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Effect of different oleogelators on lipolysis and curcuminoid bioaccessibility upon in vitro digestion of sunflower oil oleogels

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Title: Effect of different oleogelators on lipolysis and curcuminoid bioaccessibility upon in vitro digestion of sunflower oil oleogels

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Abstract: Sunflower oil enriched with curcuminoid compounds (CUs) was gelled by adding 5% (w/w) saturated monoglycerides (MG), rice bran waxes (RW) or a mixture of  $\beta$ -sitosterol and  $\gamma$ -oryzanol (PS). The resulting oleogels differed for rheological properties and firmness due to the difference in gel network structure. PS oleogel was the firmest sample followed by RW and MG ones. Upon in vitro digestion, fatty acid release as a function of digestion time was greatly affected by oleogel structure: the extent of lipolysis decreased as oleogel strength increased (PS<RW<MG). On the other hand, the nature of the oleogelator affected CUs bioaccessibility, which was lower in oleogels containing crystalline particles (MG and RW). These findings appear interesting in the attempt to develop oleogels able to control lipid digestion as well as to deliver bioactive molecules in food systems.

Dear Editor,

I would like to submit the revised manuscript entitled “*Effect of different oleogelators on lipolysis and curcuminoid bioaccessibility upon in vitro digestion of sunflower oil oleogels*” by Sonia Calligaris, Marilisa Alongi, Paolo Lucci and Monica Anese for consideration for publication in *Food Chemistry*.

We thank the Reviewers for supplying their useful observations that, in our opinion, allowed us to further improve the quality and the clarity of the manuscript. We hope that in this form it will be suitable for publication in *Food Chemistry*.

Best regards

Marilisa Alongi

**Ms. Ref. No.: FOODCHEM-D-19-03433R1**

Title: Effect of oleogelator type on lipolysis kinetics and curcuminoids bioaccessibility upon in vitro digestion of sunflower oil-based oleogels

**Answers to Reviewers' comments**

(Please find grouped text pertaining to Reviewer 1, 2, 3 or 4, and to the Editor; Reviewer text is in normal, and Answer text is italics, for each numbered item)

**Reviewer #1:**

This version of the manuscript has been improved significantly and it is thus more acceptable for publication in Food Chemistry. Most of my comments have been addressed, except for the fact that there is no rhyme or reason for the concentrations of the systems chosen. We have no idea if other concentrations have the same effect. This is not going to change with any number of revisions here. The study is also only an in vitro study. These are early days in this research, so this may be OK. The manuscript is well written, clear, and the science is sound. The scope of the work, more limited. It is OK to consider publication as is.

*The authors thank the reviewer for his/her appreciation of the general idea of the work. Indeed, further research considering also ex vivo and in vivo models is required to shed some light on this topic which is still in its infancy.*

**Reviewer #2:**

The authors did a good work on improving the manuscript, which results from excluding the samples of EC. Despite this good work, there are still some major points that should be clarified before considering the manuscript for publication.

Line 228-230. The amount of ingredient or additive depends on the material used and the food where is used. Therefore, should not be assumed that this concentration was selected because can be used in all food applications. To do so the authors should indicate the food product and explain in detail the amount allowed by EU regulations or FDA of each gelator, as example. Is rice bran wax approved to use in foods in EU and USA as texturizer?

*We agree with the reviewer that our consideration was too general. Moreover, being early days in this research, the reference to the final food application is premature. Only after the comprehension of the effect of structure on the digestive fate of oleogels, it would be possible to speculate applications in both the food and pharmaceutical fields. We modified the text accordingly (lines 220-222).*

How did the authors check the size above the 6  $\mu\text{m}$  "However, to the aim of the present work, the determination of particles up to 5.5  $\mu\text{m}$  was exhaustive. In fact, the determination was applied to digested oleogel and oil samples in which, as reported also in the literature, the biggest family expected present an average diameter below this value". The way that this was verified should be included in the manuscript. This should be taken in account for size and Zeta potential.

*In this work, we determined the particle size and zeta potential only on the micellar phase obtained after the intestinal digestion phase. This micellar phase was obtained after intense centrifugation (30,000 g x 70 min) of the sample as reported in line 156 and as suggested by the literature (Ahmed et al., 2012). These centrifugation conditions were selected after preliminary trials based on the conditions applied in the literature, being capable to remove big particles from the sample while maintaining in dispersion micelles. Only in this way, the sample was suitable for BAC assessment.*

*If we understand well the issue raised by the reviewer, he/she is suggesting studying the destructurezation of the sample during digestion, considering not only the mixed micellar phase, that was the topic of the present work but also the digested sample before centrifugation as well as the sediment recovered upon digestion and centrifugation. This is a really interesting topic that should be properly addressed with appropriate experiments in further studies.*

*To improve the clarity of our manuscript, we better explicated in the M&M and R&D sections which samples were considered in this study (line 160).*

The justification for the difference between the BAC and lipolysis is still not clear. The degradation can one of the justifications but also the extraction procedure and the gel structure. Both MG and Wax are organized in strong crystalline lamellar structures and BS in tubular structures. Can this have any influence on the CU BAC?

*The reduced BAC in MG and RW containing systems is for sure tricky and not easy to explain. Besides oxidation, it cannot be excluded a role of the matrix in entrapping CUs, as suggested by the reviewer. Being no literature on this aspect, we can only speculate the reasons for such results and more research is needed on this topic. More comments were added in the text (lines 353-362).*

Another of the doubts is how the authors considered the max lipolysis, once by the Figure is not clear that the maximum was reached at the same time for all the samples.

*We understand the issue of the reviewer. In the experiments, we considered the  $FFA_{max}$  as the maximum value reached the plateau. However, we agree with the fact that it should be better to compare samples at a defined length of time. For this reason, we repeated some experiments to confirm results and compare data at 30 min of lipolysis, improving in this way also the quality of Figure 2.*

### **Reviewer #3:**

The authors have made considerable revision to address the comments raised by all reviewers. The paper in its current state, nevertheless, requires some corrections, because after eliminating some of the data and revising the text some key issues have remained:

The authors have deleted the kinetics analysis from the text, but still refer to the effect of oil structuring on lipolysis kinetics (title, highlights, discussion (line 322), and conclusions). More proper reference would be to the total free fatty acids release.

*Suggested changes were made.*

Lines 235-237: the results are not similar (your statistical analysis showed differences).

*The text was modified accordingly (lines 230-231).*

In general, the authors did not suggest an explanation why one system showed higher G' compare to the other. Please do ... this is necessary.

Lines 250-251: why would the crystal morphology lead to different gel firmness? Justify.

Why was the PS sample firmer than the other two? Explain/justify.

*The last three reviewer considerations were carefully considered and this part of the manuscript was deeply revised (lines 234-241 and 254-258). We hope that in this version the effect of network structure on system properties is clearer than in the previous one.*

What is the source of the different surface electrical charge showed in the zeta potential analysis? Can you suggest a mechanism?

*Based on literature data we can only speculate an effect of surface-active species present in RW and PS. We modified the text accordingly (lines 308-312).*

Lines 323-325: Ashkar et al. (2019) did not show that the final strength of the oleogel is the most critical factor affecting lipolysis, they concluded that different structuring agents and gelation mechanisms demonstrate different susceptibility to digestive lipolysis. Fix.

*The text was modified as indicated (lines 325-326).*

Lines 348-350: how does the involvement in gelation/crystallization relate to exposure to oxidation? Explain in detail.

The authors should consider interactions between the MG or RW with the CU affecting the results seen in the bioaccessibility.

*Unfortunately, to our knowledge in the literature, there is no clear evidence on the effect of lipid physical state on bioactive molecule bioaccessibility. We added some comments in the text on this aspect (lines 348-350 and 353-362).*

The sample size (not thickness) is missing in the firmness method section. Add.

*Details were added to the text (line 112).*

The manuscript should be edited by an English editor.

*The manuscript was submitted to English revision.*

#### **Reviewer #4:**

Please revise the manuscript in accordance with the comments below.

1- It must be pointed out in the text, how much time was necessary to structure the <gamma>-oryzanol and <beta>-sitosterol oleogel.

*Details were added to the text (lines 104-105).*

2- The authors must provide the specifications of the compression probe used in the firmness measurements in the materials and methods section.

*Details were added to the text (line 113).*

#### Editor's Comments:

The original four referees have now commented on your revised manuscript. All indicate that great strides have been made in revising the R0. One accepted the paper as is. Unfortunately, three others still have major issues for you to address. Please consider the comments of all reviewers very carefully and either adopt or rebut each and every point made. In your rebuttal please indicate the line number in the revised manuscript corresponding to each change that has been made and possibly use yellow highlighting or color in the text to indicate the edits.

More and more these days, manuscripts are being rejected at the R1 and R2 stages for poor English. Please don't let this happen to you! Due to dwindling resources, typesetters can no longer edit the English, so there's a new category, "Reject Due to Poor Language (regardless of the scientific quality)", in the Decision Box of this EES system. At least one of the reviewers notes English problems in your submission. I strongly suggest that the paper be scrutinized very carefully by a native speaker of the language with a background in food chemistry, or pay for a reputable service to do this, before the R2 is submitted via the EES portal.

## **Highlights**

Sunflower oil was enriched with curcuminoids

Curcuminoid-enriched oil was gelled with different gelators

Obtained oleogels differed for rheological properties and firmness

Lypolysis kinetic during *in vitro* digestion was affected by gel structure

The type of gelator, instead of gel strength, affected curcuminoid bioaccessibility

1 **Effect of different oleogelators on lipolysis and curcuminoid bioaccessibility upon *in vitro* digestion of**  
2 **sunflower oil oleogels**

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6 **Abstract**

7 Sunflower oil enriched with curcuminoid compounds (CUs) was gelled by adding 5% (w/w)  
8 saturated monoglycerides (MG), rice bran waxes (RW) or a mixture of  $\beta$ -sitosterol and  $\gamma$ -oryzanol  
9 (PS). The resulting oleogels differed for rheological properties and firmness due to the difference in  
10 gel network structure. PS oleogel was the firmest sample followed by RW and MG ones. Upon *in*  
11 *vitro* digestion, fatty acid release as a function of digestion time was greatly affected by oleogel  
12 structure: the extent of lipolysis decreased as oleogel strength increased (PS<RW<MG). On the  
13 other hand, the nature of the oleogelator affected CUs bioaccessibility, which was lower in oleogels  
14 containing crystalline particles (MG and RW). These findings appear interesting in the attempt to  
15 develop oleogels able to control lipid digestion as well as to deliver bioactive molecules in food  
16 systems.

