Structure, rheology, and functionality of emulsion-filled gels: Effect of various oil body concentrations and interfacial compositions

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A B S T R A C T
The purpose of this study is to investigate the impact of varied oil body (OB) concentrations and interfacial compositions on the network topology and rheological and functional aspects of composite whey protein isolate (WPI) gels. Particle size and ζ-potential analyzes of the mixed gel solutions containing the OBs extracted at pH 6.8 (6.8-OB) and 11.0 (11.0-OB) revealed a greater aggregation in the 6.8-OB-containing mixed gel solution. 6.8-OB and 11.0-OB generated particle aggregates and oil-drop-embedded network architectures in the WPI gel, respectively. FT-IR analyses showed that OBs stabilized the protein gels’ polymeric matrix by hydrogen bonding, steric hindrance, and hydrophobic interactions. Rheology and texture showed that OBs hardened gels. Low-field nuclear magnetic resonance showed that excessive inclusion of OBs (30% of 6.8-OB and 35% of 11.0-OB) compromised gel integrity and freeze-thaw stability. This study found that OBs can be active fillers in protein gels for functional meals.

I N T R O D U C T I O N

Most foods exist as solids or semi-solids, allowing convenient packaging, transportation, and storage. A protein gel is a soft solid widely used in the field of food. Ordinary protein gels usually have an internal network structure devoid of support and can easily be destroyed by environmental factors. Fat particles have been previously filled into the network space of protein gels to improve their textures and water holding capacities (WHCs) (Oliver, Berndsen, van Aken, & Scholten, 2015; Oliver, Scholten, & van Aken, 2015). Such a gel with a protein structure containing emulsified oil drops is called an emulsion-filled gel (Torres, Murray, & Sarkar, 2016). In the absence of droplet–matrix interactions, the droplet acts as an inactive filler and weakens the texture of the gel. In contrast, when the droplet interacts with the matrix, it acts as an active filler and significantly affects the gel structure, improving the gels properties and quality of the protein-gel foods (Dickinson, 2012; Farjami & Madadlou, 2019).

Whey protein isolate (WPI) is widely used in gel-based foods because of its good gel properties and nutritional value. However, WPI production increases greenhouse gas emissions and involves significant land use. Therefore, there is an urgent need to replace animal protein with plant protein in food to meet the needs of the global population and environment. As organelles for storing lipids in plant seeds, oil bodies (OBs) contain internal triglycerides and have surfaces wrapped with monolayer phospholipid molecules and intrinsic proteins (mainly 24, 18, and 16 kDa oleosin) (Huang, 1992). Because of their unique structure, OBs can achieve natural emulsion properties without requiring emulsifiers or multiple homogenization processes, and they can be used in the food industry to replace fat droplets in food. There have been many reports on the potential of the natural emulsification of OBs in dairy product formulation (Naziri et al., 2017). Therefore, OBs can be used as a fat substitute in dairy products because of their similarity to milk fat globules.

However, there have been few reports on emulsion-filled gels incorporating OBs (Kirimlidou, Matsakidou, Scholten, Nikiforidis, & Kiosseoglou, 2017; Yang et al., 2020). These reports prove that OBs can interact with the matrix and act as active fillers. OBs are usually extracted via an aqueous extraction method. When soybean seeds are ground, a large number of extrinsic proteins (lipoxygenase, soybean globulin, β-conglycinin, γ-conglycinin, and BD 30 K) are released. Zhao,
**Preparation of OB emulsion-filled WPI gels**

The two types of OBs were dissolved in the mixed gel solutions to form 15, 20, 25, 30, and 35 wt% dispersions, and WPI was dissolved in 20 mL of each of these dispersions such that its concentration was 8 wt%. The mixed gel solutions were stirred for 30 min at 25 °C at 500 rpm. The pH of the samples was adjusted to 7.0. The mixed gel solutions were heated for 15 min in a 90 °C-water bath, cooled at 4 °C, and stored for 24 h. The gels with 6.8-OB concentrations of 15, 20, 25, 30, and 35 wt% were designated as 6.8–15, 6.8–20, 6.8–25, 6.8–30, and 6.8–35, respectively, and the gels with 11.0-OB contents of 15, 20, 25, 30, and 35 wt% as 11.0–15, 11.0–20, 11.0–25, 11.0–30, and 11.0–35, respectively. The control sample was prepared similarly but without the addition of the OB cream. An overview of the control groups of the hydrogel and emulsion-filled gels is shown in Table 1.

### Physical properties and structural characteristics

**Particle size and ζ-potential measurements**

The particle sizes of the mixed gel solutions were determined using a Zetasizer Nano-ZS90 dynamic light scattering instrument (Malvern Instrument Co., Ltd., Worcestershire, UK) at pH 7.0 in PBS (1:1000 v/v) to minimize the potential effect of multiple scattering before heating in a water bath. The refractive index of the emulsion droplets was 1.46, whereas that of the water-dispersion medium was 1.33. The volume-averaged particle sizes D_{4,3} were measured at room temperature. ζ-potential measurements were conducted using a zeta-potential analyzer (Zeta Plus, Malvern, U.K.). The mixed gel solutions were diluted with PBS at a ratio of 1:100 (v/v), and the diluted mixed gel solutions were examined at 100 V by placing the sample.

### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

To prepare the SDS-PAGE samples, 5 % (v/v) lyophilized OB-associated proteins were mixed with a buffer comprising 10 % glycercin, 0.0625 M Tris-HCl, 5 % 2-mercaptoethanol, 1 % SDS, and 0.0025 % bromophenol blue. The mixture was heated for 5 min at 100 °C in a water bath. Electrophoresis was performed using 12 % stacking gel and 8 % resolving gel.

### Preparation of OB emulsion-filled gels

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Materials and methods

**Materials**

Mature soybeans were purchased from the Great Northern Wilderness Co., Ltd. (Kenhong-34 cultivar, Harbin, China); WPI from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China); and Nile red and Nile blue from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China). All other chemicals used were of analytical grade.

**Preparation of OBs via aqueous extraction at pH 6.8 and 11.0**

Soybean OBs were isolated as described by Zhao, Chen, Yan, Kong, and Hua, (2016), with minor modifications. Soybeans (20 g) were soaked in deionized (DI) water (1:5, w/v) for 18 h at 4 °C before being homogenized in the same medium for 3 min using a tissue crusher (Multifunctional Tissue Crusher, Demashi, ltd., Beijing, China). The resulting soybean slurry was filtered through four layers of cotton gauze to remove insoluble components. Sucrose (20 %) was then added to the filtrate, and the filtrate and sucrose were stirred for 15 min at 4 °C. The filtrate after adding sucrose was divided into two equal parts, and the pH was adjusted to 6.8 and 11.0, respectively. The solution was centrifuged at 9000 rpm and 4 °C for 30 min after adjusting the pH. The centrifuges were then placed into a separatory funnel and left to settle for 5 min, after which the creams were procured. The creams were separated and cleaned by dispersing them in 1:8 (w/w) sucrose solutions to 20 % w/v, and their pH were readjusted to 6.8 and 11.0. To separate any remaining OBs, the mixtures were centrifuged at 9000 rpm for 30 min at 4 °C. This operation was performed twice to remove contaminants from the OBs. The sucrose was removed from the OB dispersion by dissolving OB samples in DI water pH-adjusted to 6.8 and 11.0. The mixture was centrifuged, and the OB layer was collected as described above. This process was repeated thrice. The OBs extracted at pH 6.8 and 11.0 were designated as 6.8-OB and 11.0-OB, respectively.

| Code       | Sample type                          | Gel matrix | Filler |
|------------|--------------------------------------|------------|--------|
| 6.8-OB     | OB cream prepared via aqueous extraction at pH 6.8 | –          | –      |
| 11.0-OB    | OB cream prepared via aqueous extraction at pH 11.0 | –          | –      |
| Control    | WPI protein hydrogel                  | 8 wt%      | WPI    |
| 6.8–15     | Emulsion-filled gel                   | 8 wt%      | 15 wt% 6.8- WPI |
|            |                                      | 8 wt%      | OB     |
| 6.8–20     | Emulsion-filled gel                   | 8 wt%      | 20 wt% 6.8- WPI |
|            |                                      | 8 wt%      | OB     |
| 6.8–25     | Emulsion-filled gel                   | 8 wt%      | 25 wt% 6.8- WPI |
|            |                                      | 8 wt%      | OB     |
| 6.8–30     | Emulsion-filled gel                   | 8 wt%      | 30 wt% 6.8- WPI |
|            |                                      | 8 wt%      | OB     |
| 6.8–35     | Emulsion-filled gel                   | 8 wt%      | 35 wt% 6.8- WPI |
|            |                                      | 8 wt%      | OB     |
| 11.0–15    | Emulsion-filled gel                   | 8 wt%      | 15 wt% 11.0- WPI |
|            |                                      | 8 wt%      | OB     |
| 11.0–20    | Emulsion-filled gel                   | 8 wt%      | 20 wt% 11.0- WPI |
|            |                                      | 8 wt%      | OB     |
| 11.0–25    | Emulsion-filled gel                   | 8 wt%      | 25 wt% 11.0- WPI |
|            |                                      | 8 wt%      | OB     |
| 11.0–30    | Emulsion-filled gel                   | 8 wt%      | 30 wt% 11.0- WPI |
|            |                                      | 8 wt%      | OB     |
| 11.0–35    | Emulsion-filled gel                   | 8 wt%      | 35 wt% 11.0- WPI |
|            |                                      | 8 wt%      | OB     |

“–” means not added.
5 % separating gel at 80 and 120 V, respectively. After electrophoresis, the 0.25 % Coomassie Brilliant Blue R-250 was used for staining gels for 30 min. Finally, the gel was destained using 10 % (v/v) acetic acid and photographed using a gel imager (Gel Doc™, Bio-Rad, California, USA) to visualize the protein bands. The molecular weights of the marker proteins (i.e., standard protein markers) ranged from 15 to 130 kDa.

