# Thermo-Reversible Oleogels Formulated with Monoglycerides or Beeswax as Oleogelator for Food Applications

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**Abstract.** Nowadays, consumers are increasingly concerned about the effects of diet on health. In this context, oleogelation has been suggested as an interesting strategy to substitute unhealthy fats and may even allow encapsulation of bioactive substances in food matrices. This suggests that the development of thermo-reversible oleogels could have a significant impact on the bioavailability and biostability of functional foods, as well as the associated beneficial outcomes. The purpose of this study was to compare oleogels using two food additives as gelling agents: E471, which consists mainly of fatty acids monoglycerides, and beeswax (E-901). To achieve this, a rheological characterization of oleogels was carried out by using Small Amplitude Oscillatory Shear tests (SAOS). Systems containing distilled commercial monoglycerides of fatty acids showed a typical gel behaviour at 20 °C, while the behaviour of beeswax oil systems corresponded to weak gels at this temperature. This was also reflected in an increase in the temperature at which the gel-sol transition occurred when the systems were heated. The transition took place at higher temperatures for the monoglyceride gels, which indicates that they are stronger gels. An increase in the elastic component of the oleogels was noted as the concentration of gelling agent increased. Oleogels with 4% distilled commercial monoglycerides and 4% beeswax show stability at body temperature, although the latter shows reversibility during heating. Conversely, systems with 2% of both gel-ling agents undergo the gel-sol transition before reaching body temperature.

**Keywords:** Oleogels · monoglycerides of fatty acids · beeswax · rheology

## 1    Introduction

In recent years, there have been ongoing improvements in the development of healthier foods demanded by consumers who are increasingly aware of the importance and impact of diet on health and wellbeing. Current preferences are shifting towards food products based on more natural and beneficial ingredients. However, modern human dietary habits, which rely heavily on the consumption of trans and saturated fats, have been linked to health disorders, including coronary heart disease, oxidative stress and excessive body weight [1]. This situation led the European Union to limit the use of trans fats to 2g per 100g of total fat (excluding trans fats naturally present in animal fats) in 2019.

In contrast, hard-stock fats make numerous contributions to the properties of end food products, including enhancing flavour, palatability, texture, and structure [2, 3] due to their solid or fully saturated form used for providing firmness [4]. Consequently, the food industry and researchers explore novel replacements to modify neither the technological nor processing nor sensory features [5]. Several ingredients can be used as substitutes for fat, including fat replacers, carbohydrate-based or protein-based fat mimetics, fat extenders, and low-calorie fats [6]. However, most of these options have cleared disadvantages. For instance, fat replacers lack the same taste properties as fats despite being calorie-free molecules. Additionally, fat imitators are vulnerable to denaturation when exposed to high temperatures during food production procedures. Furthermore, they are prone to undergoing chemical changes. Most water-soluble polar substances decrease the transportation capacity of lipid-soluble substances in contrast to regular fats because of their polar nature [7].

To convert liquid oil into solid fat, techniques such as partial hydrogenation, transesterification or fractionation have been widely utilized. Nonetheless, these techniques cannot avoid being associated with the health issues mentioned earlier, since they also contribute artificial trans fatty acids and highly saturated fats [2]. In this regard, oleogels present a promising resolution since they can imitate the functional properties of solid fats while reducing saturated fat levels and eliminating potential trans fats [8].

Therefore, the development of oleogels using food-grade ingredients has been one of the most extensively researched areas in recent years among the specialized scientific community and industry. Lipid structurants are among the most heavily researched compounds in this field due to their gelation mechanism, which allows the oil to maintain its chemical characteristics, unlike other procedures based on interesterification and hydrogenation. There are numerous applications of oleogels in the food industry, such as confectionery, spreads, emulsions, cheese, meat products, among others. In addition to providing structural benefits to food, oleogels can also enhance stability, increase shelf life, and protect bioactive compounds, which are important objectives in the food industry and can greatly expand their potential applications [9].

