1	Organogelation of extra virgin olive oil with fatty
2	alcohols, glyceryl stearate and their mixture
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23 ABSTRACT

24 The final properties of organogels based on a mixture of gelators can be tuned by taking 25 advantage of interactions between gelators. In this work, extra virgin olive oil was structured using glyceryl stearate (GS), policosanol (P) and their mixtures aiming at 26 investigating their effects on the rheological properties and microstructure of obtained 27 28 gels. Rheological tests evidenced that P yields a more consistent material with higher crystallization and gelation temperatures with respect to GS. Gels based on a mixture of 29 30 gelators exhibit intermediate properties between those of "pure" gels, even though closer to those of P organogels. The crystallization and gelation phenomena in mixed 31 gels seem to be dependent only on policosanol fraction, whereas dynamic moduli are 32 33 affected by both gelators. Intermolecular interactions were investigated with FT-IR finding that GS is able to give hydrogen bonding whereas P works mainly through the 34 formation of van der Waals interactions. Mixed gels exhibit a behavior close to that of P 35 gels, even though van der Waals interactions seem less evident than in pure P gels. As a 36 consequence, from a macroscopic point of view, the use of the mixture yields a weaker 37 38 gel than that obtained with the same concentration of pure P.

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Keywords: Organogels; Glyceryl Stearate; policosanol; intermolecular interactions;
rheology

43 **1. INTRODUCTION**

Organogels (or oleogels) are solid-like materials structured in an organic solvent thanks 44 45 to the self-assembly of structurant molecules (organogelators) which build a threedimensional network. Different organogelators, mostly Low Molecular Weight 46 (LMWO), are currently used to give stiffness to liquid solvents, in particular for foods, 47 owing to the growing necessity to obtain healthy *trans-free* hard fats or shortenings 48 without chemical modifications (Patel & Dewettinck, 2016). Among the LMWOs, 49 50 Monoacylglycerols (MAGs) are widely investigated (Co & Marangoni, 2012), because they are very used in foodstuffs or cosmetics as emulsifiers and they are cheap and 51 versatile. MAGs were used, for example for structuring cod liver oil aiming at analyzing 52 53 the potential effect of these organogels on oxidative stability of the solvent (Da Pieve et al, 2011). The same gelators were also investigated by Ojijo et al. (Ojijo et al, 2004), 54 Lopez-Martinez et al., (Lopez-Martinez et al, 2015; Lopez-Martinez et al, 2014) and by 55 Lupi et al. (Lupi et al, 2012a; Lupi et al, 2011; Lupi et al, 2012b). 56

Among other potential edible gelators, special attention was paid to waxes (Rice bran wax, candelilla wax and carnauba wax), fatty alcohols, fatty acids, sterols and sorbitan monostearate (Bot et al, 2011; Moschakis et al, 2016). In particular, fatty alcohols can be used alone to structure oil (Lupi et al, 2013), or in mixture with other components, throughout the addition of waxes extracted from vegetable sources. For example, candelilla wax is a mixture of fatty alcohols with sterols, high-melting alkanes, fatty acids and waxy esters (Mert & Demirkesen, 2016; Toro-Vazquez et al, 2013).

Recently, the effect of mixtures of gelators of different chemical nature on the rheological properties of gels has been studied, mostly aiming at tuning the final characteristics of either organogels or emulsions in which these gels are the oil phase. 67 Quite often, the investigated mixtures contain MAGs, owing to the already described68 characteristics.

Alfutimie et al. (Alfutimie et al, 2014), employing different techniques, studied the phase behavior of mixed saturated and unsaturated monoglycerides (glyceryl oleate and glyceryl stearate) to investigate whether gel phases could be formed from unsaturated monoglycerides. The authors found that there was incorporation of the saturated alkyl chains into the α -gel phase formed by the unsaturated alkyl chains of the other monoglyceride and, therefore, an interaction between gelators.

Lopez-Martinez et al. (Lopez-Martinez et al, 2015) investigated organogels prepared with different amounts of MAGs with and without ethylcellulose (EC). According to the results, the addition of EC to MAGs-canola oil organogels, gave materials with higher G* and lower oil lost during storage than those observed without EC. The authors concluded that there is a synergistic interaction between these components.

Toro-Vazquez et al. (Toro-Vazquez et al, 2013) investigated organogelled emulsions whose oil phase was structured with a mixture of candelilla wax and monoglycerides. Thanks to DSC, NMR (for Solid Fat Content measurement) and texture measurement, the authors found that the presence of MAGs affected the inter-crystal interactions in both the organogels and the emulsions, and that this phenomenon became more significant with the increase of MAGs concentration.

86 Starting from these considerations, the present paper aims at investigating the 87 rheological properties and the microstructural interactions of olive oil organogels 88 structured with GS, fatty alcohols (P) and mixtures of them.

GS is interesting because it is widely used in different areas (foods, cosmetics) and isbased on stearic acid, a saturated fatty acid that is considered "neutral" for cholesterol

and cardiovascular issues (Marangoni, 2009). On the other hand, P was recently proposed as an organogelator, whereas in the past, it was mainly known as food supplement owing to its positive effects on cholesterol and cardiovascular diseases (Xu et al, 2007). Even though more expensive than GS, it seems very effective in edible oil gelation, as a consequence, it can be interesting to investigate the properties of the mixture of this MAG and these fatty alcohols with respect to the effects of single gelators.

