ORIGINAL ARTICLE

Effect of Oil Content and Composition on the Gelling Properties of Egg-SPI Proteins Stabilized Emulsion Gels

Mengqi Zhang [1,2,3] · Yanjun Yang [1,2] · Nuria C. Acevedo [3]

Abstract
Effects of different oils on the rheological properties, textural profile, water loss (WL), oil loss (OL) and microstructure of egg-soybean protein isolate (SPI) stabilized emulsion gels were investigated at neutral pH, wherein soybean oil, olive oil and menhaden oil were used to form emulsions. The results showed that viscosity of emulsions progressively increased with the increase of oil content. Similarly, analysis of the rheological behavior of the formulated emulsion gels revealed an increase in the mechanical strength (G') with the increase in oil concentration, indicating that oil droplets played a significant role in the formation of the gel structure. In addition, at high levels of oil, the hardness and chewiness of emulsion gels were also high, while a slight decrease in springiness and cohesiveness were observed. A linear relationship between hardness and water/oil loss was found, whereas the Pearson correlation suggested that less drainage of water may slow down the outflow of oil. The microstructural images showed a more compact network as a result of the increase of oil content in the formulation. Scarce significant differences were found among emulsion gels formulated with different oil type, suggesting oil composition played a dispensable role on the gelling properties of emulsion gels.

Keywords Egg-SPI composite proteins · Oil composition · Oil-emulsion gel · Rheology · Microstructure

Introduction

Protein-stabilized emulsion filled gels, or emulsion gels, have recently attracted increasing interest because of their practical applications in food formulations [1]. For example, emulsion gel systems can be used as controlled-release carriers, especially for lipid-soluble bioactive substances, thus extending the possibilities to create foods with new and improved properties. Numerous studies have been conducted on emulsion gels formed by soybean proteins, whey proteins and milk proteins [2–10].

As an ingredient in emulsion gels, oil plays an important role in the texture of the system, imparting viscosity and taste. Oil droplets contained in gelled protein food systems have been categorized as active or inactive filler particles depending on their interaction with the surrounding gel matrix [11]. Active filler particles have hydrophilic surfaces and are integrated into the composite gel; whereas inactive particles have more hydrophobic surfaces and show little interaction with the matrix [2]. At small rheological deformation conditions of the gel, active filler particles cause the elastic modulus to increase with the increase in the volume fraction whereas inactive filler particles cause the elastic modulus to decrease [2, 11, 12], which is an easy way to distinguish active and inactive filler particles.

It is known that the textural properties of emulsion gels are dependent on many factors, such as the method used to prepare the gels, protein components, protein concentration and oil content [6, 8, 10]. The common methods for forming gels are heat treatment, acidification and enzyme treatment [13], and suitable methodologies are chosen according to the materials and the purpose of the experiment. The influence of different β-conglycinin/glycinin ratio on the properties, microstructure and gelling mechanism of soy globulin-stabilized

✉ Yanjun Yang
  yangyj@jiangnan.edu.cn

✉ Nuria C. Acevedo
  nacevedo@iastate.edu

[1] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, People's Republic of China

[2] School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, People's Republic of China

[3] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, USA

emulsion gels was studied by Chuan-He Tang, et al.. These authors found that increasing the glycinin content progressively increased the gel stiffness and led to a more inhomogeneous and porous microstructure; thus significantly decreasing the network's water-holding capacity [8]. Mao et al. [6] found that an increase in the whey protein isolate content of glucono delta-lactone (GDL)-induced emulsion gels led to higher storage modulus and water-holding capacity, whereas an increase in the oil content resulted in an earlier onset of gelation. In addition, environmental stress factors, e.g., pH, temperature and ionic strength can also affect protein structure and influence the properties of emulsion gels [14, 15]. However, limited studies are available on the effect of the oil types on the emulsion gels.

In previous studies, we found that egg-soybean protein isolate (SPI) composite protein gel showed desired gelling properties when they were formulated in a 1:3 egg:SPI ratio [16, 17]. To explore further potential applications of this type of composite gels and determine whether and how different oil composition have an effect on their characteristics, the work presented here investigates the rheological properties, texture, water/oil loss and microstructure of transglutaminase-induced egg-SPI proteins composite emulsion gels. Soybean oil, olive oil and menhaden oil, were selected to prepare the emulsions due to their differences in fatty acid composition, i.e. differences in the proportion (w/w) of saturated and unsaturated fatty acids. This research may not only expand the usage of composite protein and develop new products but also provide a reference for the study of oil types on the emulsion gels.

Materials and Methods

Materials

Fresh hen eggs (Grade A) were obtained from a local grocery store in Ames, IA. Eggs were stored in a 4 °C refrigerator. Soybean protein isolate (SPI) powder was purchased from MP Biomedicals (Santa Ana, CA, United States). Protein content of egg and SPI was 13.0% (w/w) and 90.8% (w/w) (dry basis), respectively, as determined by the Kjeldahl method (N × 6.25). Transglutaminase (TG) was purchased from Modernist Pantry (Eliot, ME, United States) (main ingredients: maltodextrin and transglutaminase, enzyme activity 100 u/g). Olive oil (O) was purchased from a local grocery store in Ames, IA. Soybean oil (S) was generously provided by ADM oils (Decatur, IL, USA). Menhaden oil (M) was generously provided by Omega Protein, Inc. (Reedville, VA, USA). Composition of the oils used in this study is shown in Table 1. All other chemicals were purchased from Fisher Scientific (Hampton, NH, United States).

