

**High-intensity ultrasound as a tool to form water in oleogels emulsions structured by lipids
oleogelators**

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Abstract

This study aims to evaluate the structuration power and physical properties of oleogel emulsions formed by monoglycerides (MG), hardfat (HF), and lecithin (LE), with and without high-intensity ultrasound (HIU). Emulsion gels with and without HIU were prepared in mono-, bi- and ternary gelator blends (10% of the lipid phase) and with different water (W) proportions (W= 1, 15, or 30%). All samples were analyzed according to their gel/emulsion formation, microstructure, melting behavior, rheology, texture, polymorphism, and oil binding capacity. In mono structured emulsions only MG was able to form structured emulsions that did not flow. Three synergic combinations of oleogels were found: MG:HF, HF:LE, and MG:HF:LE; nevertheless, the only synergic combination found in all water proportions was MG:HF. Sonicated MG:HF emulsions formed more organized and stable water droplets, and with 30W even achieved similar physical properties as MG:HF oleogel (0W). A soft structured emulsion was also found for sonicated LE and HF:LE with 30W. Multicomponent emulsion gels formed by MG, HF, and LE are a good alternative for food applications because they can form different synergic combinations with good physical properties such as hardness ($>1\text{N}$), elastic modulus ($>1 \times 10^5\text{ Pa}$), and oil binding capacity ($>99\%$), and these properties can be even improved by HIU.

Keywords: emulsion gels, high-intensity ultrasound, monoglycerides, lecithin

1. Introduction

The importance of structuring oils nowadays is undeniable not only for the food industry [1] but also for pharmaceutical [2] and personal care [3] industries. The structuration of liquids oil with high-melting-point components or oleogelators is also known as oleogel technology [4]. Oleogels formed using different routes [5–7] or different oleogelators [8–13] as well as the combinations of diverse oleogelators [14–16] have been extensively explored. The most studied and explored oleogelators combinations were formed by lipids oleogelators as monoglycerides (MG), phytosterols, fatty acids, lecithin (LE), vegetable waxes, and high melting point triacylglycerols. Those components have been mixed in order to produce synergic oleogels with good physical properties as oil binding capacity, hardness, viscoelasticity, among others [15–19].

To add an extra perspective to oleogel technology and extra knowledge on food matrices, more studies on emulsions could eventually be done, since fat and oil-based products are available to consumers in the form of water-in-oil emulsions (W/O) as spreadable margarine, or oil-in-water (O/W) emulsions as found in desserts, yogurt, mayonnaise, and dressings [20]. In both emulsion systems, solid fat plays a three key roles i) it forms a crystalline fat network in the continuous oil phase to influence the structure properties (i.e. firmness, texture, and spreadability); ii) it forms fine fat crystals that accumulate at the water-oil interfaces and stabilizes the dispersed water droplets, and iii) it contributes to the sensorial properties such as mouthfeel and flavor release [21]. Some previous research has been performed on W/O oleogels emulsions [22,23] and applying this type of systems in margarines [24–27]. They have shown that the oil structured has the potential to be applied in food emulsion products and that the network form can even entrap oil and water. Nevertheless, more studies on these oleogel emulsions are necessary to achieve final products that are similar to a spreadable fat, stable, economically viable, and sensory accepted. Most of these previous studies have used MG and/or vegetable wax to structure both phases (water and oil). More recently, LE has also showed a big potential to form oleogels emulsions when mixed with stearic acid [28], or form bigels [29–31].

High-Intensity Ultrasound (HIU) has recently become an interesting alternative for oleogels because it has shown potential to i) reduce the amount of oleogelator [32], ii) reduce the incompatibility among oleogelator blends [33], and iii) improve the physical properties of the oleogels [34,35]. Moreover, a previous study using this technology in an oleogel emulsion showed a higher stability of the emulsion even when submitted to higher temperature storage [36]. These effects were observed due to the strength of the crystalline oleogelators in the crystalline network (monoglycerides-MG, high-melting-point triacylglycerols-HF, and vegetable waxes) promoted by cavitation.

Based on previous synergic interaction of the multicomponent oleogels formed by MG, LE, and HF [33,35] sonicated or not. Moreover, on the positive effect of HIU on organizing W/O oleogels emulsion [23], we evaluated how the multicomponent system formed by MG:HF:LE would behave as a W/O oleogel emulsion, emulsified or not by HIU. Thus this study aimed to i) verify if sonication could also improve the emulsifier properties of MG, LE, HF, and their blends and ii) produce W/O oleogel emulsions based on food grade and natural lipid ingredients with improved physical properties.

2. Material and Methods

2.1. Materials

Lecithin (LE, melting point (MP) = -0.75 ± 0.1 °C) Lipoid P 100 (Phosphatidylcholine 90% from soybean (non-GMO)) was kindly donated from Lipoid AG (Switzerland). The typical fatty acid composition is palmitic acid (P, 12-17%), stearic acid (St, 2-5%), oleic acid (O, 7-12%), linoleic (L, 59-70%), linolenic (Ln, 5-8%). Monoglycerides (MG, MP 66.5 ± 0.1 °C) used was Dimodan®HP MB that was kindly donated by Royale Lacroix, Belgium. This MG is a distilled palm-based MG ($\geq 90\%$ pure), its fatty acid composition is mainly 56% P and 41% St. The fully hydrogenated rapeseed oil (HF, MP 61.0 ± 0.2 °C) was kindly donated by Cargill, Germany, this HF was formed mainly by triacylglycerol containing St (92%) and P (5.5%), and consequently by StStSt (82.3%) and PStSt (14.4%). Rapeseed oil was acquired from Royale Lacroix, Belgium, this oil was mainly composed of OOLn (12.1%), OOL (22.6%), OOO (29.3%), and POO (6.5%) triacylglycerols (TG).

2.2. Sample preparation

All samples were prepared with a total concentration of 10% (w/w) oleogelator in the lipid phase (rapeseed oil). This 10% was divided in equal proportions for each oleogelator (MG, HF, and LE) in mono, binary or ternary systems, and the emulsions were formed by adding 1, 15, or 30 % water, as shown in Table 1 with or without sonication (HIU). The total amount of oleogelator was based on recent results of our group [35]. The amount of water was used in low amounts (1%) to evaluate a possible stabilization of the crystalline network of MG and HF [23], and to form a worm like structure for the LE [28,37]. Moreover higher concentrations (15 and 30%) were used to simulate realistic O/W emulsions.

The oleogel emulsions (50mL) were prepared by firstly melting the lipid phase (110°C/30min) under magnetic stirring (350 rpm). After complete dissolution of the gelators in the oil, they were transferred to a 100mL water jacket vessel at 60 °C and fast cooled to 60°C at 10°C/min under low stirring (100 rpm) with a magnetic bar. Control samples without water phase were then poured into 100mL plastic containers (filling 25 cm of the 40 cm high container) and subjected to a second slow cooling step (cooling rate 0.5 °C/min), by placing the

samples in an incubator set at $20 \pm 0.5^\circ\text{C}$. The samples were then stored for 48h in the same incubator to stabilize the gel network at $20 \pm 0.5^\circ\text{C}$ (room temperature).

To prepare the oleogel emulsion, after the lipid phase achieve 60°C a pre-warmed water phase (60°C) was added to the lipid phase and mixed using an ultraturrax at 11000 rpm for 10s (IKA, Werke, Germany) or high-intensity ultrasound (HIU, 20kHz, CPX 750, Cole 115 PA Parmer Instruments, Illinois, USA) using 50% amplitude ($50 \pm 2 \text{ J/s}$, 1 J/s.mL), both agitations were performed for 10s using. The oleogel emulsions (50mL) with or without HIU were then statically cooled to 20°C (0.5°C/min) in 100mL plastic containers and stabilized for 48h at this temperature before further analysis. All oleogels and oleogel emulsions with or without HIU were prepared in triplicate and for each round 50 mL of the sample was made.

2.3. Methods

2.3.1. Macroscopic and Microscopy evaluation

The macroscopic visual evaluations of the oleogels and emulsions after 48h of stabilization were performed by taking about 5mL of sample with a spoon and transferred to a watch-glass. Samples were visually evaluated and pictures were taken to compare them. The microstructure using polarized and non-polarized light was analyzed with an optical microscope (Nikon Eclipse E400, Kanagawa, Japan) equipped with a digital camera (Nikon, DS-FI2, Kanagawa, Japan). One drop of the sample stabilized at 20°C for 48h was placed between a slide and a cover slide. Three different samples were collected from different points of the stabilized sample, for each process. For each sample, a total of 9 images of the three different replications were analyzed.

