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Structural and Rheological Properties of Medium-Chain Triacylglyceride (MCT) Oleogels

Running title: Medium chain triacylglyceride (MCT) oil structuring

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Summary

This research studied the effect of gelator molecules on structural properties of oleogels containing medium-chain triacylglycerides (MCTs). To this aim, a large selection of gelators (rapeseed wax RAW, rice wax RW, sunflower wax SW, beeswax BW, monoglycerides MG, and γ-oryzanol and β-sitosterol mixture γ+β) at increasing concentrations (5 to 15% w/w) was considerd.

Results showed that RAW was not able to structure MCT at any concentration. However, the addition of 5% (w/w) of MG, SW, and BW resulted to a self-standing gel. Regarding γ+β and RW, the minimum gelling concentration was observed at 10% (w/w). By increasing the concentration, it was observed a reinforcement of the network strength highlighted by the progressive increase of the rheological parameters. The strongest oleogel obtained by γ+β (w/w) and further BW and RW at 10 and 15% (w/w) concentration, respectively. These findings could provide interesting information in the choice of the best performing MCT structuring agent for intended food applications.

Keywords: Oleogel, Medium-chain triacylglyceride, Structure, Oleogelator

Introduction

The interest on health benefits of oils rich in medium-chain triacylglycerides (MCTs) increased markedly in the last years. As the name indicates, MCTs refers to mixed triacylglycerols of medium-chain fatty acids, comprising 6–10 carbons. In nature, coconut oil and palm kernel oil (>50 wt% of fatty acids) as well as bovine milk fats (4-12%) are the main sources of these fatty acids (Marten *et al.,* 2006; Nagao & Yanagita, 2010). However, industrial mixtures of synthetic MCT from hydrolyzed coconut or palm kernel oil are commercially available. The latter oil is claimed to have interesting health benefits in the treatment of several gastrointestinal and neurological disorders. This is due to the reduced length of the fatty acid chains, which do not require enzymes for digestion and thus it is further easily absorbed into the bloodstream in comparison to long-chain triacylglycerides (LCTs) typical of vegetable and fish oils (Aoyama *et al.,* 2007; Shah & Limketkai, 2017).

Hence, due to MCT peculiar properties, their application in foods, particularly for medical nutrition, could be promising. However, the direct addition of MCT oil, liquid at ambient temperature, to foods is challenging. Thus, its conversion into an oleogel could enlarge its possible food applications. By definition, an oleogel is a gel in which an oil continuous liquid phase is immobilized into a supramolecular network of self-assembled molecules known as oleogelators (Patel, 2018). Oleogelation has received in the last decay considerable interest from the academic and industrial sectors due to the high potential as a plastic fat substitute, oil migration inhibitor, as well as modulators of lipolysis and delivery system of bioactive compounds (Co & Marangoni, 2012; O'Sullivan *et al.,* 2016; Rogers 2009; Stortz *et al.,* 2012; Calligaris *et al*., 2020).

The structural properties of resulted oleogels are assumed to be largely determined by the nature of the applied gelator, oil type as well as environmental and processing conditions (Pehlivanoğlu *et al.,* 2017; Rogers, 2009). In a view of that, a number of gelators, such as low molecular component and polymeric substances, with different chemical features (lipid and non-lipid based) and building blocks systems (crystalline network formation, self-assemble, and polymeric network) have been studied in the literature (Patel & Dewettinck, 2016; Samuditha *et al.,* 2011). Nowadays, it is also known that the structuring capacity of oleogelators cannot be considered separately from the oil that has to be gelled. In this regard, researches were mainly focused on oil containing long-chain fatty acids, such as sunflower oil, corn oil, olive oil, canola oil, flaxseed oil, rice bran oil and hazelnut oil (Calligaris *et al.,* 2013; Doan *et al.,* 2015; Fayaz *et al.,* 2017; Öǐütcü & Yilmaz, 2015). On the other hand, the structuring behavior of different gelators in oils rich in medium or short-chain fatty acids has not been in-depth studied. On this topic, De Vries *et al.*, (2017) studied MCT oil, sunflower oil, and extra virgin olive oil (EVO) oleogels with the use of protein aggregate and hydrophilic/hydrophobic

silica particles as gelators. Results showed that oleogel formed by protein aggregate and hydrophilic silica resulted in stronger gels in MCT oil in comparison to other oils. Similarly, Cerqueira *et al.* (2017) and Fasolin *et al.* (2018) confirmed the remarkable influence of oil (MCT oil and sunflower oil) and gelator structure (surfactants with different hydrophilic heads and hydrophobic tails) on mechanical, thermodynamic and rheological properties of oleogels. Moreover, Valoppi *et al.* (2017) reported comparative output on oleogel containing saturated monoglyceride (MG) and different types of oils including MCT oil. In general, these authors concluded that oil properties (composition, polarity, and viscosity) and resulted gelator-oil interactions greatly affected the oleogel structure.