Preparation of Emulsions

Emulsions were prepared according to the following procedure; first, SPI dispersions were obtained by dissolving the powder in distilled water at a certain protein concentration and stirring for 4 h to ensure complete dispersion and dissolution. Then, the SPI dispersion was heated at 90 °C for 2.5 min. Egg solutions were obtained by diluting fresh liquid egg with distilled water at the same protein concentration. Second, SPI and egg dispersions were mixed together at a 3:1 (w/w) ratio by weighing corresponding dispersion quantity. Next, different oils were added to the composite dispersions and emulsified at 10,000 rpm for 2 min using a T25 digital ULTRA-TURRAX (IKA®, Germany). Three different oils were incorporated into the dispersions; soybean oil, olive oil and menhaden oil at concentrations of 5%, 10%, 15% and 20% (w/w). The final protein concentration in all emulsions was 10%. The protein to oil proportion for all the emulsions were 2.0 g protein/g oil, 1.0 g protein/g oil, 2/3 g protein/g oil and 0.5 g protein/g oil. Finally, the emulsions were adjusted to pH 7.0 with 1 M NaOH or HCl.

Preparation of Emulsion Gels

Transglutaminase (TG) was added at 10 u/g protein to the emulsions while stirring. Then, each emulsion was divided and transferred to 10 mL beakers, sealed with parafilm prior to gel for 1 h at 40 °C. Next, the TG-induced emulsion gels were heated at 85 °C for 30 min in a thermally controlled water bath and cooled to room temperature with ice water. The addition of TG did not affect the protein concentration significantly. All gels were stored at 4 °C before analysis. At least three replicates were prepared and analyzed.

Viscosity of Emulsions

The viscosity of the emulsions was determined by a DHR-2 dynamic rheometer (Waters Corporation, United States) at 25 °C using a parallel plate (40 mm diameter) geometry. The samples were loaded onto the rheometer stage (gap 1000 μm) and the extra sample around the edge of the plate was removed with paper towel. A controlled shear rate ranging from 0.01 to 100 (1/s) was selected for the runs. Three replicates were performed for each measurement.

Strain Sweep and Frequency Sweep of Emulsion Gels

Emulsion gels were cut into slices of 22 mm diameter and 3000 μm thickness. Strain sweeps (0.01–100%) at a fixed frequency of 1.0 Hz at 25 °C was performed using the same rheometer with a parallel plate (40 mm diameter) geometry. Additionally, new slices were prepared and dynamic frequency sweeps were conducted over a frequency range of 0.01–

Table 1 The proportion (w/w) of saturated and unsaturated fatty acids in oil used for this study

	Saturated fatty acid	Unsaturated fatty acids		
		Total	Monounsaturated	Polyunsaturated
Soybean oil	14.29%	85.71%	21.43%	64.28%
Olive oil	14.28%	82.14%	71.43%	10.71%
Menhaden oil	30.43%	60.89%	26.69%	34.20%

Data provided by supplier

10 Hz at a constant strain of 0.5% (within the linear viscoelastic range). Three replicates were performed for each measurement.

Texture Profile Analysis (TPA)

All samples were cylinders with 12 mm in height and 22 mm in diameter and the texture of emulsion gels was tested with a TA-XT Plus texture analyzer (SMS Co. Ltd., England). The platform of the TA-XT Plus fitted with a flat plunger of 38 mm in diameter (TA-4) was calibrated with a 2 kg load cell. The crosshead speed and compression deformation of compression tests were 2 mm/s and 50% from the initial sample height, respectively. Six replicates were performed for each measurement.

Water Loss (WL) and Oil Loss (OL) by Squeeze Tests

To test the water and oil loss of emulsion gels, each emulsion gel was placed between two round filter papers (Whatman #5, 110 mm diameter). A 1 kg weight was place on top of the samples at room temperature for 30 min; subsequently the filter papers were dried at 60 °C for 30 min. The weight of the filter papers and emulsion gels before and after pressing were recorded. Filter papers were not saturated of liquid during the experiment. Six replicates were performed for each measurement. Water loss (WL) and oil loss (OL) by squeeze tests were calculated as follows:

$$WL\ (\%) = [G_1 - G_2 - (F_2 - F_1)]/G_1 \times 100 \quad (1)$$

$$OL\ (\%) = (F_2 - F_1)/G_1 \times 100 \quad (2)$$

where G_1 and G_2 correspond to the mass of emulsion gel before and after pressing, respectively; F_1 and F_2 correspond to the mass of filter papers before pressing and after drying, respectively.

Visual Appearance

Gels were cut into slices of 22 mm diameter × 3 mm thickness. Photographs of the emulsion gels visual appearance were obtained with a high-resolution camera.

Confocal Laser Scanning Microscopy (CLSM)

The microstructural properties of the emulsion gels were analyzed by confocal laser scanning microscopy (CLSM) (Leica SP5X, Leica Microsystems Inc., Heidelberg, Germany). All the emulsion gels were observed according to a method previously described [9]. Fluorescence dyes prepared in mixed solutions to stain the protein and oil phases were Rhodamine B (0.1%, w/w) and Nile blue (0.1%, w/w), with excitation wavelengths at 488 and 633 nm, respectively. All formulated emulsions were mixed with the stock dye solution (1.0%, w/w) and then gels with stock dye solution were made and placed on microscope slides previous to observation. CLSM images were obtained with 63 × magnification lens. Three replicates were prepared and observed for each formulation. The proteins dyed with Rhodamine B are observed green, whereas the oil phase dyed with Nile blue is usually red.