2.3.2. Oil loss

The amount of oil loss in the samples was evaluated in a centrifuge 5810R Eppendorf (Hamburg, Germany) by centrifugation according to da Silva and Danthine [33]. First, a pre-weighed empty 2mL Eppendorf (w_a) was filled with approximately 1g of sample. The system sample + Eppendorf was also weighted (w_b), and centrifuged for 15min at 2950g. Subsequently, the tubes were left upside down to drain the free oil for 10min. The drained Eppendorf's were weighted once more (w_c). The test was performed in quadruplicate and oil loss was calculated according to equation 1:

$$\text{Oil loss (\%)} = \left[\left(\frac{w_b - w_c}{w_b - w_a} \right) * 100 \right] \quad (\text{Eq 1})$$

2.3.3. Rheology

Rheology experiments were performed at 20°C using a Modular Compact Rheometer MCR 302 (Rheoplus, Anton Paar, Austria). A plate-plate geometry with a diameter of 40mm was used and the gap was set to 1000 μm . Strain sweeps were conducted from 0.0008 to 100% strain at 1Hz. The linear viscoelastic region (LVR) was

calculated as the strain value where the G' was constant. Frequency sweeps were done at 0.01% constant amplitude from 0.1 – 100 Hz. The Rheoplus software (Anton Paar, Austria) was used to analyze the rheological parameters. This analysis was performed in quadruplicate.

2.3.4. Texture Profile Analysis (TPA)

A Texture Analyzer (TAXT plus, Stable Micro Systems, UK) equipped with a 5kg load cell and controlled cabinet temperature at 20 ± 0.05 °C was used to determine the mechanical properties of the oleogels. A texture profile analysis (TPA) test was performed using a 5-mm diameter cylinder probe. The probe penetrated 1mm/s for 10mm with a 10s waiting time between the two cycles. Hardness was expressed as the maximum force of the first peak (N). Analysis was performed in triplicate.

2.3.5. Melting properties

The melting curves were obtained using a Q2000 DSC (TA Instruments, New Castle, DE, USA) coupled with a refrigeration cooling system (TA Instruments, New Castle, DE, USA) and calibrated with indium and eicosane. About 5-8 mg of sample were weighted in a Tzero hermetic pan, and heated from 20°C to 80°C with 5°C/min as heating rate. A similar Tzero hermetic empty pan was used as a reference and the melting curves were analyzed using the Universal Analysis Software version 4.2 (TA Instruments, New Castle, DE, USA). The parameters evaluated were peak temperature (T_p), and enthalpy (ΔH). The analysis was performed in triplicate.

2.3.6. X-ray diffraction

The X-ray diffraction pattern (XRD) was measured at 20 °C using a Bruker D8 Advance Diffractometer (Bruker, Germany) with Cu $K\alpha$ radiation ($\lambda = 1.54178\text{\AA}$, 40 kV, 30 mA) and a LynxEye detector (LynxEye Bruker, Germany) at long spacing and short spacing ($1-27^\circ 2\theta$) using a 0.02° step size, 135.01s by step.

2.3.7. Statistical analysis

Each process and analytical measurement were performed in replicates as described above. Data shown are mean values and standard deviations of the mean. Statistical differences amongst treatments were assessed using 2-way ANOVA ($\alpha < 0.05$) and correlation analyses were performed using GraphPad Prism version 8.0 (La Jolla, CA).

3. Results and Discussion

3.1. Macroscopic and Microscopic structuration

In Figure 1 it is possible to observe the effect of each component of the system (MG, LE, HF, water, and HIU) in the structuration. All samples formed stable oleogel emulsions with no phase separation, except HF 15W HIU, 30W and 30W HIU. This result was expected because although HF and MG have a similar carbon chain

length, HF produces significantly softer gels, suggesting that the glycerol head group of MG plays a major role in strength enhancement and emulsification. This difference was already observed between free fatty acids and MG with the same carbon chain and is even more clear if the fatty acids are in the glycerol molecules [38]. Although in other water concentrations no phase separation of HF samples was observed after 48 hours, a very liquid oleogel emulsions were formed. Observing the microstructure of HF (Figures 2 and 3), we can see that HF presents spherulite crystals and both water -in small concentrations- and HIU expressly reduced the size of these crystals. The water droplets are very small with only 1% of water but with 15% their size increases, and this is probably the reason why the structure of samples with a higher water concentration broke down. In addition, HIU might have reduced even more the HF emulsification properties due to the induction of crystallization and reduction of crystal size generated by cavitation [33].

Observing Figure 1 besides the structured sample formed by the MG alone blends MG:HF, HF:LE and MG:HF:LE also formed a synergic combination, with visually well-structured oleogels. The addition of water and HIU in these synergic blends resulted in different behaviors. For MG although samples were visually structured, sample MG 30W without HIU showed some water release (filled arrow, Figure 1) when collected, suggesting that the water droplets are not well stabilized and are undergoing some coalescence. Coalescence of water droplets in a 20% monostearate emulsion with 25% of water was also observed previously [22]. The same effect was not observed for MG 30W HIU or all other emulsions structured by MG, suggesting a better water droplet stabilization. Formerly study has shown that HIU increases the capacity of the oleogel to stabilize water to form O/W emulsions, and that these emulsions are stable and hold the water droplets even after thermal variations [36]. In this previously studied system, the authors used a blend of MG, HF, and candelilla wax (CLX), suggesting that HIU might affect the emulsification properties of MG. This hypothesis was confirmed by the microscopy pictures in Figures 2 and 3. HIU samples with higher amounts of water showed a more organized crystalline structure with smaller crystals and better-strapped water droplets. In samples with MG 15 and 30W without HIU the water did not even form droplets, it formed clusters all surrounded by MG crystals which explain the shallow stability of the water phase. These might have happened because, as previously shown in MG-oleogels [32,33], the cavitation promoted by HIU generates bubbles that grow and collapse, generating heat, pressure, and shear; besides, these bubbles that do not collapse can also act as seeds [39]. These series of events were previously described as induction for crystallization by either forming primary nucleation [40] or breaking big crystals and inducing secondary nucleation [41]. In emulsions, the cavitation event is the same, but cavitation played an important role in organizing the water phase and reducing the water droplets'

size. In MG oleogels emulsions, especially MG 15W HIU and MG 30W HIU, a combination of both effects of sonication on reducing crystal size in the lipid phase and organizing the water phase could be observed. Similar visual behavior was observed in sample MG:HF (non-filled arrow, Figure 1). Nevertheless, the big water droplets + MG clusters were not observed in Figures 2 and 3, nor without sonication. On the contrary, both systems showed tiny and well-distributed crystals followed by small and well-distributed water droplets. The better structured sonicated sample could be attributed to the higher amount of crystals formed (Figure 2) due to the induced sonocrystallization promoted in the binary blend. A similar system of monostearate and canola hardfat was previously studied and the emulsion was structured by a combination of Pickering and network mechanisms [42]. When the blend was formed by rice bran wax and monostearate it was revealed that the wax crystals were generally in the continuous phase and encased the dispersed phase within a wax crystal network whereas the monostearate formed shells around the dispersed droplets, which limited droplet-droplet contact [22]. In this work a similar structure might have been formed because the MG:HF oleogel without water showed some big needles, a characteristic morphology of MG oleogels [32]. Nevertheless when water was added, only the small crystal remained, suggesting that the MG might have moved to the water interface what was already enough to stabilize the oleogel emulsion.

Synergic blend HF:LE, did not form a visually structured emulsion in the lower water content (1 and 15%), and only 1% of water changed the oleogel from solid to liquid state, with or without sonication. The changes in appearance of the HF:LE sample can also be correlated with sample microscopy. With 1 and 15% of water, a significant reduction in the number of crystals was observed, and especially in HF:LE 1W without HIU. Two crystalline forms were observed, (1) a brighter one that is probably the LE surrounding the water droplets and (2) some small spherulites in the back that are similar to what was formed in HF alone. This happened due to the higher affinity of LE for water, breaking the previously well-connected network formed between LE and HF. Nevertheless, with the increase of water content to 30%, a more visually solid structure was observed again followed by a more organized and homogeneous crystalline network (Figure 2). This could be due to the higher amount of water and a better distribution of the LE. In the sample with 1W, an excess of LE crystals was observed around water, and this might have prevented the HF:LE connection. Lecithin-containing oleogels normally result in spontaneous phospholipid self-assembly into reverse micelles [20,37]. Spatial reorganization from spheroidal to tubular reverse micelles takes place at a critical water concentration [37]. This reorganization also results in a viscosity change from non-viscous into a jelly-like state [43].

