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## Dynamic modeling of in vitro lipid digestion: Individual fatty acid release and bioaccessibility kinetics

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1 **Dynamic modeling of *in vitro* lipid digestion: Individual fatty acid release**  
2 **and bioaccessibility kinetics**

3

4

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15 **Abstract**

16 The aim of this study was to gain knowledge about the role of triacylglycerol (TAG)  
17 composition in fatty acids (FA) of o/w emulsions on both the pancreatic lipolysis kinetics and  
18 the bioaccessibility of released products (i.e. contained within the bile salt micellar phase). A  
19 mathematical model was developed and its predictions were compared to a set of  
20 experimental data obtained during an *in vitro* digestion of a whey protein stabilized emulsion.  
21 Modeling results show that FA residues of TAG were hydrolyzed at specific rates, inducing  
22 different bioaccessibility kinetics. The estimated lipolysis rate constants of the studied FA  
23 (C8:0, C10:0 >> C18:1 n-9 >> C12:0 > C14:0 > C16:0 ≈ C16:1 n-7 > C22:6 n-3) were in  
24 close agreement with the available literature on the substrate specificity of pancreatic lipase.  
25 Results also suggest that lipolysis products are very rapidly solubilized in the bile salt mixed  
26 micelles with no fractionation according to the FA carbon chain.

27

28 **Keywords:** Emulsion, Digestion, Pancreatic lipase, Substrate specificity, DHA, Simulation.

29

## 30 **1. Introduction**

31 Human digestion of lipid emulsion is influenced by both physiological parameters and  
32 emulsion properties. Triacylglycerol (TAG) digestion occurs essentially in the intestine where  
33 about 80% of the lipolysis reaction take place (Carriere, Barrowman, Verger, & Laugier,  
34 1993). The reaction is mediated by the pancreatic lipase-colipase complex at the oil-water  
35 interface and releases the *sn*-2-monoacylglycerol (MAG) and two free fatty acids (FFA)  
36 (Golding & Wooster, 2010). Lipolysis products are then incorporated into bile salt micelles  
37 before being transported to the gut wall and absorbed by the organism (Smith & Morton,  
38 2010). Hence, physiological parameters such as the concentrations of pancreatic lipase,  
39 colipase and bile salts can all modify lipid digestion. The structural characteristics of  
40 emulsions are also important to consider when evaluating lipolysis kinetics. For instance, the  
41 nature of emulsifiers influences the properties of the oil-water interface and can therefore  
42 affect lipase adsorption onto droplets. The size of the oil droplets is another key parameter  
43 since fine emulsions with high interfacial areas facilitate the enzyme activity compared to  
44 coarse emulsions with low interfacial areas (Armand et al., 1999; Golding & Wooster, 2010;  
45 Li & McClements, 2010).

46 The TAG composition in fatty acid residues (FA), characterized by their carbon chain length  
47 and degree of unsaturation, also has a strong impact. Several studies have demonstrated that  
48 human and porcine pancreatic lipases exhibit a certain fatty acid specificity (Berger &  
49 Schneider, 1991; Desnuelle & Savary, 1963; Mukherjee, Kiewitt, & Hills, 1993; Yang,  
50 Kuksis, & Myher, 1990). For instance, it is well known that pancreatic lipase is more active  
51 on medium chain TAG (MCT, up to C10) than on long chain TAG (LCT, from C12) (Armand  
52 et al., 1992; Desnuelle & Savary, 1963; Golding et al., 2011; Li & McClements, 2010), and  
53 that its activity depends on the number and positions of unsaturations (Mukherjee et al., 1993;  
54 Yang et al., 1990). Long chain polyunsaturated fatty acids, such as docosahexaenoic acid

55 (C22:6 n-3, DHA), have also been reported to be among the most resistant FA to pancreatic  
56 lipase (Bottino, Vandenburg, & Reiser, 1967; Yang et al., 1990), possibly because of the short  
57 distance of the first double bond from the ester linkage (Akanbi, Sinclair, & Barrow, 2014;  
58 Bottino et al., 1967; Lawson & Hughes, 1988).

59 Moreover, the extent of FFA and MAG that can be solubilized into the bile salt micelles also  
60 seems to be product dependent (Freeman, 1969; Hofmann, 1963). For instance, the molar  
61 saturation ratio (mole of incorporated products/mole of bile salts) in a sodium  
62 glycodeoxycholate solution at 37°C has been reported to vary from 0.07 for stearic acid  
63 (C18:0) to 1.86 for lauric acid (C12:0) with intermediate values for long chain unsaturated  
64 FFA such as oleic (C18:1 n-9) and linoleic acids (C18:2 n-6) (Freeman, 1969). Such values,  
65 which can be partly reasoned in terms of molecular polarities and amphiphilic properties, are  
66 however dependent on numerous parameters such as the type of bile acid used or the pH of  
67 the solution (Freeman, 1969; Hofmann, 1963). Moreover, if the addition of *sn*-1-monoolein  
68 within the bile salt solution has been reported to improve the bile salt solubility of FFA, the  
69 opposite has been observed with the addition of oleic acid (Freeman, 1969). Given the variety  
70 of lipolysis products and bile acids that coexist during the intestinal digestion of edible oil, it  
71 therefore seems difficult to predict the bioaccessibility of lipolysis products.