Statistical Analysis

Pearson correlation (p<0.01) between two properties as well as statistical analysis of means and standard deviations were carried out by SPSS 17.0 package. One-way analysis of variance was carried out by Duncan's Multiple Range Test (p<0.05) to detect significant difference between mean values. Figures were edited by OriginPro 9.0 software.

Results and Discussion

Viscosity of Emulsions

The viscosity of emulsions formulated with different types and oil contents before TG addition are shown in Fig. 1a. In general, all the emulsions exhibited a shear-thinning behavior at the tested shear rate range 0.01 s^{-1} to 100 s^{-1}, i.e., the viscosity progressively decreased upon increasing shear rate. This phenomenon has been attributed to the hydrodynamic forces generated by the shear rates that cause aggregates to become deformed and eventually disrupted, resulting in a reduction in the viscosity [7, 18–20]. The control sample (without oil) showed the lowest viscosity; furthermore, regardless

Fig. 1 Viscosity of emulsions (**a**) with different oil composition and content (**b**). Emulsion gel with 5% soybean oil was denoted as "S-5%" and the rest of the samples were denoted following the same format
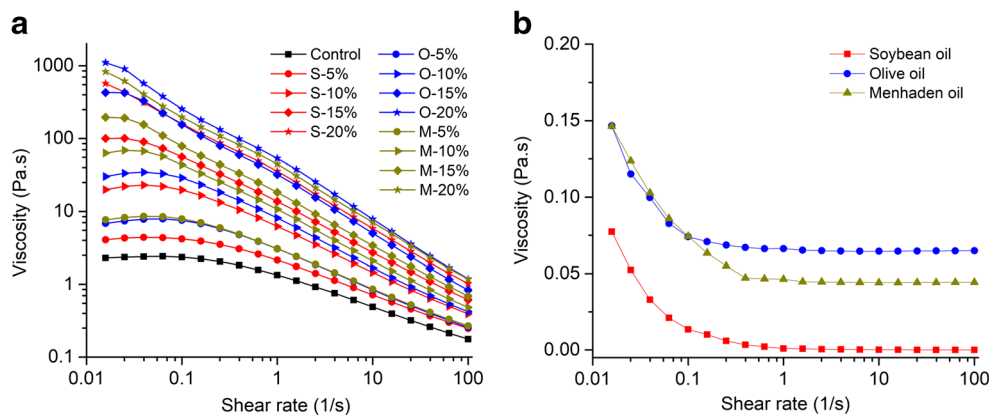
of the type of oil added to the emulsions, the higher the oil content, the higher the viscosity observed for those emulsions. These results can be ascribed to the large contact surface area between oil droplets and the continue phase that opposes to the free flow of the emulsion. At high oil contents the oil droplets are close enough to interact with one other, hence leading to an increase in the system's viscosity [7, 19, 21]. Reiffers-Magnani et al. (1990) found similar results when working whey protein-based emulsions and increasing levels of oil content [22]. In another study, Gu et al. (2009) reported an increase in the viscosity of SPI emulsions with the increase in oil concentration from 5% to 20%. The same authors found large oil droplet sizes at high oil contents, which they attributed to the increase in emulsion viscosity. They concluded that emulsion viscosity at low oil contents is the results of protein-protein interactions, while at high oil contents oil droplets are the responsible factors [3].

It is interesting to note that the viscosity of emulsions formulated with soybean oil was the lowest. Viscosity curves of olive and menhaden oil-based emulsions were not significantly different from each other at 5% oil content, nevertheless the viscosity of the 10% menhaden oil emulsion was significantly higher ($p < 0.05$) than that of the olive oil emulsion. At oil contents higher than 10%, the viscosity of emulsions with olive oil was the highest. These results can be attributed to the viscosity of oil itself (Fig. 1b). Regardless of the amount of oil added, the lowest viscosity of the soybean oil-based emulsions is most likely related to the lowest viscosity of liquid soybean oil. The viscosity of olive oil had little difference with that of menhaden oil with shear rates up to 0.1 s^{-1} whereas at shear rates higher than 0.1 s^{-1}, the viscosity of olive oil was the highest. These findings are consistent with the changes in viscosity of emulsions before and after 10% oil content. Therefore, it can be concluded that the viscosity changes of oil itself contributed to the viscosity changes of emulsions and this is more evident at high oil contents, suggesting the oil droplets may play a significant role on the rheological behavior of these emulsions [3].

Viscoelasticity of Emulsion Gels

The strain sweep of emulsion gels formed with different types and contents of oil were determined to analyze their viscoelasticity. It can be observed from Fig. 2 that the average storage modulus (G') of all gels within the linear viscoelastic region (LVR) was greater than the average loss modulus (G"), suggesting that all samples are viscoelastic materials with a predominance of an elastic behavior. Emulsion gels with more oil content showed higher G' and G" than the control. The G' within the LVR is often correlated with the sample firmness [23]. Therefore, these results are indicative of emulsion gels becoming firmer as the oil content increased; probably due to interactions between the oil droplets and the surrounding proteins. Nevertheless, there was little difference among emulsion gels prepared with different types of oil at the same oil content. These findings indicate that it is oil content rather than oil composition that has more effect on gel firmness.