**Confocal laser scanning microscopy (CLSM)**

A confocal laser scanning microscope (Leica Microsystems, Heidelberg GmbH, Germany) combined with an inverted microscope with a 400 × magnification lens was used for the protein excitation line at 633 nm and oil at 488 nm. The mixed gel solutions (5 mL) were placed in a test tube. Nile blue and Nile red were used to label the proteins and lipids, respectively. The stained sample was placed at the center of a slide with a groove and carefully covered with a cover slide. The slides were then placed in a water bath at 90 °C for 15 min, and the samples were scanned.

**Cryo-scanning electron microscopy (Cryo-SEM)**

The emulsion-filled gels were cut into slices of 1 × 1 × 3 mm³ and fixed for 24 h in 2.5 % glutaraldehyde at pH 7.4. The fixed samples were rinsed thrice in 0.1 mol/L (pH 7.4) PBS at room temperature. The samples were dehydrated in a graded series of ethanol (60 %, 70 %, 80 %, 90 %, and 100 %) for 20 min/step before critical-point drying (EM Integrated Technology, Kent, UK). The samples were soaked in tertiary butanol for 10 min thrice. The treated samples were dried at 20 °C and 80 Pa for 12 h. The emulsion-filled gels were examined using a scanning electron microscope (Se3400N, Hitachi, Japan) at 20 kV accelerating voltage. 5.0 k magnification was used for taking the pictures of each sample.

**Fourier transform infrared (FT-IR) spectroscopy**

The FT-IR spectra of each lyophilized emulsion-filled gel sample were measured using a Nicolet 6700 spectrometer (PerkinElmer, UK) in the wavenumber range 4000–400 cm⁻¹. The lyophilized samples were combined with KBr and pressed into pellets. FT-IR spectra were scanned 64 times and recorded at a resolution of 4 cm⁻¹.

**Rheological evaluation**

The rheological properties of the emulsion-filled gels were determined using the method reported by Feng, Jia, Yan, Yan, and Yin (2021), with a slight modification. The prepared emulsion-filled gels were immediately placed on two parallel plates (40 mm in diameter and 1 mm in height). The samples were then placed in a test cell for 120 s at room temperature and coated with a small amount of silicone oil to prevent the evaporation of water.

The Malvern Rotating Rheometer (Kinexus Prot, Malvern Instrument Co., Ltd., Malvern, UK) was used for measuring the viscosities and viscoelasticity of the emulsion-filled gels. The storage modulus (G’) and loss modulus (G”) were measured by the frequency sweep in the range of 0.1–10 rad/s using a strain of 0.1 %.

**Functional characteristics**

**Textural characteristics measurement**

Textural characteristic measurements can reflect different aspects of the textural properties of the gel. The textural characteristics of the emulsion-filled gels were determined using the method reported by Xu et al. (2022a), with slight modifications. Texture profile analysis (TPA) was performed using a TA-XT2i (Stable Micro Systems Ltd., UK) with a cylindrical probe 36 mm in diameter (P/36). A 10 mL sample of the OB and WPI mixture was transferred to a 25 × 25 mm (diameter × height) weighing bottle, and the emulsion-filled gel was prepared as described in Section 2.3. The samples were cut to sizes of 3.0 × 2.5 cm² (diameter × height). After the sample preparation, the hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience were analyzed: pre-test speed, 1.0 mm/s; test speed, 1.0 mm/s; pro-test speed, 1.0 mm/s; interval between two measurements, 3.0 s; compression deformation, 50 % of the sample; and thixotropic force, 1.0 g.

**Low field-nuclear magnetic resonance (LF NMR) measurements**

LF NMR measurements were performed using an LF NMR analyzer (MesoMR23-060 V-I, Niumag Corporation, Shanghai, China). The emulsion-filled gels (10 g) were placed into an NMR probe (MesoMR23-060H-160 mm) with NMR tube (25 mm in diameter). An LF NMR analyzer was used to measure the transverse relaxation time (T₂). The relaxation components, T₂1, T₂2, and T₂3, were analyzed under the following conditions: sampling frequency 200 kHz, 90° pulse width, 13 μs; 180° pulse width, 25.52 μs; and repetition time between the scans, 2500 ms. The sample was analyzed in triplicates, and each reported value was the average of at least three measurements.

**Freeze–thaw stability**

The hydrogel and emulsion-filled gels in section 2.3 were weighted and poured into culture dishes. The samples were frozen in a refrigerator at −20 °C for 24 h and then thawed at 25 °C. The freeze–thaw cycle was repeated thrice. The samples were centrifuged at 8000 rpm for 20 min to remove free water. The WHCs of the samples subjected to the freeze–thaw cycles were defined as the ratios of the centrifuged emulsion-filled gel weights to the initial emulsion-filled gel weights multiplied by 100.