Based on these considerations, the present research represents the first comprehensive study on structuring MCT oil with different widely studied gelators (rapeseed wax RAW, rice wax RW, sunflower wax SW, and beeswax BW, saturated monoglyceride MG, and mixture of γ-oryzanol and β-sitosterol γ+β) to increase the opportuninty of MCT exploitation in food . To this aim, the physical and structural properties of resulted oleogels at increasing concentrations (5, 10 and 15% w/w) were determined by using different analytical techniques, including polarized light microscopy, rheological analyses, and oil binding capacity.

Material and methods

Rapeseed wax (acid value <3 mg KOH/g, saponification value 200-240 mg KOH/g), beeswax (acid value 17-24 mg KOH/g, saponification value 87-104 mg KOH/g), rice wax (acid value <15 mg KOH/g, saponification value 65-95 mg KOH/g) and sunflower wax (acid value 2-8 mg KOH/g, saponification value 75-95 mg KOH/g) were purchased from KahlWax (Trittau, Germany); Monoglyceride (fatty acid composition: C16:0 45%, C18:0 8%, C18:1 transe 21%, C18:1 cis 20%, C18:2 2%) was purchased from Natural word S.r.l. (Lugo, Italy); γ-oryzanol (99 % purity, Nutraceutica S.r.l., Bologna, Italy) and β-sitosterol (75.5 % β-sitosterol, 12.0 % β-sitostanol, 8.4 % campesterol, 3.0 % other; Nutraceutica S.r.l., Bologna, Italy); Cremer COOR MCT 60-40 (MCT oil with fatty acid composition: C6:0 ≤ 0.5%, C8:0 55-66%, and C10:0 35-45%) was purchased from Cremer Oleo Division (Hamburg, Germany).

Oleogel preparation

Oleogels were prepared by mixing MCT oil with increasing quantity of the selected gelators (5, 10, and 15% w/w). The mixtures were heated under magnetic stirring above the melting point of each gelator until clear solutions were obtained, following the methodology reported by Calligaris *et al.* (2013), Doan *et al.* (2015), and Da Pieve *et al.* (2010). In particular, the heating temperatures were 80 °C for waxes and monoglyceride and 90 °C for a mixture of γ-oryzanol and β-sitosterol (3:2 w/w). After heating, the mixture were poured into container and subsequently cooled at 20 °C, excluding the mixture containing γ-oryzanol and β-sitosterol, which needed to be cooled at 4 °C to form gel (Calligaris *et al.,* 2014). Analyses were carried after 24 h of storage at 20 °C.

Visual appearance

The visual appearance of the samples was analyzed after preparation and storage. The samples were turned upside down and considerd as gel when it did not flow and in the case of slightly and easily flowing categorized as thickened liquid and liquid, respectively.

Polarized light microscopy

The microstructure of oleogels was observed under polarized light (PL) optical microscope (Leica DM 2000; Leica Microsystems, Heerburg, Switzerland) connected with a Leica EC3 digital camera (Leica Microsystems) with 100 × magnification at 20 °C. Micrographs obtained were processed using the application software Leica Suite LAS EZ (Leica Microsystems).

Oil binding capacity

Around one gram of molten sample was weighted into a micro tube and kept at room temperature (20 °C) for 24 h. The tubes were centrifuged at 10,000 rpm for 15 min using a microcentrifuge (Mikro 120, Hettich Zentrifugen, Andreas Hettich GmbH and Co, Tuttlingen, Germany). The tubes were turned over onto a paper and left for 5 min to drain the excess liquid oil. Finally, the oil binding capacity (OBC %) was calculated as the percentage ratio between the mass of remained oil in tube to the total mass of sample.

Rheological measurement

All the rheological measurments were carried out using a Haake Rheostress 6000 (Thermo Scientific, Rheostress, Haake, Germany) equipped with controller system (Thermo Scientific) for temperature control and application software Haake Rheowin v.4.60.0001 (Thermo Fisher Scientific). Parallel-plate geometry (Ø=40-mm and gap= 2000 µm) was used for all oscillatory measuments. Stress sweep measurment within the range of 0.01-1000 Pa was applied to determine the linear viscoelastic region (LVR) at a frequency of 1 Hz and 20 °C. The critical stress of oleogel was determined as the stress where G' value decreased to more than 10% of the values recorded in the LVR. Frequency sweep (0.1 to 10 Hz) was performed at a fixed stress value within LVR.