Frequency Sweep of Emulsion Gels

Figure 3 depicts the frequency sweep results of all the formulated emulsion gels. G' predominated over G" throughout the frequency range tested regardless of type and oil content. All emulsion gels showed solid-like behavior as all G"/G' ratios were less than 1.0 (around 0.1), which indicates the gels were real gels. Additionally, G' of all emulsion gels were practically parallel to each other and slightly dependent to the frequency within the range analyzed that reflects the viscoelastic nature of gel networks [24]. From Fig. 3a–c, we can see that gels with higher oil content showed higher G' and G", which were closely related to the results of viscosity (Fig. 1) and strain sweep (Fig. 2). Another interesting phenomenon is that there is an apparent increase of G' between 10% and 15% oil content, suggesting that 10% oil content might be a turning point on emulsion gels' property. A similar critical point was observed by other researchers. For instance, Gu et al. [3] found that the gel hardness of acidic soy emulsion gels was influenced by oil content at high concentrations (10%–20%, v/v).

Fig. 2 Storage modulus (G') (2**A**) and loss modulus (G'') (2**B**) of emulsion gels with different oil composition and content. Bars with different letters (a–h) correspond to significantly different values (*p* < 0.05)

Texture of Emulsion Gels
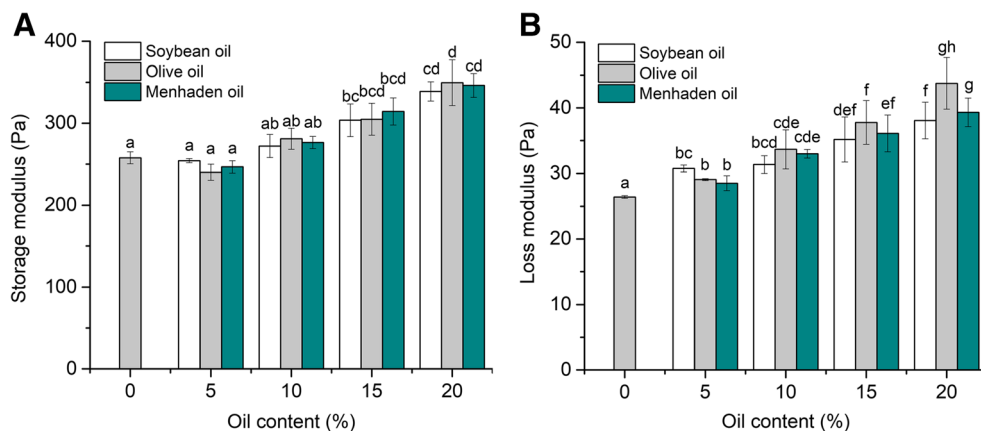
In another work, Jin et al. [25] reported that during gelation of SPI emulsions, tan δ (G''/G') initially increased with addition of 10% oil and then decreased at higher oil contents. The aforementioned authors pointed out that 10% oil content may be a critical concentration required for emulsion of 10% SPI.

Texture analysis was performed to measure the texture changes of the egg-SPI emulsion gels at large strain deformation through compression. Figure 4 shows the effect of different types of oil and oil contents on the hardness of the emulsion gels. It can be observed that the hardness of emulsion gels progressively increased with the increase in oil content regardless of the type of oil added. Therefore, the addition of oil had a positive influence on the hardness of the gels, which indicates that the emulsified oil droplets behave like 'active fillers'; i.e. oil droplets are fully assimilated or packed in the system and contribute to the reinforcement of the gel network as the oil concentration increases [2, 26]. Additionally, the continuous increase in emulsion gel hardness correlated to the increase in oil content can also be attributed to a high degree of inter-droplet covalent cross-links between the adsorbed proteins at the interface that strengthens the emulsion-filled gel network. Tang et al. (2013) reported a higher extent of TG-induced covalent cross-links between the proteins absorbed at the interface in concentrated emulsions. These authors stated that at high oil contents, the droplets are close enough to interact with one another allowing the formation of inter-droplet covalent cross-linking [9]. In this study, 15% oil added was the threshold value above which a further increase in the hardness of the emulsion gels was not observed. This is probably due to the TG-induced covalent cross-linking of the proteins adsorbed at the surface of oil droplets reaching a maximum at that oil level. Despite the observed effects of different oil contents on emulsion gel firmness, there was no significant difference in the hardness values among emulsion gels formulated with different types of oil. It is possible that since proteins are the main gelling substances, the oil exerted little effect on the gel hardness regardless of their differences in chemical composition.

Apart from hardness, other texture properties are also important sensory attributes. Table 2 shows the changes of springiness, cohesiveness and chewiness of emulsion gels. Springiness and cohesiveness, both decreased with the increase in oil content, while chewiness exhibited the opposite trend. There is no significant difference on springiness between the control gel (without oil) and gels with 5%, 10% and 15% oil content; nevertheless, a further increase in the
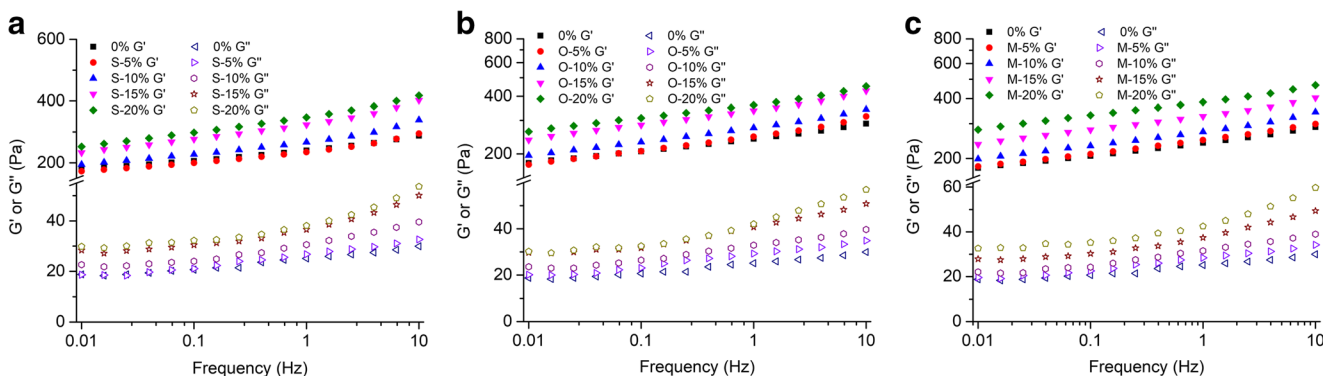