17 **Keywords**

18 Oleogel; Curcuminoids; Lipolysis; Bioaccessibility; Structure

## 19 **1. Introduction**

20 Oil gelation is a relatively novel strategy in which liquid oils are converted into semi-solid materials  
21 (Co & Marangoni, 2012; Patel & Dewettinck, 2016). Different oil gelators have been proposed in  
22 the literature, including low-molecular weight compounds (e.g. monoglycerides, waxes, sorbitan  
23 tristearate, 12-hydroxystearic acid, phytosterols, fatty alcohols, and fatty acids) and high-molecular  
24 weight polymers, such as ethylcellulose and chitin (Co & Marangoni, 2012; Nikiforidis & Scholten,  
25 2015; Patel & Dewettinck, 2016; Rogers, Wright, & Marangoni, 2009; Singh, Auzanneau, &  
26 Rogers, 2017). These compounds directly gel into liquid oil by forming a self-assembly network  
27 according to different mechanisms. Most low-molecular weight gelators (e.g. monoglycerides,  
28 waxes, fatty acids, and fatty alcohols) are able to organize themselves into crystalline networks that  
29 entrap and retain oil (Co & Marangoni, 2012). The final oleogel structure strictly depends on crystal  
30 shape, number, and size, as well as on physical molecular interactions among building blocks.  
31 Differently, mixtures of phytosterols-sterol esters promote oil gelation through the formation of a  
32 supramolecular network of hollow double-walled tubules (Bot & Agterof, 2006; Calligaris, Mirolo,  
33 Da Pieve, Arrighetti, & Nicoli, 2014; Sawalha *et al.*, 2015). Finally, ethylcellulose is able to form a  
34 network in oil *via* hydrogen bonding between polymer strands (Davidovich-Pinhas, 2016), whereas  
35 chitin develops a particle filled network by polymer aggregation (Nikiforidis & Scholten, 2015).  
36 The interest in oleogels has increased dramatically in the last decade due to their potential  
37 application as replacers of common hard stock fat (i.e. saturated and trans fatty acids) in different  
38 food products (Patel & Dewettinck, 2016; Singh *et al.*, 2017; Wang, Gravelle, Blake, & Marangoni,  
39 2016). More recently, oleogels have also been proposed as efficient tools to modulate lipid  
40 digestion and deliver nutrients and bioactive molecules (O'Sullivan, Davidovich-Pinhas, Wright,  
41 Barbut, & Marangoni, 2017; Ashkar, Laufer, Rosen-Kligvasser, Lesmes, & Davidovich-Pinhas,  
42 2019; Tan, Peh, Marangoni, & Henry, 2017a; Tan, Peh, Siow, Marangoni, & Henry, 2017b; Tan,  
43 Peh, Lau, Marangoni, & Henry, 2017c). On this topic, O'Sullivan *et al.* (2017), studying the

44 digestion of an ethylcellulose-based oleogel enriched with  $\beta$ -carotene, noted that oil structuring  
45 reduced lipolysis, probably by hindering lipase activity. Consequently, the residence time of  $\beta$ -  
46 carotene in the digestive tract increased leading to an evenly arrayed absorption over time with no  
47 spikes and drops in plasma concentration (O'Sullivan *et al.*, 2017). According to Yu, Shi, Liu, and  
48 Huang (2012), monostearin might increase the stability of curcuminoids loaded into oleogels, by  
49 preventing their recrystallization or precipitation (Yu *et al.*, 2012). However, these authors did not  
50 observe differences between the oleogel and the liquid oil in terms of curcuminoid bioaccessibility.  
51 The latter is expressed as the fraction of molecules enclosed in the micelles that can be potentially  
52 absorbed through the intestinal epithelium, thus becoming available for physiological functions  
53 (Ferruzzi, 2010).

54 Besides these studies, recently Ashkar *et al.* (2019) confirmed that oleogelation of canola oil with  
55 ethylcellulose, a  $\beta$ -sitosterol+ $\gamma$ -oryzanol mixture, or mono- and di-glycerides reduced the extent of  
56 oil lipolysis during *in vitro* digestion. As reported by these authors, such effect can be modulated by  
57 selecting the gelator type and concentration. Interestingly, also some *in vivo* studies highlighted the  
58 significant impact of lipid physical state on post-prandial plasma triglycerides, glycemia, and  
59 appetite when comparing the co-ingestion of a carbohydrate-rich meal with EC-oleogel instead of  
60 liquid oil (Tan *et al.*, 2017a, 2017b, 2017c).

61 Based on this evidence and with the final aim of designing food with tailor-made functionalities, it  
62 appears fundamental to improve the knowledge on the fate of oleogels and loaded bioactive  
63 molecules upon digestion. Therefore, the aim of the present study was to investigate the effect of  
64 gelator type and derived oleogel structure on oil lipolysis and on the bioaccessibility of  
65 curcuminoids (CUs) during *in vitro* digestion. Curcuminoids were chosen as model lipophilic  
66 bioactive molecules due to their well-known health-promoting capacity (Aggarwal, Kumar, &  
67 Bharti, 2003; Aziz *et al.*, 2013; Su, Wang, & Chi, 2017; Zheng *et al.*, 2014). CUs are extracted from  
68 turmeric and include three major compounds: bisdemethoxycurcumin, demethoxycurcumin and  
69 curcumin (Shishu & Maheshwari, 2010). Although the latter is the most abundant, representing

70 nearly 80% of total CUs, the health-promoting capacity of the mixture of the three CUs in turmeric  
71 extract is higher than that generated by the sole curcumin (Chakravarty, Chatterjee, Yasmin, &  
72 Mazumder, 2009; Račková *et al.*, 2009). In this study, sunflower-oil and oleogels containing 5%  
73 (w/w) of gelator (saturated monoglycerides, rice-bran waxes, and  $\beta$ -sitosterol+ $\gamma$ -oryzanol mixture)  
74 were enriched with CUs and characterized for mechanical and rheological properties as well as for  
75 the CUs stability during storage. Afterward, CUs-enriched oil and oleogels were *in vitro* digested to  
76 assess **lipolysis** and CUs bioaccessibility.

## 77 **2. Materials and methods**

### 78 *2.1. Materials*

79 High oleic sunflower oil (fatty acid profile reported in Table S1) was kindly provided by Olitalia srl  
80 (Forlì, Italy) and turmeric extract (NNCL2065, ext. dry conc. std 20:1) was purchased from  
81 Network Nutrition – IMCD spa (Milan, Italy). Bisdemethoxycurcumin (BDMC),  
82 demethoxycurcumin (DMC) and curcumin (C) analytical standards,  $\alpha$ -amylase from *Bacillus* sp.,  
83 porcine pepsin, porcine lipase, porcine bile extract, amyloglucosidase from *Aspergillus niger*, HCl,  
84 NaOH,  $\text{CaCl}_2(\text{H}_2\text{O})_2$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{NaHCO}_3$ , NaCl, KCl,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgCl}_2(\text{H}_2\text{O})_6$ ,  $(\text{NH}_4)_2\text{CO}_3$ , and  
85  $\text{MgSO}_4$  were purchased from Sigma Aldrich (Milan, Italy). Myverol™ saturated monoglycerides  
86 (fatty acid composition: 1.4% C14:0, 59.8% C16:0, 38.8% C18:0; melting point  $68.05 \pm 0.5$  °C)  
87 were purchased from Kerry Bioscience (Bristol, UK);  $\beta$ -sitosterol (75.5%  $\beta$ -sitosterol, 12.0%  $\beta$ -  
88 sitostanol, 8.4% campesterol, 3.0% other) and  $\gamma$ -oryzanol (99% purity) were purchased from  
89 Nutraceutica srl (Monterenzio, Italy); rice wax was purchased from Kahl GmbH & Co. KG  
90 (Reinbek, Germany). All solvents were purchased from Sigma–Aldrich (Milan, Italy). Acetonitrile  
91 and 2-isopropanol were of HPLC grade. Deionized water (System advantage A10®, Millipore  
92 S.A.S, Molsheim, France) was used for all the analyses.

## 93 2.2. Methods

### 94 2.1.1. Oil enrichment

95 The turmeric extract was added to sunflower oil (5 mg/g, w/w) and the mixture was stirred for 2 h at  
96 80 °C in the dark under nitrogen atmosphere, to avoid CUs and oil oxidation. The mixture was  
97 cooled to room temperature and filtered (Chromafil PET-20/25, 0.20 µm, 25 mm Düren, Germany)  
98 to remove insoluble particles.

### 99 2.1.2. Oleogel preparation

100 Oleogels were prepared by mixing CUs-enriched sunflower oil with 5% (w/w) of saturated  
101 monoglycerides (MG) (Da Pieve, Calligaris, Co, Nicoli, & Marangoni, 2010), rice waxes (RW)  
102 (Doan, Van De Walle, Dewettinck, & Patel, 2015), or a mixture of  $\beta$ -sitosterol and  $\gamma$ -oryzanol (PS)  
103 (2:3 w/w) (Calligaris *et al.*, 2014). The mixtures were heated under stirring in dark conditions for 30  
104 min at 80 °C for MG and RW, and for 45 min at 90 °C for PS, until melting was reached. MG and  
105 BW samples were quiescently cooled to 20 °C and stored at this temperature. PS oleoge was cooled  
106 to 4 °C and kept at this temperature for 12 hours before being stored at 20 °C. All samples were  
107 then analysed after 2 days of storage at 20 °C.

### 108 2.1.3. Oleogel storage

109 Aliquots of 5 g of CUs-enriched oil and oleogels were placed into 10 mL vials and stored at 20 °C  
110 under dark for increasing time. Samples were collected after 60 and 100 days and analyzed for CUs  
111 content.

### 112 2.1.4. Firmness

113 Oleogel firmness was determined using a texture analyzer (TA.XT Plus, Stable Micro Systems Ltd,  
114 Godalming, UK) equipped with a 5 kg load cell. Forty grams of 25-mm-thick sample were  
115 compressed with a 35-mm-diameter compression platen at a crosshead speed of 1.5 mm/s

116 (Giacintucci *et al.*, 2018) and firmness was expressed as the maximum force (N) applied to the  
117 samples.