Oleogels are semi-solid systems consisting of a hydrophobic liquid (like vegetable oil) as a continuous liquid phase and solid lipid materials (i.e., oleo-gelling agents or oleogelator), which dissolve and develop into a self-assembled network responsible for physically trapping the liquid physically [9, 10]. There are several techniques for achieving oil structuring, with the direct dispersion of the gelling agent in liquid oil being the most prevalent method. To achieve gelation, after dispersing the gelling agent, the oil phase is typically cooled to initiate nucleation and crystal growth, resulting in the creation of self-assembled structures that physically confine the oil [11]. A suitable oil-gelling agent must meet a number of specific physicochemical properties, including: (a) a strong affinity for oil (lipophilicity); (b) the ability to self-assemble and exhibit surface activity; (c) the capacity to acquire a surface disposition through supramolecular interactions; and (d) preferable exhibit thermo-reversible properties, such as crystallization [12]. Based on these considerations, distilled commercial monoglycerides of fatty acids and beeswax have been chosen as valuable options for oleogelators, being both approved additives by European legislation for use in food products.

The primary aim of this research is to acquire and analyze rheologically stable oleogels produced from refined sunflower oil and variable concentrations of oleogelator. The objective is to perform a rheological analysis on the obtained oleogels and compare the outcomes from the two oil-gelling agents employed: monoglycerides of fatty acids and beeswax. An accurate characterization of these oleogels will facilitate the identification of potential food product applications for these stable gels.

Various rheological tests have been conducted to characterize the oleogels. The study also analyzed the gel-sol transition and behaviour of the systems at body temperature.

## 2   Materials and Methods

### 2.1   Materials

The oleogels prepared for this study are monocomponent gels, which are those that contain only one oleogelator structuring the oil. They comprise a continuous liquid phase, consisting of refined commercial sunflower oil (trademark Hacendado, Spain); and a solid phase, which comprises a gelling agent. There are two gelling agents used. On the one hand, commercial monoglycerides of fatty acids distilled from RSPO-certified sustainable palm oil (MG) (Tecnoalimentación Hela S.L., Berriozar, Navarra, Spain). This gelling agent belongs to the category of emulsifiers, and it has been assigned the E-code E-471. It exhibits an ivory white hue, as well as a neutral aroma and flavour, and it is available in powder form. Their physical and chemical properties, as well as their nutritional values, are presented in Table 1.

**Table 1.** Physicochemical properties and nutritional values of commercial monoglycerides of fatty acids distilled from sustainable palm oil.

| Physicochemical properties | | Nutritional values | |
|---|---|---|---|
| Monoester content | >95.0% | Fats<br>Saturated | 100 g<br>78 g |
| Acidity index | <3.0 | Carbohydrates | 0 g |
| Iodine value | 18–26 | Protein | 0 g |
| Free glycerine | <1.0% | Sodium | 3.0 mg |
| Heavy metals | <10.0 ppm | Energy | 890 kcal |

On the other hand, yellow beeswax (BW) (Terpenic Lab S.L., Gerona, Spain), whose E-code is E-901, has also been used as gelling agent. This product belongs to the DIY category of this company, meaning that they are natural materials free of pesticides, toxins and petroleum derivatives. It is used in the form of a finely granular structure, yellow or light brown in colour, dull and non-crystalline. It exhibits a faint odour, characteristic of honey; and it is tasteless. It is practically insoluble in water and completely soluble in fatty and essential oils. Its physicochemical properties are presented in Table 2.

**Table 2.** Physicochemical properties of beeswax.

| Saponification index | 87.0–104.0 mg/g |
|---|---|
| Acidity index | 17.0–24.0 mg HOH/g |
| Ester index | 72–79 g/100 g |

Regarding composition, it is important to note that numerous researchers [13–15] have widely reported on the fatty composition of palm oil and its components. Generally, palm oil contains 50% saturated fatty acids (Palmitic, 44%; Stearic, 5%; Myristic, 1%), 40% monounsaturated fatty acids (Oleic), and 10% polyunsaturated fatty acids (Linoleic). Therefore, the monoglycerides found in E471 are likely the result of the partial esterification of these fatty acids with glycerol. Concerning beeswax, it contains 70–75% esters of $C_{24}$ to $C_{36}$ straight-chain alcohols (pairs) with straight-chain acids up to $C_{36}$ as well as $C_{18}$ hydroxy acids. Myricil palmitate is the primary component. Additionally, it contains free acids, carbohydrates, free fatty alcohols, and fatty acid stearic esters.

