaps in the adsorbed protein layer, forming a compact interfacial layer on the oil droplet surface. With 2.5 % fat addition, the soup particles seemed to aggregate, possibly due to cross-linking between dissolved substances in the soup (Guan et al., 2023).

The chicken white soup emulsion stabilised with different concentrations of fat were evaluated by AFM imaging to obtain the morphological characteristics of the emulsion (Fig. 3D). The chicken white group sample was spherical. As fat quantity increased, the spherical substance dispersed, and chicken white soup with 2.0 % fat formed a uniform and stable dispersion system. When fat addition reached 2.5 %, chicken white soup emulsion particles appeared as larger, irregular shapes, potentially because the emulsion's oil phase increased the rough surface (Liu et al., 2021).

Analyisis rheological properties

As shown in Fig. 4A, due to the interaction between protein molecules and other molecules such as fat particles, the viscosity of chicken white soup emulsion decreased steadily with shear rate until stabilizing, indicating general shear thinning behavior. At an identical shear rate, the viscosity of 2.0 % fat addition was the highest. Earlier studies demonstrated that higher emulsion viscosity reduces protein aggregate formation, slows emulsion particle settlement or aggregation, and thus enhances emulsion stability (Zhu et al., 2021). Additionally, increasing viscosity can slow oil droplet movement in the emulsion, lessen oil droplet collisions, and improve emulsion stability. Emulsion stabilization primarily stemmed from viscosity increases. With 2.5 % fat addition, viscosity decreased, possibly because excessive fat hindered chicken soup emulsification, leading to larger particle creation (Zou, Xu, Zou, & Yang, 2021). Research also suggested soup viscosity could be influenced by particle size and interactions, with finer particles disperse better in soup, resulting in higher viscosities (Zhu et al., 2021). These outcomes align with this study's microscopic imaging findings.

In Fig. 4B, chicken white soup emulsion's shear stress grew as the shear rate increased. The nonlinear relationship between shear stress

