Food Biophysics (2010) 5:211–217
DOI 10.1007/s11483-010-9162-3

ORIGINAL ARTICLE

Shear Nanostructuring of Monoglyceride Organogels

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Published online: 12 May 2010

Abstract The aim of the present research was to study the effect of shear on the crystallization behavior of monoglyceride organogels. To this end, organogels were prepared by mixing cod liver oil and saturated monoglycerides at 80°C and then crystallizing them at 20°C under shear rates ranging from 0 to 2,000 s^{-1}. The organogels were characterized using polarized light microscopy, Cryo-SEM, and X-ray diffraction. The rheological properties and the oil binding capacity of the different systems were also evaluated. Results obtained in this study showed that the introduction of shear during organogel formation greatly affects structure at the nano, micro, and macro levels. Solidification of the organogel under static conditions led to the formation of a strong gel network, with a high oil binding capacity. On the contrary, shear processing during crystallization led to the formation of a weak gel network with a low oil binding capacity.

Keywords Saturated monoglycerides · Organogel · Shear · Self-assembly structures

Introduction

In recent years much effort has been directed towards the development of novel food nanostructures obtained via the self-assembly of surfactants, colloids, and polymers. Due to their possible applications in food systems organogel materials have attracted considerable research attention.[1] Organogels are self-standing, thermoreversible, anhydrous, viscoelastic materials structured by a three-dimensional supramolecular network of self-assembled molecules (organogelators) with limited solubility in an organic liquid.[2] One of the most promising characteristic of organogel systems is the possibility of tailoring their physical properties by changing the chemical nature of the molecules used as organogelators or by modifying processing parameters. Organogels can be formed using either polymeric or low molecular weight (LMW) gelators.[3] Polymeric gelators immobilize the organic solvent by forming a network of either cross-linked or entangled chains via chemical and physical interactions, respectively.[4] The self-assembly of LMW organogelators is mediated by the physical interactions established between gelator molecules and can be further stabilized by weak inter-chain interactions such as hydrogen bonding, van der Waals forces, and π-stacking. As well as the strength of the bonding forces, thermal and entropic considerations play an important role in their assembly. If these forces can be maintained beyond the nano-scale regime into the colloidal regime, LMW organogelator aggregates can become large enough to interact and thus to induce solvent gelation.[5] These aggregates have high aspect ratios and are most commonly formed by a unidirectional growth into fiber-like structures. The length of these fibrils may range from tens of nanometers to several micrometers. Although less common, examples relevant to two-dimensional growth patterns that lead to the formation of microplatelet structures have also been described.[3,6]

A wide number of different gelators able to structure oils have been identified.[7–9] Among these structuring agents,

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saturated monoglycerides are considered to be one of those with a high potential for a wide range of application.[8] Once introduced into organic liquids these molecules in fact are able to self-assemble into inverse bilayer nanostructures. The inverse bilayers subsequently grow and organize into lamellar platelet microstructures that lead to the formation of a continuous three-dimensional network that eventually immobilizes the liquid via capillary forces.[10–12]

Monoglyceride organogels can find numerous applications as efficient structuring agents in food.[13,14] In particular, organogels may be used to solidify unsaturated oils to obtain plastic structures characteristic of hardstock fats without the attendant high levels of trans- and saturated fatty acids. These can be used to formulate low-saturated/low-trans-fat-based products with specific rheological properties.[15] This strategy would offer consumers healthier foods. In fact, it has been established that the repeated consumption of high levels of saturated and trans-fats in the diet poses a chronic threat to human health by increasing the risk of cardiovascular diseases and Type II diabetes.[16–18]

Another interesting application of organogels is their use as oil migration inhibitors in food products containing both solid and liquid lipid phases as can be found most predominantly in chocolate confections.[15] Even if only few studies on this topic have been conducted, theoretically, by immobilizing liquid oils within a gel network, the mobility of triacylglycerols out of or through the material is expected to be reduced.[19] This application is of particular interest in the reduction of fat blooming induced by oil migration in chocolate bars and in filled chocolate confections.[15]