Surprisingly, the ternary system showed a distinct behavior from all other blends. With small amounts of water, the sample was visually structured but the visual strength of the blend was lost with the increase in the amount of water. However, no significant changes were observed in the crystals, and the water droplets seemed small and stable (Figure 3).

Samples LE and MG:LE originally formed fragile liquid gels, with some lumps formations after water addition. These lumps in LE were not observed with 30W and HIU, confirming the positive effect of high amounts of water for LE emulsion gels. Moreover, sonication improved the amount of oil binding in the emulsion in LE samples with 30W (Figure 1), which could be due to the small droplets and more crystalline material formed due to cavitation (Figure 2). Usually, tiny droplets form more stable emulsions, and cavitation improves this property [36]. Nevertheless, in the MG:LE this effect was not observed and the lumps were also present at 30W with or without HIU. Moreover, the water phase was not organized in small droplets (Figure 3). This result confirms that MG and LE do not co-crystallize and the oleogel or oleogel emulsion formed sonicated or not were very liquid. Previous studies evaluated monostearate and LE as oleogelators to form bigels with different water:oil ratios (80:20 – 20:80). Even though they used 25% of monostearate and also added hydrocolloids in the water phase, in the high proportion of oil > 50 %, the samples did not form good emulsion gels or bigels, and similar lumps that we observed in our samples could be seen. Nevertheless, higher water content samples formed structured and smooth bigels with different physical properties [29]. This emphasizes the difficulty of forming W/O emulsions using natural lipid emulsifiers for food applications. Additionally, lecithin has been previously reported as a phytosterol-crystallization inhibitor, and up to 30% of the phytosterol added in the blend was not in the crystalline form after lecithin addition [44]. This suggests that LE and MG are not appropriate to form W/O oleogel emulsions, maybe because LE might be a phytosterol-crystallization inhibitor as discussed above and a MG-crystallization inhibitor. This result can be confirmed by the much lower crystalline material formed in this sample than MG-oleogel or oleogel emulsions. Furthermore, this could also be attributed to the high hydrophilic properties of both oleogelators that also favor the formation of low-fat emulsion gels. Due to the lack of structuration of this blend, it will not be discussed further.

3.2. Mechanical properties

Mechanical properties are very important physical properties for oleogels and oleogel emulsions when the final goal is food application. In Figure 4A the hardness of the structured emulsion gels can be observed. The highest hardness (5.0 ± 0.2 N) was observed for MG oleogel ($p > 0.05$), followed by the HF:LE oleogel (1.7 ± 0.4 N). The oleogel emulsion with MG (> 0.2 N) and HF:LE (> 0.1 N) were much softer than their oleogels ($p > 0.05$). A

similar profile was observed for the MG:HF:LE emulsions, which was in agreement with previously studied MG:CLX oleogel emulsions [45]. The only oleogel emulsion that showed an improvement in the hardness with water addition was the MG:HF blend. The higher hardness was found for small amounts of water (1W, 1.5 ± 0.2 N), and there were no differences between sonicated and non-sonicated samples. Although 15W (1.0 ± 0.1 N) and 30W (0.8 ± 0.2 N) showed lower hardness compared to 1W, they were similar to the MG:HF oleogels (0.9 ± 0.1 N, $p > 0.05$), which is a very positive result since they contain at least 15% of water. Da Silva et al. [23] found a similar behavior for an MG:HF:CLX oleogel emulsion, with small amounts of water (1 or 5%) a sample 3-fold harder was formed and in higher concentrations of water (10-30%) the oleogel emulsions showed similar hardness as the respective oleogel. Although HIU changed the microstructure in this study, it did not significantly improve the hardness when water was in high amount. In these samples, a change in the crystalline network was more correlated to the formation of more stable water droplets by HIU through cavitation [36]. Figure 4B shows the viscoelasticity results in a logarithmic scale. A similar behavior as observed for hardness was found. Although MG (333.3 ± 16.1 kPa) and HF:LE (100.6 ± 5.0 kPa) showed high viscoelasticity as their oleogels, the viscoelasticity of the oleogel emulsions was expressively reduced ($\sim 50\%$) after water addition even in the lowest water amount (1W). Interestingly, although not significant in view of the data magnitude in Figure 4B, HF:LE 30W HIU (3.7 ± 0.2 kPa) formed a more structured sample when compared to any other HF:LE emulsion gel (Figure 1). This confirms that cavitation has a double effect on this oleogel blend, (1) it induces crystallization of the HF (2) it organizes and better connects this oleogelator to the LE that is surrounding the water phase. MG:HF (49.9 ± 6.6 kPa) also showed a similar profile as hardness, where the addition of water improved the viscoelasticity of the material (around 2 times higher for all emulsions). Nevertheless, MG:HF:LE did not show any difference in this parameter as it showed for hardness. Figure 5 reveals the frequency sweeps, with (elastic *modulus*) G' represented by the filled markers and the relative G'' (viscous *modulus*) by the non-filled markers with same color, all samples except LE emulsions with or without HIU showed $G' > G''$, suggesting a more elastic than viscous material. Samples MG and MG:HF showed a $G' \gg G''$ as can be observed by the very well divided *modulus*, results in agreement with results in Figure 4. Sample HF:LE showed a significant difference among the oleogels and the emulsions gels, as also observed before, and the best structured emulsion was HF:LE 30W HIU. The presence of water in a gel matrix can either reduce or increase the gel strength, which depends on the interactions of the particles themselves [46]. In this

case, water droplets stabilized by crystalline MG significantly increased the gel strength of the MG and MG:HF emulsions. Moreover, HIU strengthened the interaction of the particles. On the other hand, when HF or LE were the particles, they did not interact very well in the presence of small portions of water ($\leq 15\%$), which resulted in a weak gel.

3.3. Oil Loss

The amount of oil lost in the emulsion gels can be observed in Figure 6. Samples with better oil entrapment (oil loss $< 1\%$) were MG, all MG:HF emulsion gels, HF:LE, and MG:HF:LE. These were also the gels with a high hardness, and high hardness results are often correlated with low oil loss [15,28,47]. The good oil binding capacity of the MG, HF:LE, and MG:HF:LE oleogels was not conserved in their corresponding emulsion gels. This might have happened for different reasons: first in the MG samples the very high melting point of the MG used ($66.5\text{ }^{\circ}\text{C}$) and the high concentration (10%), made the MG crystallize very fast. Although this effect generated a very good oleogel, in the presence of water they left the oil phase and agglomerated around the water droplets (Figure 2). This reduced the oil binding capacity, and although HIU distributed these crystals more uniformly in the oil and water phase and better entrapped the water (Figure 1), the oil binding capacity under stress was not improved either. On the other hand, in samples with LE, HF:LE, and MG:HF:LE, although HF:LE showed a very prospective combination for oleogels with a well-structured and stable crystalline network, the connections broke when water was added, even in small amounts as discussed above. Cavitation in this sample only improved the oil loss for 30W, which could be due to (1) the lower amount of oil for the HF to structure, plus the effect of sonocrystallization on inducing HF structuration, as already shown in a previous study of our group [33], or (2) improvements in the HF:LE connection, by either improving the HF crystalline structure and reducing the water droplets. A previous study without HIU showed an improvement in the oil binding capacity of oleogels formed by LE and stearic acid or LE and phytosterols that was attributed to the development of a more crystalline phase that created an oil entrapping network by combining stearic acid or phytosterols to LE [28,48].

3.4. Thermal profile

Figure 7 shows the thermograms of the emulsion gels, and Table 2 the melting parameters. Evaluating the melting curves of the samples structured by MG, as the amount of water increased, the respective emulsion gel showed a dislocation to lower temperatures, with a significantly lower T_p ($p < 0.05$), already with only 1% of water. The reduction was even higher with 15W, but no changes were observed between 15 and 30W ($p > 0.05$). Besides the peak dislocation, a narrow melting peak was also observed, followed by a significant reduction in

the enthalpy ($p < 0.05$). For the samples with LE, no peak appeared in the studied temperature range due to the very low melting point of LE ($-0.75\text{ }^{\circ}\text{C}$).

MG:HF samples showed a melting profile formed by a peak with two shoulders (Figure 7), the first shoulder that might be attributed to the HF was around $51\text{ }^{\circ}\text{C}$ and the second around $60\text{ }^{\circ}\text{C}$ was closer to MG oleogel, (T_p $52.3 \pm 0.1^{\circ}\text{C}$). As observed for MG emulsions, ΔH reduced with an increase in the water proportion, and this change was significant with 15W or more. T_p on the other hand increased with the increase in water content, this change was also significant with at least 15W.

