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# GELLED EGG WHITE, GELATIN, AND FIBROIN PROTEIN EMULSIONS: PHYSICO-CHEMICAL AND RHEOLOGICAL CHARACTERIZATION

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## ABSTRACT

The aim of this study was to prepare some animal protein solutions-in-oil type gelled emulsions. The 5% solutions of egg white, gelatin, and fibroin proteins were used with sunflower oil, and Span 20 as emulsifier. The prepared emulsions were treated with heat, acid, and heat+acid treatments. The samples showed centrifuge stability, and had cream-yellow color (L* values of 59.46 to 76.28, a* values of -0.42 to -1.55 and b* values of 3.13 to 9.47) tones. Their melting peak temperatures and enthalpies were ranged from 74.56 to 131.87 °C, and from 2.73 to 122.31 J/g, respectively. Rheological frequency sweep test proved that they were stable gels with G´ (storage modulus) values ranging from 900 to 1500 Pa. Further, they had structural recovery ability after exposed to high shear. Further investigations on the potential food applications (dairy, meat products, spreadable fats, etc.) of these emulsions are recommended.

**Keywords***:* Egg white protein, emulsion, gel, gelatin, fibroin, rheology

# JELLEŞTİRİLMİŞ YUMURTA BEYAZI, JELATİN VE FİBROİN PROTEİN EMÜSİYONLARI: FİZİKOKİMYASAL VE REOLOJİK KARAKTERİZASYON

## ÖZ

Bu çalışmanın amacı bazı yağ-içinde-hayvansal protein çözeltisi tipinde jelleştirilmiş emülsiyonların hazırlanması ve karakterize edilmesidir. Yumurta beyazı, jelatin ve fibroin proteinlerinin %5'lik çözeltisi, Span 20 emülsifiyeri eşliğinde ayçiçeği yağı ile emülsiye edilmiştir. Hazırlanan emülsiyonlar ısı, asit ve ısı+asit işlmeleriyle muamele edilmiştir. Örnekler santrifüj stabilitesine sahip olup, kremsi-sarı (L* değeri 59.46 - 76.28, a* değeri -0.42 – (-1.55) ve b* değeri 3.13 - 9.47) renklerdedirler. Ergime tepe sıcaklıkları ve entalpileri 74.56 - 131.87 °C ile 2.73 - 122.31 J/g arasında değişmektedir. Reolojik frekans tarama testi hepsinin dayanıklı jel yapısında olduğunu ve G´ (depo modül) değerlerinin 900 ile 1500 Pa arasında olduğunu göstermiştir. İlaveten, hepsinin yüksek kesme kuvvetine maruz kaldıktan sonra yapılarını yeniden geri kazanma yeteneğinde oldukları ortaya konulmuştur. Potansiyel gıda uygulamalarına (süt ürünleri, et ürünleri, sürülebilir yağlar gibi) yönelik yeni araştırmalar önerilmiştir.

**Anahtar kelimeler**: Emülsiyon, fibroin, jel, jelatin, reoloji, yumurta beyazı proteini

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## INTRODUCTION

Since harmful effects of *trans* and some saturated fatty acids are accepted, elimination of these fats from foods have been suggested (WHO, 2019). New technologies to structure edible liquid oils into solid or semi-solid preperates like oleogels, high internal phase emulsion (HIPE), and Pickering emulsions have been searched extensively to provide alternative solutions to partial hydrogenation, interesterification and fractionation technologies used in fats/oils industries to get solid fats. Emulsion gels are defined as the soft colloid materials that have the properties of both emulsions and gels (Liu et al., 2022; Fonseca et al., 2024; Wen et al., 2024).

Since most food proteins are hydrophilic, it is not easy to add proteins into an oil phase, which naturally is hydrophobic. In last years, several new techniques have been developed to prepare protein oleogels, sometimes in combinations with polysaccharides. Some of these new methods includes direct approaches, while some is indirect templated approaches. The most commonly used approaches are emulsion-templated, foam-templated, hydrogel-templated approaches, solvent transfer methods, and direct dispersions of freeze-dried protein aggregates (Feichtinger and Scholten, 2020). Protein hydrogel-templated approach has started to be developed in recent years. Depending upon the gelling mechanism of the bio-polymers or composite bio-polymers used, different gelling mechanisms (heat-set, cold-set, ion-set, pH-set, enzyme-set, pressure-set) have been used. Oil continuous emulsions containing different fractions of aqueous protein solutions as the dispersed phase could be structured by gelling the protein solution with different setting techniques to have stable gelled emulsions. These gelled emulsions have been shown to have certain rheological properties resembling semi-solid fats, and could be used as the hard fat source in various food aplications (Yılmaz and Keskin Uslu, 2024; Ashfaq et al., 2024; McClements, 2024).