Finally, organogels may find application as vehicle for the delivery of lipophilic bioactive compounds, such as carotenoids and essential fatty acids. In fact, encapsulation within a gelled matrix should increase their stability against oxidation as well as modulate their release, upon digestion, in the human body.[19,20] Recently, Hughes et al.[15] demonstrated the ability of a 12-hydroxystearic acid/canola oil organogel to control the release of β-carotene within the human digestive system. These results suggest that physiologically responsive organogels with the capability of delivering bioactive compounds at predefined rates or in response to certain conditions is a real possibility.

The structure of a gel is strongly affected by the thermal and perikinetic conditions established during its solidification.[5,21,22] While much is known about how thermal conditions affect the properties of an organogel, relatively little is known about the effects of shear. The scattered reports within the literature, however, all indicate that the perikinetic conditions established during solidification have an impact, albeit poorly understood, on the resultant properties of the gel. Bot and Agterof[23] reported that the agitation (via oscillatory shear) of the pro-gel solution greatly enhanced the rate of solidification for a γ-orizanol/β-sitosterol/vegetable oil organogel. This was attributed to an enhanced rate of collision and cross-sectional aggregation between the mesoscopic fibrils responsible for gel formation.[24] As well, it was observed that the time at which the oscillatory shear was applied greatly affected the magnitude of the storage modulus (G′) of the resultant gel.

The possibility of using shear to engineer organogel materials with tailored microstructures in order to obtain specific functionalities in a food application is explored in the present research. Particularly, the effect of the magnitude of the shear rate applied on the crystallization behavior of monoglyceride organogels is investigated.

Material and Methods

Gel Preparation

Myverol™ saturated monoglycerides (fatty acid composition, 1.4% $C_{14:0}$; 59.8% $C_{16:0}$; 38.8% $C_{18:0}$; melting point 68.05±0.5°C), supplied by Kerry Bioscience (Bristol, UK) was used due to its common application as ingredient in the food industry. Cod liver oil, purchased at a local pharmacy, was chosen as target lipid matrix to prepare the organogels.

Cod liver oil added to 5% (*w*/*w*) of monoglycerides was prepared by mixing the ingredients at 80°C in a temperature-controlled water bath. The samples were then crystallized till reaching 20°C at a rate of 15°C min^{-1} during which shear ranging from shear rate of 0 (static sample), 50, 100, 200, 1,000 to 2,000s^{-1} were applied using a laminar shear crystallizer.[25] The crystallizer consisted of two concentric cylinders with a gap of 2 mm in between. The internal cylinder rotates at selected shear rates while the external cylinder remain stationary.

The organogels were examined after 24 h of storage at 20°C to ensure that adequate time was given to anneal the network giving the maximum structure.

Analyses

Powder X-ray Diffraction

X-ray diffraction patterns of the gels were acquired using a Rigaku Multiflex X-ray Diffractometer (RigakuMSC Inc., The Woodlands, TX, USA) utilizing a copper source (λ= 1.54 Å) set at 44 kV and 40 mA. The setting for the divergence slit, receiving slit, and scattering slit were 0.3 mm, 0.5°, and 0.5°, respectively. A glass sample holder with an area of 20×20 mm and depth of 1 mm was used to hold approximately 0.12 g of sample during analysis. Data are shown as variations in the scattering intensity as a function of the scattering vector, q, where $q=(4\pi/\lambda)\sin\theta$,

and θ is the scattering angle. The magnitude of the scattering vector is related to the interplanar spacing d, as $q=2\pi/d$. Samples domain sizes (ξ) were stimated using the Scherrer equation:

$$\xi = \frac{0.9 \cdot \lambda}{B \cdot \cos\theta}$$

where B is the width of a peak in degrees at one-half the maximum intensity of the given peak. This equation is generally applied in crystallography to correlate the size of nanocrystals to the broadening of a peak in a diffraction pattern.