Statistical analysis

The experimental data are reported as mean value ± standard deviation of three repetitions and were analysed using Analysis of variance (ANOVA) by using R (version 3.2.3, The R Foundation for Statistical Computing, Vienna, Austria). Bartlett's test was used to check the homogeneity of variance and Tukey test was used to test for differences between means ($p<0.05$).

Results and discussion

The visual appearance and microstructure of oleogels containing increasing quantities of selected gelators (from 5 to 15% w/w) in MCT oil are shown in Figure 1. In the majority of the cases, self-standing oleogels were obtained. The only gelator unable to structure MCT at any concentrations was rapeseed wax (RAW). As well demonstrated in the literature, the composition of waxes is of utmost importance to explain their behavior in oil gelling (Samuditha *et al.,* 2011). Wax is usually defined as an ester of long-chain fatty acids with a fatty alcohol. However, it generally contains numerous other compounds, such as free fatty acids, free alcohols, and hydrocarbons, depending on the origin of the wax (Doan *et al.,* 2015). As seen from the chemical specification of gelators, among the studied waxes, RAW showed the highest saponification value (>200 mg KOH/g) and the lowest acid value (<3 mg KOH/g), indicating the short-chain of a wax ester with a low amount of free fatty acid compared to other waxes. On the bases of literature data, the gel strength of wax-based exhibited a positive correlation with a chain length of wax ester and concentration of free fatty acids; longer fatty acid chain in wax ester and high amount of free fatty acid will result in strong gel (Doan *et al.,* 2017; Hwang *et al.,* 2012). This result suggests that RAW components were unable to pack efficiently during crystallization and to form a network. Based on this result, RAW was not considered in the following experiments. Additionally, according to Figure 1, the gelling efficacy of RW and phytosterol/sterol ester mixture can only be observed at concentrations higher than 10% (w/w). The differences in structuring properties of gelators can be observed in microstructure images taken by polarized light microscopic (Figure 1). In the images, bright areas represent the crystal particles and dark zones liquid oil. MG-based oleogels revealed randomly small-dispersed needle-like crystals which are typical of MG crystallization (Da Pieve *et al.,* 2010). As well reported in the literature, MG self-organized into an inverse lamellar phase with a ß-subcell packing in oil (Da Pieve *et al.,* 2010; Valoppi *et al.,* 2017). Among waxes, beeswax and sunflower wax showed needle-like crystals, with SW crystals slightly bigger in size, in a good agreement with Doan *et al.,* (2015). On the other hand, the RW crystals appeared to crystallize as spherulites, similar to those observed in liquid paraffin and rice bran oil (Dassanayake *et al.,* 2009; Doan *et al.,* 2015). The later crystal morphology was not able to form a tight network at 5% (w/w) concentration. However, by increasing the concentrations, the possibility of creating junction points and furthermore interactions among spherical crystals increased. As extensively pointed out in the literature, the chemical feature of waxes significantly affects the crystal morphology (Doan *et al.,* 2017). It has been suggested that formation of long needle-like crystals is mainly due to the presence of the dominant wax ester (Blake *et al.,* 2018). This is in consistence with the chemical composition of SW and BW reported in the literature (96-97% and 60-70% wax ester, respectively) (Patel *et al.,* 2015). Although RW has similar high ester content to SW, it revealed significantly different morphology. This difference can be attributed to the presence of minor free

fatty acid fraction C24-COOH and C24 moiety in RW in comparison to SW (28% vs 4%, respectively) (Doan *et al.,* 2017).

Regarding the mixture of γ-oryzanol and β-sitosterol, it was not possible to observe the bulding blocks entrapping oil by using polarized light microscopy. This is due to the fact that these two molecules self-assemble into hollow tubes of nanometric size, as imaged by SEM and reported by the works of Sawalha *et al.* (2012) and (2015).