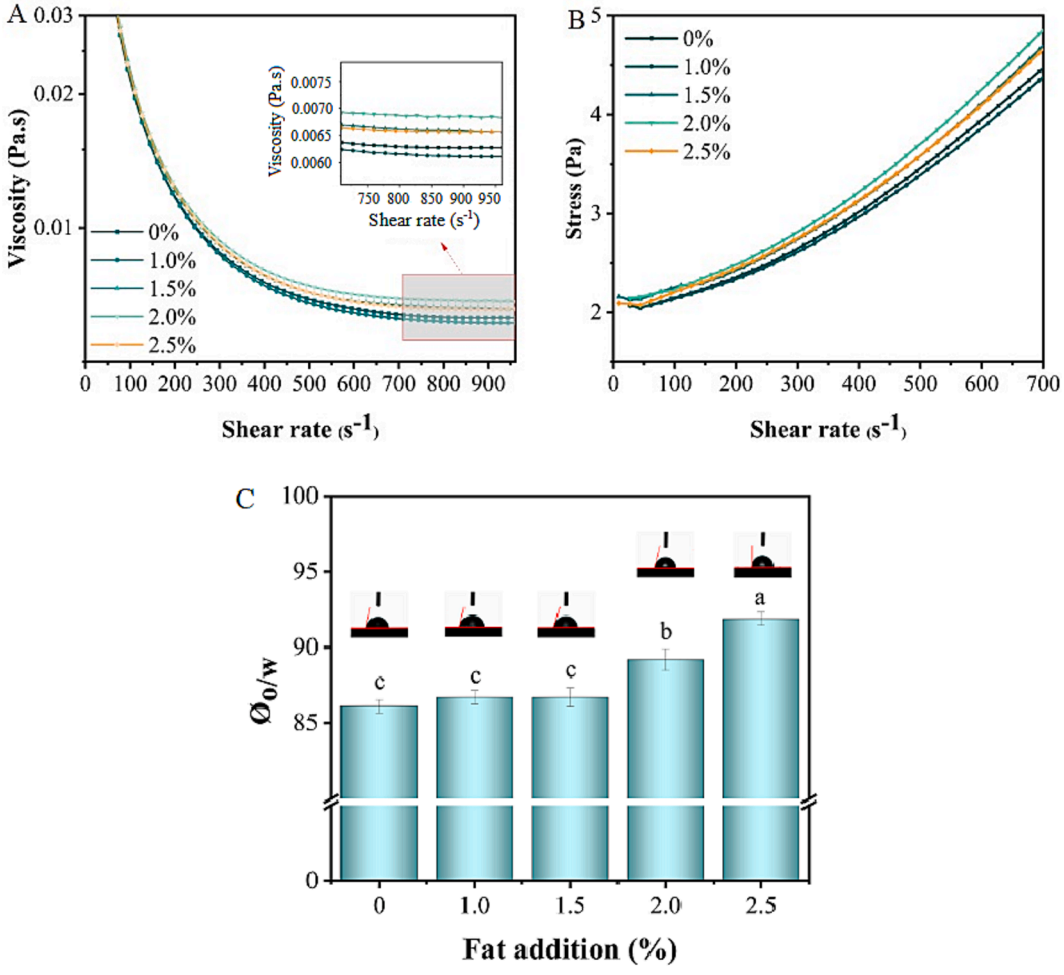

Fig. 4. Effect of different fat additions on viscosity (A), shear stress (B), and contact angle (C) of chicken white soup emulsion. Error bars represent standard errors obtained from triplicate sample analysis. Different letters indicate statistically significant differences ($P < 0.05$).

and shear rate suggests that chicken white soup is a non-Newtonian fluid (Rajinder, 1996). Additionally, the shear stress curve doesn't intersect with the origin, signifying the existence of yield stress in the chicken white soup emulsion. This implies that macromolecule interactions in the soup and particle aggregation create a weak network structure, the disruption of which requires overcoming the yield stress. At an identical shear rate, 2 % fat-added chicken white soup displayed the highest yield stress, signifying greater stability than other samples.

Contact angle denotes the angle where the gas–liquid interface tangent meets the solid–liquid interface at the gas, liquid, and solid intersection. A hydrophilic surface has a contact angle under 90°, whereas a hydrophobic one exceeds 90° (Jayaramulu et al., 2019). Owing to oil's hydrophobic qualities, adding various oils is generally believed to enhance protein membrane surface hydrophobicity (Hu et al., 2021). As shown in Fig. 4C, with the increase of fat addition, the contact angle size gradually increased. This result was consistent with the findings of Liu et al. (2021) that the contact angle of the film surface increases with the increase of oil phase in the emulsion. The contact angle of the 2.0 % fat addition treatment group was close to 90°, showing that its emulsion stability is good. A suitable wettability (close to 90°) with more hydrophobic groups on the surface favours the formation of a stronger mechanical layer around oil droplets, which could provide long-term stability of an emulsion against coalescence (Gao et al., 2023).

Analyses of interfacial properties

Analyses of interfacial expansion rheology

One of the methods used to characterize the interfacial behaviour of emulsions is interfacial tension. This method is crucial for studying the aggregation behavior and stability of chicken white soup emulsion protein at the oil–water interface. As shown in the Fig. 5A, the interfacial tension of all samples showed a decreasing trend at the measurement's initial stage, then it gradually leveled off until it became stable. This behavior might be attributed to the diffusion of small molecules from the droplet to the oil–water interface and the subsequent structural rearrangement of proteins adsorbed at the interface. Once the diffusion of molecules at the interfacial layer reached saturation, the interaction between the interfacial layers stabilized. Research indicates that during the emulsification process, a faster protein molecule adsorption rate at the oil–water interface leads to lower interfacial tension, which is more favorable for emulsification (Ravera et al., 2020). As observed in Fig. 5A, the chicken white soup with 2.0 % fat addition exhibited the most rapid decline in interfacial tension, suggesting its emulsification effect was optimal.