Peak detection and analysis were obtained using the MDI Jade 6.5 software without baseline subtraction.

Rheology

The storage (G′) and loss (G″) moduli at 20°C were determined via small amplitude dynamic measurements using an AR 1000 Rheometer (Q2000, TA Instruments, New Castle, DE, USA). A 40-mm flat plate geometry with a gap setting of 2 mm was utilized. To determine the linear viscoelastic region, a stress sweep was performed on the sample at a frequency of 1 Hz from 0.1 to 50 Pa. G′ and G″ were later obtained using a frequency sweep from 0.1 to 10 Hz using a fixed stress value of 0.5 Pa for statically crystallized sample and 0.2 Pa for the shear-crystallized samples.

Using the same geometry, viscosity measurements were carried out at 20°C. The shear rate was increased step-wise from 0 to 150 s^{-1}. Since the samples exhibited non-Newtonian flow behavior, apparent viscosity values were standardized at a shear rate of 50 s^{-1}.

Polarized Light Microscopy

The microstructure of the organogels was characterized in situ using a temperature stage with an attached shear cell (Linkam CSS450, Linkam Scientific Instruments, Surrey, UK). In particular, one drop of the melted mixture of cod liver oil and monoglyceride was placed between a stationary and moving glass plate. The sample was then crystallized at 20°C by cooling the preparation from 80°C to 20°C at a rate of 15°C min^{-1}. Shear with rates ranging from 0 to 2,000 s^{-1} were applied as the gel solidified. The gel was imaged at ×20 using a Leica DMRXA2 (Leica Micro-systems Canada Inc., Richmond Hill, Canada). Images were acquired using a CCD camera (Q-imaging Retiga, Burnaby, BC, Canada).

Cryo-SEM

A drop of the sample was placed inside a copper holder designed for the Emitech K550 Cryo-preparation unit (Ashford, Kent, UK). The copper holder was immersed in a liquid nitrogen bath and then inserted into the SEM chamber (Hitachi S-570, Tokyo, Japan) under vacuum using a transfer device. The SEM chamber was maintained at −137°C for the duration of the experiment. Images were captured digitally using the Quartz PCI imaging software (Quartz Imaging Corp., Vancouver, BC).

Oil Binding Capacity

One gram of each organogel sample was carefully weighted and centrifuged at 10,000 rpm for 15 min at 20°C using a microcentrifuge to express the oil. Subsequently, the oil expressed from the samples was separated by inversion and drainage for 3 min, and weighted. The oil binding capacity (OBC) was evaluated considering the percentage of oil released from the sample after the centrifugation:

$$\%\,\text{Oil released} = \frac{\text{Mass of expressed oil(g)}}{\text{Total mass of sample(g)}} \cdot 100$$

Statistical Analysis

Each sample was analyzed in triplicate. All results are shown as mean and standard deviation. One-way analysis of variance was carried out, and significance between means was determined using the Tukey test (Statistica 6.0, StatSoft inc., 2001).

Results and Discussion

Figure 1 shows the polarized light micrographs of organogels crystallized at various shear rates from 0 to 2,000 s^{-1}. Different microstructures were observed as a function of the applied shear rate: samples obtained under static conditions show crystalline platelets with a cross-sectional length of roughly 50 μm. Irregular clusters with mean diameters of less than 50 μm were formed as a consequence of crystallization at 50 and 100 s^{-1}. Even smaller crystalline aggregates of less than 20 μm mean diameter were observed at higher shear rates.