98 The rheological investigation of edible LMW organogels is nowadays diffusely 99 available in the literature, but a microstructural characterization based on the 100 intermolecular interaction between gelators analyzed with FTIR spectra, and focused on 101 organogels made with extremely impure solvents like commercial edible oils, is still not 102 deeply investigated. Therefore, the paper aims at giving a deep explanation on the 103 rheological properties of these complex systems related to the weak interactions typical 104 of LMW organogels.

105

106 2. MATERIALS AND METHODS

107 **2.1 Materials and samples preparation**

Organogels were prepared with an extra virgin olive oil (EVO), supplied by Gabro (Italy), as the solvent and tocopherol as an antioxidant; the adopted organogelators were policosanol (P) from rice bran wax (Lupi et al, 2013), glyceryl stearate (GS) and their mixtures. All the ingredients were supplied by A.C.E.F. (Italy).

Organogels were produced by adding tocopherol in the extra virgin olive oil to prevent oxidation (the ratio tocopherol/olive oil was kept constant and equal to $6.15 \cdot 10^{-4}$ (w/w) (Lupi et al, 2016b)), and then heating the oil in a water bath thermostated by a plate heater (VELP Scientific, Italy) up to 85°C. Afterwards, the gelator (i.e. P or GS or a
mixture of both) was added to the hot oil, and the system was continuously stirred
(stirrer RW 20, IKA-Werke, Germany). On complete melting of organogelator, mixing
was continued for at least 5 minutes before starting the rheological study.

Organogels produced with GS are indicated as 'OG' samples within the text, P organogels are called 'OP' samples, and, finally, systems made by mixing the two organogelators are indicated as 'OM' samples. Samples are labeled with a subscript indicating the total amount of gelator added to the oil (see Table 1).

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124 **2.2 Rheological characterization**

Organogels (except for samples $OG_{0.02}$ and $OP_{0.02}$, analyzed only with FTIR tests) were investigated with a controlled stress rheometer (DSR500, Rheometric Scientific, USA, parallel plates 40 mm, gap 1.0±0.2 mm) where temperature is controlled by a Peltier system.

Preliminary amplitude sweep tests were carried out at different constant temperatures from the preparation value, i.e. 85 °C, down to 20 °C almost every 10 °C to examine the potential changes in the linear viscoelastic region.

Temperature ramp tests (time cure) were, then, performed at constant frequency of 1 Hz and in the linear viscoelastic regime by cooling the sample from 85 °C down to 20 °C (or down to 10°C for OG samples) with a cooling rate of -1 °C/min. All samples were independently prepared three times and experimental results are shown in terms of average value and standard deviation, typically lower than 10%. Larger deviations were seldom observed either for data at high temperature, owing to instrument limits, or for data in the transition crystallization region, owing to the fast structuring phenomena. The onset of crystallization (T_{co}) was defined as the temperature value at which an abrupt increase of complex modulus G* (and a rapid decrease of the loss tangent curve) in temperature ramp tests is observed (Lupi et al, 2012b; Lupi et al, 2013).

142 The temperature at which clusters of crystals start to interconnect in a network structure, can be considered as the gelation temperature (T_{e}) . Even though the rigorous 143 144 determination of the gel point should be carried out according to the well-known Winter's theory (Chambon & Winter, 1987), more approximate criteria are used often 145 146 for a faster estimate of a transition temperature. Among the different proposed criteria, the crossover between dynamic moduli is often used even if, being frequency 147 dependent, it is not the real gel point (Chambon & Winter, 1987; Curcio et al, 2001; 148 149 Rao, 1999). Starting from these considerations, in the present work, for simplicity, the gelation temperature was estimated as the crossover between dynamic moduli i.e. the 150 temperature at which the loss tangent is equal to unity and, therefore, the phase angle (δ) 151 is 45° (Lupi et al, 2012b). 152

Experimental data of storage modulus, *G*', were fitted as a function of the organogelator fraction, according to a fractal model already used in the literature to describe fat crystal networks (Tang & Marangoni, 2007) and recently adopted also to analyze organogel behavior

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$$G' = \lambda \cdot X_o^{\frac{1}{3-D}} \tag{1}$$

The storage modulus is usually related to the fat crystal volume fraction (Tang & Marangoni, 2007). Nevertheless, the mass fraction of organogelator being easier to measure and proportional to the crystal volume fraction (Lupi et al, 2013), it was chosen to fit *G*' as a function of the organogelator fraction X_0 . In Eq. 1, λ is a constant determined by the links between fat crystal flocs, and *D* is the fractal dimension. A linearized form of Eq. 1 is shown in Eq. 2 and it was used to evaluate the modelparameters from experimental data.

165
$$\ln(G') = \ln(\lambda) + \frac{1}{3-D}\ln(X_o)$$
(2)

166 Rheological data were evaluated at a fixed distance from the onset of crystallization T_{co} 167 $(T_{20}=T_{co}-20)$.