Fig. 3 Frequency sweeps of emulsion gels with different oil composition and content. **a**: emulsion gels formulated with soybean oil (S); **b**: emulsion gels formulated with olive oil (O); **c**: emulsion gels formulated with menhaden oil (M)
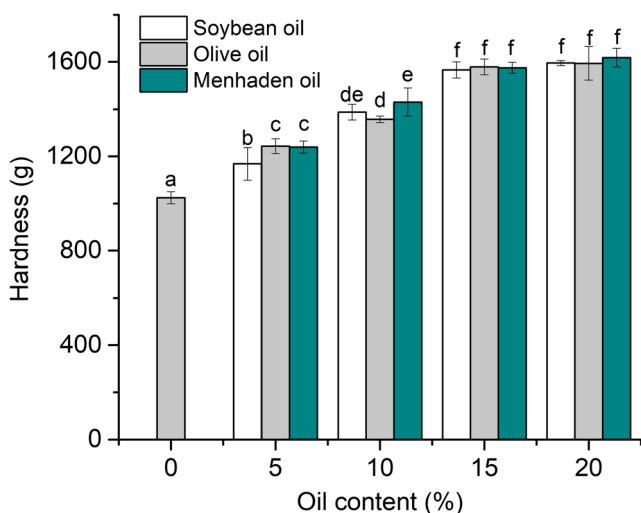
Fig. 4 Hardness of emulsion gels with different oil composition and content. Bars with different letters (a–h) correspond to significantly different values ($p < 0.05$)

oil level (20%) led to a significant decline in the springiness of emulsion gels. These results indicate that the loss in the ability of the gels to physically spring back after deformation during compression was caused by the presence of oil in amounts higher than 15%. As observed in Table 2, cohesiveness values were larger in samples with 5% oil added when compared to control gels; however, as the oil content continued raising, different trends were observed for gels formulated with different oil types. A minimum cohesiveness values was obtained when oil content was the highest (20%) for emulsion gels containing soybean or menhaden oils, whereas no significant differences were observed in cohesiveness of olive oil-based emulsion gels, regardless of the oil proportion. It has been previously demonstrated that the higher the cross-linking extent is, the greater are the hardness and cohesiveness of similar gels [27]. Thus, the measured cohesiveness is in agreement to the trends observed for gel firmness (Fig. 4), where oil

contents larger than 15% did not translate to increases in gel hardness probably because a maximum of TG-induced covalent cross-linking was reached. The differences in cohesiveness observed for emulsion gels formulated with different types of oil could be attributed to their dissimilarities in viscosity. The oil showing a more significant shear-thinning behavior (low viscosity) exhibited lower cohesiveness values (Fig. 1, Table 2). For the case of chewiness, as expected, more energy was required to chew the gels with more oil content. Similar to hardness values, there were no significant difference in the textural properties of gels with different oil composition, further evidencing that oil content rather than its composition had more of an effect on the texture of egg-SPI emulsion gels.

Water Loss (WL) and Oil Loss (OL) of Emulsion Gels

Water-holding capacity, which is the ability to effectively immobilize water through the capillary effects of gel matrices, is one of the most important properties in food systems [28]. When emulsion gels were pressed by weight, both water and oil that were mobilized could be squeezed out of the gel. Here, water and oil loss (WL and OL) were calculated to indicate the ability of emulsion gels to immobilize liquids.

Figure 5 shows the WL and OL data of emulsion gels formed with different types of oil at different oil contents. Both WL and OL progressively decreased when the oil content increased from 0% or 5% to 20% indicating that water and oil were better entrapped within the gel structure. Since WL and OL are closely associated with the structure and hardness of gel network, it can be considered that their progressive decrease can be attributed to the enhanced compact structure and strengthened gel at high oil levels. Furthermore, it has been documented in a previous study that the correlation between the improvement of the liquid oil capacity and the presence of

Table 2 Springiness, cohesiveness and chewiness of emulsion gels with different types and contents of oil

Properties	Oil type	Oil content				
		0%	5%	10%	15%	20%
Springiness	Soybean	0.981 ± 0.003 [c]	0.976 ± 0.004 [c]	0.979 ± 0.003 [c]	0.973 ± 0.005 [bc]	0.964 ± 0.008 [a]
	Olive		0.979 ± 0.002 [c]	0.976 ± 0.001 [c]	0.974 ± 0.007 [bc]	0.961 ± 0.010 [a]
	Menhaden		0.977 ± 0.004 [c]	0.976 ± 0.004 [c]	0.977 ± 0.007 [c]	0.967 ± 0.003 [ab]
Cohesiveness	Soybean	0.829 ± 0.008 [c]	0.857 ± 0.010 [de]	0.850 ± 0.008 [d]	0.859 ± 0.008 [de]	0.793 ± 0.004 [a]
	Olive		0.860 ± 0.009 [de]	0.857 ± 0.003 [de]	0.864 ± 0.006 [e]	0.854 ± 0.004 [de]
	Menhaden		0.878 ± 0.003 [f]	0.857 ± 0.005 [de]	0.861 ± 0.008 [de]	0.813 ± 0.010 [b]
Chewiness	Soybean	834 ± 35 [a]	977 ± 66 [b]	1154 ± 31 [d]	1310 ± 33 [f]	1277 ± 65 [f]
	Olive		1048 ± 37 [bc]	1136 ± 10 [d]	1330 ± 43 [f]	1315 ± 67 [f]
	Menhaden		1063 ± 23 [c]	1199 ± 46 [de]	1324 ± 23 [f]	1252 ± 71 [ef]