To better study the feature of MCT gel networks, rheological measurements such as amplitude sweep and frequency sweep tests were performed. As a representative sample, Figure 2 and 3 show the elastic modulus (G′) of oleogels at 10% (w/w) gelator content as a function of the oscillation stress and frequency, respectively. After the linear viscoelastic region (LVR), G′ sharply decreased (Figure 2). The point at which the G′ varies by 10% from LVR was generally reported as critical stress and it is an indicator of permanent deformation (Doan *et al.,* 2017). The critical stress results of all samples were reported in Table 1. The critical stress of structuring molecules was decreased in the following order: mixture of γ+β, waxes and finally MG, which is indicative of different resistance to deformation. Among waxes, SW revealed a brittle structure, supported by narrow LVR. These differences in oleogel behavior can be attributable to different intermolecular forces holding up the structure in the network of particles. As reported by Doan *et al.* (2018), the purity and chemical composition of waxes significantly impact bonding in the network and further strength. Therefore, it can be suggested that bonding in the network of SW crystalline particles is more homogenous due to the high amount of wax ester (>95% w/w), leading to broken down at the same applied force (Patel *et al.,* 2015). The plot from oscillatory frequency sweep on oleogel at 10% (w/w) was shown in Figure 3. As seen from the graphs, G′ and G″ were mostly linear as a function of frequencies with G′ > G″. This result confirms the viscoelastic gel behavior of samples as visually observed. The tan δ is a ratio of the two moduli, G″/ G′, and can be used to characterize the relative elasticity of a material. This indicator allows to classify samples into strong gels (tan $\delta < 0.1$), weak gel ($0.1 <$ tan $\delta < 1$) and viscose sole (tan $\delta \geq 1$) (Tavernier *et al.,* 2017). As shown in Table 1, all self-standing gel revealed $0.1 \leq$ tan $\delta < 1$, indicating weak gel property with different elasticity. The elasticity of MCT oleogels were depended greatly on gelling agent and concentration. For instance, at 10% (w/w) concentration, mixture of γ-oryzanol and β-sitosterol revealed the strongest MCT gel network followed by waxes and MG. However, by increasing the concentration to 15% (w/w), γ-β mixture did not show the strongest gel and the highest elastic network were associated with waxes particularly RW and BW. It should be remembered that gel structure of γ-β mixture thanks to self-assembled tubules, stack aligned as the shape of a helical ribbon with improving inter strong hydrogen bonds during time (Samuditha *et al.,* 2011). The latter suggests that structure formation of γ-β system at high concentration (>10% w/w) requires higher supercooling or longer time to be assembled. On the other hand, the ability of wax and MG particles to gel oil is

associated with crystallization in liquid oil generally results in gels where the network based on interactions of crystalline aggregates. As the structurant concentration increased, the interactions among particles increases allowing the generation of stronger networks (Doan *et al.,* 2015; Da Pieve *et al.,* 2010). This is evident in MCT oleogel containing BW, which represented the strongest gel network among waxes at 15% (w/w). This result can be due to the formation of small needle-like crystals, increasing the possibility of creating more crystal-crystal contacts. RW should be considered as a weak gel at a concentration lower than 10% (w/w), since did not form a tight network capable of entrapping a large amount of MCT oil. Interestingly, at higher concentrations (10 and 15% w/w), RW was able to reinforce the network to form stronger gel thanks to the crystallization in large aggregates. This hypothesis was confirmed by polarized light microscopy images (Figure 1).

It is interesting to note that, by performing regression analysis between gelator concentration and rheological parameters, a good linear correlation was observed between G' and critical stress and concentration of MG, SW and BW in oleogels ($R^2 > 0.90$). Even if the regression analysis was performed only with three levels of the oleogelator content, the acquired results indicate that the rheological parameters were more affected by the concentration increase in BW, followed by SW and finally MG. In other words, the increase of structurant content in BW-based oleogels effectively reinforced the gel network strength leading to a sharp increase of rheological parameters. At the same time, the same gelator content increase did not generate a so intense structure strengthen in SW and MG.

Beside microstructure and rheological properties, oil binding capacity of MCT oleogels were measured. No oil release was observed for waxes and mixture of γ-β samples. However, MG revealed lower oil binding capacity, which progressively increased with increasing the concentration (69.25, 93.82 and 98.22% for 5, 10 and 15% w/w, respectively). This may be explained by weak gel properties of MG not able to entrap the whole volume of liquid oil in the crystalline network, in consistence with microstructure and rheological properties.

To better highlight the effect of fatty acid chain length on the rheological properties of oleogels, data previously reported by our research group (Fayaz *et al.,* 2020) on the structuring behavior of considered gelators in sunflower oil were used to compute the difference (Δ) of critical stress and G′ between oleogels containing LCT and MCT at 10% (w/w) content (Table 2). It can be noted that the MCT oleogels containing the different gelators resulted in weaker gels compared to LCT (negative difference), with the only exclusions of RW and SW. This result is in agreement with the findings of Cerqueira *et al.* (2017) and Martins *et al.* (2016), reporting that oleogels prepared with LCT produced stronger oleogels than MCT. These difference behaviors can be due to the specific interactions between oil and gelator. MCT oil mainly consists of medium triacylglyceride with fatty acid in the range of 6-10 carbons with low viscosity (28.83 ± 0.08 mPa.s). On the other hand, sunflower oil is rich in linoleic acid (C18:1) and revealed higher viscosity