168 Thermo-rheological parameters (i.e. T_{co} , T_g) were compared with a statistical analysis 169 (ANOVA, MS Excel 2013, Microsoft, USA) and differences among compared samples 170 were considered significant at *p*-value < 0.05 (interval of confidence of 95%).

171

172 2.3 FT-IR characterization

In order to investigate the interactions between molecules involved in gel formation, infrared absorption spectra were obtained, at room temperature (approximately 25°C), with a Nicolet iS-10 FT-IR spectrometer (Thermo Scientific, USA) equipped with a Smart iTX ATR sampling accessory. Raw materials (P, GS and EVO oil) were investigated, as well as organogel with 0.02 and 0.08 weight fraction of gelator (both pure gelators and their mixture).

Samples were prepared slowly cooling the warm solution from 85 to 25°C in a thermostatic water bath (F25, Julabo, USA) at 25°C for the time necessary to reach thermal equilibrium. The sample was loaded into the measurement tool as soon as it reached room temperature. Spectra were collected within the range of wavenumber between 400 and 4000 cm⁻¹.

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185 **3. RESULTS AND DISCUSSION**

186 **3.1 Rheological characterization of organogels**

187 Firstly, the rheological characteristics of organogels produced by increasing the organogelator content were analyzed. As an example, time cure tests of samples 188 189 OG_{0.034}- OG_{0.125} (table 1) are shown in Fig. 1 in terms of G^* (a) and δ (b), respectively. It is worth noticing that phase angle data appears scattered because of the inertia of the 190 191 instrument which affects the measured values (Baldino et al, 2013). It is well known 192 that in stress control rheometers (the so called Combined Motor Transducer 193 instruments) where sample torque is determined from motor torque, corrections for 194 motor inertia or bearing friction are necessary (Franck, 2003). The ratio of inertia torque 195 and sample torque increases with increasing frequency and with the decreasing viscosity; therefore, for a low viscosity sample (such as the investigated material at high 196 197 temperature) inertia can be so relevant that errors in corrections can affect the measure yielding very scattered data (Franck, 2003). Moreover it is worth noticing that, owing to 198 199 the limits in minimum values of torque and strain measured by this rheometer (2 μ N·m and 4 µrad, respectively), the determination of dynamic moduli, with low viscosity 200 fluids, could be affected by some approximations that could contribute further to the 201 202 observed data scattering.

From a qualitative point of view, the trend of temperature ramp tests is quite similar for all the investigated organogels: at high temperatures, systems show a viscous behavior as confirmed by the high values of δ close to 90° (even though very scattered because of the discussed inertia effects).

207 Moreover, G^* slightly increases with decreasing temperature as the result of the 208 reduction in molecular mobility, although no structural change occurs. At T_{co} , a sudden 209 increase of G^* , corresponding to a sharp decrease in δ , is observed, as already found for 210 similar materials (Lupi et al, 2012b; Lupi et al, 2013). 211 It was observed that gelation (evidenced by δ values lower than 45°) occurs for OG samples only when the mass fraction is equal or greater than 0.040. This behavior is 212 213 different from that observed in the literature where a commercial mixture of GS and glyceryl palmitate (in equal amount), namely Myverol, is able to gel olive oil (with a 214 cooling rate of -5°C/min) at 0.015 w/w (Lupi et al, 2012b). Moreover T_{co} is always 215 significantly higher than T_g in the investigated range of GS whereas, when Myverol is 216 217 used in concentration larger than 0.003, these critical temperatures are statistically equal. Finally both dynamic moduli and critical temperatures (i.e. T_{co} and T_g) are lower 218 219 for GS with respect to the Myverol suggesting that the interesting gelator ability of this commercial mixture could be due, mainly, to the glyceryl palmitate fraction. 220

On the other hand, for P oleogels, gelation occurred for all investigated concentrations and T_{co} was found to be always statistically comparable to T_g starting from a minimum concentration of Xp = 0.034. This is in agreement with literature data where it was found that minimum fraction for gelation, within the same investigated temperature window, is approximately 0.005 (w/w) and no significant difference between T_{co} and T_g is observed for fractions larger than 0.025 (Lupi et al, 2013).

227 Samples OM exhibit a behavior very close to that of P organogels: no statistical difference (p>0.05) was observed among the critical temperatures (both T_{co} and T_{g}) of 228 229 OP and the corresponding values of OM, suggesting that both crystallization and gelation in OM samples is controlled by policosanol. This result is in agreement with 230 231 the characteristics observed for OG samples: indeed, when organogels based on pure 232 GS are considered, crystallization and gelation (when present) occur always at a temperature significantly lower than those observed for P organogels. Consequently, it 233 can be speculated that GS crystallization is hidden, in the rheological tests, by the 234

previous crystallization of policosanol. Nevertheless, the presence of GS crystals is
evidenced by the values of complex modulus, at low temperature, that in OM samples
are significantly greater than those observed in OP samples with the same policosanol
content (see for example Fig. 2).

It is worth noticing that differences in complex moduli (Fig. 2) are present even for temperatures greater than T_{co} of OG sample having the same GS content: this suggests that policosanol crystals could, probably, promote the GS crystallization.