A mechanical stirrer (IKA-Werke, Germany) and a water bath with a heating and temperature control system were used to prepare the oleogels. A determined quantity of oil was added to a beaker and heated in a water bath with continuous agitation until a temperature above the melting temperature of gelling agent, which was 65 °C for the monoglycerides oleogels and 70 °C for the beeswax oleogels. The gelling agent was then gradually added until there was complete dissolution. After adding it, the mixture was heated and stirred for an hour. Subsequently, the resulting oleogels should be refrigerated at a temperature of around 4 °C until utilization.

## 2.2 Methods

For the rheological characterization of oleogels, an AR2000 controlled stress rheometer (TA Instruments, USA) was used. This equipment controls the temperature through a Peltier plate connected to a thermostatic bath. The geometry chosen was a 40 mm diameter crosshatched plate made of stainless steel (SST PP40 mm) and the fixed gap between plates was 500 µm in all tests.

Before rheological measurements, systems were removed from refrigeration and kept at room temperature for approximately 30 min. Furthermore, in all cases, a 5 min equilibrium period was established after sample loading to allow for recovery before initiating the test in all instances. A minimum of two trial runs were carried out for each test.

**Linear Viscoelastic Properties.** Before conducting any rheological measurement, it is necessary to determine the linear viscoelastic range for each system under analysis to identify the range of stresses or deformations in which the deformation of the system is reversible (linearly dependent on the applied stresses or deformations, respectively). To accomplish this, stress sweeps were carried out at 20 °C, with a constant frequency of 1 Hz, between 0.01 and 1000 Pa. This facilitated the identification of the critical

stresses, which are within the linear viscoelastic range, to be imposed in the remaining experiments.

**Frequency Sweeps.** The purpose of the experiments was to establish the linear viscoelastic properties of oil systems, specifically regarding the storage modulus (G′) and loss modulus (G″) as a function of frequency. Frequency sweeps were conducted at 20 °C, between 0.01 and 10 Hz, using a stress lower than the critical value to ensure that the measurements remained within the linear viscoelastic range (LVE).

**Temperature Ramps.** A suitable temperature range was selected to observe the gel-sol transition (20–60 ºC). Each temperature ramp was performed using different shear stress amplitudes, which should never exceed the critical value within the corresponding temperature region. A constant frequency of 1 Hz was maintained, and heating rate was set at +1 °C /min. Furthermore, a secondary temperature ramp (20–37 °C) was carried out to assess the viscoelastic properties of each oil system at body temperature.

**Time Sweeps.** After a heating ramp from 20 °C to 37 °C, isothermal time sweeps were carried out at the latter temperature to study the behaviour of the oleogel as a function of time. The aim is to establish the behaviour of the systems at the average temperature of the human body. The stress applied to each sample was that determined by the stress sweeps, while the frequency, in all cases, was 1 Hz.

## 3   Results and Discussion

Firstly, the prepared oleogels, using either fatty acid monoglycerides (MG) or beeswax (BW), appear viscous and opaque in the gel state, whereas in the sol state they are fluid and translucent. The change in opacity can be easily observed when the oleogel is heated in the rheometer. Additionally, as the concentration of oleogelator increases, the gel becomes opaquer and firmer to the touch.

### 3.1   Determination of Linear Viscoelastic Range

Stress sweeps tests are a fundamental tool for studying the rheological response of systems under shear. They plot the values of the storage or elastic modulus (G′) and the loss or viscous modulus (G″) as a function of the applied stress. This test makes it possible to determine the linear viscoelastic range of each system, which is the range of stress or strain within which the viscoelastic properties (e.g. G′ and G″) do not depend on the applied stress (or strain). The critical stress (or strain) value delimits the onset on the non-linear region, at which G′ and G″ depend on the applied stress (or strain). The critical stress and strain values were calculated at the point at which the recorded strain showed a 5% deviation from the linear relationship between stress and strain. Table 3 shows the critical stress and strain values of the systems studied and the complex modulus achieved in linear viscoelastic region (G* = τ/γ) (Table 3).