In Fig. 5B, the dynamic change of interfacial pressure of chicken white soup protein at the O/W interface is displayed. The interfacial pressure of all samples surged within the first 1000 s of the interfacial adsorption process. Following this phase, the interfacial pressure rose slowly and remained nearly constant. This suggests that the protein adsorbed on the oil–water interface underwent a structural rearrangement, leading to saturation adsorption at the interfaces (Qi et al., 2020). The most pronounced increase in interfacial pressure was noted with a 2.0 % fat addition. This could be because an optimal fat addition enhances the attraction of adsorbed proteins at the oil–water interface, resulting in increased interfacial pressure.

Dynamic adsorption at the oil–water interface mainly involves diffusion, penetration, and reorganisation. The square root of time ($Ø^{1/2}$) dependence of interfacial pressure (π) for chicken white soup emulsion protein adsorbed layers at the oil–water interface was shown in

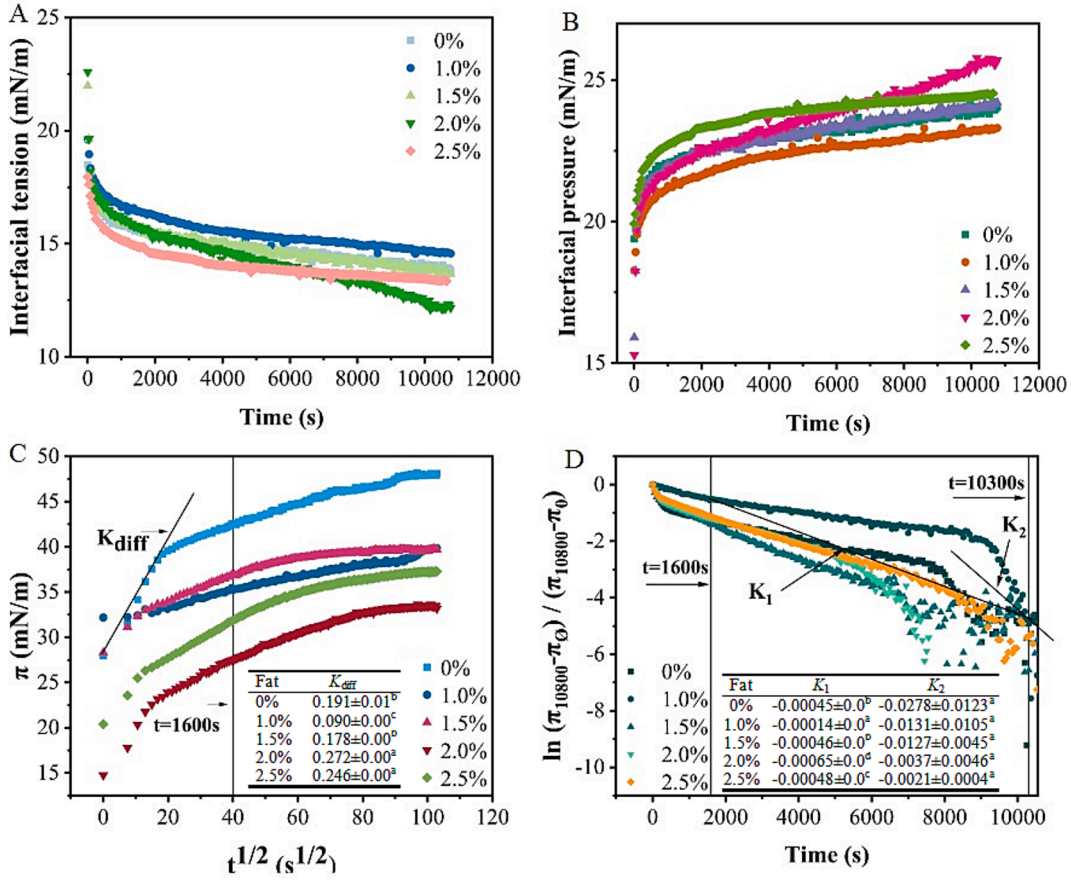

Fig. 5. Effect of different fat additions on interfacial tension (A), interfacial pressure (B), interfacial adsorption rate (C), and penetration and rearrangement rate (D) of chicken white soup emulsion.