72 Modeling has proven to be a powerful tool for a better understanding of the intestinal  
73 digestion kinetics. Various models of lipid digestion have been published over the last  
74 decades. Several models are based on the Michaelis-Menten equation. Verger and co-workers  
75 were the first to adapt such approach to the biphasic nature of the lipolysis reaction by taking  
76 into account the interfacial concentrations of the substrates and enzymes (Verger & de Haas,  
77 1976; Verger, Mieras, & de Haas, 1973). Several variants have been proposed since, as for  
78 instance by Jurado, Camacho, Luzón, Fernández-Serrano, & García-Román (2008) for a  
79 bacterial lipase. These models are however quite complex and often require parameter values

80 that are difficult to determine experimentally, in particular for the digestion of edible oils  
81 composed of different TAG species. In a much simpler approach, intestinal lipolysis can also  
82 be described by a first order kinetic model (Ye, Cui, Zhu, & Singh, 2013). If such models can  
83 be useful in comparing the initial enzyme activity in different conditions, they do not take into  
84 account the interfacial area of oil droplets. This is why Li & McClements (2010) proposed a  
85 model in which the rate constant is expressed per unit of interfacial area, and that accounts for  
86 a progressive decrease of the oil droplet size. This model provided good fits of pH-stat  
87 measurements performed on different emulsions with the assumptions that the number of  
88 droplet remains unchanged and all droplets have the same size at any given time. Recently,  
89 we resorted to a similar modeling approach to show, by integrating experimentally measured  
90 droplet sizes, that the reduction of the interfacial area induced by droplet coalescence was the  
91 main reason why our *in vitro* intestinal lipolysis seemed to stop before the substrate was fully  
92 exhausted (Giang et al., 2015).

93 Nevertheless, all the above models assume a unique reaction rate constant without  
94 considering the FA composition of the studied oils. Hence, they cannot be used to reproduce  
95 the release kinetics of individual FA. In the present study, we develop a mathematical model  
96 of intestinal lipolysis that takes the composition of the oil substrate into account. It predicts  
97 the lipolysis kinetics of each FA residue and their *in vitro* bioaccessibility, defined as the  
98 concentration of FA (FFA + *sn*-2-MAG) measured within the bile salt micellar phase. The  
99 model is applied to a set of experimental data measured during the *in vitro* gastro-intestinal  
100 digestion of a whey protein stabilized emulsion. The estimated values for the model  
101 parameters (FA specific lipolysis rate constants and bile salt micellar fractions) are compared  
102 with the available literature and discussed according to their biochemical meanings.

103

## 104 **2. Material and method**

## 105 2.1. Materials

106 The oil containing medium-chain triacylglycerols (MCT), Miglyol 812S, was purchased from  
107 Sasol GmbH, Germany. The main fatty acids contained in this oil were C8:0 (54% w/w) and  
108 C10:0 (43%). The oil containing long-chain triacylglycerols (LCT), DHAsco, was obtained  
109 from Martek, via DSM Nutritional Products Ltd, Switzerland. It contained docosahexaenoic  
110 acid (DHA, C22:6 n-3) as major fatty acid (40% w/w) along with C12:0 (4%), C14:0 (12%),  
111 C16:0 (12%) and C18:1 n-9 (24%). Whey protein powder (Prolacta 95) was purchased from  
112 Lactalis Ingredients, France. Pepsin (P7012, 2,500 U mg<sup>-1</sup>), mucin (M2378), pancreatin  
113 (P7545, 8×USP specifications), pancreatic lipase (L3126, 120 U mg<sup>-1</sup>) and bile extract (B8631)  
114 were from porcine origin and obtained from Sigma-Aldrich, France. Water was Milli-Q water.  
115 Solvents for liquid chromatography were chloroform for HPLC (Carlo Erba), methyl alcohol  
116 for HPLC (99.9%, Carlo Erba) and ammonia solution (30%, Carlo Erba). Acetone (Pure RE,  
117 Carlo Erba), sulfuric acid (RPE 96%, Carlo Erba), cyclohexane and heptadecanoic acid  
118 (Sigma) were used for the preparation of fatty acid methyl esters (FAME) of the micellar  
119 phase during intestinal digestion.

120

## 121 2.2. Emulsion preparation and digestion

122 A whey protein stabilized emulsion with droplet diameters below 1 μm was first prepared  
123 according to the procedure previously described in Giang et al. (2015). Briefly, the oil phase  
124 (20% w/w) contained 37.5% (w/w) of MCT and 62.5% of LCT, and the aqueous phase (80%  
125 w/w) contained 4% (w/w) of whey protein powder, used as emulsifier, in a 0.1M sodium  
126 phosphate buffer (pH 7.0). After high pressure homogenization and addition of maltodextrin  
127 solution to improve the freeze-dried stability of oil droplets, the emulsion was freeze-dried.  
128 On the day of the *in vitro* experiments, the dry emulsion was rehydrated in Milli-Q water to  
129 obtain a final oil concentration of 3.2% (w/w).

130 A volume of 3 mL of the rehydrated emulsion, corresponding to an oil mass of about 96 mg,  
131 was placed into hermetically sealed headspace vials (22.4 mL). The gastric and intestinal  
132 phases of the *in vitro* digestion experiments were performed sequentially at 37°C under  
133 magnetic stirring (400 rpm). The gastric phase duration was 60 min and was launched by  
134 adding 2.12 mL of simulated gastric fluid (SGF) and 40 µL of 1M HCl to reach a final pH of  
135 2.5. The SGF solution contained 3.9 g L<sup>-1</sup> of pepsin (corresponding to 10,000 U per mL of  
136 SGF using hemoglobin as substrate), 2.4 g L<sup>-1</sup> of mucin, 120 mM of NaCl, 2 mM of KCl and  
137 6 mM of CaCl<sub>2</sub>. The intestinal phase duration was then launched for 300 min maximum by  
138 adding 4.86 mL of simulated intestinal fluid (SIF) and 100 µL of 1M NaCO<sub>3</sub> to reach a final  
139 pH of 6.5. The SIF solution contained 30.8 g L<sup>-1</sup> of bile extract powder, 0.82 g L<sup>-1</sup> of  
140 pancreatin (activity: 8×USP specifications), 0.41 g L<sup>-1</sup> of pancreatic lipase (corresponding to  
141 100 U per mL of SIF using olive oil as the substrate), and the same electrolyte concentrations  
142 as the SGF.