Further examination of these gels using cryo-SEM was conducted. The reduction of particle size with increasing shear observed in the polarized light micrographs was observed also in the electron micrographs. Figure 2 shows the Cryo-SEM images of three representative samples (static, 100 s^{-1}, and 1,000 s^{-1}). Results obtained suggest that the application of shear modifies the habit of the monoglyceride crystals. In particular, the application of shear, appears to hinder the formation of well-organized platelet structures, instead promoting the random clustering of crystals domains into spherical assemblies. At higher

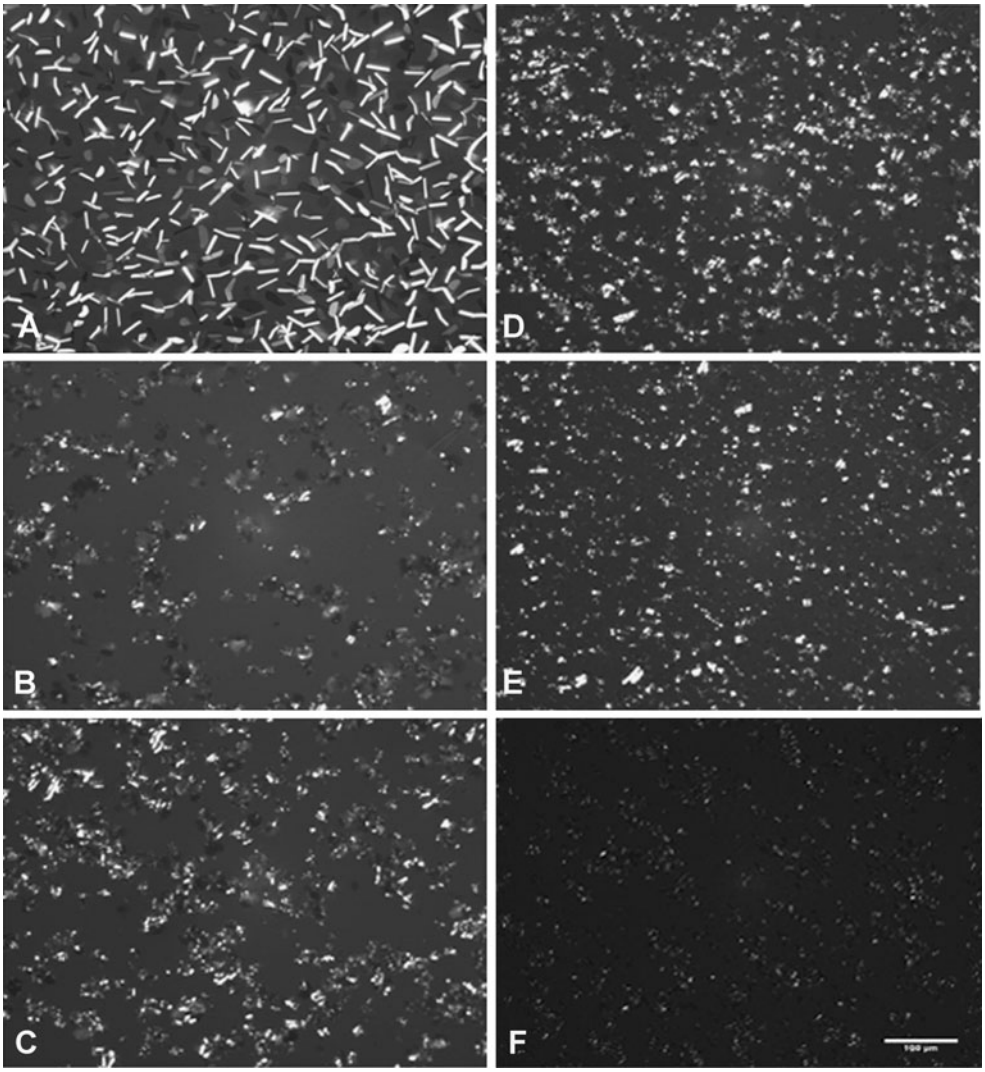

Fig. 1 Polarized light microscope images of organogels crystallized under increasing shear rates (**a** 0 s^{-1}, **b** 50 s^{-1}, **c** 100 s^{-1}, **d** 200 s^{-1}, **e** 1,000 s^{-1}, **f** 2,000 s^{-1})

shear rates the number of clusters increase with a concomitant decrease in size. This may due to a different crystallization regime that favors nucleation over growth. The reduction in cluster size may also be attributed to the break-up of previously formed clusters. The end result is a random distribution of crystal clusters throughout the gel.