In this study, egg white (EW) protein, gelatin (GL) protein, and fibroin (FB) protein were utilized. EW proteins contain ovalbumin and ovotransferrin and can form very stable emulsions due to its flexible structure, rapid interfacial film formation ability, and low interfacial tension. EW proteins are also known for their excellent gelling ability, and used extensively in food industry for both purposes (Wen et al., 2024; Garg et al., 2024). Emulsion gels prepared with grapeseed oil and cold-set egg white protein gel was used for delivery of food bio-active compounds (Ashfaq et al., 2024). Gelatin is a hydrolyzed collagen product including many hydroxyl, carboxyl, and amino groups; hence, it is a very hydrophilic polymer, and can form stable emulsions and gels upon heating (Wen et al., 2024). Gelatin-inulin complexes were used to prepare heat-set emulsion gels, and used as fat replacers in sausage type products (Ashfaq et al., 2014). Fibroin is a core protein extracted from domesticated silk worm, and consists of one heavy and one light chain connected with disulphite linkages. It is known for high mechanical property and can form emulsions due to surface film forming ability (Tang et al., 2015). Although fibroin-based hydrogels have intensively been searched for biomedical applications, food applications are rather limited (Kapoor and Kundu, 2016).

In this study, aqueous EW, GL, and FB protein solutions were used as the dispersed phase in an oil continuous phase to create emulsions with Span 20 emulsifier, and further the effects of heating, acidulation, and acidulation+heating treatments on the prepared emulsions were investigated against control samples to observe possible usability of these gelled emulsions as structured semi-solid fat sources for food applications.

## MATERIALS AND METHODS

### Materials

Commercial refined-winterized sunflower seed oil (Trakya Birlik Co., Tekirdağ, Türkiye) was purchased from a local store. Gelatin (dry dust, 200 bloom) was purchased from Yasin Teknik & Chem. Co (İstanbul, Türkiye). Pure egg white protein (98%, powder) was get from Kor Agro Organic Food Co. (İzmir, Türkiye). Fibroin protein (pure, powder) was also purchased (Yasin

Teknik & Chem. Co, İstanbul). Span 20 was purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Food-grade glucono-delta-lactone (pure white crystal) was purchased (Alfasol, İstanbul, Türkiye). All other solvents and chemicals used were procured from both Sigma-Aldrich (St. Louis, USA) and Merck (Darmstadt, Germany).

**Preparation of Protein Solutions**

The EW protein and GL protein were pure dry powders and were fairly soluble in water. Their stock solutions (5% protein) were prepared by weighing calculated amounts of the protein powder and dissolving it in pure water at room temperature by stirring for 24 h. During solubilization, microbial activity was controlled by adding 1-2 drops of 0.02% sodium azide solution into the protein slurry. After 24 h, both EW and GL solutions were heated to 35 °C to get transparent solutions, and these solutions were used as the aqueous phase in preparing the emulsions. Since fibroin (FB) was not a readily soluble protein, a prior solubilization procedure with Ajisawa technique was applied. Ajisawa solubilization technique is the most commonly used and accepted technique to solubilize fibroin, as stated in literature, and consequently used in this study (Wang et al., 2020). Briefly, fibroin dry powder (5wt% of total solution) were mixed in a 1:2:8 molar ratio with $CaCI_2$:ethanol:water mixture for 7 days at room temperature by constant stirring. Similarly several drops of sodium azide was added to prevent microbial activity. After solubilization, excess salts were removed by 4 stepwise dialysis for 6 h against ultrapure water (volume ratio of 100), while refreshing the ultrapure water each time. Finally, fibroin solution was centrifuged at 13.000 rpm for 15 min to remove any aggregates. The fibroin stock solution was used to prepare the water-in-oil emulsion similar to the other two protein solutions.

**Preparation of the Protein Emulsions and Gelling of the Dispersed Phase**

The formulations provided in Table 1 were used to prepare the animal protein emulsion gels. The calculated amounts of sunflower oil was heated to approximately 35 °C before addition of the calculated amounts of Span 20 emulsifier, and mixed at 1.000 xg with Ultra-Turrax (IKA T-25, Germany) for 1 min for homogeneous dissolution of the emulsifier. The animal protein stock solutions were also pre-heated to 35 °C before adding into the oil. While slowly adding each protein solution into the oil phase, the mixture was mixed at 1365 xg with Ultra-Turrax for 3 min. After formation of the emulsion, the sample was placed into capped glasses, and kept in refrigerator. This sample was the control (ct) sample for all kinds. To prepare the heat stabilized (hs) sample, the prepared emulsion was kept 30 min in a water bath set to 80 °C. Similarly to prepare acid stabilized (as) samples, 0.5% (w/total weight) glucono-delta-lactone was added during the emulsification phase. Finally, an acid + heat stabilized (ah) sample was prepared by adding glucono-delta-lactone as the same and the waiting of the prepared emulsion in the water bath as the same. The prepared emulsion gels could be observed in Fig. 1.

**Stability and Color of the Gelled Emulsions**

5 g of each sample was weighed into Falcon tubes, and centrifuged (Sigma 2-16 K, Postfach, Germany) at 1300 xg for 15 min at 10 °C, before visually observing the breakage of the emulsion. Stable emulsions assigned with (+) and broken emulsions assigned with (-) signs. The emulsion samples were scooped into the liquid sample holder of the Minolta CR-400 colourimeter (Konica, Minolta Sensing, Osaka, Japan) instrument, and color values (L*, a*, b*) were recorded at several points. The CIE parameters of L* specifies brightness/darkness, a* value specifies redness (+) / greenness (-), and b* value specifies yellowness (+) / blueness (-) level (Yılmaz and Toksöz, 2022).