#### 118 2.1.5. *Rheological measurement*

119 Rheological properties of oleogels were determined with a Haake Rheostress 6000 (Thermo  
120 Scientific, Rheostress, Haake, Germany). Aliquots of about 5 g of sample were transferred on a 40-  
121 mm parallel-plate geometry system thermostated at 20 °C and the measuring gap was set at 2 mm.  
122 Samples were equilibrated for 5 min before testing to allow relaxation. Stress sweep measurement  
123 in the range of 0.1 to 1000 Pa was carried out at 1 Hz frequency to determine the linear viscoelastic  
124 region. Frequency sweep was carried out by applying a fixed stress value chosen in the linear  
125 viscoelastic region with a frequency scan of 0.1 to 10 Hz. Data were acquired and managed by  
126 applying the software Haake Rheowin v.4.60.0001 (Thermo Fisher Scientific). The critical stress  
127 was computed as the stress leading to a 10%  $G'$  decrease during the stress sweep.  $G'$  and  $G''$  were  
128 compared at 1 Hz. The tangent of the phase angle ( $\text{Tan}\delta$ ) was computed as the ratio between the  
129 two moduli ( $G''/G'$ ) during the frequency sweep.

#### 130 2.1.6. *Macroscopic appearance*

131 Gel images were acquired by using an image acquisition cabinet (Immagini and Computer,  
132 Bareggio, Italy) equipped with a digital camera (EOS 550D, Canon, Milan, Italy). The digital  
133 camera was placed on an adjustable stand positioned 40 cm in front of a black cardboard base  
134 where the sample was placed. Light was provided by four 23 W frosted photographic floodlights, in  
135 a position allowing minimum shadow and glare. Other camera settings were: shutter time 1/250 s,  
136 F-Number F/2,8 and focal length 60 mm. Images were saved in jpeg format resulting in pictures of  
137  $5184 \times 3456$  pixels,  $72 \times 72$  dpi.

138 2.1.7. *Polarized light microscopy*

139 Polarized light microscopy was carried out by using a Leica DM 2000 optical microscope under  
140 polarized light conditions (Leica Microsystems, Heerbrugg, Switzerland). A small portion of gel  
141 was placed on a glass slide, covered with a cover slide and observed at 20 °C. Images were taken at  
142 200× magnification using a Leica EC3 digital camera and elaborated by the Leica Suite Las EZ  
143 software (Leica Microsystems, Heerbrugg, Switzerland).

144 2.1.8. *In vitro digestion*

145 *In vitro* digestion was carried out according to the protocol proposed by Minekus *et al.* (2014).  
146 Briefly, the simulated salivary (SSF), gastric (SGF) and intestinal (SIF) fluids were prepared and  
147 stored at 4 °C. The fluids were preheated to 37 °C just before *in vitro* digestion. The oral phase was  
148 started by adding to 0.25 g sample (oil or oleogel), 6 μL of CaCl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> (0.3 M), 194 μL of water  
149 and 800 μL of a 6.4 mg/mL α-amylase solution, prepared in SSF and providing 75 U/mL activity in  
150 the final mixture. The sample was maintained at 37 °C under stirring for 2 min. At the end of the  
151 oral phase, the pH was adjusted to 3.0 with 40 μL HCl (1 M). Subsequently, 140 μL water and 1.82  
152 mL of a 0.31 mg/mL pepsin solution, prepared in SGF and providing 2,000 U/mL activity in the  
153 final mixture, were added to start the gastric phase. The mix was stirred at 37 °C for up to 2 h. At  
154 the end of the gastric phase, the pH was adjusted to 7.0 with 30 μL NaOH (1 M). The intestinal  
155 phase was initiated by adding 8 μL CaCl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> (0.3 M), 262 μL of water, 3.2 mL of 22.15 mg/mL  
156 lipase solution, prepared in SIF and providing 100 U/mL activity in the final mixture, and 0.5 mL of  
157 160 mM bile extract prepared in SIF. The mix was stirred at 37 °C for up to 2 h. At the end of the  
158 intestinal phase, samples were centrifuged at 30,000 g for 70 min at 4 °C (Beckman Avanti tm J-25,  
159 Beckman Instruments Inc., Palo Alto, CA, USA) and the supernatant, i.e. the mixed micellar phase,  
160 was collected.

161 2.1.9. Particle size and zeta potential of digested samples

162 The particle size distribution of the mixed micellar phase of digested oil and oleogels was measured  
163 by dynamic laser light scattering (Zetasizer NanoZS, Malvern Instruments, Worcestershire, UK).  
164 Samples were diluted 1:100 (v/v) with deionized water and placed in a cell where the laser light, set  
165 at 173 ° angle, was scattered by the particles. Particle size was reported as volume-weighted mean  
166 diameter in nm. The  $\zeta$ -potential was also measured by placing the diluted sample in a capillary cell  
167 equipped with two electrodes to assess particle electrophoretic mobility.

168 2.1.10. Free fatty acid release

169 The amount of free fatty acids (FFA) released from the sample during the intestinal phase of *in vitro*  
170 digestion was measured by using a titration method (Ahmed, Li, McClements, & Xiao, 2012).  
171 Immediately after the addition of lipase, the pH of the digestion mixture was monitored and  
172 maintained at 7.00 by adding 0.25 M NaOH. The volume of NaOH added to the sample was  
173 recorded and used to calculate the percentage of FFA released during lipolysis (Equations 1 and 2):

174 
$$V_t = 2 \times \left[ \frac{m_{oil}}{MW_{oil}} \frac{1000}{C_{NaOH}} \right] \quad \text{Equation 1}$$

175 
$$\text{FFA (\%)} = \frac{V_e}{V_t} \times 100 \quad \text{Equation 2}$$

176 where  $V_e$  was the experimental volume of NaOH used for the titration,  $V_t$  was the theoretical  
177 volume required to titrate the fatty acids released by complete hydrolysis of triglycerides in the  
178 reaction vessel, assuming 2 FFA are produced for each triacylglycerol molecule (L) (Li, Hu, Du,  
179 Xiao, & McClements, 2011),  $m_{oil}$  was the mass of oil in the reaction vessel (g),  $MW_{oil}$  was the  
180 average molecular weight of sunflower oil ( $\text{g mol}^{-1}$ ) and  $C_{NaOH}$  was the concentration of the sodium  
181 hydroxide ( $\text{mol L}^{-1}$ ). The maximum value of free fatty acids released during digestion was  
182 determined after the plateau was reached, i.e. after 30 min from the beginning of intestinal phase.  
183 This value was considered as an indication of the maximum lipolysis and was reported as  $\text{FFA}_{\text{max}}$ .

184 2.1.11. Curcuminoid quantification and bioaccessibility computation

185 CUs-enriched oil and oleogels were diluted in isopropanol (100  $\mu\text{g}/\text{mL}$ ) and transferred to glass  
186 vials for UHPLC analysis, while the mixed micellar phase recovered after *in vitro* digestion, was  
187 submitted to extraction. Briefly, 2 mL of water was added to the digested samples followed by 2  
188 mL of isopropanol. The sample was vigorously hand- shaken for 1 min and vortexed for 15 s. A  
189 mixture of salts ( $\text{MgSO}_4/\text{NaCl}$  2.0:1.5, w/w) was then added and shaking was repeated under the  
190 same conditions. The resulting mixture was centrifuged at 5000 g for 15 min and the supernatant  
191 was recovered for UHPLC analysis.

192 To validate the extraction procedure for CUs analysis in digested samples, accuracy was evaluated  
193 by means of recovery experiments, analyzing digested samples fortified with three different  
194 amounts of CUs (5, 10 and 20  $\mu\text{g}$  for each CUs), whereas precision, expressed as the repeatability  
195 of the method, was determined in terms of relative standard deviation (RSD) from recovery  
196 experiments at each fortification level. In all cases, the average recovery ranged from 92 to 100%  
197 with repeatability (CV%) lower than 2%, revealing the suitability of the procedure for the  
198 quantitative extraction of CUs from digested samples.

199 Different chromatographic conditions were employed to obtain a baseline separation of BDMC,  
200 DMC, and C. Fig. S1 shows the chromatogram of CUs in enriched oil samples obtained by applying  
201 the optimized chromatographic conditions. The UHPLC system used was a Shimadzu Prominence  
202 LC-20A coupled with a RF-20A fluorescence detector (Shimadzu, Milan, Italy). The column was  
203 an Agilent Poroshell C18 column (150 mm x 4.6 mm x 2.7  $\mu\text{m}$ ) thermostated at 30 °C. The mobile  
204 phase was a mixture of 0.2% aqueous phosphoric acid water and acetonitrile (85:15, v/v) at 0.45  
205 mL/min flow rate. The excitation wavelength ( $\lambda_{\text{ex}}$ ) and emission wavelength ( $\lambda_{\text{em}}$ ) were set a 430  
206 and 524 nm, respectively. Standard stock solutions of BDMC (0.25 mg/mL), DMC (0.25 mg/mL)  
207 and C (0.5 mg/mL) were prepared in acetonitrile and intermediate working CUs solutions were  
208 prepared weekly from the stock standard solution by appropriate dilution with acetonitrile and

209 stored in the dark at 4 °C. Calibration curves were obtained for each curcuminoid (0.1 - 500 ng on  
210 column) and presented  $R^2 > 0.998$  in all cases.

211 CUs bioaccessibility was calculated as the percentage ratio between the concentration of compound  
212 incorporated in the micelles after *in vitro* digestion and its concentration in the undigested sample.

#### 213 2.1.12. Statistical analysis

214 Results are averages of three measurements carried out on two replicated experiments and are  
215 reported as means  $\pm$  standard deviation. Analysis of variance (ANOVA) was performed using R  
216 (version 3.2.3, The R Foundation for Statistical Computing, Vienna, Austria). Bartlett's test was  
217 used to check the homogeneity of variance and the Tukey test was used to test for differences  
218 between means ( $p < 0.05$ ).