143 Samples were taken at t = 0 min of the intestinal phase using a modified SIF that contained all  
144 constituents except pancreatin and lipase, and at 15, 30, 60, 120 and 300 min of intestinal  
145 digestion. One vial was used for one sampling time and one type of measurement  
146 (quantification of LCT and MCT by HPLC, quantification of the lipolysis products within the  
147 bile salt micellar phase by GC, or droplet size by laser granulometry) so that the contents of  
148 18 vials in total were analyzed (3 methods times 6 sampling times) for one digestion. Three  
149 independent digestion experiments, further denoted as replicates, were performed.

150

### 151 2.3. Quantification of LCT and MCT by HPLC

152 HPLC paired with an evaporative light scattering detector (ELSD) was used to quantify the  
153 decrease in both LCT and MCT masses during the time course of the *in vitro* digestions. Total  
154 lipids were extracted according to Bligh and Dyer method with minor modifications in the



155 ratio CHCl<sub>3</sub>/CH<sub>3</sub>OH/H<sub>2</sub>O 1/2/1, and dissolved in CHCl<sub>3</sub> to obtain a final lipid concentration  
 156 of 0.7 mg mL<sup>-1</sup>. HPLC operating conditions were similar to those described in Kenmogne-  
 157 Domguia, Meynier, Viau, Llamas, & Genot (2012) using a Uptip-prep Strategy column (2.2  
 158 μm SI, 150×4.6 mm, Interchim, Montluçon, France) and 30 μL of injected lipid extract. The  
 159 signal of LCT and MCT (retention times of 1.21 and 1.32 min, respectively) were  
 160 deconvoluted using a specifically developed algorithm running with the Matlab™ software  
 161 (The MathWorks Inc., Natick, MA), and converted into masses using a pre-established  
 162 calibration curve. LCT and MCT masses were finally converted into lipolysis percentages  
 163 using Equation 1:

$$lipolysis(t) = \frac{m_{TAG_0} - m_{TAG}(t)}{m_{TAG_0}} \times 100\% \quad (1)$$

164 where  $m_{TAG_0}$  and  $m_{TAG}(t)$  are the masses (mg) of LCT or MCT initially present in the vials  
 165 and measured by HPLC at time  $t$ , respectively.

166

#### 167 2.4. Droplet size measurement

168 The volume-based distribution of oil droplet sizes was measured using a Mastersizer S  
 169 (Malvern Instruments Ltd., Worcestershire, UK) equipped with a 2 mW He-Ne laser of  $\lambda =$   
 170 633 nm and the 300RF lens with detection limits of 0.05 and 900 μm. The refractive index  $n_0$   
 171 of the aqueous phase was 1.33 and the properties of the dispersed phase were 1.457 for the  
 172 refractive index and 0.001 for the absorption. Samples were diluted with distilled water to  
 173 reach an oil volume concentration near 0.01% (w/w) for the circulation in the measurement  
 174 cell. The surface weighted mean diameter,  $d_{32}$ , was calculated using Equation 2:

$$d_{32} = \frac{\sum n_i^3 d_i^3}{\sum n_i^2 d_i^2} \quad (2)$$

175 where  $n_i$  is the number of droplets of diameter  $d_i$ .

176

177 2.5. Quantification of lipolysis products within the bile salt micellar phase by GC  
178 Intestinal aliquots were frozen immediately after sampling. The day after, they were  
179 centrifuged at 50,000 rpm for 45 min at 4°C with Aventi J26-XP (Beckman-Coulter). Three  
180 phases were observed after centrifugation: a lipidic phase at the top, a pellet at the bottom,  
181 and a major intermediate phase containing the bile salt mixed micelles and which will be  
182 further referred as the micellar phase. 500 µL of the micellar phase were sampled for the  
183 determination of its FA composition by direct trans-methylation as described previously by  
184 Berton, Genot, & Ropers (2011). Briefly, 500 µL of the micellar phase was trans-methylated  
185 in presence of 100 µL of internal standard (heptadecanoic acid 1mg/mL in acetone/MeOH 2/1  
186 v/v). 2 mL of methanol and 400 µL of H<sub>2</sub>SO<sub>4</sub> were then introduced. The mixture was mixed;  
187 the tube screw capped and then heated à 100°C for 1 h. After cooling at room temperature,  
188 500 µL of water and 1 mL of cyclohexane were added. After mixing, and decantation, the  
189 organic phase was removed for later analysis by GC. Fatty acid methyl ester (FAME)  
190 analyses were performed on a gas chromatograph paired with a splitless injector and a FID  
191 (Clarus, Perkin Elmer). They were separated on a capillary column (DB 225, 30 m X 0.25  
192 mm, film thickness 0.25 mm) after splitless injection. The carrier gas (H<sub>2</sub>) was set at 2 m min<sup>-1</sup>  
193 <sup>1</sup>. Temperature gradient was programmed as follows: 50°C for 3 min, increase to 180°C at  
194 15°C min<sup>-1</sup>, 7 min at 180°C, increase to 220°C at 10°C min<sup>-1</sup> and finally 10 min at 220°C. The  
195 temperature of the injector and the detector were maintained at 250°C. Individual FA were  
196 identified by comparison of their retention time with those of standards FAME mix (cat  
197 47885-U 37, Supelco); n-3 PUFA from menhaden oil (cat 4-7085 U-14, Supelco), and marine  
198 oil standard of the AOCS. Peak surfaces were integrated and corrected by response factors of  
199 individual fatty acids.