Fig. 5C. When the adsorption process is governed by protein diffusion, the plot of π versus $t^{1/2}$ is linear, with the slope representing the diffusion rate (K_{diff}) (Perez et al., 2009). In Fig. 5C, as adsorption time progressed, the π values rose, indicating a growing quantity of protein at the oil–water interface. Concerning the correlation between interfacial pressure (π) and adsorption time ($t^{1/2}$) at the oil–water interface, the addition of fat had a significant effect on the diffusion rate of chicken white soup emulsion protein at the interface, especially the K_{diff} at 2.0 % fat addition was markedly higher than other groups, signifying a considerable enhancement in the diffusion rate.

Furthermore, as shown in Fig. 5D, there are two linear segments. Typically, the first segment corresponds to the molecular permeation phase, while the second pertains to the molecular rearrangement phase. The first gradient is generally perceived as the first-order rate constant for permeation (K_1), which relates to inhibition the permeation and swelling of protein molecules at the oil–water interface. In contrast, the second gradient represents the first-order rate constant for molecular rearrangement (K_2) and correlates with the molecular repositioning of protein molecules at the interface (Perez et al., 2009). The first linear region has a negative rate constant (K_1), indicating that the extension of the chicken white soup emulsion protein at the oil–water interface diminishes over time (Qu, Wang, Zhao, Liu, & Jiang, 2021). The rate constants in both linear segments are negative in the permeation and molecular rearrangement phases, with the absolute value of K_1 being notably smaller than K_2. This points to increased diffusion, reduced rearrangement time, and augmented interfacial stability (Ravera, Loglio, & Kovalchuk, 2010). The K_{diff} value of chicken white soup emulsion protein at 2.0 % fat addition were larger indicating that the protein diffusion rate is fast at these fat addition.

Dilatational rheological properties of interfacial membranes

The interfacial rheology of adsorbed layers serves as an indicator of the surfactants and proteins adsorbed at the oil–water interface and their interactions. The viscoelastic properties of protein adsorbed layers at the oil–water interface predict the emulsion's stability. The surface dilatational modulus (E) reflects the mechanical strength of the absorbed layer at the protein interface. The dynamic dilatational properties of chicken white soup emulsions with varied fat additions are illustrated in Fig. 6A. The E values for all samples rose with increasing π, suggesting that more proteins were adsorbed at the oil–water interface and more interactions took place (Wang et al., 2019). The slopes of the E-π curves can inform about the adsorption quantity and molecular interaction degree (Maldonado-Valderrama and Patino, 2010). The slopes of the E-π curves were all greater than 1, indicating a strong interaction between the protein and the oil–water interface at this time, reflecting the non-ideal behaviour of the strong interaction between the proteins adsorbed at the oil–water interface. However, the slopes of the E-π curves of the chicken white soup emulsion protein solutions increased with the increase of fat from 0 % to 2.0 %, and then decreased with the continuous increase of fat from 2.0 % to 2.5 %. Among all smaples, the modulus with a 2.0 % fat addition surpassed those of other groups, suggesting the thickest interfacial membrane.

Fig. 6B displays the variation curves of the modulus of dilatational elasticity in the interfacial layer of chicken white soup emulsion protein over time for different fat levels. The E_d values for all samples showed a consistent rise with extended adsorption time, indicating a steady enhancement in the interfacial membrane's elasticity. This increase is largely attributed to protein adsorption at the interface and the formation of intermolecular interactions. Research indicates that intensified interactions among molecules at the oil–water interface, such as electrostatic repulsion, spatial resistance and hydrophobic interactions, can alter the proteins' native conformation in the molecules (Zhu et al., 2020b). This alteration facilitates their unfolding, rearrangement, and cross-linking at the interface, producing an interfacial membrane of specific thickness and viscoelasticity, thereby augmenting interfacial viscoelasticity. As evident from Fig. 6B, fat addition significantly influenced interfacial elasticity, with the 2.0 % fat addition exhibiting greater elasticity than other groups.