To obtain information on the molecular organization of monoacylglyceride (MAGs) in the solid state, powder X-ray diffraction (XRD) analyses of the gels were performed. The application of such technique is feasible because the certain uniformity in shape and size of crystallites has been assured by previous results. Figure 3 displays an XRD pattern of a MAG organogel crystallized under static conditions at 20°C. The sample shows broad diffraction peaks at angles corresponding to spacings of 4.50 and 24.20 Å. The broadness of the peak is due to the amorphous scattering associated with the liquid-state triacylglicerol molecules that comprise approximately the 95% of the gel. The single diffraction peak (001 reflection) in the small angle region corresponds to the interplanar distance of monoglyceride bilayers.[26] These results indicate that the system consist of lamellae with an approximate width of 46.4 Å. The other peaks observed at higher q values correspond to higher order reflections of the same repeating distance. In the wide angle region, is evident a main peak corresponding to a distance of 4.55 Å with a number of other peaks along the shoulder of the amorphous band. These peaks are due to the in-plane ordering of monoglyceride aliphatic chains into the β phase, as suggested by Chen et al.[11] and Chen and Terentjev.[12] Shear-crystallized samples did not show different XRD patterns (data not shown) indicating that this processing condition did not affect either inter-plane spacing or the subcell packing of the monoglyceride crystals.

Even if no differences in XRD patterns were observed among static and shared samples, the domain sizes (ξ), as estimated using the Scherred equation considering the B value of the 001 plain reflection (d=46.4 Å), were smaller for samples crystallized under shear compared to the statically crystallized samples (Table 1). Interestingly,

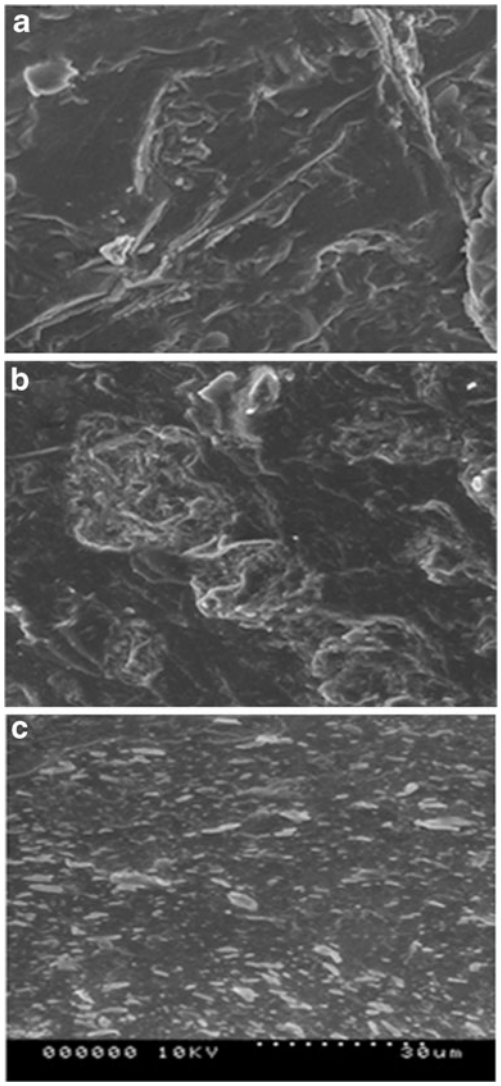

Fig. 2 Cryo-SEM images of organogels crystallized under static conditions (**a**), and under shear at 100 s^{-1} (**b**) and 1,000 s^{-1} (**c**)

however, an increase in the domain size was observed with increasing shear rates. The difference between sheared and statically crystallized samples can be explained by an increased incidence of nucleation. As shear is known to enhance the nucleation of materials,[27,28] it is not surprising that the creation of a larger number of crystallites would lead to smaller crystal domain sizes. The increase in domain size as a function of increasing shear rate, however, remains unexplained.