200 The FA composition of the native emulsion was also quantified by GC. As summarized in  
201 Table 1, 8 fatty acids representing 95.6% of the total FA mass, were considered for the

202 modeling approach: C8:0, C10:0, C12:0, C14:0, C16:0, C16:1 n-7, C18:1 n-9, and C22:6 n-3.  
203 These 8 FA showed coefficients of variation of less than 10.26% over the three replicate  
204 measurements. Since the emulsion was prepared from a mixture of two oils, the last 2  
205 columns of Table 1 provide the fractional contributions of LCT ( $\alpha_{FA_i}$ ) and MCT ( $1-\alpha_{FA_i}$ ) for  
206 each FA residue. Results show that almost all C8:0 and C10:0 came from the MCT oil,  
207 whereas all the other fatty acids came essentially from LCT. These fractions will be used for  
208 TAG mass calculations in the model.

209

### 210 **3. Mathematical modeling**

#### 211 3.1. Model assumptions and equations

212 The model was built to predict the lipolysis kinetics and the concentration profiles of the  
213 lipolysis products in the bile salt micellar phase, knowing that pancreatic lipase produces two  
214 FFA and one *sn*-2-MAG per TAG molecule. However, if the GC analyses of the samples  
215 make it possible to quantify the different acyl chains, they cannot be used to discriminate their  
216 molecular origin, *i.e.* free or esterified fatty acids. In the rest of this paper, the term fatty acid  
217 (FA) will therefore refer to FA carbon chains in any kinds of molecular products. The main  
218 modeling assumptions were as follows:

219 A1: the hydrolysis rate of a given fatty acid residue  $FA_i$  was independent of the *sn*-  
220 position within TAG molecules, with  $FA_i$  referring to the fatty acid considered (C8:0, C10:0,  
221 C12:0, C14:0, C16:0, C16:1 n-7, C18:1 n-9, or C22:6 n-3).

222 A2: the hydrolysis rate of a given fatty acid residue  $FA_i$  was proportional to the  
223 interfacial area occupied by this acyl chain.

$$\frac{dm_{FA_i}^{lip}(t)}{dt} = -k_{FA_i} \cdot A_{FA_i}(t) \quad (3)$$

224 where  $m_{FA_i}^{lip}$  is the mass of  $FA_i$  in the oil droplets (mg),  $k_{FA_i}$  is the lipolysis rate constant of  
 225  $FA_i$  ( $\text{mg m}^{-2} \text{min}^{-1}$ ), and  $A_{FA_i}(t)$  is the interfacial area occupied by  $FA_i$  ( $\text{m}^2$ ) at time  $t$  (min).

226 A3: the interfacial area occupied by  $FA_i$  at time  $t$  was assumed to be proportional to its  
 227 molar fraction within the oil droplets.

$$A_{FA_i}(t) = \frac{n_{FA_i}^{lip}(t)}{\sum_i n_{FA_i}^{lip}(t)} \cdot A_{TAG}(t) \quad (4)$$

228 where  $n_{FA_i}^{lip}(t)$  is the number of moles of  $FA_i$  in the oil droplets (mol) at time  $t$  and  $A_{TAG}(t)$  is  
 229 the interfacial area of oil droplets ( $\text{m}^2$ ) at time  $t$ . This equation can be rewritten as

$$A_{FA_i}(t) = \frac{\frac{m_{FA_i}^{lip}(t)}{M_{FA_i}}}{\sum_i \frac{m_{FA_i}^{lip}(t)}{M_{FA_i}}} \cdot A_{TAG}(t) \quad (5)$$

230 where  $M_{FA_i}$  is the molar mass of  $FA_i$  ( $\text{g mol}^{-1}$ ).

231 A4: oil droplets in the emulsion were considered as spheres. The droplet interfacial area  
 232 is thus:

$$A_{TAG}(t) = 6 \cdot \frac{m_{TAG}(t)}{\rho \cdot d_{32}(t)} \quad (6)$$

233 where  $m_{TAG}(t)$  is the total mass (mg) of TAG at time  $t$ ,  $\rho$  is the mean mass density of the  
 234 TAG (taken as  $0.92 \text{ mg mm}^{-3}$ ) and  $d_{32}(t)$  is the surface weighted mean droplet diameter  
 235 measured by laser diffraction (nm).

236 Combining assumptions A1-A4, Eq. (3) can be rewritten:

$$\frac{dm_{FA_i}^{lip}}{dt} = -k_{FA_i} \frac{\frac{m_{FA_i}^{lip}(t)}{M_{FA_i}}}{\sum_i \frac{m_{FA_i}^{lip}(t)}{M_{FA_i}}} \cdot 6 \cdot \frac{m_{TAG}(t)}{\rho \cdot d_{32}(t)} \quad (7)$$

237

238 A5: our experimental results show that only a fraction of hydrolyzed products were  
 239 transferred into the bile salt micellar phase. To calculate the masses of  $FA_i$  within this phase,  
 240 a “micellar fraction”,  $f_{FA_i}^{mic}$ , was therefore introduced with the assumption that the transfer of  
 241 lipolysis products into the micellar phase is instantaneous:

$$\frac{dm_{FA_i}^{mic}}{dt} = -f_{FA_i}^{mic} \frac{dm_{FA_i}^{lip}}{dt} \quad (8)$$

242 where  $m_{FA_i}^{mic}$  is the mass of  $FA_i$  in the micellar phase (mg).