242 Starting from temperature ramp tests, fractal model was used to fit the rheological data as a function of total organogelator mass fraction within the organogel and parameters λ 243 244 and D were estimated (Table 2). Complex moduli were evaluated at a relevant distance from the onset of crystallization ($\Delta T=20^{\circ}$ C) with the aim of taking into account the 245 246 effects of both gelators. It appears (Fig. 3) that the mixture of gelators gives intermediate properties between those of the systems produced with pure 247 organogelators, even though a non-linear trend is evidenced, and it seems that samples 248 OM approach the properties of OP gels. It is worth noticing that significant differences 249 250 are observed among parameter values of λ parameters whereas the fractal dimension of 251 OM samples is statistically comparable to that of OP gels, confirming again that 252 "mixed" samples exhibit a behavior very close to that of P systems.

If the mixed gels would follow the rules of an ideal mixture, their properties could be estimated as the weight-average of the corresponding properties of single pure gels obtaining equations similar to those proposed for polymer composites (Rao, 1999); for instance if storage modulus at T_{20} would be considered, it would be obtained:

257
$$(G'_{OM})^{id} = yG'_{OP} + (1 - y)G'_{OG}$$
(3)

258 where the superscript "id" refers to the "ideal mixture" behavior and "y" is the fraction 259 of OP gel in the mixture. Usually the volume fraction is used in Eq. 3 (Rao, 1999), 260 anyway, in the present case it can be reasonably assumed that density of all tested gels 261 is the same and, therefore, weight fractions can be used instead of the volume ones (in the present case y=0.5 for all investigated systems). The comparison between 262 263 experimental G' values and those estimated according to Eq. 3, is shown in Fig. 4 for 264 available concentrations. It can be seen that experimental values are always lower than 265 "ideal" ones, even though percentage differences (shown in the figure for each concentration) do not seem to have a clear trend. This result suggests a complex 266 interaction between gelators (and formed gels) different from a simple "overlapping" of 267 268 single gels where one structure is dispersed in the other one.

On the basis of the observed behavior it could be speculated that the main structure of OM organogels is based on policosanol crystallization (occurring at high temperature) and the subsequent aggregate formation (yielding the same fractal dimension of policosanol organogels); on the other hand GS crystals seem to participate in a network, already formed, affecting mainly its "strength" and consistency (therefore modifying the parameter λ with respect to the OP gels).

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276 **3.2 FT-IR analysis**

Aiming at understanding the molecular interactions occurring in organogels produced by mixture of different gelators, and, consequently, aiming at interpreting rheological results, FT-IR tests were carried out on raw materials alone, firstly, and on samples with constant fractions of gelators (both pure and mixed), later on. FT-IR spectra are used in this case to investigate solvent/gelator or gelator/gelator intermolecular interactions orbonds occurring during gelation.

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284 *3.2.1 – Pure EVO Oil*

Figure 5a shows the FT-IR spectrum of pure oil. The most prominent peaks appearing 285 in the range of wavenumbers 500-4000 are: C-H stretching mode peak, in the 286 wavenumber region of 2800-3100 cm⁻¹, C=O stretching peak in the region of 1700-287 1800 cm⁻¹ and C–O–C stretching and C–H bending in the region of 900–1400 cm⁻¹ (Tay 288 et al, 2002). In more detail, the major peaks representing triglyceride functional groups 289 were observed around 2922 cm⁻¹ and 2853 cm⁻¹ (symmetric and asymmetric stretching 290 vibration of the aliphatic CH₂ group), 1743 cm⁻¹ (ester carbonyl functional group of 291 triglycerides), 1465 cm⁻¹ (bending vibrations of the CH₂ and CH₃ aliphatic groups), 292 1238 cm⁻¹ and 1163 cm⁻¹ (stretching vibration of the C=O ester groups) and 709 cm⁻¹ 293 294 (C-H bending) (Vlachos et al, 2006). EVO oil spectrum exhibited also a small peak at around 3005 cm⁻¹, assigned to the C-H stretching vibration of cis-double bond (=CH), 295 296 that is specific for each vegetable oil: virgin and extra-virgin olive oil contains a high proportion of oleic acyl groups; therefore, this peak appears at around 3005-3006 cm⁻¹, 297 whereas for other vegetable oils, this peak is shifted to higher wavenumbers. 298

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 $300 \quad 3.2.2 - Pure \ GS$

The most interesting region for examining intermolecular interactions for MAGs is the high-energy region from 3000 to 4000 cm⁻¹ (Chen & Terentjev, 2009; Oğütcü & Yılmaz, 2014). In fact, this zone corresponds to the OH-stretching modes (Lopez-Martinez et al, 2014) to which H-bonds between molecules are attributed. In general,

MAGs have two OH groups in the hydrophilic portion of the molecule corresponding to 305 2 and 3-OH groups respectively, which are bonded by hydrogen bonds (Alfutimie et al, 306 307 2014). Figure 5b shows IR spectrum for GS. Hydrogen bonds formed by each hydroxyl group can be detected by the peak at 3307 cm⁻¹, followed by a second one, slightly 308 hidden by a shoulder appearing at 3241 cm⁻¹. In the low-energy region, two more 309 noticeable peaks, appear at 2914 and 2849 cm⁻¹, representing the typical CH₂ stretch of 310 alkyl carbon chains; finally, another prominent peak, at 1730 cm⁻¹, is attributable to the 311 312 C=O stretching mode.