**Statistical analysis**

All tests were repeated at least thrice, and the results were expressed as mean ± standard deviation. All figures were drawn using Origin 2022. The IBM SPSS 23 software was used to analyze the gel data, and the significant differences were analyzed at a p-level of 0.05.

**Results and discussion**

**Characteristics of the mixed gel solutions**

We characterized the mixed gel solutions before gelation, which helped improve the operability of the emulsion-filled gels during processing and application. Fig. 1 shows the particle size distribution (Fig. 1A and B), average particle size (Fig. 1C), and ζ-potential (Fig. 1D) of the mixed gel solution. In the absence of the OB cream, the particle size distribution of the control sample exhibited a bimodal uneven distribution. The particle size (285.3 nm) and absolute value of the ζ-potential (9.7 mV) were low for the control group. Upon the addition of 6.8-OB, the average particle size and absolute value of the potential increased significantly, and the particle size distribution of the mixed gel solution shifted from the multimodal distribution of the control group to a unimodal distribution. These results indicate that the mixed gel solution was well dispersed at pH 7.0 after the addition of the OB, owing to strong electrostatic repulsive interactions. When the concentration of the 6.8-OB exceeded 25 %, the average particle size increased significantly (p < 0.05), and the absolute value of the potential decreased slightly (Fig. 1C and D). This may be attributed to the flocculation of the excess OB in the continuous phase. Yang et al. (2021) proved that a WPI-OB mixture formed a mixed interface surrounded by whey protein clusters with a trend of interaction and interference. When the 11.0-OB was added, the particle size distribution of the mixed gel solution resembled that obtained by the addition of the 6.8-OB; however, the mixed gel solution containing the 11.0-OB had a lower average particle size and higher absolute value of the potential than that containing the 6.8-OB due to the different number of proteins bound to the OB (Zhao, Chen, Yan, Kong, & Hua, 2016). Zhao, Chen, Can, Kong, and Hua (2013) found that the intrinsic proteins of the OB were mainly oleosins (24 and
Fig. 1. Particle size distributions of the mixed gel solutions with (A) 6.8-OB and (B) 11.0-OB. (C) Average particle size and (D) ζ-potential of the mixed gel solutions with 6.8-OB and 11.0-OB (Different lower-case letters on the bars denote significant differences with the control group and 6.8-OB, \( p < 0.05 \); the various capital letters on the bars denote significant differences without the control group and 11.0-OB, \( p < 0.05 \)) (E) SDS-PAGE profiles of the surface proteins of the marker, 6.8-OB, and 11.0-OB.
18 kDa), while the other subunits (lipoygenase, \( \alpha, \gamma, \beta \)-amylase, \( \beta, A_3, \alpha, \) and Bd 30 k) corresponded to extrinsic proteins (Fig. 1E). The extrinsic-protein content of the 6.8-OB has been previously observed to be more than that of the 11.0-OB (Fig. 1E). Yan, Zhao, Kong, Hua, and Chen (2016) proposed that hydrophobic interactions between the extrinsic proteins on the 6.8-OB and other molecules formed an “adhesive”, which was conducive to the coalescence of the OB. As expected, the increase in the degree of aggregation of the sample droplets depended on the amount of OB added and the content of the OB surface proteins. The 6.8-OB could increase the particle size of the mixed solution more effectively than the 11.0-OB, resulting in an increase in the aggregation degree of the mixed gel solutions.

**Structural characterization of the emulsion-filled gels**

**Microstructure: CLSM**

Confocal laser scanning fluorescence microscopy (Fig. 2A) was used to further understand the microstructure of the emulsion-filled gels with different OB contents and types. There was little to no aggregation of proteins in the control group, and the droplet size was consistent with the average particle size. This was because the WPI concentration did not reach the minimum gel concentration of 12%. Therefore, the protein–protein interaction was not sufficient to form a network structure (Hongsprabhas & Barbut, 1997). After the addition of different protein content and concentrations of the OB cream to the WPI, the emulsion-filled gels exhibited different network structures. With increasing 6.8-OB concentration (15%–35%), the sizes of the gels increased over the control group. The network appeared to transform from a protein network to an oil-droplet-aggregation network. The oil-network structure was the most evident when the concentration of the 6.8-OB reached 25%. A high concentration of the 6.8-OB made the network more prominent, as the pores became smaller and the red oil droplet gradually covered the green protein.