The binary blend HF:LE, showed a melting curve also formed by 2 small shoulders, but these shoulders were less separated than in MG:HF. The addition of water removed the shoulders and formed only one melting peak with 1 and 15W. Moreover, T_p significantly increased already at only 1W, and this increase was even higher ($\sim 15\text{ }^{\circ}\text{C}$) with 30W and 30W HIU. The higher increase with 30W might have happened because a fractionation of the melting peak into two smaller melting peaks can be observed (HF:LE 30W). This was nevertheless only observed for non-sonicated samples, since the same sample when sonicated showed only one peak. A result that confirms the new network formed due to HIU in high amounts of water. Higher melting and crystallization temperatures were also observed for LE and stearic acid oleogel emulsions compared to the reference oleogel. Authors attributed this change to the formation of a more stable network, and the presence of both crystalline LE and stearic acid materials in these oleogel emulsions [28]

The ternary combination only formed one melting peak, and similar to HF:LE also showed an increase in the T_p with water addition from $43.2 \pm 0.9\text{ }^{\circ}\text{C}$ MG:HF:LE to $49.7 \pm 0.2\text{ }^{\circ}\text{C}$ for MG:HF:LE 1W. However, further increase in the amount of water did not change this parameter ($p > 0.05$). No changes were observed due to the sonication.

3.5. Polymorphism

Figure 8 presents the X-ray diffraction profile of the oleogel emulsions. MG oleogel samples exhibited a characteristic peak at $48.0\text{ }\text{\AA}$ in the small-angle that represents the MG double-layer. In the wide-angle, the peaks $4.6\text{ }\text{\AA}$, $4.5\text{ }\text{\AA}$, $4.35\text{ }\text{\AA}$, and $3.9\text{ }\text{\AA}$ were found and confirmed the β -form for MG-oleogels, as also found in previous studies [19,33]. In general, the addition of water did not change the polymorphic form, neither did HIU, except for the sample with 30W without HIU, that had an extra peak in the small-angle at $63.0\text{ }\text{\AA}$.

LE had a main peak in the small-angle at $47.5\text{ }\text{\AA}$ for all samples. A previous study found that a soybean lecithin micelle can be anywhere from 40 to $56\text{ }\text{\AA}$, which fits the peak $47.5\text{ }\text{\AA}$ within the range [49]. Gaudino and others [28] also found the presence of reverse micelles indicated by a d-spacing of $48.5 \pm 0.1\text{ }\text{\AA}$ in the LE oleogels, and

when water was added to form LE oleogel emulsions, a small dislocation to 49.5 Å was found, but the same micelle structure was attributed to it. In the wide-angle, no peaks were found, and water or HIU did not change this profile. Results that corroborated with literature [28].

The binary blend MG:HF showed similar peaks, 48.0 Å was the only peak in the long spacings, and 4.6 Å was the strongest peak in the short spacings. This was expected since this peak was predominant for MG, which corresponds to MG:HF in β -form, as also shown previously [33]. The addition of water or sonication did not change this profile.

HF:LE showed similar short spacing as the previous MG:HF sample, and consequently the same polymorphic form. The small-angle was at 48.5 Å, as a unique peak was formed it might confirm the interaction between the two crystalline structures HF + LE. Similar combination was previously found it for LE with stearic fatty acids oleogels and oleogels emulsions, where a combination of double layers (fatty acids) and reverse micelle (LE) was found [28]. After the addition of water, some characteristics reflections of LE alone were observed at 11 and 15.8 Å. These suggested that in oleogel emulsions, HF was the primary structure of the oil immobilization by crystals, and that the LE formed some worm-like reverse micellar fibers around the water.

The ternary blend showed two peaks at the small-angle, one at 56.0 Å and another at 48.0 Å. The peak 48.0 Å was more visible in small amounts of water, but the peaks in the small-angle were reduced and barely seen after 15W. In the short spacing's, although peaks had a lower intensity, they were similar to previous samples at 4.6, 4.35, and 3.9 Å, which confirmed that all oleogels were in the β -form, and no changes were observed due to HIU. Gaudino and others also observed that an increase in the water amount did not affect the wide-angle profile of oleogel emulsions [28].

4. Conclusion

The combinations of MG, HF, and LE, formed different kinds of oleogel emulsions, and the formation of W/O oleogel emulsions was more successful using only MG and HF with HIU. When using the MG alone in higher amounts of water, sonication and cavitation were necessary to reduce and organize the crystal and consequently the water droplets, avoiding coalescence and water release. This type of oleogel emulsion formed very hard materials with different water contents (hardness of approximately 5N and elastic modulus higher than 10^5 Pa), which makes possible the use of this lipid base for harder food applications.

The combination MG:HF was the most organized and stable, and showed the lowest oil loss (<1%) among all samples in all concentrations of water. The oleogelators alone showed a very organized crystalline

network formed by small crystals and stabilized the water in small droplets even without HIU. In the presence of sonication, in another hand the water droplets were more stable.

When LE was present in the blend, the oleogel emulsions were not as good, mainly for small amounts of water. In the presence of MG, the samples were even more liquid. Since MG alone has a very strong structuration power it was assumed that LE inhibited MG structuration power, since a significantly less crystalline material was observed in the MG:LE oleogel.

In the HF:LE sample, the oleogel emulsions started to form a structured network only in high amounts of water (30W) and HIU. These suggested that LE was not able to form stable W/O emulsions when water was in lower amounts (1-15%), and although sonication improved this, the material formed was still very soft. Although they had a substantial water entrapment, the oil binding capacity after shear was not very good.

With this, we can assume that it is possible to form W/O oleogels emulsions and that sonication is a reliable tool to improve water droplets' stability when the oleogelators MG and HF are used. In this type of emulsion, HIU has a positive effect even if used for a short time (10s) compared to previously reported (3min) [36], and was even able to reduce the amount of MG and HF compared to previously studied [42]. Moreover, there was no need to use waxes in the system [22,23,36] what could solve the sensory defect in food application as margarine previously found on MG:CLX oleogel emulsions [26]. When LE is present, the stability of the emulsions is not achieved in the water amounts of this study (30% or less). Although cavitation improves the water and oil entrapment in LE samples, it is not enough to achieve good physical properties such as hardness, viscoelasticity, and oil binding capacity.

5. Statements and Declarations

Conflict of Interest: The authors declare no conflict of interest.

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Figure Captions

Figure 1 Visual appearance of the samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please vide Table 1

Figure 2 Polarized light microscopy image of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please vide Table 1

Figure 3 Non-polarized microscopy image of samples structured by MG, HF, LE and their combinations, with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please vide Table 1

Figure 4 Hardness (A) and viscoelasticity (B) of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please vide Table 1

Figure 5 Frequency sweeps of the oleogels emulsions structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. Elasticity (G' , filled marker) and viscous modulus (G'' , non-filled marker). For nomenclature of the samples please see Table 1

Figure 6 Oil loss (%) of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please vide Table 1

Figure 7 Melting profile of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please vide Table 1

Figure 8 X-ray profile of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please vide Table 1.

Table 1. Oleogels and oleogel emulsions proportions used in the study.

Sample name	Components				
	MG* (%)	LE (%)	HF (%)	Water (%)	Oil (%)
MG**	10	0	0	0	90
MG 1W	10	0	0	1	89
MG 15 W	10	0	0	15	75
MG 30W	10	0	0	30	60
LE	0	10	0	0	90
LE 1W	0	10	0	1	89
LE 15W	0	10	0	15	75
LE 30W	0	10	0	30	60
HF	0	0	10	0	90
HF 1W	0	0	10	1	89
HF 15W	0	0	10	15	75
HF 30W	0	0	10	30	60

MG:LE	5	5	0	0	90
MG:LE 1W	5	5	0	1	89
MG:LE 15W	5	5	0	15	75
MG:LE 30W	5	5	0	30	60
MG:HF	5	0	5	0	90
MG:HF 1W	5	0	5	1	89
MG:HF 15W	5	0	5	15	75
MG:HF 30W	5	0	5	30	60
HF:LE	0	5	5	0	90
HF:LE 1W	0	5	5	1	89
HF:LE 15W	0	5	5	15	75
HF:LE 30W	0	5	5	30	60
MG:HF:LE	3.333	3.333	3.334	0	90
MG:HF:LE 1W	3.333	3.333	3.334	1	89
MG:HF:LE 15W	3.333	3.333	3.334	15	75
MG:HF:LE 30W	3.333	3.333	3.334	30	60