**Thermal Properties of the Gelled Emulsions**

The melting onset and peak temperatures, and enthalpies of the gelled emulsions were assessed with a Perkin-Elmer 4000 Series Differential Scanning Calorimetry (Groningen, The Netherlands). The instrument was regularly calibrated with Indium and Zinc. Around 10 mg of samples were weighed into aluminum pans and sealed hermetically. The temperature program was set by heating from 20 °C to 100 °C by 10

°C/min rate. The Pyris 1 Manager Software of the instrument was used for the calculations (Yılmaz and Toksöz, 2022).

Table 1. The formulations used to prepare gelled animal protein emulsions.

| | Oil (%) | 5% Animal Protein Solution | Span 20 Emulsifier (%) | Glucono-δ-Lactone (%) |
|---|---|---|---|---|
| EW-ct | 70.0 | 27.0 | 3.0 | - |
| EW-hs | 70.0 | 27.0 | 3.0 | - |
| EW-as | 70.0 | 26.5 | 3.0 | 0.5 |
| EW-ah | 70.0 | 26.5 | 3.0 | 0.5 |
| GL-ct | 70.0 | 27.0 | 3.0 | - |
| GL-hs | 70.0 | 27.0 | 3.0 | - |
| GL-as | 70.0 | 26.5 | 3.0 | 0.5 |
| GL-ah | 70.0 | 26.5 | 3.0 | 0.5 |
| FB-ct | 70.0 | 27.0 | 3.0 | - |
| FB-hs | 70.0 | 27.0 | 3.0 | - |
| FB-as | 70.0 | 26.5 | 3.0 | 0.5 |
| FB-ah | 70.0 | 26.5 | 3.0 | 0.5 |

EW-ct: Egg white protein-control emulsion gel, EW-hs: Egg white protein-heat stabilized emulsion gel, EW-as: Egg white protein-acid stabilized emulsion gel, EW-ah: Egg white protein-acid+heat stabilized emulsion gel, GL-ct: Gelatin protein-control emulsion gel, GL-hs: Gelatin protein-heat stabilized emulsion gel, GL-as: Gelatin protein-acid stabilized emulsion gel, GL-ah: Gelatin protein-acid+heat stabilized emulsion gel, FB-ct: Fibroin protein-control emulsion gel, FB-hs: Fibroin protein-heat stabilized emulsion gel, FB-as: Fibroin protein-acid stabilized emulsion gel, FB-ah: Fibroin protein-acid+heat stabilized emulsion gel

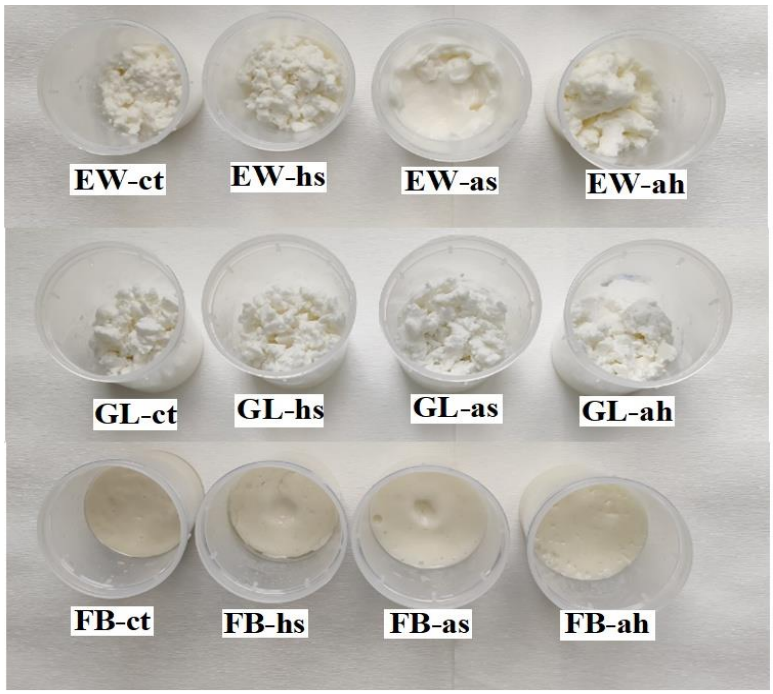



Fig. 1. The gelled animal protein emulsions prepared (For nomenclature of the sample names please see Table 1).

**Polyphormic Forms of the Gelled Emulsions**

The polymorphic forms of the prepared samples were determined by the PANalytical Empyrean model (The Netherlands) X-ray diffractometer according to the AOCS Cj 2-95 method (AOCS, 2012). The sample was placed in the sample holder, and the measurements were completed at room temperature (20±3 °C) with 2.0° to 50° by 2°/min scan rate angular scans (2θ) performed under a Cu source X-ray tube (λ = 1.54056 Å, 40 kV and 40 mA). Data analysis was performed with X'Pert HighScore Plus software (Malvern Panalytical Ltd., Royston, UK) (Keskin Uslu and Yılmaz, 2021).