313



It has already been specified the chemical nature of P, which is different from MAGs or olive oil. P is a mixture of long alkyl chain fatty alcohols, characterized by just one hydroxyl group. FT-IR spectrum for pure P, in figure 5c, shows some typical peaks observed for fats: 2955 and 2915 cm⁻¹ (stretching vibration of the aliphatic CH₂ group), 1462 and 1472 cm⁻¹ (bending vibrations of the CH₂ and CH₃ aliphatic groups), 1378cm⁻¹ (bending vibrations of CH₂ groups), whereas the peak at 3292 cm⁻¹ indicates the OHstretching mode (Vlachos et al, 2006).

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323 *3.2.4 Organogels with pure gelators*

In the low-energy region, from 500 to about 3000 cm⁻¹, the shape of the peaks appearing in the FTIR spectra of organogels are quite similar to those already described for pure oil. A prominent peak is in the region between of $1700-1800 \text{ cm}^{-1}$ (C=O stretching peak). Moreover, the peaks corresponding to C–H bending can be identified in the region of 900–1400 cm⁻¹. In particular, the C=O stretching peak appears at 1743 cm⁻¹ in 329 oil and in organogels. On the other hand, in high energy region the trend can be330 different, according to the specific sample.

331 The spectrum of $OG_{0.02}$ (Fig. 6a) is very similar to that of pure oil even in the high energy region, evidencing the absence of peaks potentially related to hydrogen bonding; 332 this is quite expected because this sample does not exhibit, down to 25°C, 333 crystallization phenomenon. When sample $OG_{0.08}$ is considered (Fig. 6b), differences 334 emerge and, in particular, a small and broad peak at 3366 cm⁻¹ is present, suggesting the 335 336 hydrogen bonding formation. From a rheological point of view, this results in both 337 crystallization phenomenon and gelation of the sample. These data seem to confirm that GS is able to gel the oil thanks to the formation of hydrogen bonding, in agreement with 338 339 literature works on MAGs (Chen & Terentjev, 2009; Lupi et al, 2016a). It is worth noticing that MAGs organogels, usually, exhibit two peaks, corresponding to 2-OH and 340 3-OH hydrogen bonding (Chen & Terentjev, 2009). Nevertheless, according to the 341 literature (Alfutimie et al, 2014), the presence of a single peak in monoglycerides can 342 also occur when fresh samples are considered: the single peak, gradually, evolves in two 343 344 bands when a sufficient annealing time is allowed as shown by Lupi et al. (Lupi et al, 2016a) for a mixture of glyceryl stearate and glyceryl palmitate. 345

When organogels containing pure P are considered, a behavior similar to OG samples is observed: at low fraction (i.e. 0.02), the spectrum is almost equal to that of pure oil (Fig. 6c), whereas at larger fractions (i.e. $OP_{0.08}$, Fig. 6d), a very small peak is present in the region of hydrogen bonding. Nevertheless, it is worth noticing that, according to previous works on the same materials (Lupi et al, 2013), sample with P fraction of 0.02 ($OP_{0.02}$) exhibits a T_{co} equals to 47.65±0.01°C and a gelation point of 45.6±0.3°C: therefore, the apparent absence of interactions seems in contrast with the observed rheological behavior. These results confirm similar data obtained by the same authors (Lupi et al, 2016a) for gelation of virgin olive oil with policosanol: it was observed that this gelator is mainly able to create van der Waals interactions evidenced by the shift in the absorption bands of symmetric and anti-symmetric CH₂ stretching vibrational modes.

In the present work CH_2 bands appear at 2922 cm⁻¹ and 2853 cm⁻¹ for pure oil whereas they are recorded at 2921 cm⁻¹ and 2852 cm⁻¹ and at 2918 cm⁻¹ and 2849 cm⁻¹ for samples containing 0.02 and 0.08 of P, respectively. Although peak values are close to each other, literature works suggest that very slight changes in wavenumbers (Lupi et al, 2016a; Ren et al, 2015) could be attributed to van der Waals interactions.

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364 *3.2.5 – Organogels with mixtures of gelators*

FT-IR spectra of organogels prepared with mixtures of gelators exhibit a behavior closeto that already described in the previous section.

Sample $OM_{0.04}$ (Fig. 7a) shows, in the low-energy region, a small broad peak at 3469 cm⁻¹ in similarly as shown by pure oil for which a small peak appears at 3466 cm⁻¹. This could indicate the potential presence of weak intermolecular hydrogen bonding of solvent molecules. It is worth recalling that pure P shows a broad peak at 3292 cm⁻¹, whereas GS has a broad peak with a shoulder, corresponding to OH groups, at 3307 and 3241 cm⁻¹.

373 On the other hand, $OM_{0.16}$ (Fig. 7b) shows a twin peak at the same wavenumbers 374 already observed for pure GS in the low-energy region. Therefore, it can be speculated 375 that intermolecular H-bonds, firstly attributable to GS, can be detected in the sample 376 containing the highest amount of organogelators. It is worth noticing that potential H- bonding because of P, evidenced in sample $OP_{0.08}$, could be hidden by peaks attributed to GS.