In contrast, with an increase in the 11.0-OB concentration, the network structure shifted from oil-droplet to protein aggregation. The oil droplets in the sample appeared to be larger than those in the control group. This was confirmed by the aggregation of the green proteins in the control group and the droplet size was consistent with the average particle size. This was because the WPI concentration did not reach the minimum gel concentration of 12%. Therefore, the protein–protein interaction was not sufficient to form a network structure (Hongsprabhas & Barbut, 1997). After the addition of different protein content and concentrations of the OB cream to the WPI, the emulsion-filled gels exhibited different network structures. With increasing 6.8-OB concentration (15%–35%), the sizes of the gels increased over the control group. The network appeared to transform from a protein network to an oil-droplet-aggregation network. The oil-network structure was the most evident when the concentration of the 6.8-OB reached 25%. A high concentration of the 6.8-OB made the network more prominent, as the pores became smaller and the red oil droplet gradually covered the green protein.

**Microstructure: Cryo-SEM**

After the characterization of the emulsion-filled gels on the macroscopic level, Cryo-SEM images were used to observe the microstructure of the gels. Cryo-SEM micrographs show the gel matrix structure of the samples. However, the control group did not form a gel due to a low protein concentration (8%), and its structure was mostly in the form of regular protein tablets (Fig. 2B). A spherical aggregation structure with different pore sizes could be seen in the micrographs after the addition of the OBs. With an increase in the 6.8-OB concentration (6.8–15 and 6.8–20%), the gel began to exhibit a well-filled network structure. When the concentration of 6.8-OB exceeded 25%, the spherical droplet structure became more prominent, the network structure gradually disappeared, and the number of pores gradually increased. However, most of the particles were separated from the protein gel matrix, and the filler seemed to be bound weakly to the WPI matrix (Gravelle, Barbut, & Marangoni, 2015). Unlike the 6.8-OB, the 11.0-OB penetrated the polymerization chain and became a scaffold embedded in the protein network. This may be because the WPI gel network makes it easier for small oil droplets to enter the gap of its network and exhibit a filler effect (Wu et al., 2022). Notably, the aggregation degree of the gel droplets formed by the 6.8-OB was higher than that formed by the 11.0-OB. This may be attributed to the interaction between the interfacial protein film and the continuous phase of proteins, along with the aggregation trend of the droplets. Heat treatment leads to a greater protein denaturation and hydrophobic domain exposure, resulting in the extensive aggregation of molecules. Therefore, the heat treatment could have enhanced the interconnection between the droplets and the adsorbed and un-adsorbed proteins (Tang, Chen, & Foegeding, 2011). After heating, the aggregation of the two OB droplets differed significantly (Section 3.2.1). Therefore, the two OB emulsion droplets, with different extrinsic protein contents, existed in different forms in the gel matrix.

**FT-IR spectroscopy**

FT-IR analysis was performed after the emulsion-filled gel was prepared and freeze-dried to reveal the possible structural changes in it. Figs. S1A and B show the characteristic peaks of the WPI after heating. The amide A absorption peak of the WPI corresponds to the stretching vibrations of the O–H and N–H groups at 3293 cm\(^{-1}\), indicating hydrogen bonding (Xiao et al., 2020). The other characteristic peaks of the WPI were 2926 cm\(^{-1}\) (C–H stretching), 1656 cm\(^{-1}\) (C=O stretching vibrations and amide I), and 1536 cm\(^{-1}\) (amide II) (Bai, Chen, & Qi, 2022). The 6.8-OB exhibited a typical band at 3312 cm\(^{-1}\) (O–H and N–H stretching), 2926 cm\(^{-1}\) (C–H stretching), 1746 cm\(^{-1}\) (C=O stretching vibration), 1657 cm\(^{-1}\) (amide I), 1548 cm\(^{-1}\) (amide II), and 722 cm\(^{-1}\) (C–H vibration) (Acevedo et al., 2014). After gelation, the characteristic peaks of the emulsion-filled gels were similar to those of the OB. With an increase in the OB concentration, the characteristic peaks of the gel changed uniformly. Fig. S1A shows that the characteristic peaks of the amide I and II bands became sharper and more prominent with increasing 6.8-OB concentration. The O–H and N–H stretching peaks of the emulsion-filled gels shifted to 3290 cm\(^{-1}\), compared to those of the control group and the 6.8-OB. The amide I and II bands were red-shifted to 1655 cm\(^{-1}\) and 1644 cm\(^{-1}\), respectively. These absorption changes may be attributed to the hydrophobicity of the gel and the hydrogen bonding between the WPI and 6.8-OB (Bai, Chen, & Qi, 2022). The proteins adsorbed on the surface of the oil droplets were expected to interact with those in the aqueous phase. Herrero, Carmona, Pintado, Jiménez-Colmenero, and Ruiz-Capillas (2011) showed that emulsions can affect the properties of gels by changing the local protein environment and promoting the exposure of the hydrophobic groups of the tryptophan residues. When the gels were filled with 11.0-OB, their characteristic peaks were similar to those mentioned above, indicating that static hindrance and hydrophobic interactions between the particles in the emulsion-filled gel system maintained the stability of the gels. Notably, there was no new characteristic peak in the FT-IR spectra of the gels containing different OBs. This confirms the previous conclusion that physical entanglement and noncovalent interactions are the most prominent interactions between the WPI and OB.