Values with different letter are statistically different ($p < 0.05$)

Fig. 5 Water loss (WL) (5**A**) and oil loss (OL) (5**B**) of emulsion gels with different oil composition and content. Bars with different letters (a–h) correspond to significantly different values ($p < 0.05$)
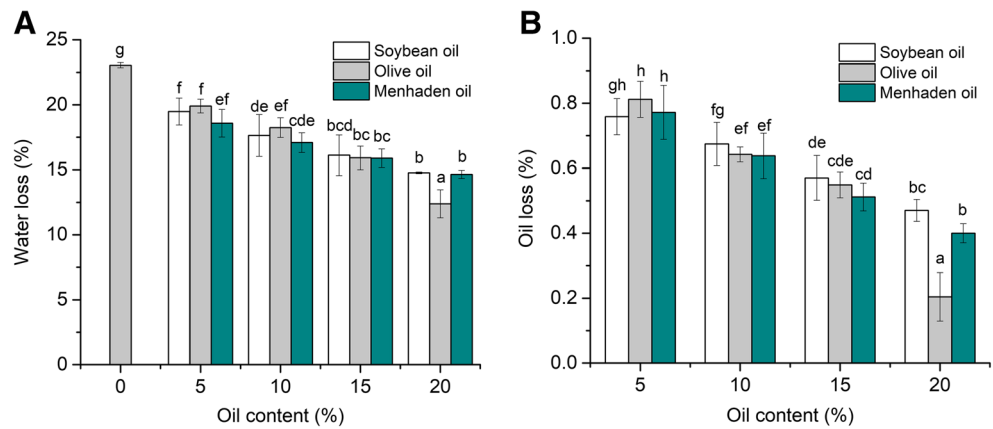
colloidal interactions among droplets as a result of heat-induced unfolding and subsequent enzymatically induced crosslinking of protein molecules adsorbed at the oil water interface [29]. In addition, in order to maintain a constant protein concentration, the increase of oil content was accompanied by a reduction in water content during emulsion gels preparation and it is hypothesized that to some extent, less outflow of water may slow down the outflow of oil. Therefore, it is not surprising to find a positive correlation between WL and OL (Table 3). On the other hand, a negative linear relationship between WL/OL and hardness of emulsion gels was found (Table 3), which agrees with previous observations where gels with higher hardness exhibited inferior WL/OL. Similar results on the relationship between the hardness/strength and water-holding capacity have been reported for emulsion gels [10, 30, 31]. Nevertheless, the WL/OL difference among gels with different types of oil was little except for the gel with 20% olive oil. These results may be related to the cohesiveness of emulsion gels (Table 2). In other words, higher cohesiveness of emulsion gels reduced the leakage of water and oil.

Structural Characteristics of Emulsion Gels

All of the emulsion gels, regardless of the oil type and content used in the formulation, showed structural stability after preparation as they retained their shape and did not flow. Figure 6 shows representatives images of the emulsion gels' discs. In terms of visual appearance, all the oil-containing emulsion

gels appeared more opaque and yellow than the control (no oil added). The opacity of the samples indicates a superior emulsification, whereas the yellow-like color can be attributed to the presence of liquid oil. The more oil present, the more yellow-like the gel appeared, particularly for olive oil-based emulsion gels. An interesting observation is the presence of moisture on the gels surface that becomes less evident, and then disappears, as the oil concentration increases. These findings correlate to the enhanced water and oil binding capacity which occurred as result of the strengthening of the gel network and that were previously discussed (Fig. 5). As observed in the images in Fig. 6, concentrated emulsion gels showed well visible pores or air pockets dispersed throughout the matrix. As the oil content increased the emulsification degree was higher and thus; the incorporation of air into the network was more important causing the observed non-uniformity. As expected, no significant differences were observed among gels formulated with different types of oil.

The microstructure of the emulsion gels prepared with different types and contents of oil is displayed in Fig. 7. In all systems, proteins were stained with Rhodamine B and the oil phase was marked with Nile Blue. Most oil droplets were entrapped by proteins as a result of the homogenization step; thus, the perimeter of the protein-coated oil droplets and the gel network were predominantly green-yellow in appearance in the overlay images. All the emulsion gels exhibited a similar microstructural network showing a trend toward smaller pore sizes and a denser gel structure as the oil content raised from 5% to 20%. This phenomenon also suggested that for concentrated emulsion gels, although the structure was uneven at the macroscopic level, the microstructure of these gels was more compact with a tight molecular packing (Fig. 7a–d). According to the results for OL (Fig. 5B), the proportion of leaked oil decreased as the oil ratio increased indicating an improvement of the oil binding capacity. Therefore, as depicted in Fig. 7a–d, the proportion of protein-coated oil droplets gradually increased when oil concentration increased. At low oil contents, the gel network was formed mainly through cross-linking between proteins unadsorbed to the