When the region corresponding to symmetric and anti-symmetric CH_2 stretching is considered, a shift is observed also for samples $OM_{0.04}$ and $OM_{0.16}$. In fact, these peaks appear at 2921 and 2852 cm⁻¹ for sample $OM_{0.04}$, 2919 and 2851 cm⁻¹ for sample $OM_{0.16}$, and 2922 and 2853 cm⁻¹ in the case of EVO oil. The observed shift suggests, also in this case, the presence of van der Waals forces promoting the network formation. It is worth noticing that CH_2 peak values in OM organogels are close to those observed in OP gels.

Therefore, finally, it can be speculated that studied organogels, prepared with mixtures 386 387 of fatty alcohols and GS show both H-bonding and van der Waals interactions. At low concentrations, van der Waals forces, caused by P presence, are the main factor 388 responsible for gel formation whereas, at high concentration, both interactions seem to 389 act. Nevertheless, apparently, the presence of GS hinders the P van der Waals 390 interactions and yields weaker systems as observed in rheological tests which evidenced 391 that OM gels are close to OP ones, even though both dynamic moduli and critical 392 temperatures are lower. 393

394

395 4. CONCLUSIONS

Fatty alcohol (Policosanol, P) and Glyceryl Stearate (GS) organogels were produced either with pure organogelators (samples OG and OP) or with mixture of them (samples OM).

The rheological characterization of OG samples evidenced that glyceryl stearate is a lesseffective gelator with respect to monoglycerides mixtures (glyceryl palmitate and

401 stearate) already studied in the literature (such as the commercial product Myverol), as402 shown by the lower dynamic moduli and crystallization temperatures.

Experimental results showed an intermediate behavior of organogels OM with respect to OP and OG, with a non-linear trend which resulted in moduli values more influenced by the amount of P. It was observed that onset of crystallization and gelation temperature (estimated in approximate way as G'-G'' cross-over in a temperature ramp test) of OM samples depend only on policosanol content whereas dynamic moduli (at temperature far from crystallization) are affected also by GS presence and are larger than those measured for OP samples having the same policosanol fraction.

410 Moreover experimental *G* ' values of OM gels are greater than those computed assuming
411 an "ideal mixture" behavior based on the simple overlapping of single gels.

412 With the aim of better understanding the behavior of these gelators, alone and in mixture, FTIR spectra were obtained for pure components (oil, P and GS) and 413 organogels produced with different levels of pure and mixed gelators. According to the 414 results, GS seems mainly responsible for H-bonding interactions, whereas P is able to 415 416 build van der Waals interactions and, at high concentrations, H-bonding. No interactions are observed when GS is used in low concentration, confirming the 417 rheological results that evidenced the absence of gelation in the investigated 418 419 temperature range, for concentration lower than 0.040.

According to the FTIR spectra, when mixed gels are produced, van der Waals forces are
responsible for gel formation for a low amount of gelators, whereas both van der Waals
and hydrogen interactions are present when gelator concentration increases.

423 Combining information from rheological and FT-IR measurements, it can be speculated424 that policosanol crystals (forming at high temperature) build the three-dimensional

425 network of the organogel whereas GS crystals (forming at lower temperature)426 strengthen the network yielding a more consistent gel at low temperature.

A deeper analysis of microstructure in mixed gels at different OP/OG ratio can be useful in better understanding the potential interactions among gelators and the crystallization phenomena occurring in mixed systems. Nevertheless, the obtained results are a useful starting point in better understanding the gelation phenomena in these systems and in tuning the rheological properties of final materials according to the desired application.

432 **REFERENCES**

- Alfutimie, A., Curtis, R. & Tiddy, G. J. T. (2014) Gel phase (Lβ) formation by mixed
 saturated and unsaturated monoglycerides. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 456, 286-295.
- 436 Baldino, N., Gabriele, D., Lupi, F. R., Oliviero Rossi, C., Caputo, P. & Falvo, T. (2013)
- 437 Rheological effects on bitumen of polyphosphoric acid (PPA) addition. *Construction*
- 438 *and Building Materials*, 40, 397-404.
- 439 Bot, A., den Adel, R., Regkos, C., Sawalha, H., Venema, P. & Flöter, E. (2011) 440 Structuring in β-sitosterol+ γ -oryzanol-based emulsion gels during various stages of a 441 temperature cycle. *Food Hydrocolloids*, 25(4), 639-646.
- Chambon, F. & Winter, H. H. (1987) Linear Viscoelasticity at the Gel Point of a
 Crosslinking PDMS with Imbalanced Stoichiometry. *Journal of Rheology*, 31(8), 683697.
- Chen, C. H. & Terentjev, E. M. (2009) Aging and Metastability of Monoglycerides in
 Hydrophobic Solutions. *Langmuir*, 25(12), 6717-6724.
- Co, E. D. & Marangoni, A. G. (2012) Organogels: An Alternative Edible OilStructuring Method. *Journal of the American Oil Chemists Society*, 89(5), 749-780.
- Curcio, S., Gabriele, D., Giordano, V., Calabro, V., de Cindio, B. & Iorio, G. (2001) A
 rheological approach to the study of concentrated milk clotting. *Rheologica Acta*, 40(2),
 154-161.
- Da Pieve, S., Calligaris, S., Panozzo, A., Arrighetti, G. & Nicoli, M. C. (2011) Effect of
 monoglyceride organogel structure on cod liver oil stability. *Food Research International*, 44(9), 2978-2983.