Conclusion

The fat and some water-soluble proteins in the chicken skeleton dissolved upon boiling, while the emulsifying protein served as an emulsifier, creating a stable and homogeneous dispersion of fine particles and forming an oil-in-water emulsion. The degree of emulsification of the soup correlates with its fat and protein content, the chicken white soup emulsion showed high stability with an addition of 2.0 % fat. Additionally, the effect of protein additions on the stability of chicken soup emulsion will be reported in another manuscript. This study provides a practical means to improve the stability of chicken white soup. Meanwhile, it also offers a comprehensive reference for promoting the high-value utilization of livestock bone. However, the process of interfacial membrane formation in the chicken white soup emulsion warrants further investigation.

CRediT authorship contribution statement

Haining Guan: Software, Methodology, Conceptualization. **Chunmei Feng:** Writing – original draft, Formal analysis. **Yanli Tian:** Software, Data curation. **Siqi Leng:** Investigation, Formal analysis. **Shifa Zhao:** Software, Formal analysis. **Dengyong Liu:** Supervision, Funding acquisition. **Xiaoqin Diao:** Writing – review & editing, Methodology.

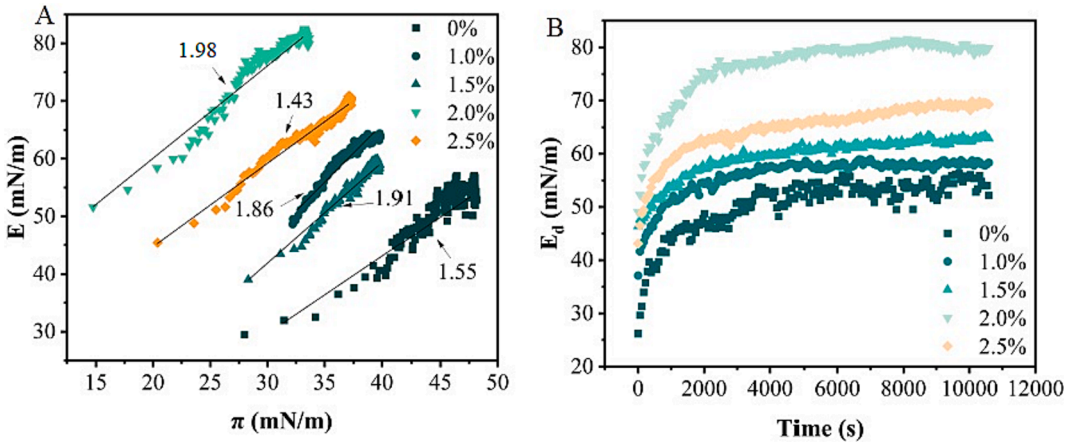

Fig. 6. Effect of different fat additions on surface dilatational modulus (E) as a function of surface pressure (π) (A) and timedependent dilatational elasticity (Ed) (B) in chicken white soup emulsion.

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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References

Cai, X. R., Du, X. F., Zhu, G. L., & Cao, C. (2020). Induction effect of nacl on the formation and stability of emulsions stabilized by carboxymethyl starch/xanthan gum combinations. *Food Hydrocolloids, 105*, Article 105776. https://doi.org/10.1016/j.foodhyd.2020.105776

Dong, X. B., Li, X., Zhang, C. H., Wang, J. Z., Tang, C. H., Sun, H. M., & Chen, L. L. (2014). Development of a novel method for hot-pressure extraction of protein from chicken bone and the effect of enzymatic hydrolysis on the extracts. *Food Chemistry, 157*, 339–346. https://doi.org/10.1016/j.foodchem.2014.02.043

Duan, N. N., Zhang, T. R., Liao, Y. H., & Zhou, X. H. (2019). Study on relativity of polypeptides sequence and emulsifying properties in white chicken broth. *Journal of Food Science and Technology, 37*(1), 41–46. https://doi.org/10.3969/j.issn. 2095-6002, 2019.01.007

Gao, J. B., Qiu, Y. H., Chen, F., Zhang, L. J., Wei, W., An, X. Y., & Zhu, Q. M. (2023). Pomelo peel derived nanocellulose as Pickering stabilizers: Fabrication of pickering emulsions and their potential as sustained-release delivery systems for lycopene. *Food Chemistry, 415*, Article 135742. https://doi.org/10.1016/J. FOODCHEM.2023.135742

Gavahian, M., Chen, Y. M., Khaneghah, A. M., Barba, F. J., & Yang, B. B. (2018). In-pack sonication technique for edible emulsions: understanding the impact of acacia gum and lecithin emulsifiers and ultrasound homogenization on salad dressing emulsions stability. *Food Hydrocolloids*, 83, 79-87. https://doi.org/10. 1016/j.foodhyd.2018.04.039.