   Table 3 shows that an increase in the concentration of MG leads to an increase in both the critical stress and strain values, while maintaining the value of the complex modulus. As for beeswax oleogels, when BW content becomes higher, there is an increase in

**Table 3.** Critical stresses ($\tau_{cry}$) and strain ($\gamma_{cry}$) values and complex modulus in the linear region ($G^*$) of the systems studied at 20 ℃ and a frequency of 1 Hz.

| Oil system | Critical stress ($\tau_{cry}$) (Pa) | Critical strain ($\gamma_{cry}$) (%) | Complex modulus ($G^*$) (Pa) |
|---|---|---|---|
| 2% MG | 0.2 | 0.0012 | 4052 |
| 4% MG | 3.7 | 0.035 | 224663 |
| 2% BW | 0.1 | 0.060 | 6 |
| 4% BW | 0.1 | $6.9 \bullet 10^{-4}$ | 179 |

consistency, represented by an increase of G*. A decrease in the critical strain of the LVE range can also be observed for the output of, suggesting that the system in addition to more consistent becomes more sensitive to shear forces.

## 3.2  Frequency Sweeps

Figure 1 shows the LVE properties (G′ and G″) of the systems studied as a function of frequency at 20 ℃. The variation of the phase angle tangent (tan δ) in the same frequency range is also presented. The phase angle tangent is a ratio of the two viscoelastic moduli G′ and G″ (tan δ = G″/G′) and it can be used to characterize the relative elasticity of a material [16]. Oleogels with higher tan δ values exhibit higher damping mechanics, that is, they are more liquid [8].

It is observed that, at 20 °C, systems with MG present values of G′ higher than G″ in the whole frequency range, both moduli showing a low frequency dependence. This response is characteristic of a gel behavior, with tan δ values lower than 0.2 over the whole frequency range. The 4% MG-containing oleogel shows much higher values for G′ and G″ (almost two decades higher) than that one containing 2% MG, with lower tan δ values, reflecting an enhanced network structure.

The 4% BW-containing oleogel also shows G′ values higher than G″ and tan δ values lower than 1, but the response corresponds to weaker gel-like behavior, with much lower G′ and G″ values and higher frequency dependence compared to the MG-containing systems. This response corresponds to the plateau region of the mechanical spectrum and has been referred to as the formation of a pseudogel or a temporary entanglement network. The 2% BW system shows a clearly smaller plateau region, delimited by a crossover point between G′ and G″ at high frequency, which corresponds to a weakly structured pseudogel.

In relation to the oleo-gelling agent, viscoelastic modulus values are higher in systems containing distilled commercial monoglycerides, so a higher elasticity of the gel is observed when they are used as oleogelator compared to beeswax. Some authors [17, 18] explain this increase in gel elasticity as the result of a more uniform distribution of solids throughout the system, with more crystal-crystal interactions.
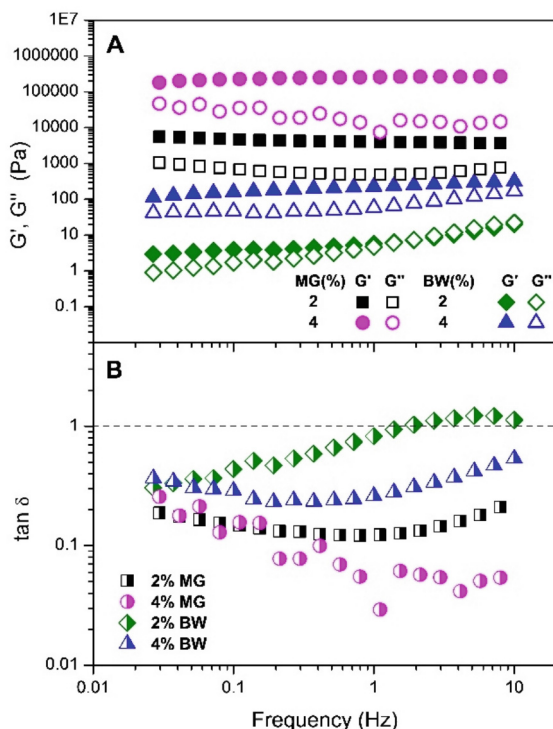
**Fig. 1.** Linear viscoelastic properties of oil systems containing MG or BW at 20 ℃, as a function of frequency: (A) Storage modulus (G′) and loss modulus (G″); (B) Loss tangent (tan δ).