**Rheological characteristics**

The shear moduli were used for the rheological characterization of the hydrogels and emulsion-filled gels. The \( G' \) and \( G'' \) modulus of the samples in the dynamic frequency scanning test are shown in Fig. 3A and B. In the frequency range shown in the figures, the value of \( G' \) for all gels exceeded that of \( G'' \), indicating elasticity (Tedros, 1996). In the absence of filler droplets, the value of \( G' \) in the control group was relatively low. With an increase in the OB concentration, the \( G' \) of the emulsion-filled gels was significantly higher than that of the control group (Fig. 3E), indicating that the presence of the OB enhanced the network structure. Dickinson and Chen (1999) showed that active fillers can increase gel
Fig. 2. (A) CLSM and (B) Cryo-SEM images of the emulsion-filled gels.
strength, while inactive particle fillers decrease it. In the present study, the values of $G'$ increased with the OB concentration for the two emulsion-filled gels, indicating that the dispersed OB emulsion acted as active fillers during the interaction with the gel matrix. Chen and Dickinson (1998) showed that aggregated droplets increase the effective volume fraction of a gel matrix more than dispersed droplets. According to Section 3.1, the 6.8-OB increased the gel strength more than the 11.0-OB. However, the emulsion formed by the 6.8-OB was stronger than that formed by 11.0-OB, which could be attributed to the degree of aggregation and particle size of the filler (Sala, van Vliet, Cohen Stuart, van de Velde, & van Aken, 2009). In contrast, as discussed in Section 3.1, the 6.8-OB had more surface extrinsic proteins than 11.0-OB, which helped enhance the network structure of the gels. This can be attributed to the release of extrinsic proteins on the surface of 6.8-OB during

Fig. 3. Rheological properties of the emulsion-filled gels with different OB concentrations and interface compositions. Frequency-sweep curves with frequencies ranging from 0.1 to 10 Hz for the emulsion-filled gels with (A) 6.8-OB and (B) 11.0-OB. Apparent viscosities of the emulsion-filled gel samples containing (C) 6.8-OB and (D) 11.0-OB, with shear rates ranging from 0 to 10 s$^{-1}$. (E) $G'$ at a constant frequency of 5 Hz. (F) Viscosities at a constant shear rate of 0.4 s$^{-1}$. (The different letters indicate that there were significant differences in the data of the different samples, $p < 0.05$.).
heating treatment, which contributed to the formation of the gel (Yan, Zhao, Kong, Hua, & Chen, 2016).

Fig. 3C and D show the change in viscosity of different emulsion-filled gels with increasing shear rate. A noticeable shear-thinning behavior can be observed, because the viscosity of the gels decreased with increasing shear rate, which also destroyed the cluster of oil droplets in the emulsion-filled gels, resulting in a reduced shear resistance (Niknam, Ghanbarzadeh, Ayaseh, & Rezagholi, 2018). An increase in the OB concentration at a constant shear rate can significantly increase the viscosity of the gel ($p < 0.05$). This is because the oil content increases the probability of the oil being wrapped in the proteins, thus affecting the network structure of the gel matrix (Zhao, Bhandari, Gaianti, & Prakash, 2021). It is worth noting that 6.8-OB can increase the viscosity of the gel more than 11.0-OB can (Fig. 3F, $p < 0.05$). This is because the possibility of increasing the viscosity of the gels increases with the flocculation degree of the droplets, which is consistent with the conclusions presented in Section 3.1. In addition, there was no significant difference between the apparent viscosities of 6.8-30 and 6.8-35 (Fig. 3F), which may be due to the saturation of the gel structure at 30% and 35% of the 6.8-OB emulsion.