### 219 3. Results and discussion

#### 220 3.1. Oleogel physical and chemical properties

221 Oleogels containing sunflower oil enriched with CUs were prepared by using 5% (w/w) of saturated  
222 monoglycerides (MG), rice waxes (RW) and a mixture of  $\beta$ -sitosterol and  $\gamma$ -oryzanol (PS). This  
223 concentration was selected being effective in gelling vegetable oils as previously reported by  
224 different authors (Da Pieve *et al.*, 2010; Calligaris *et al.*, 2014; Doan *et al.*, 2015).

225 Table 1 shows firmness, rheological parameters (critical stress,  $G'$ ,  $G''$  and  $\text{Tan}\delta$ ), and micro- and  
226 macroscopic images of oleogels prepared by using MG, RW, and PS as oil gelators. The original  
227 stress sweep and frequency sweep curves are reported in Fig. S2. **From the macroscopic images it  
228 can be noted that all samples were self-standing materials, even if with different structures. In  
229 particular, MG and RW oleogels were opaque systems with cream-like structure, whereas the PS  
230 oleogel appeared as a transparent solid.** From a rheological point of view, the gel behavior was  
231 confirmed for all samples, being the storage modulus ( $G'$ ) higher than the loss modulus ( $G''$ ) and  
232  $\text{Tan}\delta < 1$  (Zetzl *et al.*, 2014). **Considering  $G'$  and  $G''$  at 1 Hz, the MG-based sample showed the  
233 lowest values, followed by PS and RW.** On the other hand, PS demonstrated the highest critical

234 stress value, which represents the beginning of the non-linear region and accounts for the structure  
235 breakdown required to onset flowing (Doan *et al.*, 2015).

236 As well known,  $G'$  and  $G''$  are indexes of the elastic and viscous behavior of the matrix,  
237 respectively; whereas the critical stress is an indicator of the stress needed to break the gel network.  
238 Thus, from the rheological parameters it can be concluded that, in spite of the reduced elastic  
239 behavior, PS presented the highest stress resistance. This is also consistent with firmness values that  
240 followed the order PS>RW>MG (Table 1).

241 These results are in agreement with those reported by Fayaz, Calligaris & Nicoli (2019). These  
242 authors compared the ability of different molecules to gel sunflower oil, and attributed the different  
243 gel properties to the peculiarities of the network structure formed by each oil gelator. As well  
244 reported by Sawalha *et al.* (2013, 2015),  $\beta$ -sitosterol+ $\gamma$ -oryzanol self-assemble into a network by  
245 alignment of cross-linked tubules into helical ribbons strongly stabilized by hydrogen bonds. The  
246 dimension of these tubules is around 10 nm, which is smaller than the wavelength of visible light  
247 accounting for system transparency (Bot & Agterof, 2006; Bot, Den Adel, & Roijers, 2008; Bot *et*  
248 *al.*, 2011). or this reason, tubules are not detectable by using polarised light microscopy and they  
249 can be imaged by SEM solely (Sawalha *et al.*, 2013). The peculiar network arrangement of  $\beta$ -  
250 sitosterol+ $\gamma$ -oryzanol conferred to the system a higher stress resistance in comparison to the  
251 crystalline networks formed by MG and RW. It should be remembered that the ability of these  
252 molecules to gel into oil is associated to their self-assembly into crystals (Da Pieve *et al.*, 2010,  
253 Doan *et al.*, 2015). As can be observed in the polarized light microscopy images (Table 1), the  
254 morphology of crystals (bright areas) was different in MG and RW-based oleogels: small needle-  
255 like crystals were formed in MG containing system, whereas large dendritic crystals were observed  
256 in RW oleogel, in well agreement with the literature (Da Pieve *et al.*, 2010; Doan *et al.*, 2015;  
257 Fayaz *et al.*, 2019). Both MG and RW crystals further aggregated mainly by hydrogen bonds, van  
258 der Waals and hydrophobic forces to finally develop into three-dimensional gel network. As

259 demonstrated by Fayaz *et al.* (2019), the physical molecular interactions among crystal aggregates  
260 are expected to be stronger in rice wax-containing sample than in MG-based oleogel.

### 261 3.2. *Curcuminoid stability*

262 After the characterization of oleogels, further research was carried out to investigate the impact of  
263 oleogel preparation conditions on CUs content. As already mentioned, oleogels were prepared by  
264 heating oil at temperatures higher than the melting temperatures of the gelators. Being CUs  
265 sensitive to heat, their degradation could occur during oleogel preparation. The concentration of  
266 BDMC, DMC, and C in turmeric extract, enriched oil and oleogels was thus determined by  
267 UHPLC. BDMC, DMC, and C concentration in turmeric extract accounted for  $10,142 \pm 10 \mu\text{g/g}$ ,  
268  $53,277 \pm 79 \mu\text{g/g}$  and  $147,761 \pm 249 \mu\text{g/g}$ , respectively. As expected, C was the most abundant  
269 compound, followed by DMC and BDMC (Yu & Huang, 2012). Based on CUs concentration in the  
270 turmeric extract and in freshly prepared oil (Table 2), it can be observed that these compounds were  
271 completely solubilized during oil enrichment. BDMC, DMC and C concentration in oleogels did  
272 not differ significantly from those found in freshly prepared oil and ranged between 39 and 41;  
273 and 274;  $1,049$  and  $1,112 \mu\text{g/g}_{\text{oil}}$ , respectively. These results suggest that the preparation  
274 methodology applied to produce oleogels did not induce CUs degradation. It can be noted that  
275 BDMC, DMC, and C accounted for nearly 2%, 14% and 84% of CUs, respectively, in oil and  
276 oleogels.

277 Samples were further analyzed for CUs content during storage at 20 °C. As shown in Table 2, CUs  
278 concentration did not change up to 60 days of storage, whereas a significant decrease was recorded  
279 at 100 days of storage in all samples. In any case, the measured differences did not allow  
280 highlighting a clear effect of oleogel structure on CUs stability.

### 281 3.3. *Physical properties, lipolysis and bioaccessibility of in vitro digested oleogels*

282 The aim of the second part of the research was to study the effect of oil gelator type, and thus  
283 oleogel structure, on the digestive fate of oil and CUs. The particle size distribution of the micellar

284 phases obtained after *in vitro* digestion of oil and oleogels was firstly investigated (Fig. 1a). A  
285 multimodal particle size distribution was observed for all samples, revealing the presence of small  
286 and large particles. The smallest particle family detected in oil, PS and RW showed an average  
287 diameter ranging from 90 to 190 nm, attributable to the presence of mixed micelles formed upon  
288 digestion (Salvia-Trujillo *et al.*, 2017). By observing particle size distributions, it is evident that  
289 RW and PS produced broader distributions than that observed in oil. This result suggests the  
290 formation of micelles with unhomogeneous size probably due to the fact that gel structure interferes  
291 with the digestion process (O'Sullivan *et al.*, 2017). In particular, the gel network might represent a  
292 physical barrier to the access of lipase to its substrate or could directly interfere with lipid digestive  
293 components (McClements, Decker, & Park, 2009), resulting in a less efficient micelle formation. In  
294 this regard, it is important to highlight that micellarization plays a pivotal role in determining the  
295 absorption of loaded lipophilic bioactive compounds, but no correlation has been demonstrated  
296 between size and bioaccessibility (Salvia-Trujillo *et al.*, 2017).

297 Differently, the major particle family in MG presented an average diameter of 28 nm. Such  
298 difference could be attributed to the ability of MG to act as surfactants during *in vitro* digestion,  
299 thus promoting the formation of smaller micelles (Reis *et al.*, 2008).

300 Beside the presence of particles smaller than 200 nm, as shown in Fig. 1a, all analyzed samples also  
301 presented a particle family with an average diameter of 4,800-5,500 nm. This family is attributable  
302 to the presence of undigested lipid droplets surrounded by anionic species, such as free fatty acids  
303 and bile salts (Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013; Singh, Ye, & Horne,  
304 2009; Zou *et al.*, 2016), and resulting in complex colloidal structures with negative charge (Zhang *et al.*, 2016).

305

306 The negative charge of particles in the micellar phase was confirmed by  $\zeta$ -potential measurements  
307 (Fig. 1b). As well known, this parameter provides information about the surface electrical charge of  
308 particles and depends on the adsorbed species at the oil-water interfaces (Salvia-Trujillo *et al.*,  
309 2017). All digested oleogels presented negatively charged particles, with a  $\zeta$ -potential ranging from

310 -50 to -62 mV. Unstructured oil and MG-containing oleogel were characterized by the most  
311 negatively charged micelles upon digestion, whereas RW- and PS-containing samples presented a  
312 higher  $\zeta$ -potential, confirming the ability of different structuring agents to affect not only micelle  
313 size but also their surface charge (Fig. 1). To explain these results it can be speculated that some  
314 surface active species contained in RW and PS might play a role in modifying the total surface  
315 charge of the micelles. Moreover, some authors observed a relationship between the electrical  
316 charge upon digestion and the lipolysis degree (Qian, Decker, Xiao, & McClements, 2012). Lipid  
317 digestion was thus monitored by measuring free fatty acid (FFA) release during the intestinal phase  
318 of *in vitro* digestion (Fig. 2). It can be observed that the FFA release increased during digestion for  
319 all analyzed samples and reached a plateau within 30 min of intestinal digestion. This behavior is  
320 in agreement with literature data (McClements & Li, 2010; O'Sullivan, Barbut, & Marangoni,  
321 2016). However, the extent of lipid digestion was significantly affected by oil structure. The  
322 unstructured oil presented the highest value of lipid digestion ( $FFA_{max} = 54\%$ ) in comparison with  
323 oleogels. This value agreed with literature findings, reporting a maximum lipolysis for unstructured  
324 sunflower oil around 57% (Ye *et al.*, 2019). Among oleogels, MG and RW presented similar  
325 lipolysis extents (nearly 45%), whereas PS-based oleogel showed the lowest lipolysis upon *in vitro*  
326 digestion, being 20% lower than that of oil.