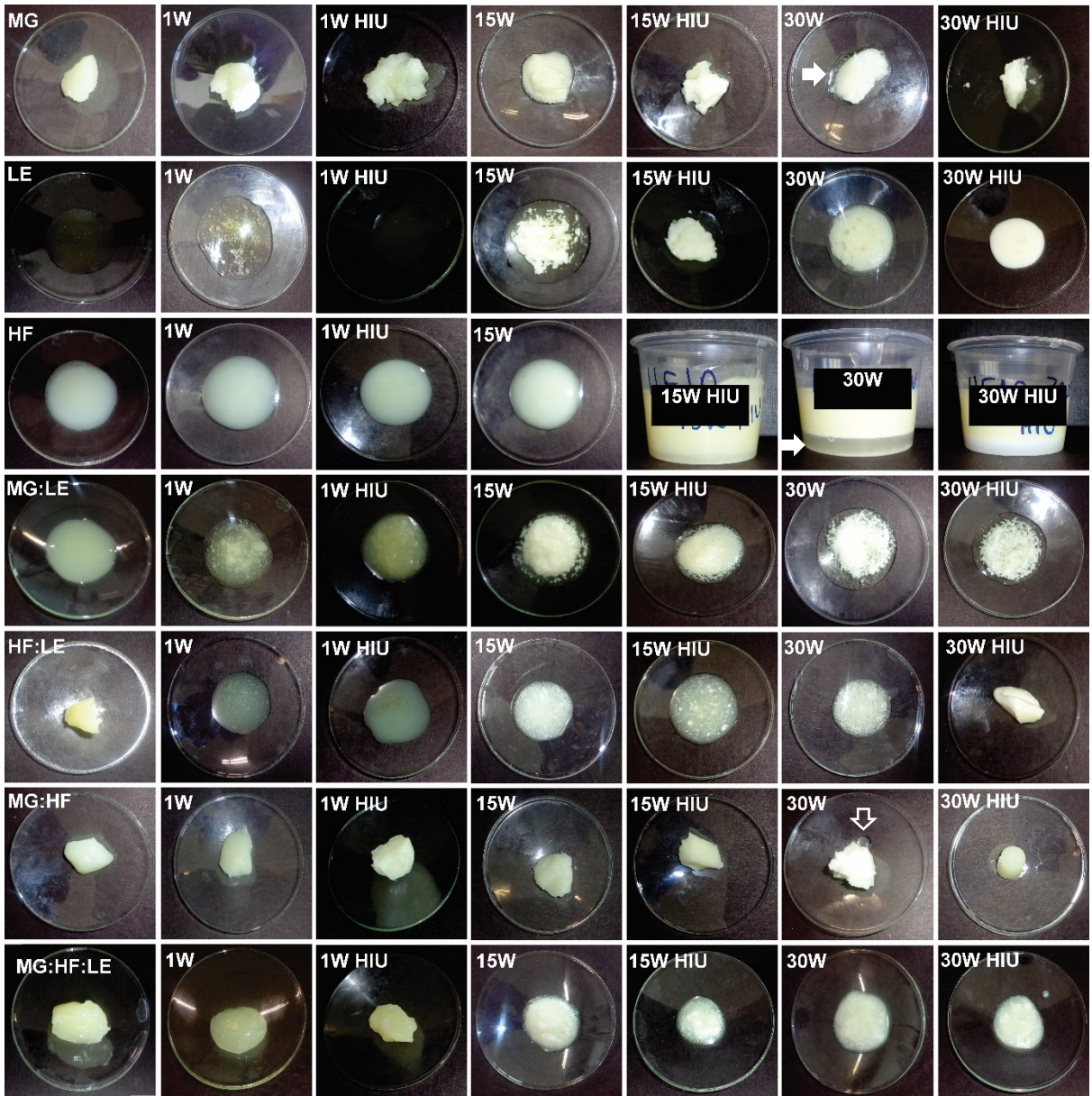
* MG: monoglycerides, LE: lecithin, HF: hardfat, W:water

** All samples were prepared with or without high-intensity ultrasound.

Table 2. Melting peak temperature (T_p), and enthalpy (ΔH) of the oleogels and emulsion gels with and without HIU after stabilization at 20°C for 24h.

Sample name	Melting parameters			
	Without HIU		With HIU	
	T_p (°C)	ΔH (J/g)	T_p (°C)	ΔH (J/g)
MG	64.7 ± 0.8^a	18.0 ± 1.6^A	-	-
MG 1W	59.4 ± 1.1^b	14.8 ± 1.8^{BCD}	57.8 ± 0.3^b	10.1 ± 1.7^{EFG}
MG 15W	54.8 ± 0.2^c	9.6 ± 1.1^{EFGH}	54.8 ± 0.1^c	12.3 ± 2.8^{DEF}
MG 30W	55.0 ± 0.1^c	5.3 ± 0.2^{HIJ}	55.0 ± 0.1^c	7.9 ± 1.1^{GHI}
LE	-	-	-	-
LE 1W	-	-	-	-
LE 15W	-	-	-	-
LE 30W	-	-	-	-
MG:HF	52.3 ± 0.4^{de}	16.6 ± 2.0^{ABC}	-	-
MG:HF 1W	51.8 ± 0.5^{de}	16.7 ± 2.4^{AB}	52.0 ± 0.1^{de}	14.7 ± 1.4^{BCD}
MG:HF 15W	54.6 ± 0.7^c	12.3 ± 1.1^{DEF}	53.7 ± 1.6^{cd}	15.6 ± 3.0^{ABCD}
MG:HF 30W	55.2 ± 0.4^c	12.4 ± 1.5^{BCD}	50.8 ± 0.7^{ef}	13.5 ± 2.7^{BCDE}
HF:LE	48.2 ± 1.3	7.9 ± 0.7^{GHI}	-	-
HF:LE 1W	53.1 ± 0.5^{cd}	6.2 ± 1.1^{GHI}	53.7 ± 1.2^{cd}	8.6 ± 0.1^{FGHI}
HF:LE 15W	51.7 ± 1.0^e	5.6 ± 0.1^{HI}	53.4 ± 0.1^{cd}	7.3 ± 0.1^{GHI}
HF:LE 30W	64.1 ± 0.4^a	5.6 ± 1.7^H	63.6 ± 0.2^a	1.4 ± 0.2^J
MG:HF:LE	43.2 ± 0.9^g	6.6 ± 2.1^{GHI}	-	-
MG:HF:LE 1W	49.8 ± 0.2^f	12.6 ± 2.4^{CDEF}	49.3 ± 0.7^f	9.7 ± 1.3^{EFG}
MG:HF:LE 15W	50.6 ± 0.4^{ef}	6.5 ± 2.1^{GHI}	49.1 ± 1.0^f	4.5 ± 0.7^{IJ}
MG:HF:LE 30W	49.7 ± 0.2^f	5.0 ± 0.6^{HIJ}	51.0 ± 1.4^{ef}	6.6 ± 1.2^{GHI}

* samples followed by same letter lowercase or uppercase are not statistically different from each other based on Tukey test ($\alpha=0.05$).



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472 **Figure 1** Visual appearance of the samples structured by MG, HF, LE and their combinations with and without
473 aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please see Table 1