## 3.3 Temperature Ramps

In order to draw conclusions on the degree of structuring of each system and its evolution as a function of temperature, the variation of the values of the storage (G′) and loss (G″) moduli over the range from 20 to 60 °C was studied. All temperature ramps were carried out within the LVE range, at a constant frequency of 1 Hz and a heating rate of 1 °C /min.

Figure 2 shows that at 20 °C all systems, except the oil containing 2% BW, exhibit gel-like behaviour with G′ values above G″. Initially, there is a decrease in both moduli as the temperature rises, reaching a crossover point between them which denotes the gel-sol transition of the system in question. This decrease in the moduli G′ and G‴ starts at a lower temperature in the case of the 4% BW system, whereas the 4% MG system has an initial temperature range in which both moduli remain practically constant (gel remains stable).

From the point of intersection of the viscoelastic moduli, a zone is reached where the behaviour of the systems is characteristic of a sol, with values of G″ higher than G′. This behaviour is similar for all samples, and it can be related to the predominant role of the oil beyond that point.
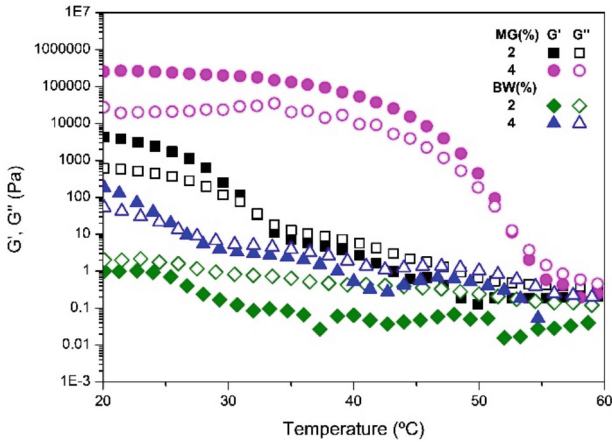
**Fig. 2.** Temperature ramps from 20 to 60 ºC of oil systems containing MG or BW at 1 Hz.

As for the 2% BW system, it shows a predominant value of $G''$ over $G'$ throughout the temperature range studied, with both values being closer at 20 ºC.

The values of the crossover temperature ($T_c$) and modulus ($G_c$) obtained from the heating ramps are included in Table 4 as a function of the gelling agent and its content. System with 2% BW is not presented because it does not show crossover point between viscoelastic moduli in the heating temperature ramp, $G''$ remains above $G'$ throughout the temperature range. However, it is worth mentioning that the crossover temperature for this system must be very close to 25 ºC (i.e. the 2% BW system shows in Fig. 1A a crossover point at a frequency slightly higher than 1Hz at 25 °C).

**Table 4.** Linear viscoelastic parameters $G_c$ and $T_c$ obtained from heating temperature ramp tests performed, as a function of gelling agents content.

| Oil system | $G_c$ (Pa) | $T_c$ (ºC) |
|---|---|---|
| 2% MG | $36.7 \pm 1.6$ | $33.4 \pm 0.2$ |
| 4% MG | $17.5 \pm 5.0$ | $52.2 \pm 0.4$ |
| 4% BW | $13.8 \pm 0.9$ | $26.3 \pm 0.7$ |

As it can be seen in Table 4, beeswax system has a lower crossover temperature than MG systems, the latter being stronger gels that can withstand higher temperatures in the gel state. In MG oleogels, the higher the concentration of gelling agent, the higher the crossover temperature. In other words, the higher the amount of oleogelator, the more thermal energy is required to melt the oleogel, as the system is more structured. Regarding the values of the crossoverpoint (Gc) all of them are in the same order of magnitude but the system containing beeswax shows a slightly lower value.