The differences observed between the shear-crystallized and statically crystallized samples go beyond the micro-structural level to the macroscopic properties of the material, as is clearly demonstrated by the rheological properties of the gel (Table 2). As expected, all samples show typical gel-like behavior due to the presence of a monoglyceride crystal network immobilizing liquid oil. However, the magnitude of the mechanical properties of sheared and statically crystallized samples differ. Sheared samples show lower G′ and G″ values than statically crystallized samples, indicating the presence of a weaker gel network. On the contrary, the rheological properties of the sheared systems do not appear to exhibit a dependence on the shear rate.

Considering the organogel's flow characteristic, two points must be noted. The apparent viscosity of the static sample is significantly higher than that of the sheared samples. The only sample with a significantly different ($p<0.05$) viscosity was the gel sheared at 2,000 s^{-1}. These results are most certainly related to the different microstructures of the given samples (Figure 1). The rheological properties of the static samples could be explained by the presence of a network of large platelets with possibly permanent junction zones between the crystals. The introduction of shear induces the formation of clusters of aggregated crystals that interact only via transient interactions, resulting in the formation of a weaker gel

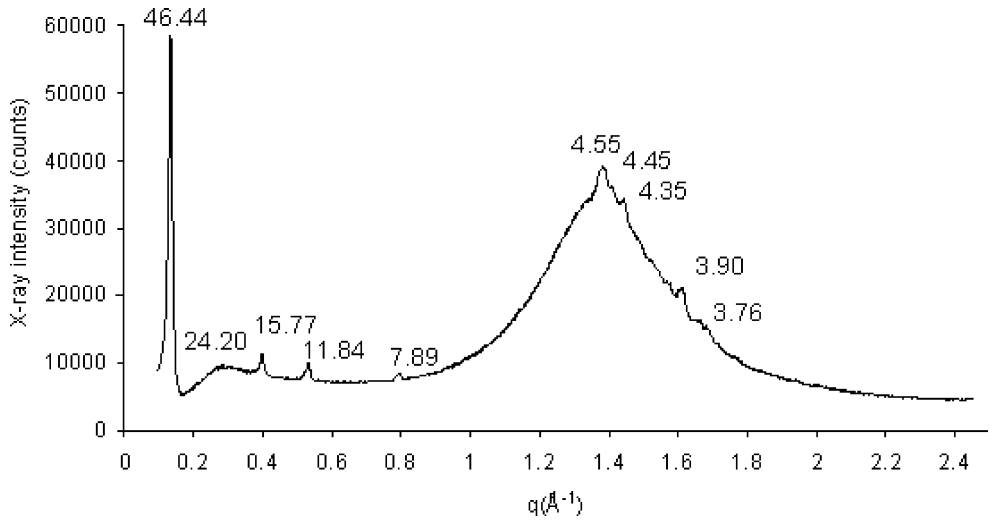

Fig. 3 Powder X-ray diffraction pattern of a 5% monoglyceride organogel crystallized statically at 20°C

Table 1 Domain size of the 001 plane for samples crystallized at increasing shear rates

Shear rate (s^{-1})	Crystallite size (Å)
0	259±15a
100	366±17b
500	340±17b
1,000	314±16bc
2,000	310±18c

Values with the same letter are not significantly different ($p>0.05$)

network. The transience of these interactions may possibly result in a material that is more fluid than solid.