Guan, H. N., Yang, C., Tian, Y. L., Feng, C. M., Gai, S. M., Liu, D. Y., & Diao, X. Q. (2023). Changes in stability and volatile flavor compounds of self-emulsifying chicken soup formed during the stewing process. *LWT-Food Science and Technology, 175*, Article 114520. https://doi.org/10.1016/J.LWT.2023.114520

Guo, H. H., Hong, Z., & Yi, R. Z. (2015). Core-shell collagen peptide chelated calcium/calcium alginate nanoparticles from fish scales for calcium supplementation. *Journal of Food Science, 80*(7), N1595–N1601. https://doi.org/10.1111/1750-3841.12912

He, W., Zhu, W. H., Bu, Y., Wang, W. H., Li, X. P., Li, J. R., & Zhang, Y. Y. (2022). Formation of colloidal micro-nano particles and flavor characteristics of Greenland halibut bone soup. *Journal of Food Science, 87*(1), 216–230. https://doi.org/10.1111/1750-3841.15979

Hebishy, E., Buffa, M., Guamis, B., Blasco-Moreno, A., & Trujillo, A. J. (2015). Physical and oxidative stability of whey protein oil-in-water emulsions produced by conventional and ultra high-pressure homogenization: Effects of pressure and protein concentration on emulsion characteristics. *Innovative Food Science & Emerging Technologies, 32*, 79–90. https://doi.org/10.1016/j.ifset.2015.09.013

Hu, Y. Y., Shi, L. F., Ren, Z. Y., Hao, G. X., Chen, J., & Weng, W. Y. (2021). Characterization of emulsion films prepared from soy protein isolate at different preheating temperatures. *Journal of Food Engineering, 309*(8), Article 110697. https://doi.org/10.1016/j.jfoodeng.2021.110697

Jayaramulu, K., Geyer, F., Schneemann, A., Kment, S., Otyepka, M., Zboril, R., & Fischer, R. A. (2019). Hydrophobic metal-organic frameworks. *Advanced Materials, 31*(32), 1900820. https://doi.org/10.1002/adma.201900820

Liu, Z., Shen, R., Yang, X. B., & Lin, D. H. (2021). Characterization of a novel konjac glucomannan film incorporated with Pickering emulsions: Effect of the emulsion particle sizes. *International Journal of Biological Macromolecules, 179*, 377–387. https://doi.org/10.1016/j.ijbiomac.2021.02.188

Lu, J. Y., Wang, Y., Cao, W. H., Yan, Y., Guo, F. X., Li, J. L., & Li, W. W. (2024). Stability and gastrointestinal behavior of curcumin-loaded emulsion stabilized by multi-conformation soy proteins: Influence of oil volume fraction. *Food Chemistry, 440*, Article 138215. https://doi.org/10.1016/j.foodchem.2023.138215

Maldonado-Valderrama, J., & Patino, J. M. R. (2010). Interfacial rheology of protein–surfactant mixtures. *Current Opinion in Colloid & Interface Science, 15*(4), 271–282. https://doi.org/10.1016/j.cocis.2009.12.004

Perez, A. A., Carrara, C. R., Sánchez, C. C., Santiago, L. G., & Rodríguez Patino, J. M. (2009). Interfacial dynamic properties of whey protein concentrate/ polysaccharide mixtures at neutral pH. *Food Hydrocolloids, 23*(5), 1253–1262. https://doi.org/10.1016/j.foodhyd.2008.08.013