Table 3 Pearson correlation (R) of hardness, water loss (WL) and oil loss (OL) of emulsion gels with different types of oil ($p < 0.01$)

	Soybean oil	Olive oil	Menhaden oil
Hardness and WL	−0.895	−0.934	−0.960
Hardness and OL	−0.798	−0.823	−0.919
WL and OL	0.948	0.979	0.952

R > 0 represents the relationship is a positive correlation; R < 0 represents the relationship is a negative correlation

Fig. 6 Visual appearance of emulsion gels with different oil composition (S: soybean oil; O: olive oil; M: menhaden oil) and content
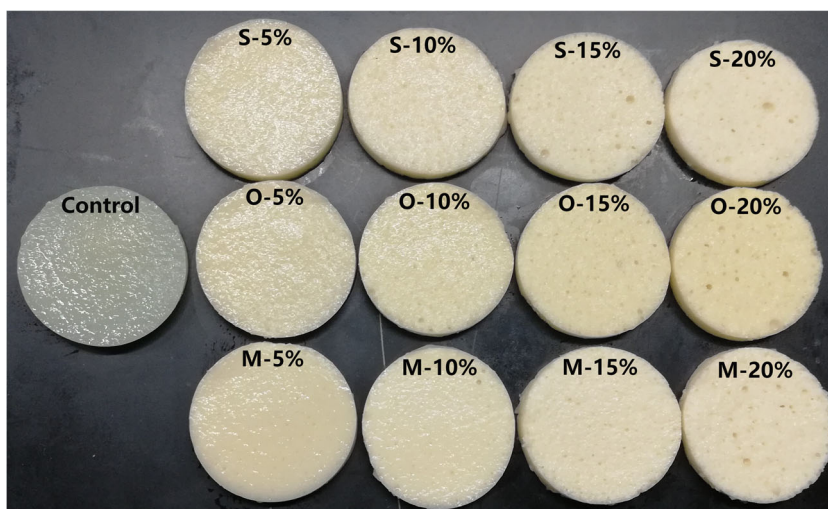

surface of oil droplets in the continuous phase. On the other hand, at high oil contents, plenty of proteins would be adsorbed at the interphase and these proteins' structure would unfold and even rearrange, which greatly facilitated the enzymatic cross-linking between proteins adsorbed on different neighboring droplets [32]. As a result, the structure of gels became more compact, leading to the increase of G' and hardness of gels (Fig. 2, 3 and 4). The influence of oil composition on the microstructure of emulsion gels can be observed when comparing micrographs of Fig. 7d, e and f. Only a few protein-uncoated oil droplets can be observed in the gels with menhaden oil (Fig. 7f), whereas the emulsion gel with soybean oil (Fig. 7d) showed the largest number of protein-uncoated oil droplets. This phenomenon is in good agreement with the result of OL studies (Fig. 5B), that suggested the

emulsion gels formulated with soybean oil had the highest OL though the difference was small. All these findings support the previously proposed hypothesis that protein-coated oil droplets acted as active filler in emulsion gels and that they participated in the formation of the gel network.

Conclusion

In this study, the effects of different composition and content of oil on the gelling properties of egg-SPI composite protein stabilized emulsion gels were investigated. The viscosity of the emulsions progressively increased as the oil content increased from 5% to 20% and emulsions with soybean oil had the lowest viscosity, which were related with the viscosity

Fig. 7 Typical CLSM images of emulsion gels with different oil composition and content (protein phase: green/yellowish color; oil phase: red color). **a**: 5% soybean oil; **b**: 10% soybean oil; **c**: 15% soybean oil; **d**: 20% soybean oil; **e**: 20% olive oil; **f**: 20% menhaden oil. Scale bar: 25 μm
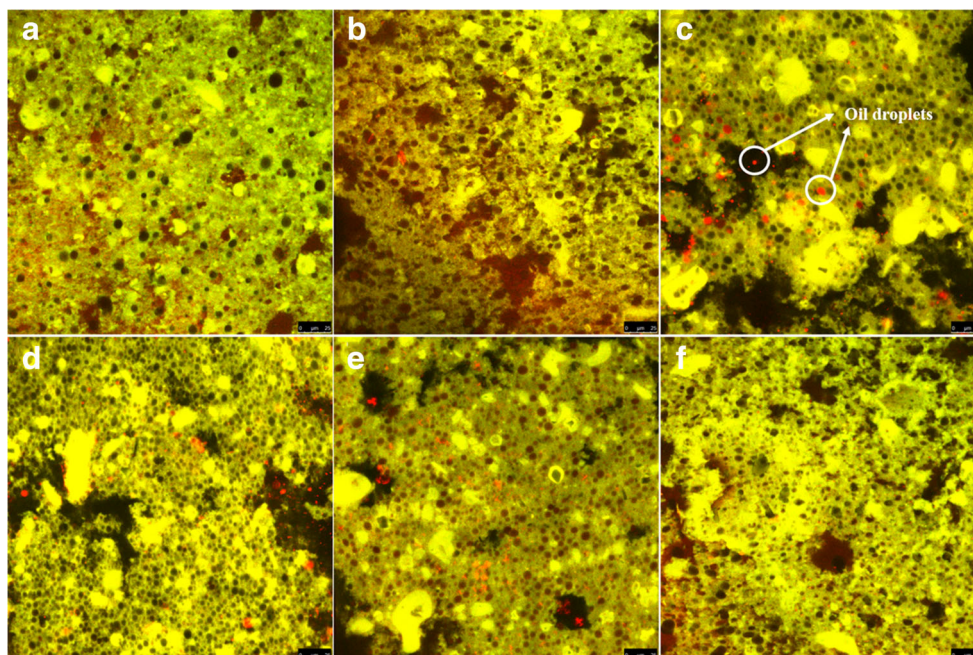
of oil itself. Both G' and G" of emulsion gels rose with the increase of the oil content and there was a larger progression between G' of gels with 10% oil and that with 15% oil. In terms of texture, an increase in the oil content led to an increase in hardness and chewiness but a decrease in springiness and cohesiveness. Moreover, WL and OL declined when the oil content increased and a strong linear relationship was observed between these two properties and hardness. Slight differences among emulsion gels with various types of oil were reflected, which indicated that oil composition had little influence on the gelling properties of egg-SPI protein composite emulsion gels. In addition, the analysis of the microstructure revealed that all the emulsion gels exhibited similar network structural features characterized by the presence of smaller pore sizes and a denser structure as the oil content increased from 5% to 20%. The changes of microstructure among gels with various types of oil agreed with the trend of hardness and OL. All the findings above indicated that protein-coated oil droplets acted as active filler in emulsion gels and they participated in the formation of the gel network.