(62.77 ± 0.44 mPa.s). The latter difference in the nature of oil as well as the nature of gelling agent affects both particle-particle and particle-solvent interaction and finally network strength. In this regard, De Vries *et al.* (2017) and Sawalha *et al.* (2012) showed that the strength of oleogel and emulsion-gel decreases when the interaction between the gelator molecules and solvent are enhanced. For instance, hydrophilic silica particles resulted in weaker gel by increasing particle-solvent interaction as a function of increased the polarity changing from MCT oil to extra virgin olive oil (De Vries *et al.,* 2017). Similarly, oryzanol and sitosterol tubular microstructure formed stronger emulsion-gel by reducing the water activity and/or by using low polarity oil (Sawalha *et al.,* 2012). It can be inferred that more particle-solvent interaction led to higher solubility of gelators which cannot participate in formation of gel network. This is in agreement with Valoppi *et al.* (2017), showing higher solubility of MG in MCT oil and castor oil and subsequently weaker gel in comparison to cod liver oil. Therefore, the gel network strength depends on gelator solubility in a solvent and the proper interaction between gelator-gelator and gelator-solvent.

Conclusions

Result obtained in this study revealed the possibility to gel oils containing medium chain triacylglycerols by exploiting the oleogelation properties of different gelators, whose structuring performances are strictly dependent on their chemical features. For instance, the addition of 5% (w/w) of MG, SW, and BW resulted enough to generate self-standing gels; whereas the minimum gelling concentration of γ+β and RW resulted 10% (w/w). Finally, the addition of RAW was not able to structure MCT at any concentration considered. These results appear interesting in the attempt to select the proper gelator based on the specific food application of MCT oil and open new possibility to develop functional foods enriched with MCT oil for medical nutrition.

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Table 1. Tan δ, storage modulus (G′) recorded at 1 Hz, and critical stress of MCT oleogels containing 5, 10 and 15% (w/w) of monoglyceride (MG), rice bran wax (RW), sunflower wax (SW), beeswax (BW), and mixture of γ-oryzanol and β-sitosterol (γ-β) at 20 °C

Gelator	Tan δ			G′ ×10^4 (Pa)			Critical stress (Pa)		
	5% (w/w)	10% (w/w)	15% (w/w)	5% (w/w)	10% (w/w)	15% (w/w)	5% (w/w)	10% (w/w)	15% (w/w)
MG	$^{B}0.34\pm0.00^{a}$	$^{B}0.36\pm0.03^{a}$	$^{A}0.39\pm0.01^{a}$	$^{C}0.58\pm0.01^{c}$	$^{C}4.13\pm0.22^{b}$	$^{D}6.61\pm0.79^{a}$	$^{B}2.85\pm0.63^{b}$	$^{D}10.13\pm2.25^{ab}$	$^{D}19.11\pm4.26^{a}$
RW	-	$^{D}0.10\pm0.00^{a}$	$^{B}0.10\pm0.03^{a}$	-	$^{B}28.66\pm0.30^{b}$	$^{A}136.00\pm7.49^{a}$	-	$^{B}333.20\pm74.25^{b}$	$^{A}2541\pm704.28^{a}$
SW	$^{A}0.47\pm0.03^{a}$	$^{A}0.47\pm0.01^{a}$	$^{A}0.35\pm0.08^{a}$	$^{A}20.77\pm0.45^{c}$	$^{B}34.89\pm0.43^{b}$	$^{C}54.52\pm0.39^{a}$	$^{AB}26.26\pm5.85^{b}$	$^{CD}68.09\pm15.17^{ab}$	$^{C}126.05\pm25.10^{a}$
BW	$^{C}0.25\pm0.01^{b}$	$^{B}0.31\pm0.02^{a}$	$^{AB}0.26\pm0.00^{ab}$	$^{B}18.65\pm0.07^{c}$	$^{B}37.56\pm2.52^{b}$	$^{AB}107.85\pm0.07^{a}$	$^{A}36.08\pm8.03^{b}$	$^{C}176.50\pm39.32^{b}$	$^{B}575.40\pm64.49^{a}$
γ- β	-	$^{C}0.20\pm0.04^{a}$	$^{AB}0.22.\pm0.05^{a}$	-	$^{A}71.39\pm4.24^{b}$	$^{B}90.27\pm5.84^{a}$	-	$^{A}917.40\pm9.81^{b}$	$^{A}2088.0\pm90.51^{a}$

Upper case letters (A–D) indicate significant differences among different gelator within the same concentration ($p<0.05$).
Lowercase letters (a-c) indicate significant differences among different concentration of same gelator ($p<0.05$).