**Rheological Properties of the Gelled Emulsions**

Rheological measurements were done with a DHR 2 rheometer (TA Instruments, USA) including a Peltier system (±0.1 °C) beneath lower plate. All analyses were completed with cross-hatched parallel plate (ϕ = 40 mm, gap 0.9±0.1 mm) geometry. All tests were conducted at 10 °C, since at that temperature, the samples were stable and solid. An amplitude sweep test with 0.01-100% strain and 1 Hz frequency was completed at first to determine the linear viscoelastic region (LVR). The LVR is defined as the region in which a plateau of storage (G´) and loss (G´´) moduli prevail (Mezger, 2014). The LRV strains were in the range of 0.09-2.51%, respectively. The frequency sweep tests were completed at the determined LVR strains and angular frequencies from 1.0 to 1000 rad/s. Finally, time sweep tests were completed to assess the structural recovery ability. In this test, the structural recovery abilities of the samples exposed to stress and released from the stress were determined. Three strain gradient regions were applied to the samples at 10 °C with a 1 Hz frequency. In the first time region, strain at LVR strain values determined for each sample was applied for 200 s to simulate standard resting condition. Then, stronger shear (LVRstrain≤ Strain) was applied to simulate structural breakdown (higher force region) for 200 s; and finally, in the last region (1000 s), very low shear (LVRstrain≥ Strain) was applied to simulate structural recovery.

**Statistical Analysis**

All samples were prepared two times as two replicates, and analyses for each replicate were completed in two times. The mean data with standard deviations were given from four measurements. Comparison of the samples was accomplished with a one-way analysis of variance (ANOVA) of Tukey's test. The independent variables were the protein types and the gel treatment techniques, and the dependent variables were the measured analytical values. The statistical test were conducted with Minitab statistical software (Minitab, 2010) under 95% confidence level.

## RESULTS AND DISCUSSION

**Stability and Color Properties**

The centrifuge stability and instrumental color values of the prepared gelled emulsion samples are given in Table 2. The control sample of egg white (EW-ct) protein emulsion was broken after applying the centrifuge force of 1300 xg for 15 min, the rest of the samples were fairly stable. This test is aimed to predict the mechanical stability of the samples when exposed to transport, mixing, whipping or other processes yielding force. Eventually, heat, acid and heat+acid treatments of the emulsions enhanced the stability of EW emulsion gels. These emulsions included 70% oil phase, and gel state was provided by the solubilized and gelled animal proteins within the dispersed aqueous droplet phase. Clearly treatments (heat, acid, and both together) enhanced gelling of the proteins to enhance stability. Since these gelled emulsions were more solid-like samples, they might be used as oleogel-like preparates. Consequently, their rheological characterizations were completed in this study.

The L* component of color indicates the level of brightness or luminosity. The L* values were ordered among the three protein gels as GL > EW > FB (Table 2). The samples could also be observed in Fig. 1. The highest L* value (76.28) was measured in GL-hs sample, and the lowest one (59.46) was in FB-hs sample. Generally, the samples were not dark, and suitable to be used in foods. Similarly, a* values showed some small

differences, and usually heat application enhanced this value. Clearly, applied heat caused formation of some green colored materials to enhance the a* value in negative direction. In this complex matrix including proteins, oil and emulsifier, which compounds formed by heat are not known, but they yielded trace amount of greenness. The a* value indicates level of redness-greenness, and all samples had some small levels of green tones, since all were in negative directions (Fig. 1). The +b* value shows the level of yellowness and –b* value indicates the levels of blueness. Clearly all samples had some yellow pigments, and yellowness were highest in FB samples and lowest in GL emulsions. Overall, color of the samples were creamy-yellow and quite suitable for food products. The samples could be entagrated into formulated food products as semi-solid fat source without any color discrepancy.

Table 2. Physical properties of the gelled animal protein emulsions.

| | | Instrumental Color Values | | |
|---|---|---|---|---|
| | Centrifuge Stability | L* | a* | b* |
| EW-ct† | - | $75.16 \pm 0.38^{b}$†† | $-1.29 \pm 0.06^{c}$ | $6.37 \pm 0.27^{d}$ |
| EW-hs | + | $74.91 \pm 0.20^{b}$ | $-1.55 \pm 0.04^{a}$ | $6.37 \pm 0.10^{d}$ |
| EW-as | + | $74.43 \pm 0.14^{b}$ | $-1.45 \pm 0.03^{b}$ | $6.51 \pm 0.12^{d}$ |
| EW-ah | + | $74.67 \pm 0.39^{b}$ | $-1.55 \pm 0.02^{a}$ | $6.05 \pm 0.21^{d}$ |
| GL-ct | + | $74.62 \pm 0.13^{b}$ | $-0.98 \pm 0.06^{e}$ | $3.81 \pm 0.29^{f}$ |
| GL-hs | + | $76.28 \pm 0.45^{a}$ | $-1.11 \pm 0.09^{d}$ | $4.30 \pm 0.21^{e}$ |
| GL-as | + | $75.10 \pm 0.68^{a}$ | $-0.94 \pm 0.04^{e}$ | $3.09 \pm 0.19^{f}$ |
| GL-ah | + | $75.90 \pm 0.55^{a}$ | $-0.91 \pm 0.03^{e}$ | $3.13 \pm 0.08^{f}$ |
| FB-ct | + | $62.49 \pm 0.36^{d}$ | $-0.42 \pm 0.05^{f}$ | $9.47 \pm 0.04^{a}$ |
| FB-hs | + | $59.46 \pm 0.58^{e}$ | $-1.10 \pm 0.02^{d}$ | $7.22 \pm 0.06^{c}$ |
| FB-as | + | $65.94 \pm 0.81^{c}$ | $-0.94 \pm 0.01^{e}$ | $8.10 \pm 0.06^{b}$ |
| FB-ah | + | $61.38 \pm 0.51^{d}$ | $-1.15 \pm 0.03^{d}$ | $8.03 \pm 0.03^{b}$ |