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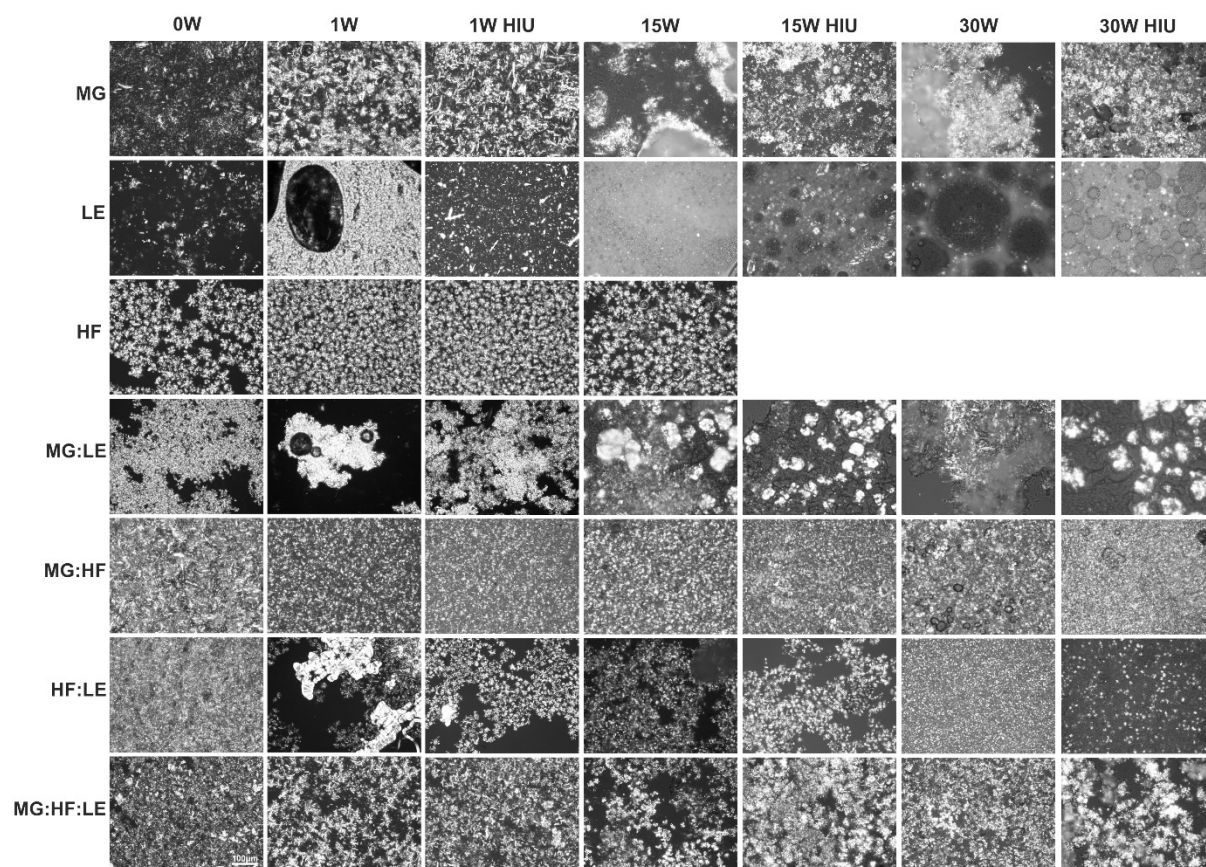


Figure 2 Polarized light microscopy image of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please see Table 1

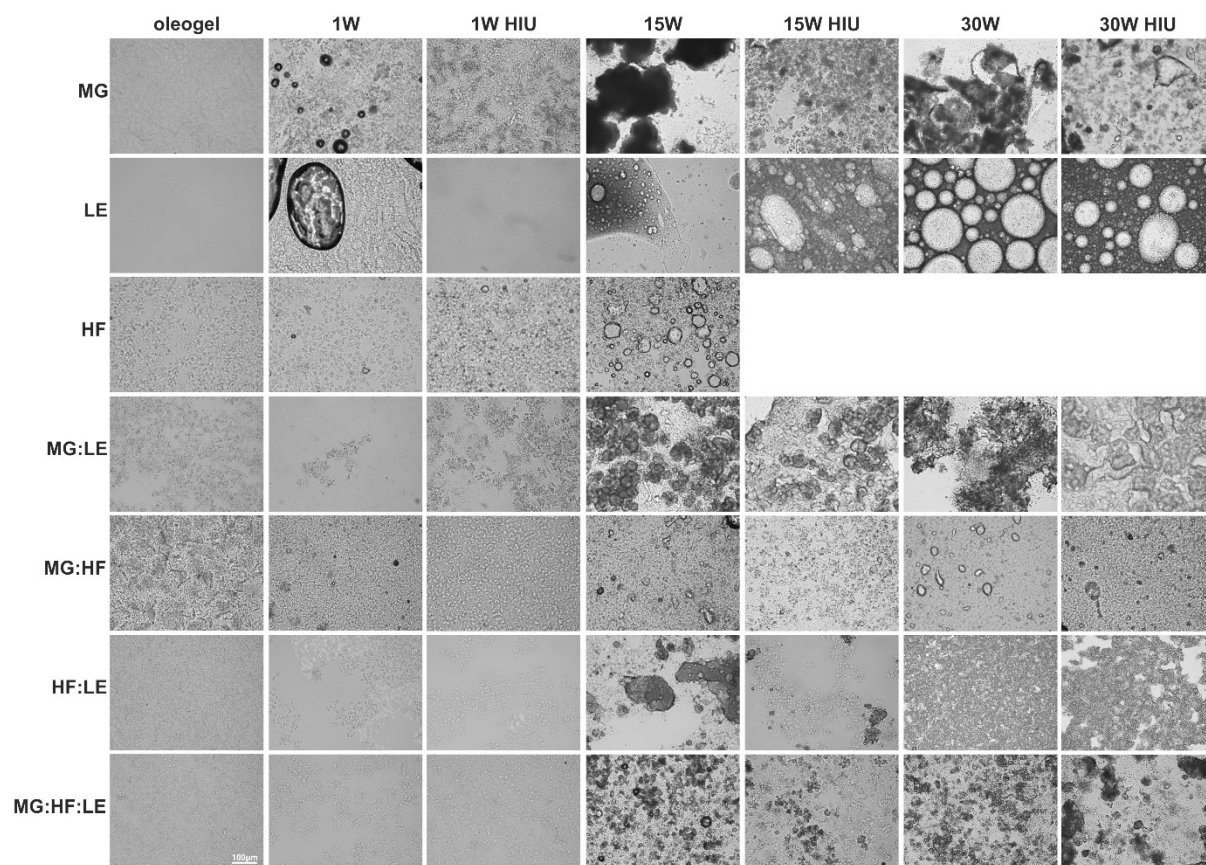
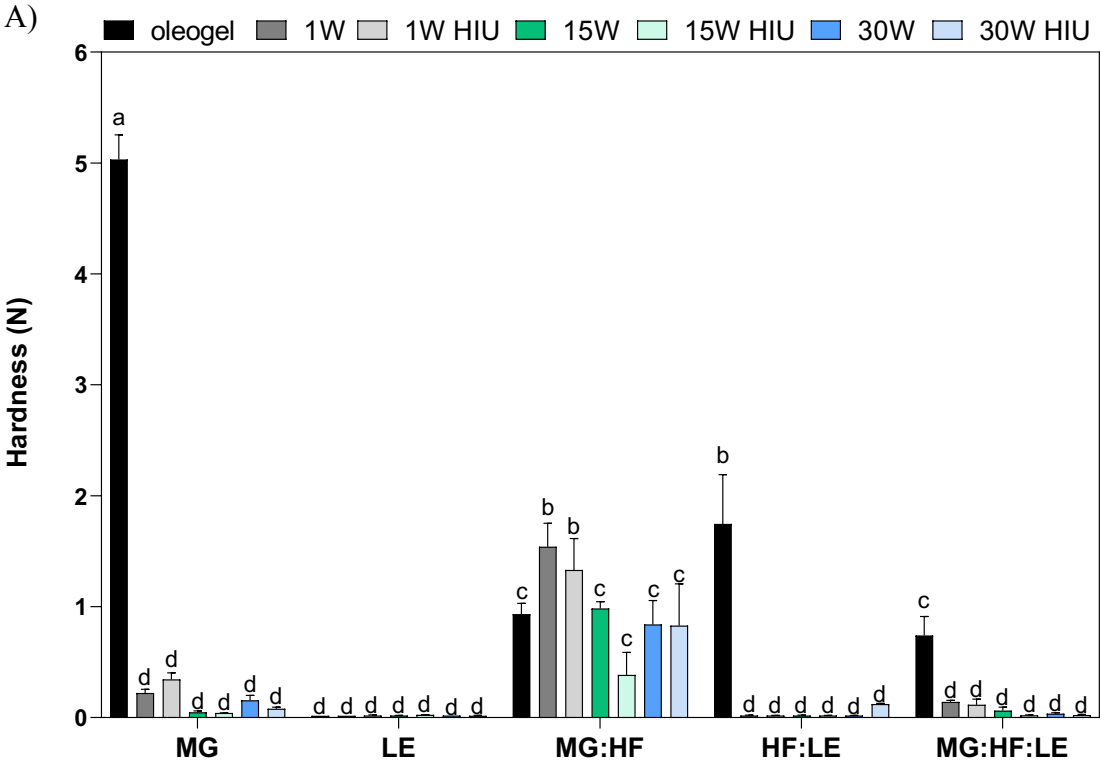
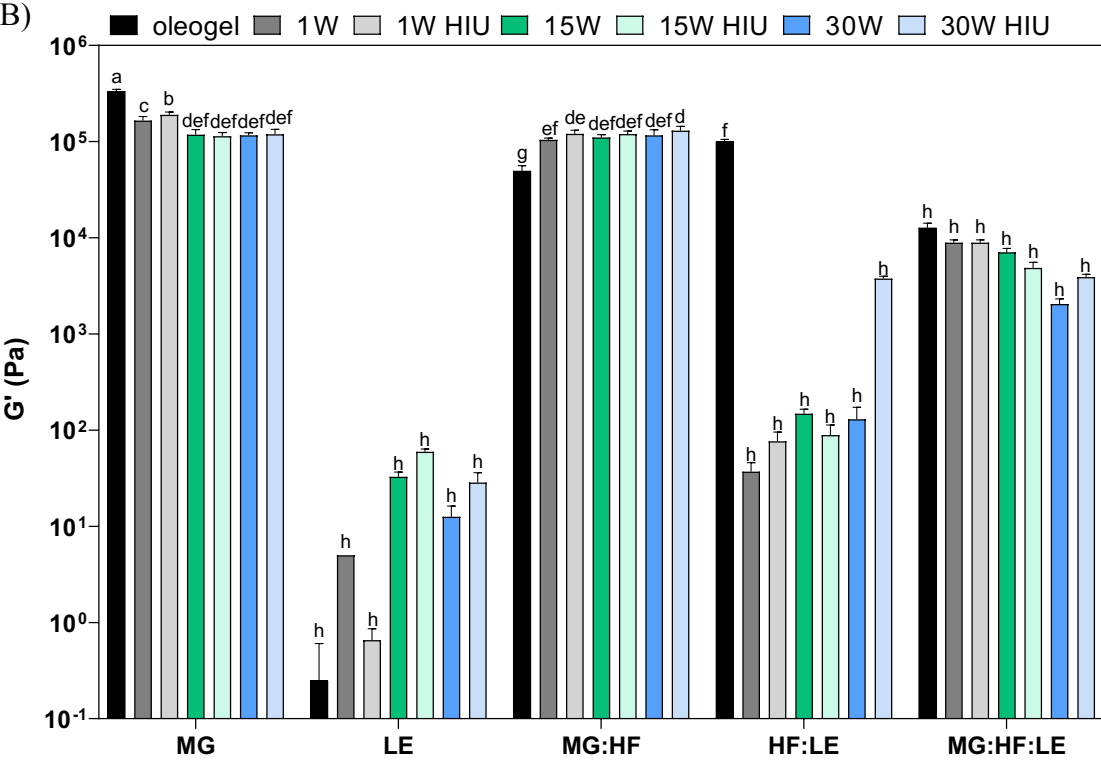