Table 2. Regression equation and coefficients of determination (R^2) among gelator concentration and rheological properties (Critical stress and storage modulus G') of MCT oleogels as well as difference between rheological properties of MCT oleogel and sunflower oleogel ($\Delta_{(MCT-SUN)}$) containing 10% (w/w) monoglyceride (MG), rice bran wax (RW), sunflower wax (SW), beeswax (BW), and mixture of γ-oryzanol and β –sitosterol (γ-β) at 20 °C

Gelator	Critical stress (Pa)		G' ×10^4 (Pa)		$\Delta_{(MCT-SUN)}$ Critical stress (Pa)	$\Delta_{(MCT-SUN)}$ G'×10^4 (Pa)
	Equation	R^2	Equation	R^2		
MG	y=1.63x-5.56	0.99	y=0.23x-1.28	0.93	-41.16	-12.19
RW	-		-	-	212.66	3.73
SW	y=10.02x-26.62	0.99	y=3.37x+2.98	0.99	3.01	3.24
BW	y=53.73x-273.99	0.93	y=10.17x-51.21	0.95	-300.7	-97.04
γ- β	-	-	-	-	-629.6	-104.51

Figure 1

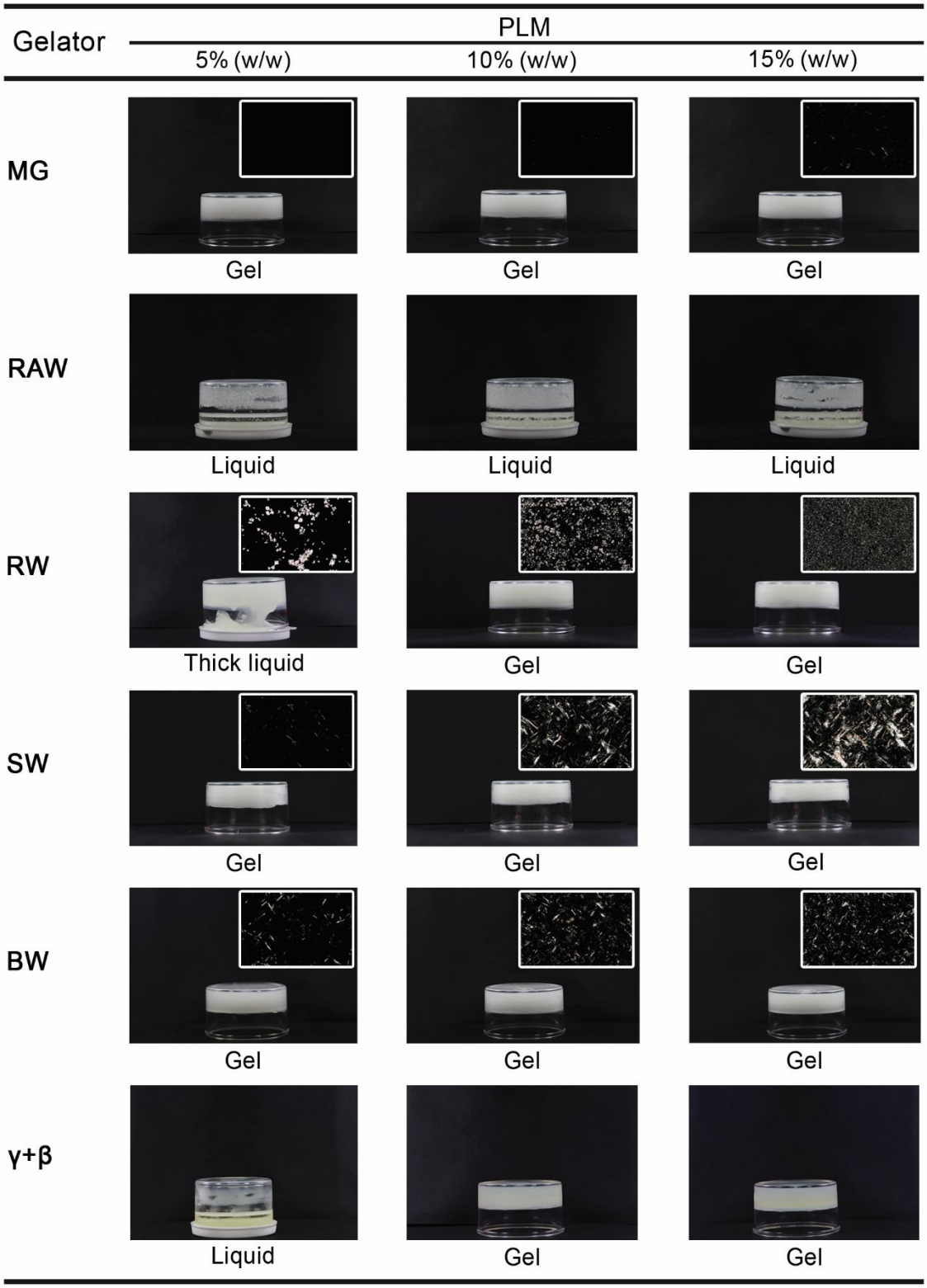

Gelator
PLM
5% (w/w)
10% (w/w)
15% (w/w)
MG
Gel
Gel
Gel
RAW
Liquid
Liquid
Liquid
RW
Thick liquid
Gel
Gel
SW
Gel
Gel
Gel
BW
Gel
Gel
Gel
γ+β
Liquid
Gel
Gel