243 The micellar fraction  $f_{FA_i}^{mic}$  and the lipolysis rate  $k_{FA_i}$  are the unknown parameters of the  
 244 model. They were estimated for each  $FA_i$  by adjusting the model to the experimental data (see  
 245 section 3.2). To this end, model predictions for TAG masses were calculated as follows.

246 The total TAG mass,  $m_{TAG}(t)$ , is the sum of the medium chain TAG (MCT) and long chain  
 247 TAG (LCT) masses:

$$m_{TAG}(t) = m_{MCT}(t) + m_{LCT}(t) \quad (9)$$

248 where  $m_{MCT}(t)$  and  $m_{LCT}(t)$  are the MCT and LCT masses (mg) at time t, respectively,  
 249 calculated according to their fractional mass contributions ( $\alpha_{FA_i}$  and  $1 - \alpha_{FA_i}$ ) shown in Table  
 250 1 and by adding the adequate mass of glycerol moiety:

$$m_{MCT}(t) = \sum_i m_{FA_i}^{lip}(t) \cdot (1 - \alpha_{FA_i}) + m_{Glycerol_{MCT}}(t) \quad (10)$$

$$m_{LCT}(t) = \sum_i m_{FA_i}^{lip}(t) \cdot \alpha_{FA_i} + m_{Glycerol_{LCT}}(t) \quad (11)$$

251 where  $m_{Glycerol_{LCT}}(t)$  and  $m_{Glycerol_{MCT}}(t)$  are the masses of glycerol moiety coming from  
 252 MCT and LCT, respectively. They were calculated via a molar balance equation by  
 253 considering that there is one mole of glycerol moiety per three moles of  $FA_i$ .

254

255 3.2. Parameter estimation

256 The differential equations of the model (Eq. 7 and Eq. 8) were solved numerically using  
257 Matlab<sup>®</sup> software (The MathWorks Inc., Natick, MA) equipped with the Statistics Toolbox.  
258 The surface weighted mean diameter  $d_{32}(t)$  was integrated into the model using linear  
259 interpolations of the experimental values. Model outputs, namely the evolution of the MCT  
260 and LCT masses and masses of hydrolyzed products within the micellar phase, were  
261 confronted to the corresponding experimental results measured by HPLC and GC,  
262 respectively, to estimate the unknown model parameters:  $k_{FA_i}$  and  $f_{FA_i}^{mic}$ . Standard errors,  
263 coefficients of variation and 95% confidence intervals of the estimated parameter values were  
264 computed using the nonlinear regression software in the Matlab<sup>®</sup> Statistics Toolbox and it  
265 was checked that estimated values were statistically significant at 0.05 level.

266

## 267 **4. Results and Discussion**

### 268 4.1. Experimental results

269 Results related to the evolution of droplet sizes during the gastric and intestinal phases were  
270 described in detail previously (Giang et al., 2015). It was found that droplet flocculation took  
271 place during the gastric phase but these aggregates were re-dispersed after the subsequent  
272 addition of bile. Fig. 1 summarizes the results related to the evolution of oil droplet sizes  
273 ( $d_{32}$ ) during the intestinal phase together with the corresponding TAG lipolysis kinetics. Fig.  
274 1A shows that the lipolysis rate was much higher for MCT than for LCT as the hydrolysis of  
275 MCT was completely finished after 15 min, whereas about 20% of LCT could still be  
276 detected after 300 min of intestinal digestion. In good agreement with many studies (Li &  
277 McClements, 2010; Ye et al., 2013; Zhu, Ye, Verrier, & Singh, 2013), the LCT lipolysis  
278 curve also showed two stages with a fast initial reaction rate that markedly slowed down after  
279 about 30 min. As previously shown in (Giang et al., 2015), this can be attributed to the sudden  
280 increase of the surface weighted mean diameter induced by the coalescence of oil droplets,

281 and which led to a sharp reduction of the interfacial area available for lipase adsorption (Fig.  
282 1B).

283 Fig. 2 presents the bioaccessibility of the 8 fatty acids considered, *i.e.* the lipolysis product  
284 concentrations measured within the micellar phase, expressed as percentages of FA initially  
285 present in the oil phase. Three main observations can be made. First, fatty acids can be  
286 divided into two groups according to the evolution of their bioaccessibility. The first group  
287 contains the FA arising from lipolysis of MCT, C8:0 and C10:0. They were released more  
288 quickly than the second group which corresponds to the FA arising from the lipolysis of LCT  
289 (Table 1). One may also notice that the long chain FA showed two-stage shapes with a slower  
290 rate of appearance within the micellar phase after about 30 min. These results are therefore  
291 consistent with the MCT and LCT lipolysis kinetics deduced from the HPLC measurements  
292 (Fig. 1A). Second, both the rate of appearance and the concentration measured at the end of  
293 the experiment (300 min) depended on the FA considered. The rate and extent of FA  
294 appearance in the micellar phase tended to be higher for shorter FA. This confirms that all  
295 fatty acids do not behave in the same way in terms of lipolysis kinetics and/or incorporation  
296 into the bile salt micelles. Finally, since MCT were fully hydrolyzed in less than 15 min  
297 according to the HPLC measurements (Fig. 1A), the plateau value of about 65% for C8:0 and  
298 C10:0 (Fig. 2) demonstrates that only a fraction of hydrolyzed products was recovered within  
299 the micellar phase. This is in accordance with the study of Sek, Porter, Kaukonen, & Charman  
300 (2002) who showed that lipolysis products can also be found in the oil and pellet phases after  
301 the centrifugation step used to separate the micellar phase.