References

- Franck, A. (2003) Measuring structure of low viscosity fluids in oscillations using
 rheometers with and without a separate torque transducer. *Annual Transactions of the Nordic Rheology Society*, 11, 95-100.
- Lopez-Martinez, A., Charo-Alonso, M. A., Marangoni, A. G. & Toro-Vazquez, J. F.
 (2015) Monoglyceride organogels developed in vegetable oil with and without
 ethylcellulose. *Food Research International*, 72, 37-46.
- Lopez-Martinez, A., Morales-Rueda, J. A., Dibildox-Alvarado, E., Charo-Alonso, M.
 A., Marangoni, A. G. & Toro-Vazquez, J. F. (2014) Comparing the crystallization and
 rheological behavior of organogels developed by pure and commercial monoglycerides
 in vegetable oil. *Food Research International*, 64, 946-957.
- Lupi, F. R., Gabriele, D. & de Cindio, B. (2012a) Effect of Shear Rate on
 Crystallisation Phenomena in Olive Oil-Based Organogels. *Food and Bioprocess Technology*, 5(7), 2880-2888.
- Lupi, F. R., Gabriele, D., de Cindio, B., Sánchez, M. C. & Gallegos, C. (2011) A
 rheological analysis of structured water-in-olive oil emulsions. *Journal of Food Engineering*, 107(3-4), 296-303.
- Lupi, F. R., Gabriele, D., Facciolo, D., Baldino, N., Seta, L. & de Cindio, B. (2012b)
 Effect of organogelator and fat source on rheological properties of olive oil-based
 organogels. *Food Research International*, 46(1), 177-184.
- Lupi, F. R., Gabriele, D., Greco, V., Baldino, N., Seta, L. & de Cindio, B. (2013) A
 rheological characterisation of an olive oil/fatty alcohols organogel. *Food Research International*, 51(2), 510-517.
- Lupi, F. R., Greco, V., Baldino, N., de Cindio, B., Fischer, P. & Gabriele, D. (2016a)
 The effects of intermolecular interactions on the physical properties of organogels in
- 479 edible oils. *Journal of Colloid and Interface Science*, 483, 154-164.

References

- Lupi, F. R., Shakeel, A., Greco, V., Oliviero Rossi, C., Baldino, N. & Gabriele, D.
 (2016b) A rheological and microstructural characterisation of bigels for cosmetic and
 pharmaceutical uses. *Materials Science and Engineering: C*, 69, 358-365.
- Marangoni, A. G. (2009) Novel strategies for nanostructuring liquid oils into functional
 fats, *5th International Symposium on Food Rheology and Structure ISFRS*. Zurich:
- 485 Lappersdorf: Kerschensteiner Verlag GmbH.
- 486 Mert, B. & Demirkesen, I. (2016) Evaluation of highly unsaturated oleogels as
 487 shortening replacer in a short dough product. *LWT Food Science and Technology*, 68,
 488 477-484.
- 489 Moschakis, T., Panagiotopoulou, E. & Katsanidis, E. (2016) Sunflower oil organogels
- 490 and organogel-in-water emulsions (part I): Microstructure and mechanical properties.
- 491 *LWT Food Science and Technology*, 73, 153-161.
- Ojijo, N. K. O., Neeman, I., Eger, S. & Shimoni, E. (2004) Effects of monoglyceride
 content, cooling rate and shear on the rheological properties of olive oil/monoglyceride
 gel networks. *Journal of the Science of Food and Agriculture*, 84(12), 1585-1593.
- Oğütcü, M. & Yılmaz, E. (2014) Oleogels of virgin olive oil with carnauba wax and
 monoglyceride as spreadable products. *Grasas y Aceites*, 65(3), e040, 1-11.
- 497 Patel, A. R. & Dewettinck, K. (2016) Edible oil structuring: an overview and recent
 498 updates. *Food & Function*, 7(1), 20-29.
- Rao, M. A. (1999) *Rheology of fluid and semisolid foods Principles and Applications*.
 Gaithersburg: Aspen Publishers, Inc.
- Ren, Y., Wang, B. & Zhang, X. (2015) Synthesis of photoresponsive cholesterol-based
 azobenzene organogels: dependence on different spacer lengths. *Beilstein Journal of Organic Chemistry*, 11, 1089-1095.