Qi, J., Jia, C. K., Zhang, W. W., Yan, H. M., Cai, Q. Y., Yao, X. N., & Li, M. Q. (2023). Ultrasonic-assisted stewing enhances the aroma intensity of chicken broth: A perspective of the aroma-binding behavior of fat. *Food Chemistry, 398*, Article 133913. https://doi.org/10.1016/J.FOODCHEM.2022.133913

Qi, J., Wang, H. H., Zhang, W. W., Deng, S. L., Zhou, G. H., & Xu, X. L. (2018). Identification and characterization of the proteins in broth of stewed traditional Chinese yellow-feathered chickens. *Poultry Science, 97*(5), 1852–1860. https://doi.org/10.3382/ps/pey003

Qi, J., Xu, Y., Xie, X. F., Zhang, W. W., Wang, H. H., Xu, X. L., & Xiong, G. Y. (2020). Gelatin enhances the flavor of chicken broth: A perspective on the ability of emulsions to bind volatile compounds. *Food Chemistry, 333*, Article 127463. https://doi.org/10.1016/j.foodchem.2020.127463

Qi, J., Yan, H. M., Zhang, W. W., Yao, X. N., Cai, Q. Y., Xu, K., & Xiong, G. Y. (2023). Thermal-assisted stirring as a new method for manufacturing o/w emulsions stabilized by gelatin-arginine complexes. *Journal of Food Engineering, 339*, Article 111261. https://doi.org/10.1016/j.jfoodeng.2022.111261

Qu, D. N., Wang, S. N., Zhao, H. K., Liu, H., Zhu, D. S., & Jiang, L. Z. (2021). Structure and interfacial adsorption behavior of soy hull polysaccharide at the oil/water interface as influenced by pH. *Food Hydrocolloids, 116*, Article 106638. https://doi.org/10.1016/j.foodhyd.2021.106638

Rajinder, P. (1996). Effect of droplet size on the rheology of emulsions. *AIChE Journal, 42*(11), 3181–3190. https://doi.org/10.1002/aic.690421119

Ravera, F., Dziza, K., Santini, E., Cristofolini, L., & Liggieri, L. (2020). Emulsification and emulsion stability: The role of the interfacial properties. *Advances in Colloid and Interface Science, 288*, Article 102344. https://doi.org/10.1016/j.cis.2020.102344

Ravera, F., Loglio, G., & Kovalchuk, V. I. (2010). Interfacial dilational rheology by oscillating bubble/drop methods. *Current Opinion in Colloid & Interface Science, 15*(4), 217–228. https://doi.org/10.1016/j.cocis.2010.04.001

Sullivan, A. P., & Kilpatrick, P. K. (2002). The effects of inorganic solid particles on water and crude oil emulsion stability. *Industrial & Engineering Chemistry Research, 41*(14), 3389–3404. https://doi.org/10.1021/ie010927n

Tang, Y., Gao, C. C., Zhang, Y., & Tang, X. Z. (2021). The microstructure and physiochemical stability of Pickering emulsions stabilized by chitosan particles coating with sodium alginate: Influence of the ratio between chitosan and sodium alginate. *International Journal of Biological Macromolecules, 183*, 1402–1409. https://doi.org/10.1016/j.ijbiomac.2021.05.098

Urbina-Villalba, G. (2009). An algorithm for emulsion stability simulations: Account of flocculation, coalescence, surfactant adsorption and the process of ostwald ripening. *International Journal of Molecular Sciences, 10*(3), 761–804. https://doi.org/10.3390/IJMS10030761

Wang, Y. N., Wang, R. C., Li, Y., & Zhang, L. B. (2023). Beef tallow/lard blends in O/W emulsions: Characterization of fat crystals, partial coalescence, rheology, and aeration performance. *Food Research International, 172*, Article 113140. https://doi.org/10.1016/J.FOODRES.2023.113140

Wang, S., Yang, J., Shao, G., Qu, D., Zhao, H., Zhu, L., & Zhu, D. (2019). Dilatational rheological and nuclear magnetic resonance characterization of oil-water interface: Impact of pH on interaction of soy protein isolated and soy hull polysaccharides. *Food Hydrocolloids, 99*, Article 105366. https://doi.org/10.1016/j.foodhyd.2019.105366