Since oil migration within organogels could lead to substantial quality defects in food formulations were they may be possibly used, the oil binding capacity of samples was evaluated by measuring the oil released after centrifugation (Figure 4). It is evident that the static system is characterized by a high capability to entrap oil and a low bulk diffusivity of the oil through the gel. When increasing shear is applied during crystallization, the amount of oil expressed increases noticeably, resulting in a lower ability to entrap oil. The static sample, characterized by the strongest gel network as well as the highest viscosity, shows the lowest oil loss, in agreement with previous work.[29]

Interestingly, the OBC of the sheared samples only was well related to the crystalline domain size: larger domain sizes at lower shear rates translated to a greater OBC in agreement with what observed by Marty and Marangoni.[30] It was hypothesized that the dimensions of the nanocrystallites affected the mobility of the liquid oil phase. Under static conditions, a smaller number of thinner nanocrystals presumably translates into significantly larger lengths in the other two dimensions relative to the sheared samples. This would result in a thin nanocrystal with large cross-sectional area. It is assumed that the solid-state crystalline domains are impermeable to the passage of liquid oil and that liquid oil can only move through the space in between nanoplatelets. A high cross-sectional surface area would mean that the path of diffusion for the liquid oil is more tortuous for a thin but wide-spanning nanoplatelet than for a thick but relatively small nanoplatelet. The result would be a lower effective bulk diffusion coefficient. It is conceivable that, by modifying the dimension of the constituent nanoplatelets and extending the tortuousity of the diffusive path, the oil diffusion through a crystalline triacylglicerol or MAG material can be modulated.

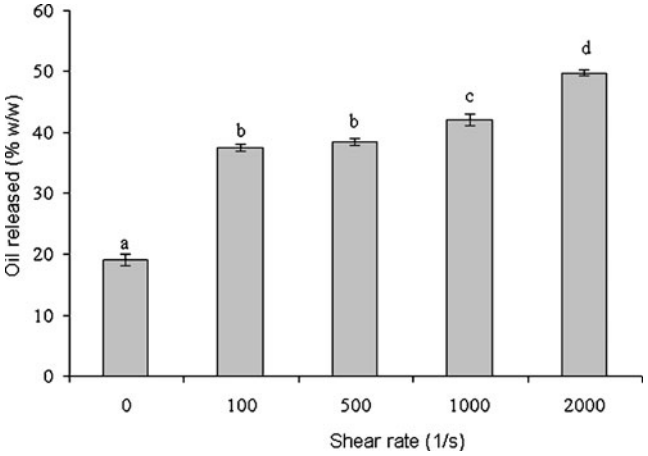

Fig. 4 Percentage of oil released after centrifugation as a function of shear rate applied during the crystallization of the samples. *Bars with the same letter* are not significantly different ($p>0.05$)

Conclusion

Results obtained in this study show that the introduction of shear during the organogel crystallization greatly affects the structure at the nano, micro, and macro levels. In particular, static and sheared samples have widely different structures at all levels. Static conditions lead to the formation of a strong gel network rich in junction zones between monoglyceride crystals and characterized by a high oil binging capacity. On the contrary, shear processing causes the formation of weak gels made of small crystal clusters weakly interacting among each other. However, the domain sizes of the sheared crystals were increased. These systems have a low oil binding capacity.

Table 2 Storage moduli (G′), loss moduli (G″), and apparent viscosity (η) of organogels crystallized at increasing shear rates

	Shear rate (1/s)				
	0	100	500	1,000	2,000
G′ (Pa)	3427.5±147.0a	155.6±4.3b	161.5±11.6b	212.7±23.0b	200.4±10. 2b
G″ (Pa)	607.2±32.2a	51.7±2.4b	48.7±2.4b	62.3±1.4b	53.8±1.5b
η (Pa s)	1.54±0.60a	0.35±0.04b	0.30±0.01b	0.37±0.05b	0.14±0.01c

Values with the same letter across each row are not significantly different ($p>0.05$)

These results may provide useful information for the applications of monoglyceride organogels in food formulation. By applying shear during the crystallization of a monoglyceride organogel, it is possible to engineer its structure and thus obtain tailored functionalities for specific food applications.

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