References

- Tang, D. & Marangoni, A. G. (2007) Modeling the rheological properties and structure
 of colloidal fat crystal networks. *Trends in Food Science & Technology*, 18(9), 474-483.
- 506 Tay, A., Singh, R. K., Krishnan, S. S. & Gore, J. P. (2002) Authentication of Olive Oil
- 507 Adulterated with Vegetable Oils Using Fourier Transform Infrared Spectroscopy. LWT -
- 508 *Food Science and Technology*, 35(1), 99-103.
- Toro-Vazquez, J. F., Mauricio-Pérez, R., González-Chávez, M. M., Sánchez-Becerril,
 M., Ornelas-Paz, J. d. J. & Pérez-Martínez, J. D. (2013) Physical properties of
- 511 organogels and water in oil emulsions structured by mixtures of candelilla wax and
- 512 monoglycerides. *Food Research International*, 54(2), 1360-1368.
- 513 Vlachos, N., Skopelitis, Y., Psaroudaki, M., Konstantinidou, V., Chatzilazarou, A. &
- 514 Tegou, E. (2006) Applications of Fourier transform-infrared spectroscopy to edible oils.
- 515 Analytica Chimica Acta, 573–574, 459-465.
- Xu, Z., Fitz, E., Riediger, N. & Moghadasian, M. H. (2007) Dietary octacosanol reduces
 plasma triacylglycerol levels but not atherogenesis in apolipoprotein E–knockout mice.
- 518 *Nutrition Research*, 27, 212-217.
- 519

TABLES CAPTIONS

522	Table 1	Organogels samples ID and compositions. The light grey background
523		indicates samples for which there are no significant differences between
524		T_{co} and T_g . Data are based on three repetitions. *No rheological results
525		are available for samples $OG_{0.02} ** T_{co}$ and T_g data are taken from (Lupi
526		et al, 2013)
527	Table 2	Parameters for Eq. (2) at $\Delta T=20^{\circ}C$. Different letters within the same
528		column refer to parameters statistically different (α =0.05)

529 FIGURES CAPTIONS

Figure 1 Complex modulus (a) and phase angle (b) for samples $OG_{0.034}(\bullet)$, $OG_{0.04}(\bullet)$, $OG_{0.068}(\bullet)$, $OG_{0.08}(\bullet)$ and $OG_{0.125}(\triangle)$. The dotted line in figure 1 (b) refers to the phase angle value of 45°. Data are based on three repetitions. Phase angle curves do not display error bars (deviation typically lower than 10%) in order to allow a better readability of the plot.

- Figure 2 Complex modulus for samples $OP_{0.034}$ (white circles) and $OM_{0.068}$ (black circles) having the same policosanol content (i.e. 0.034 w/w).
- 537 Figure 3 lnG' versus lnX (mass fraction of organogelators) at T_{20} for samples
- 538 'OG' (white circles), 'OM' (grey circles) and 'OP' (black circles). Fitting lines refers to
- fractal model detailed in Eq. (2) for the respective kind of organogel.
- Figure 4 Comparison between experimental values (white bars) and "ideal" estimated values (Eq. 3, grey bars) of storage modulus for OM samples. Numerical values represent the percentage difference between them for each concentration.
- 543 Figure 5 FTIR spectra for EVO oil (a), P (b), GS (c),
- 544 Figure 6 FTIR spectra for $OG_{0.02}$ (a), $OG_{0.08}$ (b), $OP_{0.02}$ (c), $OP_{0.08}$ (d)
- 545 Figure 7 FTIR spectra for $OM_{0.04}$ (a) and $OM_{0.16}$ (b)

Sample	Olive Oil	GS	Р		
Name	Xo (w/w)	$X_{GS}(w/w)$	$X_P(\%w/w)$	$T_{co}(^{\circ}\mathbf{C})$	$I_g(^{\circ}\mathbf{C})$
OG _{0.02} *	0.980	0.020	-	-	-
OG _{0.034}	0.966	0.034	-	27.03±0.01	-
OG _{0.04}	0.960	0.040	-	30.04±0.01	15±1
OG _{0.068}	0.932	0.068	-	35.8±0.3	25.8±0.3
OG _{0.08}	0.920	0.080	-	37.53±0.01	31±1
OG _{0.125}	0.875	0.125	-	41.8±0.4	32±1
OP _{0.02} **	0.980	-	0.020	47.65±0.01	45.6±0.3
OP _{0.034}	0.966	-	0.034	53.3±0.4	51.9±0.2
OP _{0.04}	0.960	-	0.040	55.3±0.4	53.1±0.2
OP _{0.068}	0.932	-	0.068	60.0±0.7	58±1
OP _{0.08}	0.920	-	0.080	61.7±0.3	59.5±0.4
OP _{0.125}	0.875	-	0.125	64.3±0.4	62.13±0.07
OM _{0.034}	0.966	0.017	0.017	46±1	42.62±0.08
OM _{0.04}	0.960	0.020	0.020	47.54±0.01	44.11±0.02
OM _{0.068}	0.932	0.034	0.034	54.1±0.1	50.3±0.1
OM _{0.16}	0.840	0.080	0.080	61.5±0.7	58.4±0.4
OM _{0.25}	0.750	0.125	0.125	65±1	61±2

Samples	$\ln(\lambda), \lambda[=] kPa$	D (-)
OG	$22.2{\pm}~0.7^{\rm a}$	2.831 ± 0.008^a
OP	25.7 ± 0.3^{b}	$2.785 \pm 0.006^{\mathrm{b}}$
OM	$23.47 \pm 0.02^{\circ}$	2.788 ± 0.003^{b}















Figure 6 Click here to download high resolution image