327 These results suggest that the presence of differently structured supramolecular networks in oil  
328 affected the free fatty acid release, probably by hindering lipase access to triacylglycerol digestion  
329 sites. Thus, different oil gelators and gelation mechanisms seem to critically affect lipolysis, in  
330 agreement with results reported by Ashkar *et al.* (2019) with reference to canola oil-based oleogels  
331 containing MG and PS. It should be noted that no universal conclusion on the relation between the  
332 gel strength and lipolysis can be gathered, as both gel strength (Yang *et al.*, 2018; Sawalha *et al.*,  
333 2015) and lipolysis extent (Ye *et al.*, 2019) may be differently affected by fat composition. Still, it  
334 can be inferred that it may be possible to modulate fat digestibility and availability by selecting a  
335 proper oleogel structure. Several authors observed an interdependence between lipolysis efficiency

336 and micelle characteristics (Qian *et al.*, 2012; Salvia-Trujillo *et al.*, 2017). In fact, besides resulting  
337 in the highest lipolysis extent (Fig. 2), MG-containing oleogel also presented the smallest and most  
338 negatively charged mixed micelles (Fig. 1), comparable with those observed in the unstructured oil.  
339 Since these differences could affect the bioaccessibility of loaded lipophilic bioactive compounds,  
340 to get an insight into the effect of different structuring agents on this feature, CUs concentration and  
341 bioaccessibility in the *in vitro* digested oil and oleogels were assessed (Table 3). As well known,  
342 bioaccessibility measures the percent transfer of the bioactive molecules from the lipid phase to the  
343 aqueous one, in which CUs are incorporated into mixed micelles (Ferruzzi, 2010). Only CUs  
344 enclosed in the micellas are then available to be absorbed by the intestinal epithelium cells. A  
345 significant decrease ( $p < 0.05$ ) in the concentration of CUs was observed for both oil and oleogels  
346 in the micellar phase after *in vitro* digestion, as compared to undigested samples (Table 2).  
347 Regarding the susceptibility of different CUs upon digestion, curcumin presented the lowest BAC,  
348 being more susceptible to oxidation as compared to BDMC and DMC (Gordon Luis, Ashley,  
349 Osheroff, & Schneider, 2015), in agreement with the results reported by Yu *et al.* (2011, 2012). In  
350 addition, the gelator significantly affected CUs concentration and bioaccessibility in digested  
351 samples, in contrast with the results found for the undigested oleogels (Tables 2 and 3). **The**  
352 **bioaccessibility of CUs included into PS oleogel was comparable to that of oil ( $p > 0.05$ ),**  
353 **suggesting that the tubular network did not compromise the release of CUs into digestive fluids**  
354 **impairing their bioaccessibility.** On the other hand, oleogels structured through crystalline networks  
355 (MG- and RW-based oleogels) presented the lowest CUs content and bioaccessibility upon *in vitro*  
356 digestion. It can be speculated an effect of the presence of crystalline particles on CUs BAC. **In**  
357 **particular, CUs could be involved in the gelation/crystallization process, being more exposed to**  
358 **oxygen and/or other oxidants present in the digestive mixture. To our knowledge, in the literature**  
359 **there are no indications on this aspect. However, some evidences show an effect of the lipid**  
360 **crystalline network structure on  $\beta$ -carotene oxidative degradation. On this regards, some authors**  
361 **(Calligaris, Valoppi, Barba, Anese, & Nicoli, 2018; Martins, Cerqueira, Cunha, & Vicente, 2017)**

362 demonstrated the critical role of biomolecule location inside the lipid crystal network. Even if more  
363 research is needed on this aspect, it can be inferred that also during digestion the presence of fat  
364 crystals could impact not only the chemical stability of bioactive molecules, but also their release  
365 into the digestive fluids and thus their bioaccessibility.

#### 366 **4. Conclusions**

367 Results reported in the present study confirm that oleogelation could be a profitable strategy to  
368 modulate lipid digestion while delivering bioactive molecules. Oleogel structure seems to affect the  
369 lipolysis extent and the bioaccessibility of loaded lipophilic bioactive compounds. Regarding lipid  
370 digestion, gel strength resulted critical in affecting the rate and extent of lipolysis. On the contrary,  
371 the choice of the gelling agent had an impact on CUs bioaccessibility.

372 Based on this knowledge, it can be suggested that oleogel development would not only offer the  
373 food industry a plastic fat replacer but could represent a strategy to modulate lipid digestion and  
374 deliver health benefits. Matching the reduction of fat uptake and the improved bioaccessibility of  
375 bioactive molecules might provide the consumers with functional foods potentially able to tackle  
376 the risk of obesity and cardiovascular diseases, currently representing major issues for public health.

#### 377 **Abbreviations**

378 BDMC = bisdemethoxycurcumin; C = curcumin; CUs = curcuminoids; DMC =  
379 demethoxycurcumin; MG = monoglycerides; PS = mixture of  $\beta$ -sitosterol and  $\gamma$ -oryzanol; RW =  
380 rice wax.

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#### 383 **Conflict of interest**

384 There are no conflicts of interest to declare.

#### 385 **References**

- 386 Aggarwal, B. B., Kumar, A., & Bharti, A. C. (2003). Anticancer potential of curcumin: preclinical  
387 and clinical studies. *Anticancer Research*, *398*, 363–398.
- 388 Ahmed, K., Li, Y., McClements, D. J., & Xiao, H. (2012). Nanoemulsion- and emulsion-based  
389 delivery systems for curcumin: encapsulation and release properties. *Food Chemistry*, *132*,  
390 799–807.
- 391 Ashkar, A., Laufer, S., Rosen-Kligvasser, J., Lesmes, U., & Davidovich-Pinhas, M. (2019). Impact  
392 of different oil gelators and oleogelation mechanisms on digestive lipolysis of canola oil  
393 oleogels. *Food Hydrocolloids*, *97*, 105218.
- 394 Aziz, M. T. A., El Ibrashy, I. N., Mikhailidis, D. P., Rezaq, A. M., Wassef, M. A. A., Fouad, H. H.,  
395 ... Hussein, R. E. (2013). Signaling mechanisms of a water soluble curcumin derivative in  
396 experimental type 1 diabetes with cardiomyopathy. *Diabetology and Metabolic Syndrome*, *5*,  
397 1–12.
- 398 Bot, A., & Agterof, W. G. M. (2006). Structuring of edible oils by mixtures of  $\gamma$ -oryzanol with  $\beta$ -  
399 sitosterol or related phytosterols. *Journal of the American Oil Chemists' Society*, *83*, 513–521.
- 400 Bot, A., Den Adel, R., & Roijers, E. C. (2008). Fibrils of  $\gamma$ -oryzanol +  $\beta$ -sitosterol in edible oil  
401 organogels. *Journal of the American Oil Chemists' Society*, *85*, 1127–1134.
- 402 Bot, A., den Adel, R., Regkos, C., Sawalha, H., Venema, P., & Flöter, E. (2011). Structuring in  $\beta$ -  
403 sitosterol +  $\gamma$ -oryzanol-based emulsion gels during various stages of a temperature cycle. *Food*  
404 *Hydrocolloids*, *25*, 639–646.
- 405 Calligaris, S., Mirolo, G., Da Pieve, S., Arrighetti, G., & Nicoli, M. C. (2014). Effect of oil type on  
406 formation, structure and thermal properties of  $\gamma$ -oryzanol and  $\beta$ -sitosterol-based organogels.  
407 *Food Biophysics*, *9*, 69–75.
- 408 Calligaris, S., Valoppi, F., Barba, L., Anese, M., & Nicoli, M. C. (2018).  $\beta$ -Carotene degradation  
409 kinetics as affected by fat crystal network and solid/liquid ratio. *Food Research International*,  
410 *105*, 599–604.
- 411 Chakravarty, A. K., Chatterjee, S. N., Yasmin, H., & Mazumder, T. (2009). Comparison of efficacy

412 of turmeric and commercial curcumin in immunological functions and gene regulation. *IJP -*  
413 *International Journal of Pharmacology*, 5, 333-345.

414 Co, E. D., & Marangoni, A. G. (2012). Organogels: an alternative edible oil-structuring method.  
415 *Journal of the American Oil Chemists' Society*, 89, 749–780.

416 Commission Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16  
417 December 2008 on food additives.

418 Da Pieve, S., Calligaris, S., Co, E., Nicoli, M. C., & Marangoni, A. G. (2010). Shear  
419 nanostructuring of monoglyceride organogels. *Food Biophysics*, 5, 211–217.

420 Davidovich-Pinhas, M. (2016). Oleogels: a promising tool for delivery of hydrophobic bioactive  
421 molecules. *Therapeutic Delivery*, 7, 7–9.

422 Doan, C. D., Van De Walle, D., Dewettinck, K., & Patel, A. R. (2015). Evaluating the oil-gelling  
423 properties of natural waxes in rice bran oil: rheological, thermal, and microstructural study.  
424 *Journal of the American Oil Chemists' Society*, 92, 801–811.

425 Doan, C. D., Tavernier, I., Okuro, P. K., & Dewettinck, K. (2018). Internal and external factors  
426 affecting the crystallization, gelation and applicability of wax-based oleogels in food industry.  
427 *Innovative Food Science and Emerging Technologies*, 45, 42–52.

428 Fayaz, G., Calligaris, S., Nicoli, M.C. (2019). Comparative study on the ability of different  
429 oleogelators to structure sunflower oil. *Food Biophysics*, in press, DOI 10.1007/s11483-019-  
430 09597-9.

431 Ferruzzi, M. G. (2010). The influence of beverage composition on delivery of phenolic compounds  
432 from coffee and tea. *Physiology & Behavior*, 100, 33–41.

433 Giacintucci, V., Di Mattia, C. D., Sacchetti, G., Flamminii, F., Gravelle, A. J., Baylis, B., ... Pittia,  
434 P. (2018). Ethylcellulose oleogels with extra virgin olive oil: the role of oil minor components  
435 on microstructure and mechanical strength. *Food Hydrocolloids*, 84, 508–514.

436 Gordon, O. N., Luis, P. B., Ashley, R. E., Osheroff, N., & Schneider, C. (2015). Oxidative  
437 transformation of demethoxy- and bisdemethoxycurcumin: products, mechanism of formation,

438 and poisoning of human topoisomerase II $\alpha$ . *Physiology & Behavior*, 28, 989–996.

439 Gravelle, A. J., Davidovich-Pinhas, M., Zetzl, A. K., Barbut, S., & Marangoni, A. G. (2016).  
440 Influence of solvent quality on the mechanical strength of ethylcellulose oleogels.  
441 *Carbohydrate Polymers*, 135, 169–179.