302

#### 303 4.2. Modeling results

304 The model was designed to simulate the lipolysis kinetics as well as the bioaccessibility of  
305 individual FA, and considers 8 fatty acids representing 95.6% of the total FA mass. The

306 parameters to be estimated were the reaction rate constant ( $k_{FA_i}$ ) and the micellar mass  
307 fraction ( $f_{FA_i}^{mic}$ ) for each FA.

308 It appeared that the fatty acids coming from MCT, C8:0 and C10:0, were fully hydrolyzed in  
309 less than 15 min of intestinal digestion (Fig. 1A and Fig. 2), *i.e.* before the first sampling time.

310 The lipolysis rate constants,  $k_{C8}$  and  $k_{C10}$ , could therefore not be estimated due to lack of  
311 intermediate data. After preliminary model simulations, they were set at the minimum value  
312 enabling to reproduce a sufficiently fast MCT lipolysis, which was found to be  $10 \text{ mg m}^{-2}$   
313  $\text{min}^{-1}$ . According to the above considerations, 6 lipolysis rates  $k_{FA_i}$  and 8 micellar fractions  
314  $f_{FA_i}^{mic}$  remained to be determined. In a first attempt, all parameters were estimated by fitting  
315 the model to the experimental data. This model version provided very good fits (data not  
316 shown) but it appeared that the  $f_{FA_i}^{mic}$  values were similar for all FA except C8:0. In other  
317 words, the bile salt solubilization ratio of the lipolysis products was about the same for FA  
318 longer than C8:0. To reduce the number of unknown parameters, it was therefore decided to  
319 keep only two  $f_{FA_i}^{mic}$  parameters, one for C8:0 and the other one common to all the other FA.

320 Hence, the final version of the model had 6 unknown lipolysis rates  $k_{FA_i}$  and 2 unknown  
321 micellar fractions  $f_{FA_i}^{mic}$ . Fig. 3 shows that this model version still provided very good fits of  
322 the experimental data, and Table 2 shows that the parameter values were properly estimated  
323 (coefficients of variation smaller than 10%). These results are further discussed according to  
324 their biological meanings.

325

#### 326 4.3. Fractions of hydrolyzed products recovered within the bile salt micellar phase

327 Estimated values for the micellar fraction parameters (Table 2) can be interpreted as follows.

328 About 70% (w/w) of the C8:0 lipolysis products were released in the bile salt micellar phase,  
329 and about 56% for the other FA. The remaining amounts of products were retained in the oil  
330 and pellet phases during the centrifugation step. The order of magnitude of these values is



331 consistent with studies in which centrifugation was also employed to measure the micellar  
332 content in lipolysis products (Sek et al., 2002). Note also that the model assumes an  
333 instantaneous transfer of the products into the micellar phase. The very good fits of the  
334 bioaccessibility kinetics (Fig. 3) obtained under this assumption therefore strongly suggest  
335 that the solubilization of the lipolysis products in the bile salt micelles was not rate limiting.  
336 Similar micellar fraction values for all the lipolysis products considered may seem in  
337 contradiction with previously published data. Several studies have indeed shown that lipolysis  
338 products behave differently in terms of bile salt solubility (Freeman, 1969; Hofmann, 1963),  
339 with molar saturation ratios that can vary from 0.07 up to 1.86 (Freeman, 1969). In fact, the  
340 experimental protocol used in these studies was quite different from an *in vitro* digestion  
341 protocol since mixtures of pure molecules were used to obtain these results. During the  
342 digestion of complex natural oils, a great number of interactions take place between the  
343 different lipolysis products and bile acids, and this can greatly modify the solubility of the  
344 products in the bile salt mixed micelles. In our study, the products/bile salts molar ratio was of  
345 about 1.32 at the end of the experiment, thereby indicating that the saturation ratio for our  
346 mixture of lipolysis products was even greater.

347 One may also wonder why the micellar fraction obtained for C8:0 is different from those  
348 obtained for the other FA products. This can probably be attributed to the greater water  
349 solubility of this fatty acid. Indeed, using the formula proposed by Tzocheva et al. (2012), it  
350 was found that C8:0 was the only free fatty acid with a significant water solubility ( $0.64 \text{ g L}^{-1}$ ).  
351 More precisely, within our experimental conditions, it can be estimated that about 6 mg of  
352 caprylic acid could dissolve if our reaction medium was made of pure water. This corresponds  
353 to 31% by weight of the total C8:0 mass introduced within the digestive tubes. According to  
354 our modeling results, a common micellar fraction of 56% provides good results for all FA  
355 except C8:0, for which a micellar fraction of 70% was estimated. The additional 14% for

356 C8:0 (70 = 56 + 14) are therefore compatible with the contribution of a water soluble fraction,  
357 knowing that the micellar phase is not pure water and that the total C8:0 mass accounts for  
358 both FFA and *sn*-2-MAG molecular forms. According to this interpretation, the solubility of  
359 the lipolysis products in the bile salt mixed micelles would therefore be approximately the  
360 same for all the FA considered, C8:0 included.