Wang, Q., Zhu, Y., Ji, Z. C., & Chen, J. S. (2021). Lubrication and sensory properties of emulsion systems and effects of droplet size distribution. *Foods, 10*, 3024. https://doi.org/10.3390/foods10123024

Xiong, W. F., Ren, C., Li, J., & Li, B. (2018). Characterization and interfacial rheological properties of nanoparticles prepared by heat treatment of ovalbumin-carboxymethylcellulose complexes. *Food Hydrocolloids, 82*, 355–362. https://doi.org/10.1016/j.foodhyd.2018.03.048

Yan, H. M., Song, Y., Yu, M. M., Yao, X. N., Zhang, W. W., Xu, Y., & Hu, Y. (2024). Aspartic acid/arginine enhance the stability of gelatin emulsions. *Journal of Food Engineering, 361*, Article 111735. https://doi.org/10.1016/j.jfoodeng.2023.111735

Yang, H. J., Han, M. Y., Wang, H. F., Cao, G. T., Tao, F., Xu, X. L., & Shen, Q. (2021). HPP improves the emulsion properties of reduced fat and salt meat batters by promoting the adsorption of proteins at fat droplets/water interface. *LWT-Food Science and Technology, 137*, Article 110394. https://doi.org/10.1016/j.lwt.2020.110394

Yue, J. Y., Wang, J. Z., Zhang, C. H., Jia, W., Li, X., & Sun, Z. (2017). Effects of hot-pressure extraction time on composition and gelatin properties of chicken bone extracts. *Journal of Food Science, 82*(5), 1066–1075. https://doi.org/10.1111/1750-3841.13687

Zhang, J., Yao, Y., Ye, X., Fang, Z., Chen, J., Wu, D., & Hu, Y. (2013). Effect of cooking temperatures on protein hydrolysates and sensory quality in crucian carp (Carassius auratus) soup. *Journal of Food Science and Technology, 50*, 542–548. https://doi.org/10.1007/s13197-011-0376-2

Zhao, Y., Ren, Z. Y., Shi, L. F., & Weng, W. Y. (2023). Effect of W/O pre-emulsion prepared with different emulsifiers on the physicochemical properties of soy protein isolate-based emulsion films. *Food Hydrocolloids, 139*, Article 108440. https://doi.org/10.1016/J.FOODHYD.2022.108440

Zhou, F., Sun, D., Xu, S. Q., Liu, D. Y., & Shao, J. H. (2018). Effect of unsaturated fatty acids on rheological and physical properties of pork protein-stabilized emulsions. *Food Science, 39*(24), 34–40. https://doi.org/10.7506/spkx1002-6630-201824006

Zhu, C. Z., Jin, H. Q., Yin, F., Cui, W. M., Zhang, Q. H., & Zhao, G. M. (2021). Emulsion-forming properties of heat-induced pork myofibrillar protein affected by NaCl. *International Journal of Food Science & Technology, 56*(6), 3016–3025. https://doi.org/10.1111/IJFS.14945

Zhu, L. J., Xu, Q. Y., Liu, X. Y., Xu, Y. Y., Yang, L. N., Wang, S. N., ... Liu, H. (2020 a). Soy glycinin-soyasaponin mixtures at oil–water interface: Interfacial behavior and O/W

emulsion stability. *Food Chemistry*, 327, 127062. Doi: 10.1016/j.foodchem.2020.127062.

Zhu, L. J., Xu, Q. Y., Liu, X. Y., Xu, Y. Y., Yang, L. N., Wang, S. N., ... Liu, H. (2020 b). Oil-water interfacial behavior of soy β-conglycinin-soyasaponin mixtures and their effect on emulsion stability. *Food Hydrocolloids*, *101*(3), 105531. Doi: 10.1016/j.foodhyd.2019.105531.

Zou, J., Xu, M. J., Zou, Y. F., & Yang, B. (2021). Chemical compositions and sensory characteristics of pork rib and Silkie chicken soups prepared by various cooking techniques. *Food Chemistry, 345*, Article 128755. https://doi.org/10.1016/j.foodchem. 2020.128755