442 Gravelle, Andrew J., Davidovich-Pinhas, M., Barbut, S., & Marangoni, A. G. (2017). Influencing  
443 the crystallization behavior of binary mixtures of stearyl alcohol and stearic acid (SOSA) using  
444 ethylcellulose. *Food Research International*, 91, 1–10.

445 Li, Y., Hu, M., Du, Y., Xiao, H., & McClements, D. J. (2011). Control of lipase digestibility of  
446 emulsified lipids by encapsulation within calcium alginate beads. *Food Hydrocolloids*, 25,  
447 122–130.

448 Martins, A. J., Cerqueira, M. A., Cunha, R. L., & Vicente, A. A. (2017). Fortified beeswax  
449 oleogels: effect of  $\beta$ -carotene on the gel structure and oxidative stability. *Food and Function*,  
450 8, 4241–4250.

451 McClements, D. J., Decker, E. A., & Park, Y. (2009). Controlling lipid bioavailability through  
452 physicochemical and structural approaches. *Critical Reviews in Food Science and Nutrition*,  
453 49, 48–67.

454 McClements, D. J., & Li, Y. (2010). Structured emulsion-based delivery systems: controlling the  
455 digestion and release of lipophilic food components. *Advances in Colloid and Interface*  
456 *Science*, 159, 213–228.

457 Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., ... Brodkorb, A.  
458 (2014). A standardised static *in vitro* digestion method suitable for food – an international  
459 consensus. *Food and Function*, 5, 1113–1124.

460 Mohsenin, N. N., & Mittal, J. P. (1977). Use of rheological terms and correlation of compatible  
461 measurements in food texture research. *Journal of Texture Studies*, 8, 395–408.

462 Nikiforidis, C. V., & Scholten, E. (2015). Polymer organogelation with chitin and chitin  
463 nanocrystals. *RSC Advances*, 5, 37789–37799.

- 464 O'Sullivan, C. M., Barbut, S., & Marangoni, A. G. (2016). Edible oleogels for the oral delivery of  
465 lipid soluble molecules: composition and structural design considerations. *Trends in Food*  
466 *Science and Technology*, 57, 59–73.
- 467 O'Sullivan, C. M., Davidovich-Pinhas, M., Wright, A. J., Barbut, S., & Marangoni, A. G. (2017).  
468 Ethylcellulose oleogels for lipophilic bioactive delivery-effect of oleogelation on *in vitro*  
469 bioaccessibility and stability of beta-carotene. *Food and Function*, 8, 1438–1451.
- 470 Patel, A. R., & Dewettinck, K. (2016). Edible oil structuring: an overview and recent updates. *Food*  
471 *and Function*, 7, 20–29.
- 472 Peyronel, F., & Campos, R. (2012). *Methods used in the study of the physical properties of fats*. In  
473 A. G. Marangoni (Ed.), *Structure-function analysis of edible fats* (pp. 262–277). Urbana:  
474 AOCS Press.
- 475 Pinheiro, A. C., Coimbra, M. A., & Vicente, A. A. (2016). *In vitro* behaviour of curcumin  
476 nanoemulsions stabilized by biopolymer emulsifiers - Effect of interfacial composition. *Food*  
477 *Hydrocolloids*, 52, 460–467.
- 478 Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012). Nanoemulsion delivery systems:  
479 influence of carrier oil on beta-carotene bioaccessibility. *Food Chemistry*, 135, 1440–1447.
- 480 Račková, L., Košťálová, D., Bezáková, L., Fialova, S., Bauerova, K., Toth, J., ... Obložinský, M.  
481 (2009). Comparative study of two natural antioxidants, curcumin and *Curcuma longa* extract.  
482 *Journal of Food & Nutrition Research*, 48, 148-152.
- 483 Reis, P. M., Raab, T. W., Chuat, J. Y., Leser, M. E., Miller, R., Watzke, H. J., & Holmberg, K.  
484 (2008). Influence of surfactants on lipase fat digestion in a model gastro-intestinal system.  
485 *Food Biophysics*, 3, 370–381.
- 486 Rogers, M. A., Wright, A. J., & Marangoni, A. G. (2009). Oil organogels: the fat of the future? *Soft*  
487 *Matter*, 5, 1594–1596.
- 488 Salvia-Trujillo, L., Qian, C., Martín-Belloso, O., & McClements, D. J. (2013). Influence of particle  
489 size on lipid digestion and beta-carotene bioaccessibility in emulsions and nanoemulsions.

490 *Food Chemistry*, 141, 1475–1480.

491 Salvia-Trujillo, L., Verkempinck, S. H. E., Sun, L., Van Loey, A. M., Grauwet, T., & Hendrickx,  
492 M. E. (2017). Lipid digestion, micelle formation and carotenoid bioaccessibility kinetics:  
493 influence of emulsion droplet size. *Food Chemistry*, 229, 653–662.

494 Sawalha, H., Margry, G., den Adel, R., Venema, P., Bot, A., Flöter, E., & van der Linden, E.  
495 (2013). The influence of the type of oil phase on the self-assembly process of  $\gamma$ -oryzanol +  $\beta$ -  
496 sitosterol tubules in organogel systems. *European Journal of Lipid Science and Technology*,  
497 115, 295–300.

498 Sawalha, H., Venema, P., Bot, A., Flöter, E., Den Adel, R., & Van Der Linden, E. (2015). The  
499 phase behavior of  $\gamma$ -oryzanol and  $\beta$ -sitosterol in edible oil. *JAOCS, Journal of the American*  
500 *Oil Chemists' Society*, 92, 1651–1659.

501 Shishu, & Maheshwari, M. (2010). Comparative bioavailability of curcumin, turmeric and  
502 Biocurcumax in traditional vehicles using non-everted rat intestinal sac model. *Journal of*  
503 *Functional Foods*, 2, 60–65.

504 Singh, A., Auzanneau, F. I., & Rogers, M. A. (2017). Advances in edible oleogel technologies – A  
505 decade in review. *Food Research International*, 97(March), 307–317.

506 Singh, H., Ye, A., & Horne, D. (2009). Structuring food emulsions in the gastrointestinal tract to  
507 modify lipid digestion. *Progress in Lipid Research*, 48, 92–100.

508 Su, L., Wang, Y., & Chi, H. (2017). Effect of curcumin on glucose and lipid metabolism, FFAs and  
509 TNF- $\alpha$  in serum of type 2 diabetes mellitus rat models. *Saudi Journal of Biological Sciences*,  
510 24, 1776–1780.

511 Tan, S.-Y., Peh, E., Marangoni, A. G., & Henry, C. J. (2017a). Effects of liquid oil vs. oleogel co-  
512 ingested with a carbohydrate-rich meal on human blood triglycerides, glucose, insulin and  
513 appetite. *Food and Function*, 8, 241–249.

514 Tan, S. Y., Peh, E., Siow, P. C., Marangoni, A. G., & Henry, C. J. (2017b). Effects of the physical-  
515 form and the degree-of-saturation of oil on postprandial plasma triglycerides, glycemia and

- 516 appetite of healthy Chinese adults. *Food and Function*, 8, 4433–4440.
- 517 Tan, S.-Y., Peh, E., Lau, E., Marangoni, A. G., & Henry, C. J. (2017c). Physical form of dietary fat  
518 alters postprandial substrate utilization and glycemic response in healthy chinese men. *The*  
519 *Journal of Nutrition*, 147, 1138–1144.
- 520 Verkempinck, S. H. E., Moens, L. G., Charleer, L., Loey, A. M. Van, & Hendrickx, M. E. (2018).  
521 Emulsion stability during gastrointestinal conditions effects lipid digestion kinetics. *Food*  
522 *Chemistry*, 246, 179–191.
- 523 Wang, F. C., Gravelle, A. J., Blake, A. I., & Marangoni, A. G. (2016). Novel trans fat replacement  
524 strategies. *Current Opinion in Food Science*, 7, 27–34.
- 525 Yang, S., Zhu, M., Wang, N., Cui, X., Xu, Q., Saleh, A. S. M., ... Xiao, Z. (2018). Influence of oil  
526 type on characteristics of  $\beta$ -sitosterol and stearic acid based oleogel. *Food Biophysics*, 13,  
527 362–373.
- 528 Ye, Z., Cao, C., Li, R., Cao, P., Li, Q., & Liu, Y. (2019). Lipid composition modulates the intestine  
529 digestion rate and serum lipid status of different edible oils: a combination of *in vitro* and *in*  
530 *vivo* studies. *Food and Function*, 10, 1490–1503.
- 531 Yu, H., Li, J., Shi, K., & Huang, Q. (2011). Structure of modified e-polylysine micelles and their  
532 application in improving cellular antioxidant activity of curcuminoids. *Food and Function*, 2,  
533 373–380.
- 534 Yu, H., & Huang, Q. (2012). Improving the oral bioavailability of curcumin using novel organogel-  
535 based emulsions. *Journal of Agricultural and Food Chemistry*, 60, 5373–5379.
- 536 Yu, H., Shi, K., Liu, D., & Huang, Q. (2012). Development of a food-grade organogel with high  
537 bioaccessibility and loading of curcuminoids. *Food Chemistry*, 131, 48–54.
- 538 Zetzl, A. K., Gravelle, A. J., Kurylowicz, M., Dutcher, J., Barbut, S., & Marangoni, A. G. (2014).  
539 Microstructure of ethylcellulose oleogels and its relationship to mechanical properties. *Food*  
540 *Structure*, 2, 27–40.
- 541 Zhang, R., Zhang, Z., Zou, L., Xiao, H., Zhang, G., Decker, E. A., & McClements, D. J. (2016).

542 Impact of lipid content on the ability of excipient emulsions to increase carotenoid  
543 bioaccessibility from natural sources (raw and cooked carrots). *Food Biophysics*, *11*, 71–80.

544 Zheng, A., Li, H., Feng, Z., Wu, J., Cao, K., Wang, X., ... Zhou, B. (2014). Anticancer effect of a  
545 curcumin derivative B63: ROS production and mitochondrial dysfunction. *Current Cancer*  
546 *Drug Targets*, *14*, 156–166.

547 Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., ... McClements, D. J. (2016). Influence  
548 of lipid phase composition of excipient emulsions on curcumin solubility, stability, and  
549 bioaccessibility. *Food Biophysics*, *11*, 213–225.