Figure 3 Non-polarized microscopy image of samples structured by MG, HF, LE and their combinations, with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please see Table 1

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Figure 4 Hardness (A) and viscoelasticity (B) of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please see Table 1

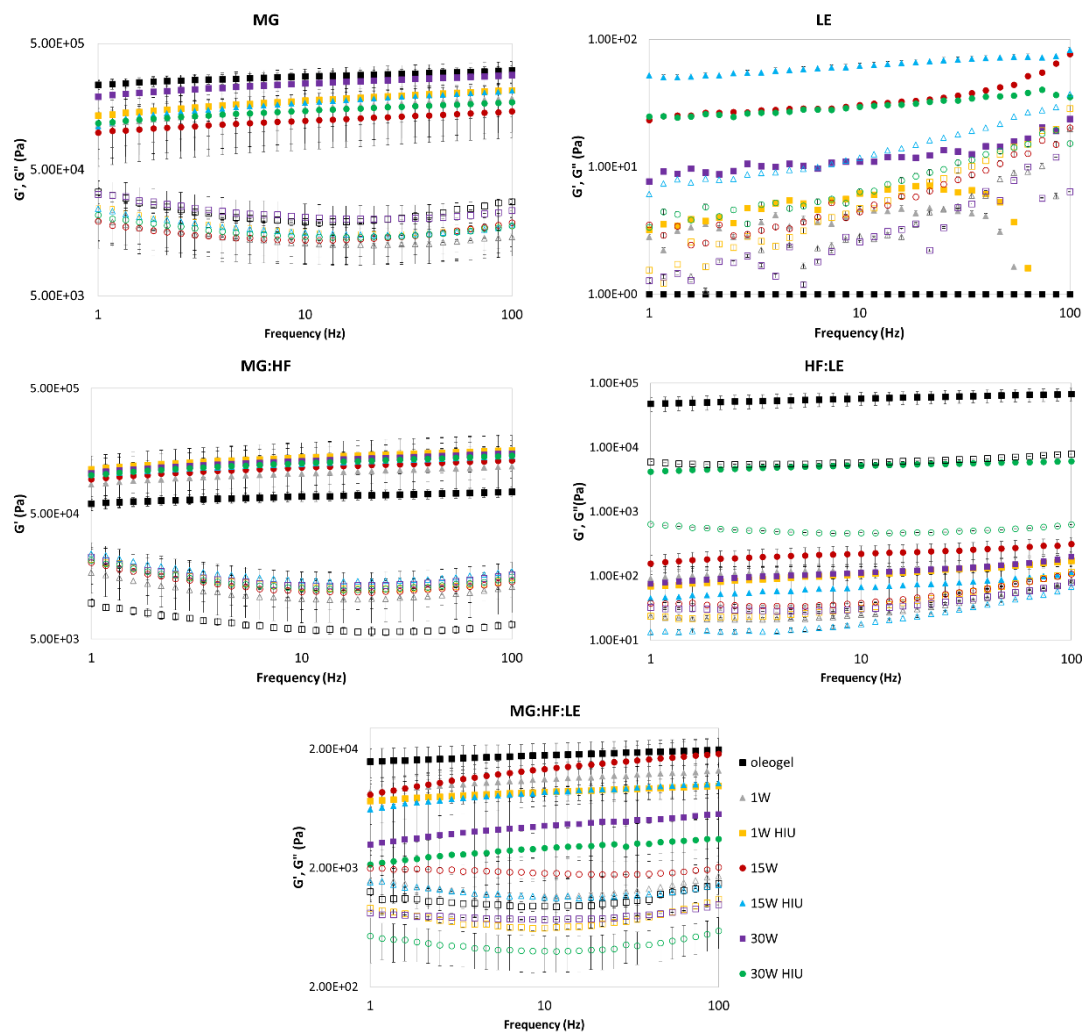


Figure 5 Frequency sweeps of the oleogels emulsions structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. Elasticity (G' , filled marker) and viscous modulus (G'' , non-filled marker). For nomenclature of the samples please see Table 1