361

#### 362 4.4. FA specific lipolysis rate constants

363 The lipolysis rate constants estimated with the model are given in Table 2. The same data, but  
364 expressed as normalized values relatively to oleic acid, are also presented in Fig. 4 for  
365 comparison purposes with the available literature. We may first highlight the very good  
366 agreement of our results with those obtained by Berger & Schneider (1991) for C12:0, C14:0,  
367 C16:0 and C18:1n-9 in spite of very different experimental conditions. Indeed, if our results  
368 arise from the modeling of *in vitro* intestinal digestion of emulsified lipids made of a mixture  
369 of different TAG, Berger & Schneider (1991) used a protocol based on a lipase-catalyzed  
370 reaction in an organic solvent with a mixture of TAG that contained 3 identical acyl residues.  
371 We may also stress that, according to our results, C8:0 and C10:0 presented rate constants that  
372 were at least 3 times the one estimated for oleic acid (Table 2), hence confirming the early  
373 study of Desnuelle & Savary (1963) who found the highest pancreatic lipase activity for C4:0  
374 and reported a strong decrease in activity (by a factor of about 2) between C10:0 and C12:0.  
375 Altogether, our findings are therefore in agreement with the reported tendency of pancreatic  
376 lipase to be less active on longer saturated fatty acid residues. These variations are usually  
377 ascribed to substrate dependent catalytic lipase activity, although other reaction steps, such as  
378 interfacial or diffusive mass transfers of substrates and products, may play a role (Verger &  
379 de Haas, 1976; Verger, Mieras, & de Haas, 1973).

380 Besides, if DHA (C22:6 n-3) was not studied by Berger & Schneider (1991), slower rates of  
381 pancreatic lipolysis for long-chain polyunsaturated fatty acids have been reported by many  
382 authors (Akanbi et al., 2014; Bottino et al., 1967; Zhu et al., 2013). For instance, the lipolysis  
383 rate constant of DHA should be about 0.17 times that obtained for C18:1 n-9 according to  
384 Yang et al. (1990), and of about 0.6 times that obtained for C14:0 according to Mukherjee et  
385 al. (1993). These values are represented by a triangle and a circle in Fig. 4, respectively, to  
386 show that our estimated rate constant for DHA is consistent with these findings. This higher  
387 resistance of long-chain polyunsaturated fatty acids has been attributed to an inhibitory effect  
388 induced by the presence of a double bond near the carboxyl group (Akanbi et al., 2014;  
389 Bottino et al., 1967; Lawson & Hughes, 1988).

390

#### 391 4.5. Evolution of the TAG composition during lipolysis

392 Fig. 5 presents the oil-water interfacial areas occupied by each FA residue of TAG molecules  
393 as given by the model. They were calculated by assuming proportionality with their molar  
394 concentrations within the lipid phase (Eq. 4). Therefore, the evolution of these FA specific  
395 interfacial areas is also representative of the predicted changes of the oil droplet composition  
396 during the course of digestion. Results show that about 50% of the oil surface area was  
397 occupied by MCT fatty acid residues (C8:0 and C10:0) at the beginning of the experiment.  
398 After 15 min, MCT had totally disappeared (Fig. 1A) due to higher lipolysis rate constants for  
399 C8:0 and C10:0. This induced a considerable change in the composition of the remaining oil,  
400 with only long-chain fatty acid residues remaining. At the end of the experiment, DHA was  
401 by far the dominant FA residue (more than 70% by mole) because it had the lowest lipolysis  
402 rate constant (Table 2).

403 As a final remark, we may highlight that our previous model (Giang et al., 2015) assumed an  
404 average lipolysis rate for the entire LCT oil fraction, which somewhat overestimated the LCT

405 lipolysis rate at long times. By taking into account FA specific lipolysis rate constants, the  
406 newly developed model predicts an accumulation of most resistant FA residues in the  
407 remaining oil (Fig 5), which leads to a progressive decrease of the average reaction rate and a  
408 better agreement with experimental measurements of the remaining LCT at 300 min (Fig 3).

409

## 410 **5. Conclusion**

411 In the present study, our previous model (Giang et al., 2015) was extended to take into  
412 account the fatty acid composition of the oil substrate, and to enable the modeling of the *in*  
413 *vitro* bioaccessibility of the lipolysis products. The model provided very good fits of the  
414 experimental data and shows that the differences observed in the bioaccessibility kinetics of  
415 the studied FA originated from different FA lipolysis rate constants. It was also used to  
416 simulate the compositional evolution of the remaining oil during the course of digestion.  
417 Results related to the FA specific reaction rates were in good agreement with the available  
418 literature and confirm a general tendency towards a greater pancreatic lipase activity on  
419 shorter fatty acid residues. Our results also support the idea that lipolysis products are rapidly  
420 and equally solubilized (*i.e.* no fractionation) within the bile salt mixed micelles, with a  
421 probable additional contribution of water soluble products for fatty acids  $\leq$  C8. The present  
422 model provides a significant improvement in comparison with models which assume an  
423 average lipolysis rate with a better representation of the intestinal lipid digestion mechanisms.

424

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429 properties of food by sharing our knowledge on the digestive process (INFOGEST)’.

430

431 **Nomenclature**

<b><i>Symbols</i></b>	<b><i>Meaning</i></b>
$A_{TAG}$	Interfacial area of the oil droplets ( $m^2$ )
$A_{FA_i}$	Interfacial area occupied by the $i^{th}$ FA residue ( $m^2$ )
$d_{32}$	Surface weighted mean droplet diameter (nm)
$k_{FA_i}$	Lipolysis rate constant of the $i^{th}$ FA residue ( $mg\ m^{-2}\ min^{-1}$ )
$f_{FA_i}^{mic}$	Micellar mass fraction of the $i^{th}$ FA
$M_{FA_i}$	Molecular mass of the $i^{th}$ FA ( $g\ mol^{-1}$ )
$m_{TAG}$	Mass of TAG (mg)
$m_{FA_i}^{lip}$	Mass of the $i^{th}$ FA residue in oil droplets (mg)
$m_{FA_i}^{mic}$	Mass of the $i^{th}$ FA in micellar phase (mg)
$n_{FA_i}^{lip}$	Quantity of the $i^{th}$ FA residue in oil droplets (mmol)
$n_{FA_i}^{mic}$	Quantity of the $i^{th}$ FA in aqueous phase (mmol)
$t$	Time (min)
$\rho$	Average mass density of TAG ( $mg\ mm^{-3}$ )
$\alpha$	Fraction of FA residue coming from LCT
$1-\alpha$	Fraction of FA residue coming from MCT

432

433

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508



509 **Figure Captions**

510

511 **Fig 1.** Lipolysis of MCT (squares) and LCT (triangles) during intestinal digestion (A).  
512 Surface weighted mean diameters,  $d_{32}$ , (circles) and interfacial areas (diamonds) of oil  
513 droplets (B). Solid lines are guide for the eyes. Means and standard deviations (smaller than  
514 symbol size) were calculated over 3 replicates.