550

551 **Captions for figures**

552 Fig. 1. Particle size distribution (a) and  $\zeta$ -potential (b) of oil and oleogels obtained by using 5%  
553 (w/w) of monoglycerides (MG), rice wax (RW) and phytosterols (PS), after *in vitro* digestion.  
554 Different letters (a-c) mean significant differences ( $p < 0.05$ ) among samples.

555 Fig. 2. Concentration of free fatty acids (FFA) during *in vitro* digestion of oil and oleogels  
556 containing 5% (w/w) of monoglycerides (MG), rice wax (RW) or phytosterols (PS). Different  
557 letters (a-c) mean significant differences ( $p < 0.05$ ) among samples.

558 Fig. S1. UHPLC trace of CUs enriched oil. 1: bisdemethoxycurcumin; 2: demethoxycurcumin; 3:  
559 curcumin.

560 Fig. S2. Stress and frequency sweep curves of monoglyceride- (MG), rice wax- (RW) and  
561 phytosterol- (PS) based oleogels.

## DECLARATION OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

Udine, 15<sup>th</sup> May 2019

, Signed by all authors as follows:

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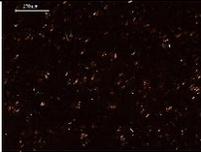
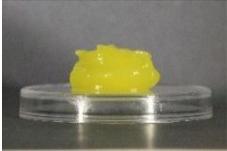


**Author contribution**

Sonia Calligaris: Conceptualization, Writing - Review & Editing, Supervision; Marilisa Alongi: Investigation, Formal analysis, Writing - Original Draft; Paolo Lucci: Methodology, Validation, Writing - Review & Editing; Monica Anese: Writing - Review & Editing, Supervision.

Table 1

Firmness,  $G'$ ,  $G''$ ,  $\text{Tan}\delta$ , critical stress, and microscopic and macroscopic images of oleogels obtained by using 5% (w/w) of monoglycerides (MG), rice wax (RW) and phytosterols (PS).

| Gelling agent | Firmness (N)      | $G'$ (Pa)             | $G''$ (Pa)          | $\text{Tan}\delta$ | Critical stress (Pa) | Microscopic appearance  | Macroscopic appearance  |
|---------------|-------------------|-----------------------|---------------------|--------------------|----------------------|---|---|
| MG            | $0.91 \pm 0.08^c$ | $25090 \pm 4044^b$    | $3075 \pm 513^b$    | $0.15 \pm 0.01^b$  | $6.6 \pm 0.1^b$      |  |  |
| RW            | $3.49 \pm 0.24^b$ | $37413 \pm 2591^a$    | $4384 \pm 539^{ab}$ | $0.15 \pm 0.01^b$  | $16.7 \pm 3.1^b$     |  |  |
| PS            | $5.65 \pm 0.42^a$ | $29410 \pm 4646^{ab}$ | $6026 \pm 808^a$    | $0.21 \pm 0.01^a$  | $128.5 \pm 28.6^a$   | n.a.  |  |

Different letters (a-c) mean significant differences ( $p < 0.05$ ) of structural properties among oleogels.

n.a. Images not acquired.

Table 2

Bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC) and curcumin (C) contents in oil and oleogels obtained by using 5% (w/w) of monoglycerides (MG), rice wax (RW) and phytosterols (PS), during storage at 20 °C under dark.

| Sample | BDMC ( $\mu\text{g}/\text{g}_{\text{oil}}$ ) |                               |                               | DMC ( $\mu\text{g}/\text{g}_{\text{oil}}$ ) |                             |                             | C ( $\mu\text{g}/\text{g}_{\text{oil}}$ ) |                             |                             |
|--------|--|-------------------------------|-------------------------------|---|-----------------------------|-----------------------------|---|-----------------------------|-----------------------------|
|        | 0 days                                       | 60 days                       | 100 days                      | 0 days                                      | 60 days                     | 100 days                    | 0 days                                    | 60 days                     | 100 days                    |
| Oil    | 41.2 $\pm$ 1.8 <sup>A,a</sup>                | 38.5 $\pm$ 0.2 <sup>A,a</sup> | 31.6 $\pm$ 0.1 <sup>B,a</sup> | 274 $\pm$ 12 <sup>A,a</sup>                 | 251 $\pm$ 1 <sup>A,a</sup>  | 193 $\pm$ 3 <sup>B,b</sup>  | 1112 $\pm$ 48 <sup>A,a</sup>              | 974 $\pm$ 5 <sup>B,a</sup>  | 820 $\pm$ 5 <sup>C,b</sup>  |
| MG     | 39 $\pm$ 1.5 <sup>A,a</sup>                  | 38.9 $\pm$ 1.8 <sup>A,a</sup> | 32.1 $\pm$ 0.3 <sup>A,a</sup> | 268 $\pm$ 10 <sup>A,a</sup>                 | 250 $\pm$ 12 <sup>A,a</sup> | 192 $\pm$ 2 <sup>A,b</sup>  | 1049 $\pm$ 38 <sup>A,a</sup>              | 974 $\pm$ 49 <sup>A,a</sup> | 812 $\pm$ 6 <sup>B,bc</sup> |
| RW     | 41.0 $\pm$ 2.8 <sup>A,a</sup>                | 37.3 $\pm$ 0.3 <sup>A,a</sup> | 29.8 $\pm$ 0.2 <sup>B,b</sup> | 272 $\pm$ 15 <sup>A,a</sup>                 | 246 $\pm$ 1 <sup>A,a</sup>  | 200 $\pm$ 2 <sup>B,ab</sup> | 1104 $\pm$ 57 <sup>A,a</sup>              | 968 $\pm$ 2 <sup>A,a</sup>  | 794 $\pm$ 3 <sup>B,c</sup>  |
| PS     | 39.7 $\pm$ 2.3 <sup>A,a</sup>                | 38.2 $\pm$ 0.3 <sup>A,a</sup> | 31.3 $\pm$ 0.2 <sup>B,a</sup> | 265 $\pm$ 15 <sup>A,a</sup>                 | 247 $\pm$ 2 <sup>A,a</sup>  | 207 $\pm$ 1 <sup>B,a</sup>  | 1079 $\pm$ 58 <sup>A,a</sup>              | 1007 $\pm$ 8 <sup>A,a</sup> | 853 $\pm$ 3 <sup>B,a</sup>  |

Different capital letters (A-C) mean significant differences of curcuminoid concentration during storage. Different lowercase letters (a-c) mean significant differences ( $p < 0.05$ ) of curcuminoid concentration between oil and oleogels.

Table 3

Bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC) and curcumin (C) content and bioaccessibility (BAC) in oil and oleogels obtained by using 5% (w/w) of monoglycerides (MG), rice wax (RW) and phytosterols (PS) after *in vitro* digestion.

| Sample | BDMC  |                             | DMC   |                              | C   |                             |
|--------|---|-----------------------------|---|------------------------------|---|-----------------------------|
|        | Concentration ( $\mu\text{g}/\text{g}_{\text{oil}}$ ) | BAC (%)                     | Concentration ( $\mu\text{g}/\text{g}_{\text{oil}}$ ) | BAC (%)                      | Concentration ( $\mu\text{g}/\text{g}_{\text{oil}}$ ) | BAC (%)                     |
| Oil    | 22.9 $\pm$ 3.8 <sup>a</sup>                           | 55.6 $\pm$ 9.1 <sup>a</sup> | 141.9 $\pm$ 22.8 <sup>a</sup>                         | 51.7 $\pm$ 8.3 <sup>a</sup>  | 520.4 $\pm$ 85.9 <sup>a</sup>                         | 50.4 $\pm$ 7.7 <sup>a</sup> |
| MG     | 13.1 $\pm$ 0.5 <sup>c</sup>                           | 33.6 $\pm$ 1.4 <sup>b</sup> | 87.3 $\pm$ 5.5 <sup>b</sup>                           | 32.5 $\pm$ 2.1 <sup>c</sup>  | 333.9 $\pm$ 31.5 <sup>b</sup>                         | 31.8 $\pm$ 3.0 <sup>b</sup> |
| RW     | 13.7 $\pm$ 3.4 <sup>bc</sup>                          | 33.3 $\pm$ 8.4 <sup>b</sup> | 94.0 $\pm$ 22.6 <sup>b</sup>                          | 34.5 $\pm$ 8.3 <sup>bc</sup> | 376.5 $\pm$ 93.0 <sup>ab</sup>                        | 29.2 $\pm$ 8.4 <sup>b</sup> |
| PS     | 20.5 $\pm$ 2.0 <sup>ab</sup>                          | 51.4 $\pm$ 5.0 <sup>a</sup> | 130.5 $\pm$ 11.1 <sup>ab</sup>                        | 49.1 $\pm$ 4.2 <sup>ab</sup> | 489.3 $\pm$ 39.7 <sup>ab</sup>                        | 45.2 $\pm$ 3.7 <sup>a</sup> |

Different letters (a-c) mean significant differences ( $p < 0.05$ ) of curcuminoid concentration or bioaccessibility among samples.