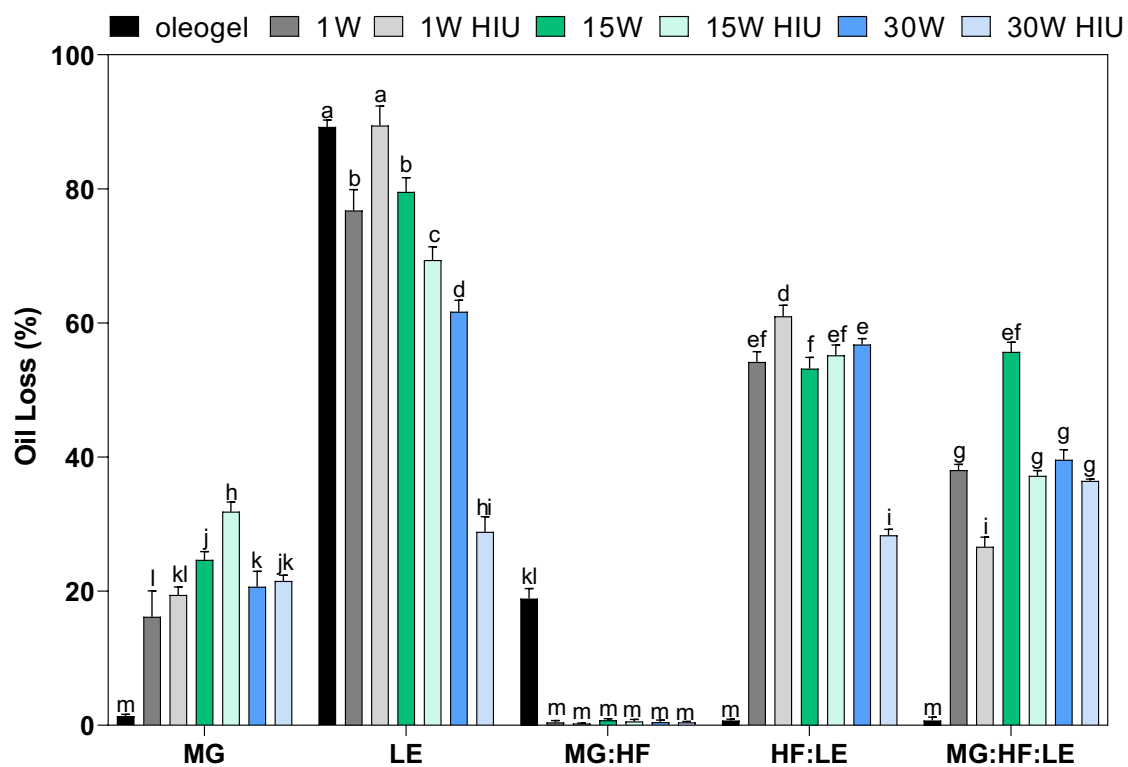
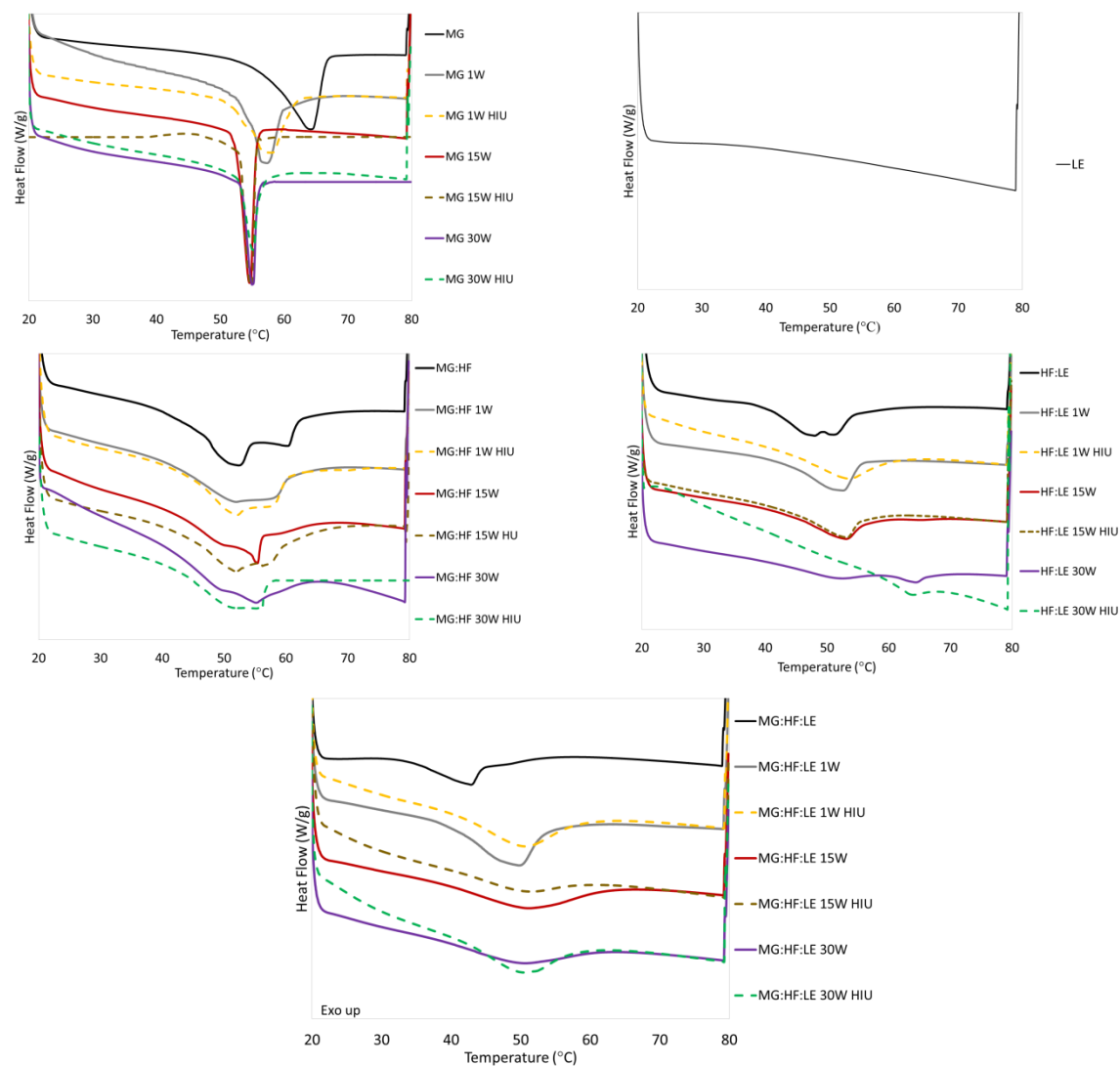


Figure 6 Oil loss (%) of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please see Table 1

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Figure 7 Melting profile of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please see Table 1

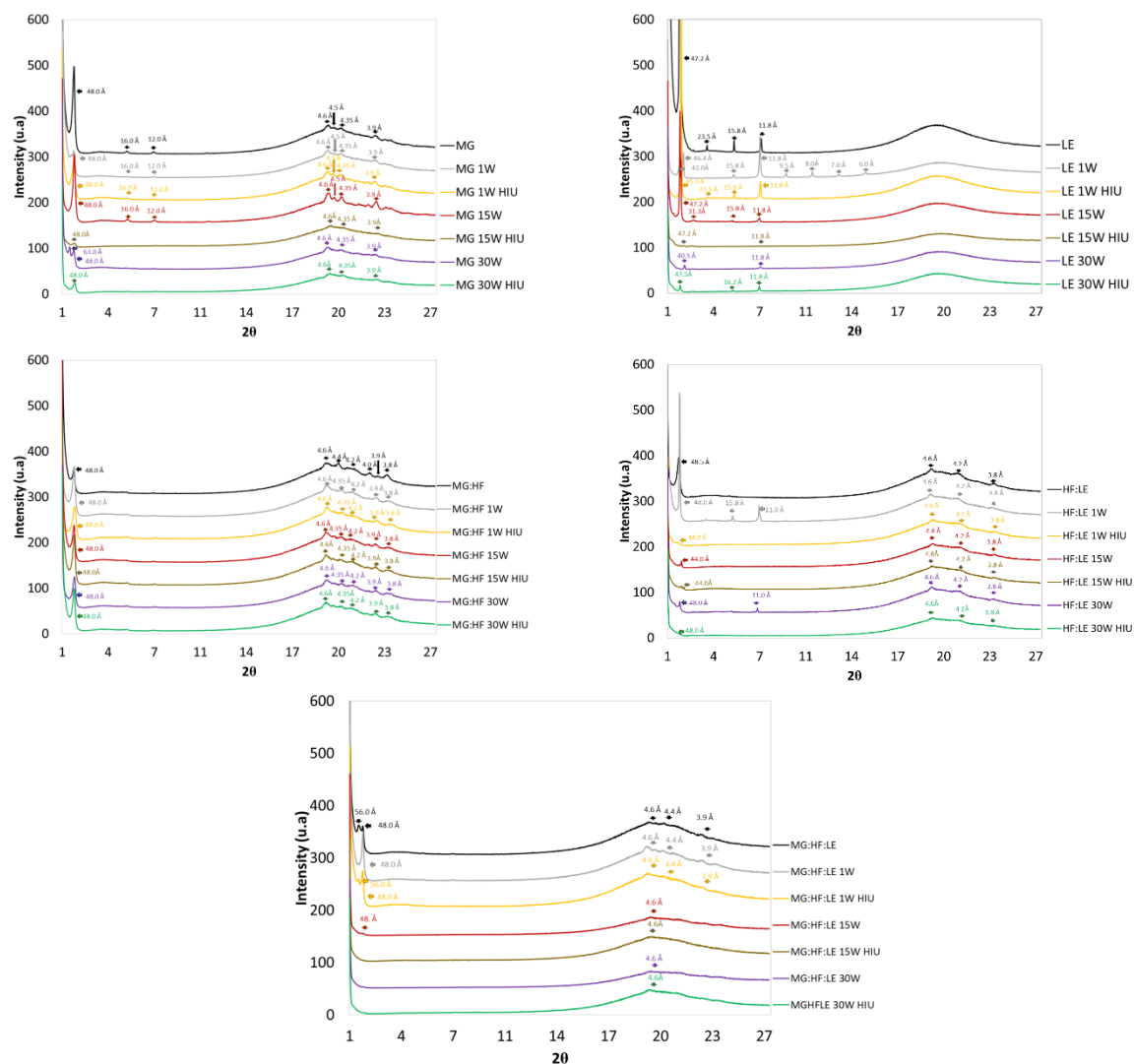


Figure 8 X-ray profile of samples structured by MG, HF, LE and their combinations with and without aqueous phase, and with and without HIU at 20°C. For nomenclature of the samples please see Table 1