†For nomenclature of the sample names please see Table 1.
††Lowercase supercript letters in columns indicate statistically significant differences among the samples ($p < 0.05$)

**Thermal Properties**

DSC determined melting onset, peak temperatures and ethalpies of the gelled animal protein emulsion samples are presented in Table 3. The melting peak temperatures ranged from 74.56 to 92.76 $^{o}$C for EW protein emulsion samples, whereas the same range were 99.47-109.54 $^{o}$C for GL protein and 90.52-118.40 $^{o}$C for FB protein emulsion samples. Clearly, FB protein emulsion gels had a wider range and highest peak melting points followed by GL protein and EW protein emulsions. There were two fractions yielding doubled peaks in fibroin protein-based samples (Table 3). This might be due to protein composition of the samples, and most probably fibroin contained two distinct proteins. In addition, acid, heat, and combined treatments have caused little enhancements of the peak melting temperatures, but the trend was not the same in all samples. These results indicate that the prepared gelled emulsion samples are fairly heat-stable. In a study (Garg et al., 2024), lowering pH and heating of ovalbumin protein enhanced the emulsion formation ability and stability, just similar to the findings of this study. Overall, fairly good thermal stability of the samples could be a beneficial property to also utilize these samples in prepared foods requiring heat treatments.

Table 3. Thermal melting properties of the gelled animal protein emulsions.

| | Melting | | |
|---|---|---|---|
| | Onsetm (°C) | Peak (Tm) (°C) | ΔHm (J/g) |
| EW-ct† | 73.24 ± 0.14$^{f}$ | 74.56 ± 0.01$^{g}$ | 4.41 ± 0.22$^{h}$ |
| EW-hs | 86.26 ± 0.02$^{e}$ | 88.40 ± 0.23$^{f}$ | 2.73 ± 0.01$^{i}$ |
| EW-as | 90.28 ± 0.23$^{d}$ | 92.76 ± 0.16$^{e}$ | 11.18 ± 0.10$^{g}$ |
| EW-ah | 66.07 ± 0.08$^{g}$ | 91.92 ± 0.06$^{e}$ | 4.23 ± 0.03$^{h}$ |
| GL-ct | 99.08 ± 0.06$^{d}$ | 99.47 ± 0.27$^{d}$ | 68.82 ± 0.10$^{b}$ |
| GL-hs | 108.77 ± 0.30$^{c}$ | 108.96 ± 0.04$^{c}$ | 122.31 ± 0.35$^{a}$ |
| GL-as | 97.59 ± 0.10$^{e}$ | 99.57 ± 0.13$^{d}$ | 19.76 ± 0.17$^{d}$ |
| GL-ah | 109.06 ± 0.00$^{c}$ | 109.54 ± 0.18$^{c}$ | 17.60 ± 0.74$^{e}$ |
| FB-ct-Fr.1 | 101.98 ± 0.01$^{c}$ | 102.95 ± 0.86$^{d}$ | 14.70 ± 0.21$^{f}$ |
| FB-ct-Fr.2 | 131.57 ± 0.02$^{a}$ | 131.87 ± 0.01$^{a}$ | 16.52 ± 0.04$^{e}$ |
| FB-hs | 103.29 ± 0.37$^{c}$ | 105.47 ± 0.27$^{cd}$ | 69.26 ± 0.05$^{b}$ |
| FB-as-Fr.1 | 89.64 ± 0.03$^{d}$ | 90.52 ± 0.42$^{e}$ | 5.35 ± 0.18$^{h}$ |
| FB-as-Fr.2 | 117.36 ± 0.01$^{b}$ | 118.40 ± 0.23$^{b}$ | 3.63 ± 0.05$^{i}$ |
| FB-ah | 99.33 ± 0.45$^{d}$ | 101.24 ± 0.02$^{d}$ | 56.33 ± 0.01$^{c}$ |

†For nomenclature of the sample names please see Table 1.
††Lowercase supercript letters in columns indicate statistically significant differences ($p < 0.05$). Fr: fraction

**Polyphormic Forms**

The X-ray diffraction (X-RD) pattern data of the samples were given in Table 4. Generally, the X-RD patterns of solid lipid samples show the crystalline types of triacylglycerol crystals within a sample. If there are other crystallized sources in the sample, they could have different 2-teta values. By observing and comparing the d spacings, it would be possible to determine the polymorphic types present in the samples. According to AOCS method Cj 2-95 (AOCS, 2012), the main peaks observed in a sample provide the most meaningful clue to define the fat crystal polymorphs. If a sample shows a single peak at around 4.2 Å, its polymorphic form is α, if a sample contains 3.8 and 4.2 Å peaks, it must be β' polymorph, and if the peak appears at 4.6 Å position, then it should be β polymorphic form. Clearly, most of the samples contain peaks at around 4.42-4.56 Å, and consequently they contain β polymorphic forms. The intensities of the peaks were also similar among the samples. Further, some samples had a peak at around 19-20 Å, indicating the presence of some liquid or amorph fat. In edible oil technology, each fat polyphorm is characterized with some macroscopic properties, and preferred according to these properties. It is well known that the β' polymorphic forms looks like creamy, smooth, and very fine crystals with technological properties of dispersibility, smooth texture, optimum melting, and good mouthfeel. Consequently, this form is preferred for breakfast margarine and other spreadable fat products, while β polymorph is the preferred polyphorm for chocolate, confectionery, and shortening fats, and it is characterized with a sandy, hard, and rough texture (Chrysam, 1996). It was also indicated that after storage at certain temperatures, the fat crystal polyphorms can undergo transformations (Chrysam, 1996; Yılmaz and Toksöz, 2022). There were no observed effects of the treatments (heat, acid, and heat+acid) on the polymorphic forms of the lipid crystals. Overall, the prepared gelled emulsion samples could be used as solid fat source replacers in bakery, dairy, and spreadable margarine products.