Figure 1  
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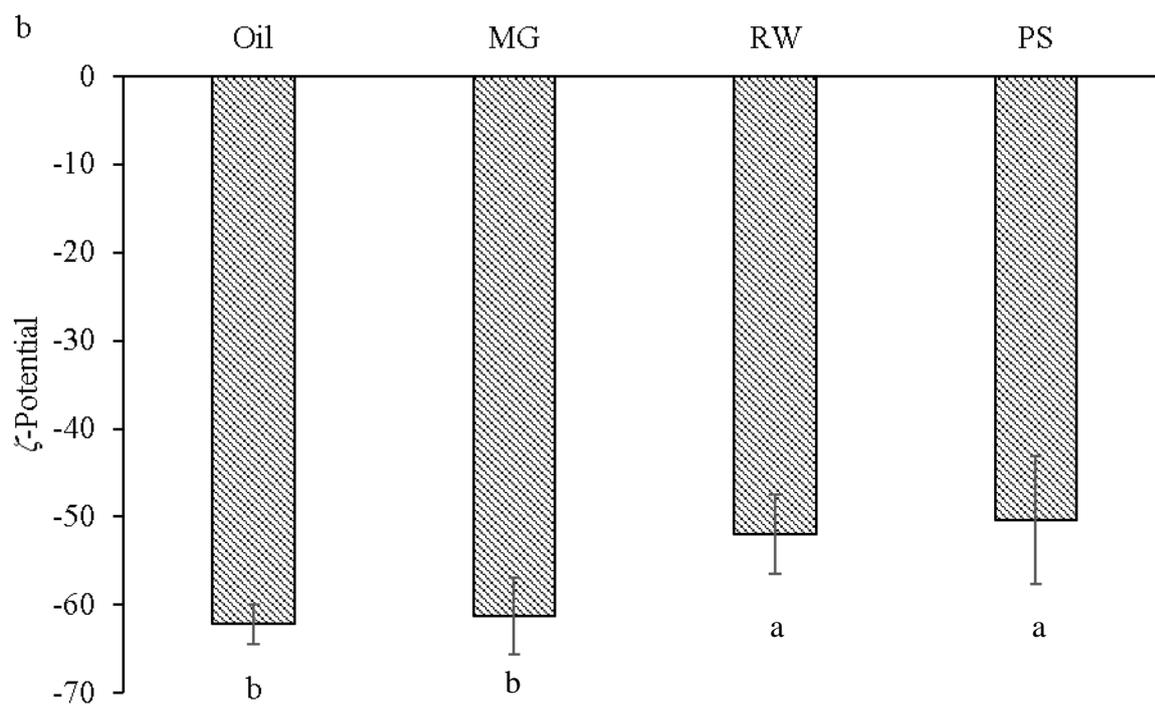
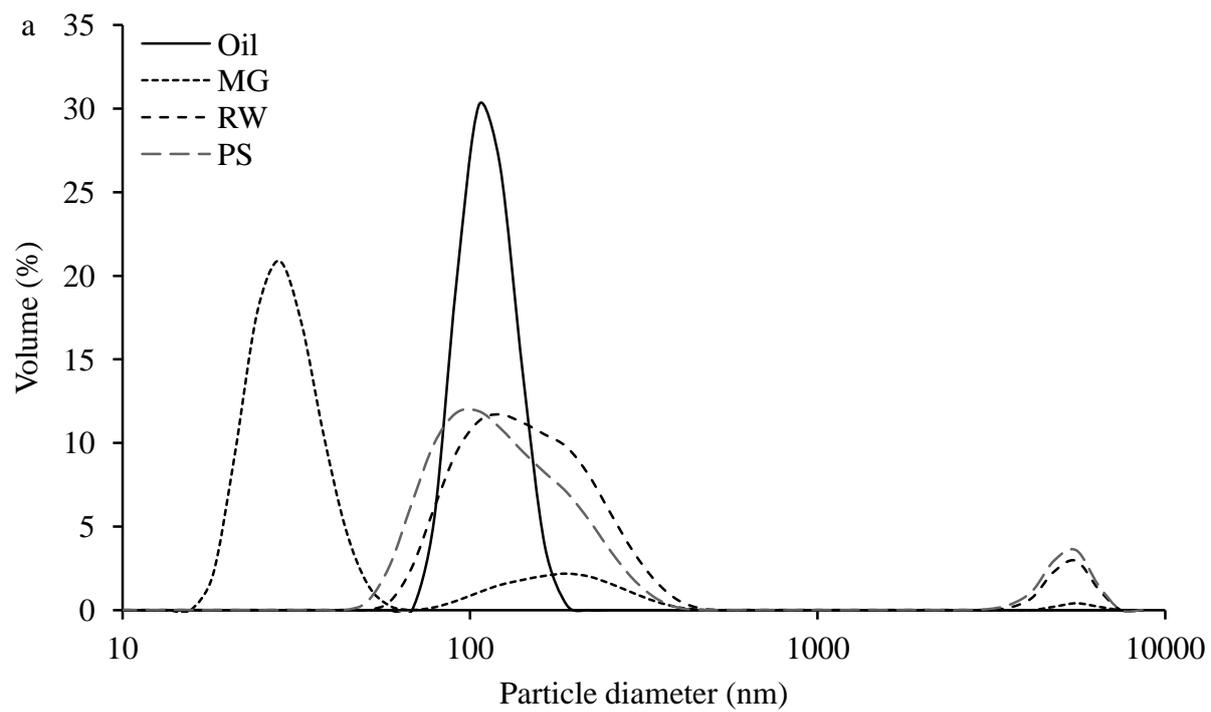


Fig. 1

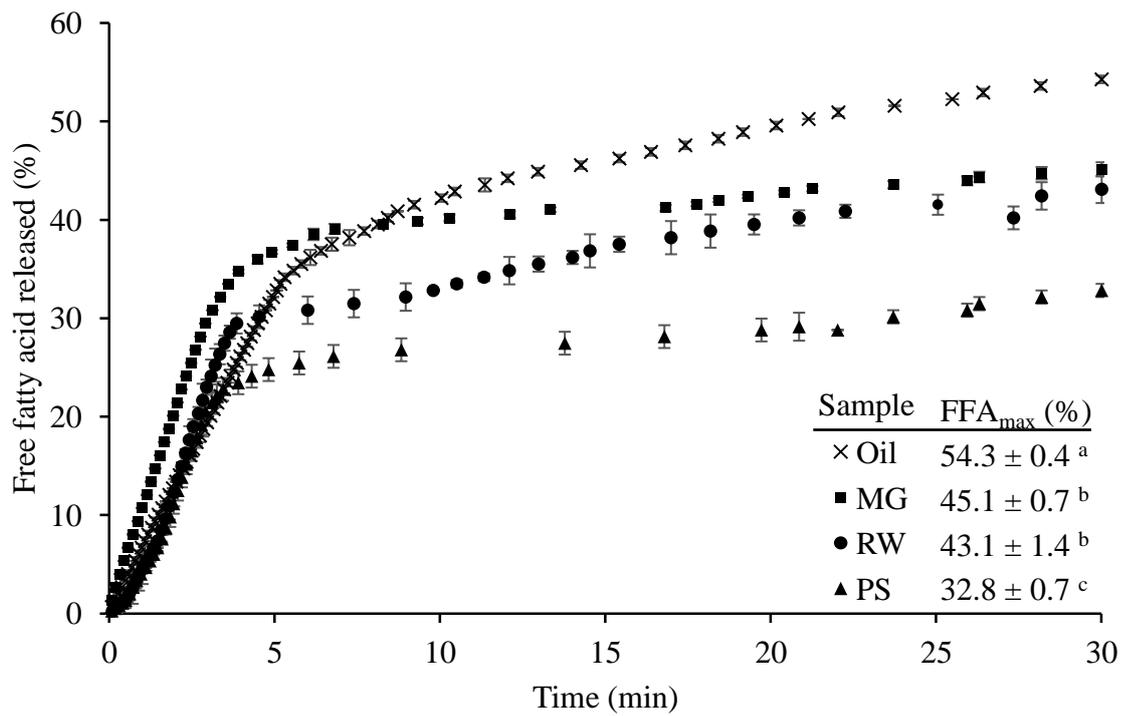


Fig. 2.

Table S1

Total fatty acid profile of high-oleic sunflower oil

| Fatty acid                | Relative abundance (%) |
|---------------------------|------------------------|
| C16:0                     | 5.1 ± 1.0              |
| C16:1 $\Delta^9c$         | 0.2 ± 0.0              |
| C18:0                     | 3.0 ± 0.1              |
| C18:1 $\Delta^9c$         | 81.6 ± 1.5             |
| C18:2 $\Delta^9c,12c$     | 8.3 ± 0.8              |
| C18:3 $\Delta^9c,12c,15c$ | 0.2 ± 0.0              |
| C20:0                     | 0.3 ± 0.0              |
| C20:1 $\Delta^{11}$       | 0.3 ± 0.1              |
| C22:0                     | 1.1 ± 0.2              |

Legend for fatty acids – m:n  $\Delta_x$ , m=number of carbon atoms, n= number of double bonds, x= position of double bonds.

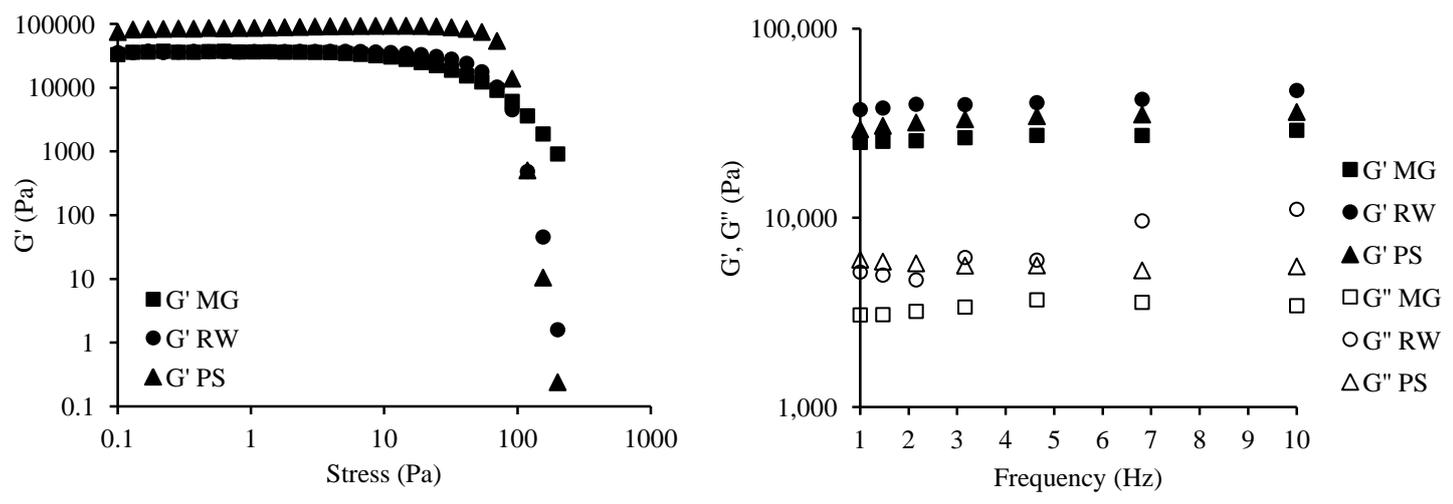


Fig. S2