Compliance with Ethical Standards

Conflict of Interest   The authors declare no conflict of interest.

References

1. L. Chen, G.E. Remondetto, M. Subirade, Trends Food Sci. Technol. **17**(5), 272–283 (2006)
2. J. Chen, E. Dickinson, Colloids Surf. B **12**(3), 373–381 (1999)
3. X. Gu, L.J. Campbell, S.R. Euston, Food Res. Int. **42**(8), 925–932 (2009)
4. H. Khalesi, B. Emadzadeh, R. Kadkhodaee, Y. Fang, Int. J. Biol. Macromol. **125**, 17–26 (2019)
5. F. Li, X. Kong, C. Zhang, Y. Hua, Food Hydrocoll. **29**(2), 347–355 (2012)
6. L. Mao, Y.H. Roos, S. Miao, J. Agric, Food Chem. **62**(47), 11420–11428 (2014)
7. C.-H. Tang, F. Liu, Food Hydrocoll. **30**(1), 61–72 (2013)
8. C.-H. Tang, L.-J. Luo, F. Liu, Z. Chen, Food Res. Int. **51**(2), 804–812 (2013)
9. C.-H. Tang, M. Yang, F. Liu, Z. Chen, LWT - food Sci. Technol. **53**(1), 15–21 (2013)
10. M. Yang, F. Liu, C.-H. Tang, Food Res. Int. **52**(1), 409–418 (2013)
11. T. van Vliet, Colloid Polym. Sci. **266**(6), 518–524 (1988)
12. E. Dickinson, Colloids Surf. B **20**(3), 197–210 (2001)
13. T. van Vliet, C.M.M. Lakemond, R.W. Visschers, Curr. Opin. Colloid Interface Sci. **9**(5), 298–304 (2004)
14. E. Dickinson, Food Hydrocoll. **28**(1), 224–241 (2012)
15. T. Farjami, A. Madadlou, Food Hydrocoll. **62**, 262–272 (2017)
16. M. Zhang, J. Li, Y. Su, C. Chang, X. Li, Y. Yang, L. Gu, Food Hydrocoll. **97**, 105191 (2019)
17. M. Zhang, Y. Yang, N.C. Acevedo, Food Chem. **318**, 126421 (2020)
18. H. Liu, X.M. Xu, S.D. Guo, LWT - food Sci. Technol. **40**(6), 946–954 (2007)
19. K. Manoi, S.S.H. Rizvi, Food Hydrocoll. **23**(7), 1837–1847 (2009)
20. D. J. McClements, in Food emulsions –Principles, practice and techniques, ed. by D. J. McClements. (Washington, 1999), pp. 235–266
21. F. Liu, C.-H. Tang, Food Chem. **127**(4), 1641–1647 (2011)
22. C.K. Reiffers-Magnani, J.L. Cuq, H.J. Watzke, Food Hydrocoll. **13**(4), 303–316 (1999)
23. M. Primacella, T. Wang, N.C. Acevedo, Food Hydrocoll. **96**, 529–536 (2019)
24. X.D. Sun, S.D. Arntfield, Food Hydrocoll. **25**(1), 25–31 (2011)
25. I.-H. Jin, J.-E. Kim, J.-H. Seo, S.-P. Lee, Food Sci. Biotechnol. **22**(1), 129–136 (2013)
26. J. Chen, E. Dickinson, M. Langton, A.-M. Hermansson, LWT - food Sci. Technol. **33**(4), 299–307 (2000)
27. C.-H. Tang, L. Li, J.-L. Wang, X.-Q. Yang, LWT - food Sci. Technol. **40**(4), 579–586 (2007)
28. M. Wu, Y.-L. Xiong, J. Chen, X. Tang, G. Zhou, J. Food Sci. **74**(4), E207–E217 (2009)
29. C.-H. Tang, L. Chen, E.A. Foegeding, J. Agric. Food Chem. **59**(8), 4071–4077 (2011)
30. V.L. Sok Line, G.E. Remondetto, M. Subirade, Food Hydrocoll. **19**(2), 269–278 (2005)
31. X. Wang, Z. He, M. Zeng, F. Qin, B. Adhikari, J. Chen, Food Chem. **221**, 130–138 (2017)
32. M. Færgemand, B.S. Murray, E. Dickinson, J. Agric. Food Chem. **45**(7), 2514–2519 (1997)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.