Table 4. The X-ray diffraction patterns of the gelled animal protein emulsions.

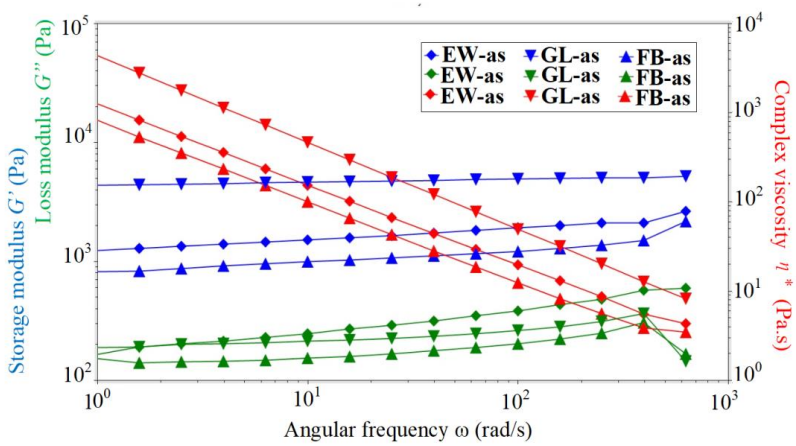
| | 2-Theta | d (Å) |
|---|---|---|
| EW-ct† | 2.04, 19.64 | 43.16, 4.51 |
| EW-hs | 19.54 | 4.53 |
| EW-as | 18.32, 20.05 | 4.83, 4.42 |
| EW-ah | 7.80, 20.05 | 11.31, 4.42 |
| GL-ct | 4.71, 8.51, 19.95 | 18.71, 10.31, 4.44 |
| GL-hs | 7.80, 14.67, 20.05 | 11.31, 6.03, 4.42 |
| GL-as | 19.58 | 4.53 |
| GL-ah | 20.01 | 4.55 |
| FB-ct | 20.05 | 4.42 |
| FB-hs | 3.12, 19.44 | 28.27, 4.56 |
| FB-as | 8.21, 19.64 | 10.75, 4.51 |
| FB-ah | 8.00, 20.05 | 11.03, 4.42 |

†For nomenclature of the sample names please vide Table 1.

**Rheological Properties**

The frequency sweep test graphics with the storage modulus (G´), loss modulus (G´´), and complex viscosities (η*) of the samples against applied angular frequencies ranging from 1 to 1000 rad/s are shown in Fig. 2. This test provides information about how the sample response to increasing strains, and describes time-dependent behavior. Practically, results provide information about the inner structure and stability of the gels.

The storage modulus (G´) represents the elastic portion and describes the sample's solid-like properties, while the loss modulus (G´´) represents the viscous portion, and describes the liquid-like properties of the sample. In fact, these two parameters (G´ and G´´) are the two components of the complex viscosity (η*), which describes the entire viscoelastic behaviour (Mezger, 2014).