Figure 2

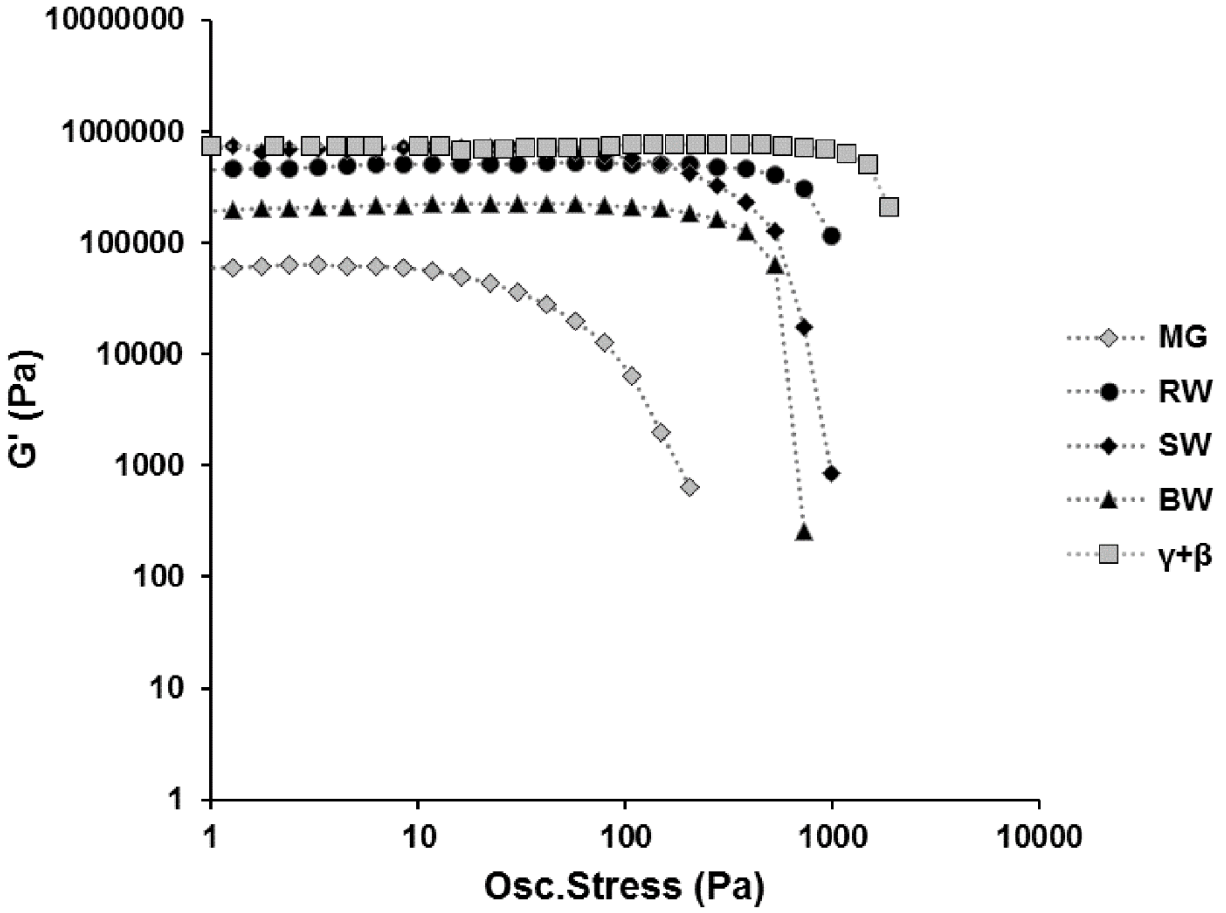
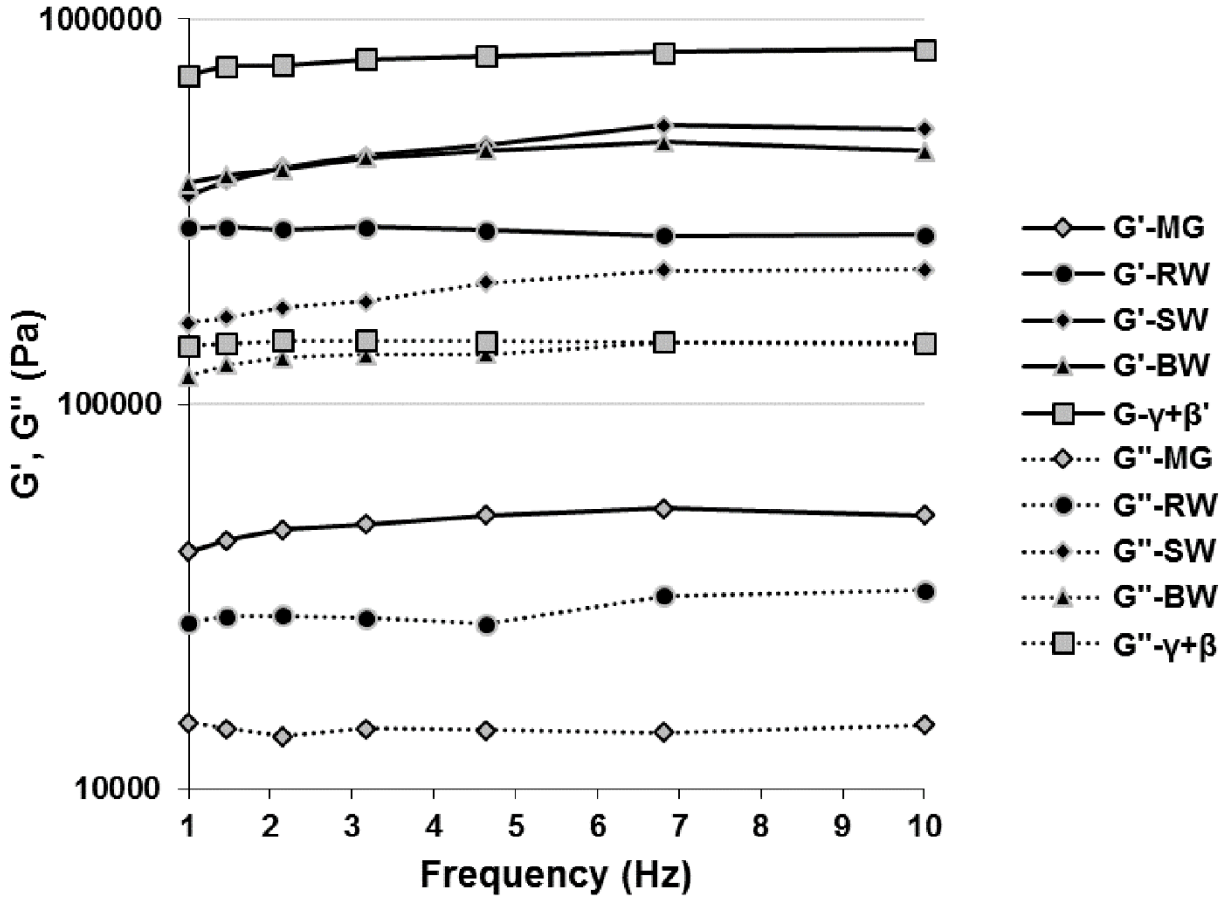
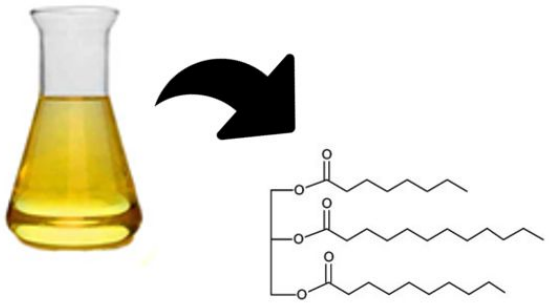


Figure 3


1000000
100000
10000
G', G'' (Pa)
1
2
3
4
5
6
7
8
9
10
Frequency (Hz)
G'-MG
G'-RW
G'-SW
G'-BW
G-γ+β'
G''-MG
G''-RW
G''-SW
G''-BW
G''-γ+β



Medium-Chain Triacyglyceride (MCT)

↓

MCT oleogels

Rice wax
5% (w/w)

Rapeseed wax
10% (w/w)

Mixture of γ-oryzanol
and β-sitosterol
15% (w/w)