**Functional characteristics**

**Textural characteristics**

TPA was used to simulate the effects of human oral chewing and swallowing on gels, and various texture parameters such as hardness, cohesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience were obtained. As shown in Table 2, the TPA could not be performed for the control group due to its fluidity and the lack of a strong gel-network structure. Hardness is the force required to achieve a certain deformation and is an important parameter in TPA. The addition of the OB significantly affected the structural parameters of the emulsion-filled gels. When the concentrations of the 6.8-OB and 11.0-OB reached 35%, the hardness of the gel formed was the highest, compared to other concentrations. This was because the density of the physical bonds, which enhanced the emulsion-filled gel network to avoid damage, increased with the OB content of the gel (Feng, Jia, Yan, Yan, & Yin, 2021). However, the 6.8-OB appeared to be more effective than the 11.0-OB at higher gel strengths, which is consistent with the conclusions presented in Section 3.3. Springiness determines the degree of sample recovery during the compression cycle. As shown in Table 2, the change in the springiness of the emulsion-filled gel was similar to that in the hardness. Cohesiveness indicates the ability of a gel to maintain its overall network structure during the two deformation processes. With an increase in the OB concentration in the emulsion-filled gel, the cohesiveness of the gel first increased and then decreased. According to Section 3.2.2, a high concentration of the OB can reduce the cohesiveness of the gels due to the formation of large network structures. Li et al. (2020) demonstrated that reduced cohesiveness makes the gel brittle when swallowed or homogenized, facilitating the preparation of fat analogs. Gumminess and chewiness were the secondary TPA parameters, representing the energy required to decompose semi-solid food and chew solid food into states ready for swallowing. The emulsion-filled gels were semi-solid; therefore, gumminess was the more appropriate measurement. The gumminess and hardness of the gel followed the same trend, mainly because the gumminess of the gel was the product of the hardness and cohesiveness. The resilience of the gel indicates its ability to be restored to its original shape and size. Compared to the gel containing a low concentration of the OB, the gel containing a high concentration of the OB consumed more energy while being restored to its original shape and size ($p < 0.05$). The gumminess and chewiness of the emulsion-filled gels were high, but their springiness, cohesiveness, and resilience were low, indicating that these gels had low elasticity but high acceptability of chewing properties (Guo et al., 2021). The network structure of the emulsion-filled gels changed with increasing amounts of the added OB. We believe that the OB gradually controlled gelation, thus contributing to the gel texture.

**LF NMR measurements**

The longer the $T_2$ relaxation time in a gel system, the easier is the flow of the corresponding water molecules (Xu et al., 2022b). Figs. S2A and D show the relaxation time distribution of the $T_2$ measured for the gel samples. $T_{21}$ (0.1–5 ms) and $T_{22}$ (1.5–20 ms) were assigned to the bound water in the macromolecular structure. $T_{23}$ (20–250 ms) was fixed to the network structure and was called immovable water. $T_{24}$ (300–4000 ms) was called mobile water.

As shown in Fig. S2, T21, T22, and T23 of the emulsion-filled gel gradually blue-shifted compared to the control group with an increase in the OB concentration (15%–35%), indicating that the OB can make gel samples solid and well-structured (Yasui, Ishioroshi, Nakano, & Saimjina, 1979). This was due to the OB acting as the active filler material in the cavity of the WPI matrix and a water migration barrier for the gel network, resulting in the interception of water (Yu, Song, Xiao, Xue, & Xue, 2022). In contrast, at the same OB concentration, $T_{23}$ and $T_{22}$ of the 6.8-OB-filled gels were lower than those of the 11.0-OB-filled gels. However, the $P_{21}$ of the gel filled with 30% 6.8-OB increased by 177% and that of the gel filled with 30% 11.0-OB increased by 219% compared to the control group (Table S1). This indicates that the 6.8-OB can increase the bound-water content of the gel more effectively than the 11.0-OB. The surface protein content of 6.8-OB was high, and soybean protein can bind to water molecules (Hansen, 1976). Therefore, the 6.8-OB could bind more water than the 11.0-OB. After adding the OB, a new $T_{24}$ peak appeared and the $T_{24}$ peak disappeared. The free water of the hydrogel in the control group was captured in the gel structure and transformed to bound water after the addition of the OB. Notably, when the concentration of 6.8-OB reached 30% and that of 11.0-OB reached 35%, the $T_{24}$ peak reappeared. This shows that excessive emulsion filling may also destroy the integrity of the gels, causing the pressure to partially dehydrate the gel network.

### Table 2

| Sample  | Hardness (g) | Springiness | Cohesiveness | Gumminess | Chewiness | Resilience |
|---------|-------------|-------------|--------------|-----------|-----------|------------|
| 6.8-15  | 95.75 ± 5.68^ef| 0.92 ± 0.06 ^cd| 0.79 ± 0.04 ^cd| 76.12 ± 1.43 ^f| 68.45 ± 1.32 ^f| 0.47 ± 0.01 ^f|
| 6.8-20  | 106.17 ± 10.36^def| 1.03 ± 0.14 ^f| 0.86 ± 0.02 ^def| 88.46 ± 5.83 ^ef| 85.86 ± 2.26 ^ef| 0.53 ± 0.02 ^d|
| 6.8-25  | 128.72 ± 24.35^cd| 1.23 ± 0.25 ^d| 0.92 ± 0.02 ^d| 120.64 ± 4.97 ^d| 152.67 ± 4.78 ^d| 0.62 ± 0.04 ^d|
| 6.8-30  | 153.27 ± 14.68^bc| 1.58 ± 0.19 ^c| 0.90 ± 0.06 ^bc| 142.60 ± 7.98 ^bc| 220.88 ± 2.34 ^bc| 0.64 ± 0.03 ^c|
| 6.8-35  | 183.40 ± 23.12^a| 1.64 ± 0.06 ^a| 0.82 ± 0.12 ^a| 158.89 ± 2.56 ^a| 252.21 ± 3.18 ^a| 0.68 ± 0.05 ^a|
| 11.0-15 | 80.06 ± 3.56^i| 0.75 ± 0.01 ^i| 0.64 ± 0.04 ^i| 46.32 ± 6.44 ^f| 33.14 ± 5.76 ^f| 0.52 ± 0.02 ^d|
| 11.0-20 | 98.09 ± 5.82^ef| 0.81 ± 0.03 ^ef| 0.73 ± 0.02 ^ef| 73.21 ± 3.66 ^ef| 55.53 ± 1.12 ^ef| 0.58 ± 0.02 ^d|
| 11.0-25 | 112.96 ± 10.36^de| 0.83 ± 0.03 ^de| 0.78 ± 0.01 ^de| 85.84 ± 7.99 ^de| 69.34 ± 5.40 ^de| 0.64 ± 0.01 ^de|
| 11.0-30 | 121.13 ± 12.88^bc| 0.92 ± 0.06 ^bc| 0.84 ± 0.02 ^bc| 106.24 ± 8.54 ^bc| 95.65 ± 3.56 ^bc| 0.67 ± 0.06 ^bc|
| 11.0-35 | 156.08 ± 20.60^b| 0.90 ± 0.12 ^b| 0.82 ± 0.03 ^b| 134.32 ± 3.50 ^b| 127.20 ± 7.76 ^b| 0.72 ± 0.08 ^b|