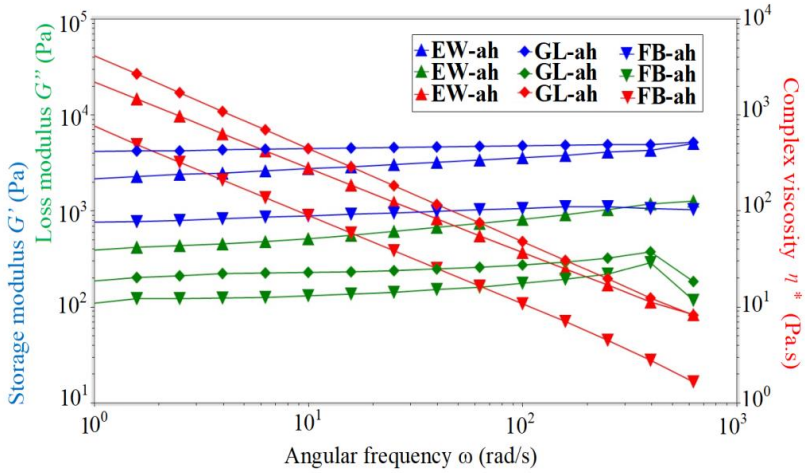
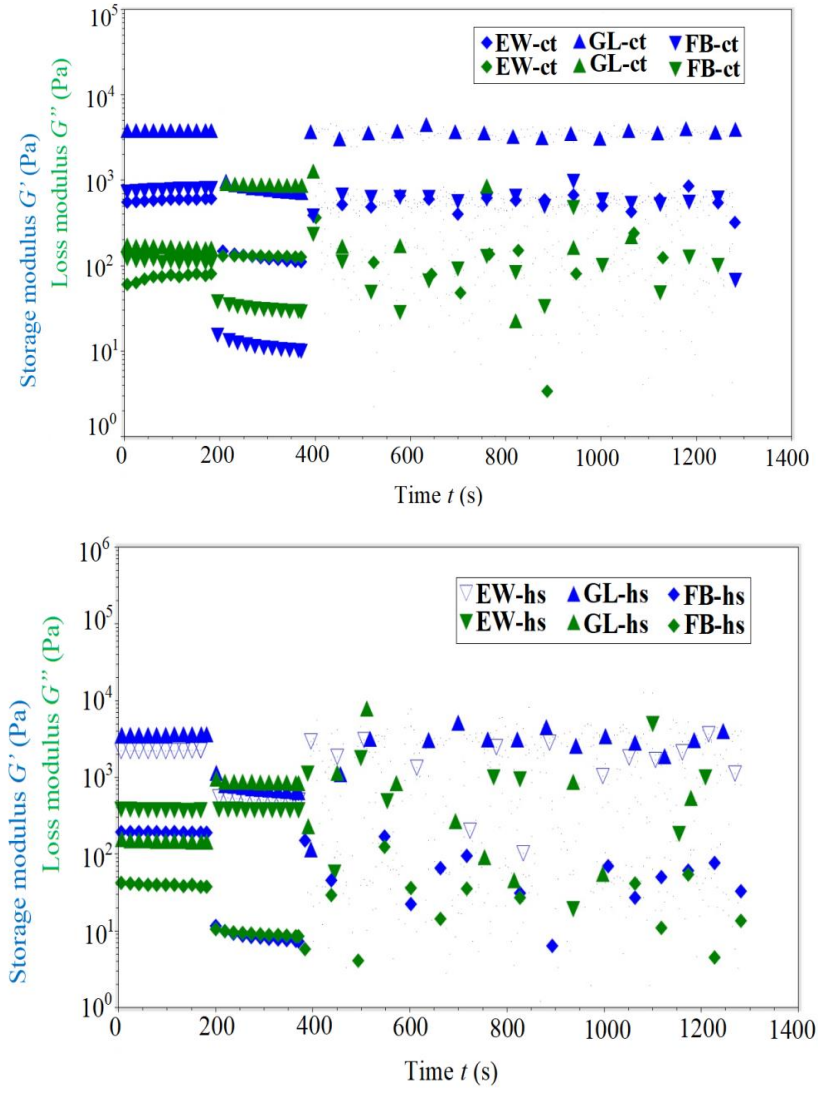



Fig. 2. The frequency sweep test graphics of the gelled animal protein emulsions (For nomenclature of the sample names please see Table 1).

All samples had the G´ values always higher than the G´´ values, indicating that within the applied angular frequency range, the samples were more solid-like, or in gelled state. In all samples, the G´ modulus values were much higher than the G´´ values, indicating that the gels were fairly stable. Further, heat, acid, and heat+acid treatments have changed the hardness of the gels. The EW gels had around 900, 1100, 1100, and 1200 Pa G´ values for the ct (control), hs (heat stabilized), as (acid stabilized), and ah (heat and acid stabilized) treatments, respectively. Around 3000, 1200, 1300, and 1400 Pa G´ values for GL samples, and around 1000, 200, 900, and 1100 Pa G´ values for the FB samples could be observed from Fig. 2, respectively. Clearly, amog all samples, the highest G´ values (highest gel hardness) were measured in GL protein samples, while lowest values were measured in FB samples. Differences in gel strenghts with different proteins indicate that choice of protein and also technique of gel setting could influence rheological properties. The gel strenght can be result of covalent interactions among polypeptide chains, and setting techniques affect network formation by arranging peptide unfolding and cross-linking interactions. Consequently, through election of different proteins and setting techniques, gel strenghts could be altered (Feichtinger and Scholten, 2020). Gelatin have been used in food sector as a common stabilizer. EW proteins gels enhanced their hardness after the treatments. Utilization of FB proteins in food sector is almost unknown, but this study pointed out that there was a potential. The FB protein emulgels treated with acid lowered the G´ value, but treatment with acid+heat stabilization have yielded enough hardness to be used as structured oil emulsion sample. This finding was supported by the results of Tang et al. (2015), in which, lowered pH in silk fibroin protein emulsions reduced emulsion stability and yielded larger droplets. In a previous study (Zhang et al., 2022), peanut protein gels were effectively stabilized with 0.5% glukono-δ-lactone as acidifier. Except FB, acid treatments were enhanced gel strenght in this study.