## 3.4  Stability of the Systems Studied at 37 °C

As the final objective is to use the oleogels obtained in food applications, it may be interesting to analyze, as a first approximation, their behaviour at the body temperature, to which they will be subjected after ingestion. For this purpose, heating ramps within the LVE region were performed from 20 to 37 °C (+1 °C /min rate) followed by a time sweep at this temperature. Viscoelastic moduli and tan δ are represented as a function of time in Fig. 3. A constant frequency of 1 Hz was maintained throughout the test. This test was only carried out on the 4% gelling agent systems.
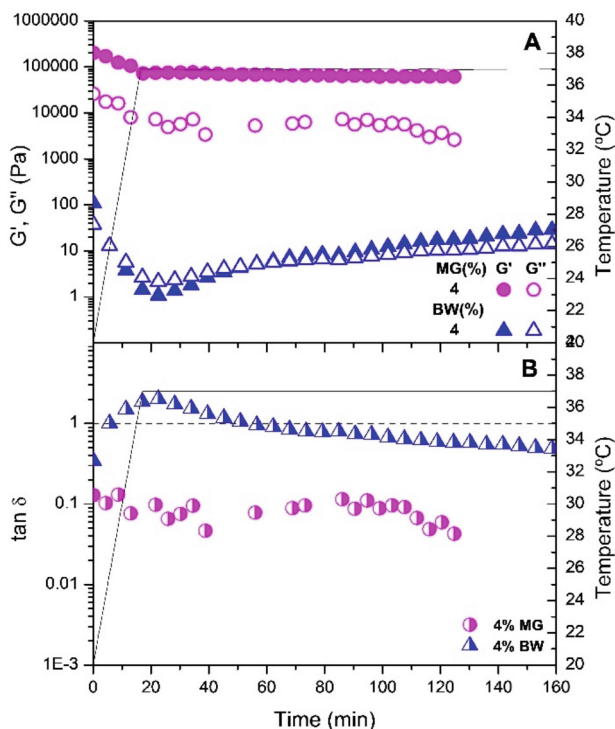


**Fig. 3.** Heating temperature ramp from 20 to 37 ºC and time sweep at 37 ºC of 4% MG and 4% BW systems: (A) Storage moudulus (G′) and loss modulus (G″); (B) Loss tangent (tan δ).

The results of these tests show that oleogel containing 4% MG does not degrade when kept at 37 °C for up to 120 min. Throughout this period both viscoelastic moduli remain constant, with G′ always higher than G″ and tan δ less than 1 (gel behaviour). This indicates that the system is stable at body temperature.

For the 4% beeswax oleogel system, reversibility is observed. The system initially shows a weak gel behaviour (20 °C). However, as the temperature increases, it undergoes a process leading to a gel-sol transformation (G″ higher than G′ and tan δ greater than 1). Then, by keeping the system at 37ºC for a period of time, an increase in both viscoelastic functions (particularly G′) takes place and a new crossover occurs after about 60 min.

This is followed by a slight evolution over time in which the difference between G′ and G″ is slowly but progressively increasing.

## 4    Conclusions

Stable oleogels have been prepared by adding beeswax or a fatty acid monoglyceride to an oil phase consisting of sunflower oil. Both oil-gelling agents are able to form a gel-like network when are added at low concentration (2–4% w/w), except for the system containing the lower amount of beeswax which exhibited a fairly weak pseudogel behaviour. Moreover, both linear viscoelastic functions increased as oil-gelling agent concentration was higher.

An increase in temperature leads to melting of the oleogel network resulting in a fluid behavior. The amount of energy required to melt the oleogel (taking into account time and temperature) is higher as the oleogel strength is greater.

At body temperature (i.e., 37 °C), the 4% MG-containing oleogel showed high stability, maintaining a predominantly elastic gel behaviour over time. Conversely, the 4% beeswax system exhibited thermo-reversibility at temperatures below 37 °C, which may be valuable for encapsulation of lipophilic compounds as it would be possible to switch to the sol state when subjected to body temperature conditions. But, on the other hand, a greater stability and viscoelastic properties of the oleogel at body temperature can be considered favorable for modulating the resistance of the oleogel during its transit through the gastrointestinal tract. Therefore, further research is needed to evaluate the convenience of using MG or beeswax as well as its optimal concentration to develop oleogels able to overcome the various conditions to which they will be subjected in the different stages of the gastrointestinal tract (e.g., residence time, reduced pH, presence of enzymes, bile salts, etc.).

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