All measured values have been expressed as the mean ± standard deviation (n = 3). Different letters in the superscripts indicate significant differences at the level of $p < 0.05$. 

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Freeze–thaw stability

The appearances, morphologies, and WHCs of the hydrogel and emulsion-filled gel after three freeze–thaw cycles are shown in Fig. 4. The sample network was disrupted with increasing freeze–thaw cycles due to the formation of ice crystals, which increased the pore sizes (Zhang et al., 2021). Therefore, the samples showed a noticeable collapse and a mesh-like structure after being frozen and thawed (Fig. 4A). As expected, the WHC of the emulsion-filled OB gels before and after the freeze–thaw cycles was much higher than that of the control protein hydrogel (Fig. 4A, $p < 0.05$). This indicated that the presence of a certain amount of the oil phase could increase the density of the network structure and that water was locked in the pores of the gel. However, this effect was limited: when 6.8-OB and 11.0-OB reached 30 % and 35 %, respectively, the WHC and freeze–thaw stability decreased. This was attributed to the presence of an excess of the OB, which led to pore enlargement and capillary-force reduction, making it difficult for the gel matrix to retain more moisture. This was consistent with the conclusion of Section 3.2.2, and a high concentration of the OB enlarged the internal pores of the gels. In contrast, 6.8-OB was more effective than 11.0-OB at improving the WHC of gels at the same concentration, which is consistent with the conclusions of Section 3.3. Overall, these results further proved that the OB effectively made the emulsion-filled gels denser and more uniform, which helped improve their freeze–thaw stability and WHC.

Conclusions

In conclusion, the mixing of OB and WPI solution contributed to the gelation of the network formed after heat treatment. 6.8-OB is more prone to thermally induced coalescence and can form a stronger network structure than 11.0-OB due to the different extrinsic protein contents and aggregation trends of the two. Microstructure analysis indicates that 6.8-OB-containing gels are mainly composed of an oil-droplet network, forming a particulate gel matrix and that the 11.0-OB-containing gels are mainly composed of a protein network, forming an oil-drop-embedded-network structure. Therefore, OBs can be used as active fillers to change the microstructure of protein gels. As confirmed by FT-IR spectroscopy, the OBs maintain the stability of the polymeric gel matrix through hydrogen bonding and hydrophobic interactions, forming a denser gel structure and increasing the hardness of the gels. LF NMR results reveal that free water in the gels is transformed to bound water by the addition of OBs. The disappearance of the peak corresponding to mobile water in the LF NMR spectra upon initial addition of the OBs, and its subsequent reappearance at 6.8-OB and 11.0-OB concentrations of 30 % and 35 %, respectively, indicates that excessive OB addition destroys the integrity of the gels, resulting in dehydration of the gel network under pressure, which reduces the

Fig. 4. (A) The appearances, morphologies, and (B and C) WHCs of the hydrogel and emulsion-filled gels after three freeze–thaw cycles.
freeze–thaw stability of the gel. Overall, the findings of this study suggest that oil can be an excellent nutritional substitute for traditional fat droplets and may be incorporated in protein gels for the development of functional foods.

**CRediT authorship contribution statement**

**Yi Liao:** Writing – original draft. **Yufan Sun:** Supervision. **Zhenxiao Wang:** Formal analysis. **Mingming Zhong:** Data curation. **Runnan Li:** Methodology. **Shizhang Yan:** Writing – review & editing. **Baokun Qi:** Project administration, Writing – review & editing. **Yang Li:** Funding acquisition.

**Declaration of Competing Interest**

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

**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.2022.100509.

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