However, for food applications, not only the gel strength, but also other rheological properties are important. Especially, structural recovery ability is relevant when samples are used on an industrial scale, where pumping through pipes are required. During pipe pumping or mixing, whipping processes, the samples loss their gel state due to high shear. When a material has the ability to recover its structure, it will be less susceptible to changes occurring during high shear conditions (Feichtinger and Scholten, 2020). Consequently, time sweep tests have completed (Fig. 3). The aim of this test was to observe the structural recovery abilities of the gel samples exposed to and released from high shear. In the first region, the resting beheviour was simulated by applying low strains. At this region, the samples had higher G´

values indicating their gelled state. In the second region, 10-times higher shear than LVR shear was applied to get a structural disruption. In fact, in all samples the storage modulus (G´) values were lowered below the loss modulus (G´´) values showing a real structural deformation. In the last region, the recovery of gel state was observed after removing the applied high shear in the second region. Clearly, the samples returned to their original gelled state proved by re-enhanced G´ values. As observed from Fig. 3, the G' values enhanced to almost to their resting state (region one) level, indicating that a partial recovery of the structure was attained. This means that during the destruction region (second region), the emulsion was not broken, but the gel state was lost. This behavior is called thixotropy in rheology science, and samples with this property is preferred as they provide structural flexibility to let high shear food operation be applied without a final loss of gel structure. Quite similar findings were found in previous oleogel (Yılmaz and Toksöz, 2022) and plant protein emulsion gel samples (Yılmaz and Keskin Uslu, 2024).



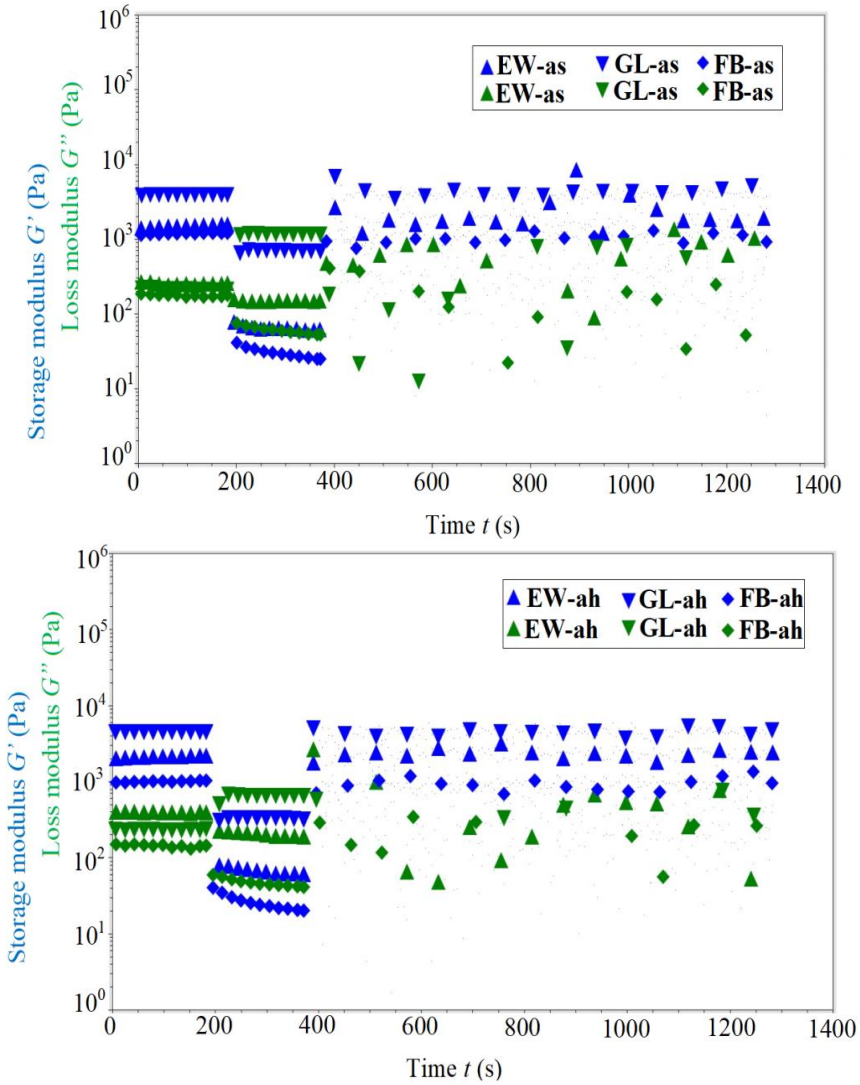



Fig. 3. The time sweep test graphics of the gelled animal protein emulsions (For nomenclature of the sample names please see Table 1).

## CONCLUSIONS

In this study, aqueous protein solution-in-oil type emulsions were prepared and treated with heating, acidulation and combination of both against control to get gelled protein emulsions as semi-solid fat sources. Egg white protein, gelatin and fibroin protein were utilized. Results indicated that all proteins yielded stable gelled emulsions, although gel strenght was highest in gelatin and lowest in fibroin samples. Further, heat and acid treatments were usually enhanced both structural and thermal stabilities. The prepared samples looked quite suitable to be used in processed foods as semi-solid fat sources. This study were provided piooner data for the fibroin protein emulgels as food samples. Further studies for food applications were suggested.

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## CONFLICT OF INTERESTS

The author declares that for this article they have no actual, potential, or perceived conflict of interests.

## AUTHOR CONTRIBUTIONS

Manuscript writing: Emin Yılmaz, planning the experiments: Emin Yılmaz and Eda Keskin Uslu, laboratory experiments: Eda Keskin Uslu, the idea of the study: Emin Yılmaz, designing the study: Emin Yılmaz, editing original draft: Emin Yılmaz. All authors have read and approved the final manuscript.

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