515

516 **Fig 2.** Bioaccessibility of individual lipolysis products (lines are guides for eyes). Means and  
517 standard deviations were calculated over 3 replicates.

518

519 **Fig 3.** Comparison between calculated and experimental evolutions of the MCT, LCT, and  
520 bioaccessible product masses during intestinal digestion. Symbols are means over 3 replicated  
521 experiments (squares for MCT, triangles for LCT). Solid lines show the mass evolution  
522 calculated using the model. Dotted lines show the asymptotic values calculated using the  
523 model.

524

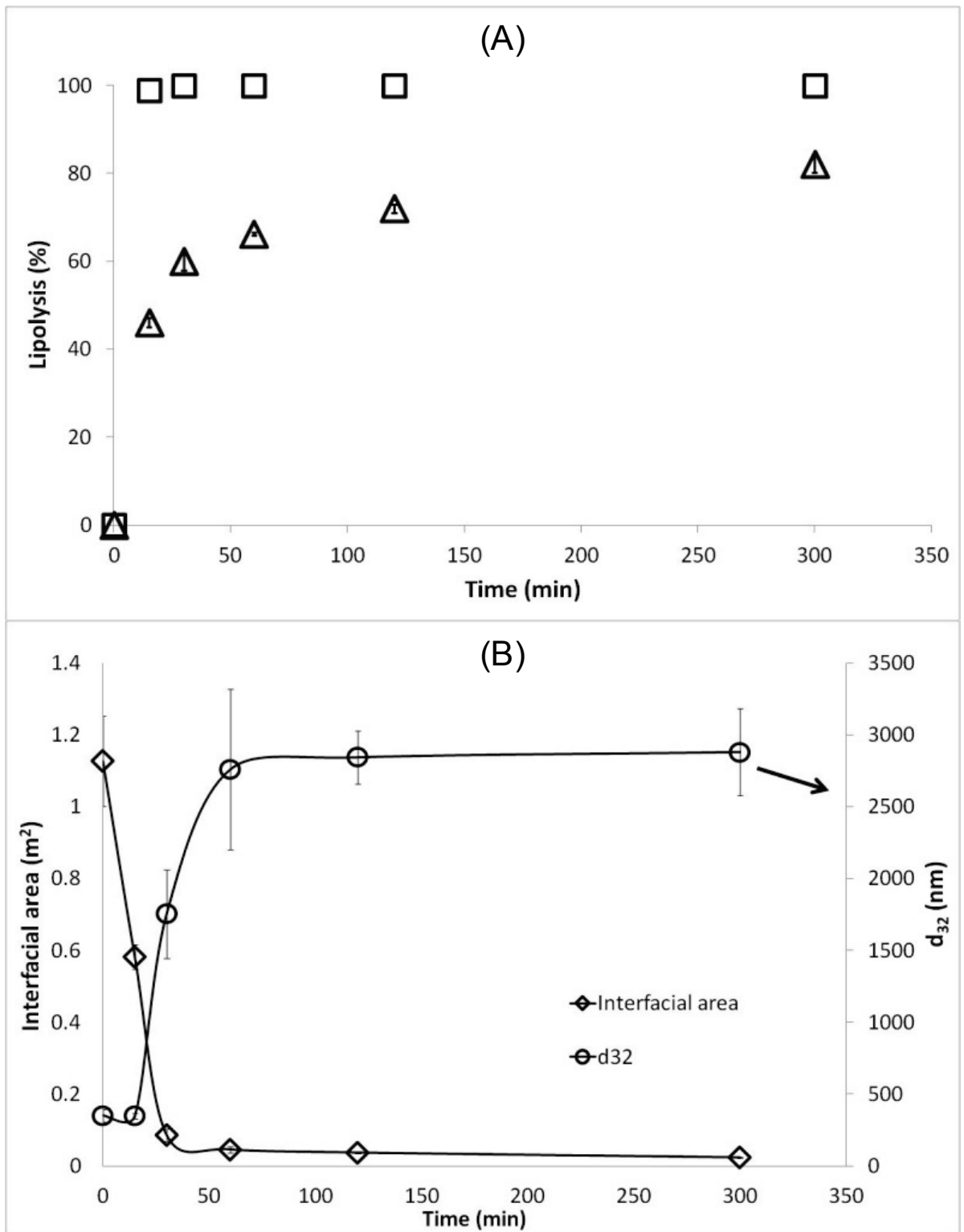
525 **Fig 4.** Lipolysis rate constants for the different fatty acid residues according to the carbon  
526 chain length (expressed as normalized values relatively to C18:1 n-9). Crosses represent the  
527 values determined in this study by model fitting. Squares correspond to values reported by  
528 Berger & Schneider (1991). The circle and the triangle for C22:6 n-3 were estimated from the  
529 studies of Mukherjee *et al.* (1993) and Yang *et al.* (1990), respectively.

530

531 **Fig 5.** Evolution of the interfacial areas (normalized values) occupied by each fatty acid  
532 residue during intestinal digestion (A). Zoom on the first 15 min (B).

533

534 **Figure 1**

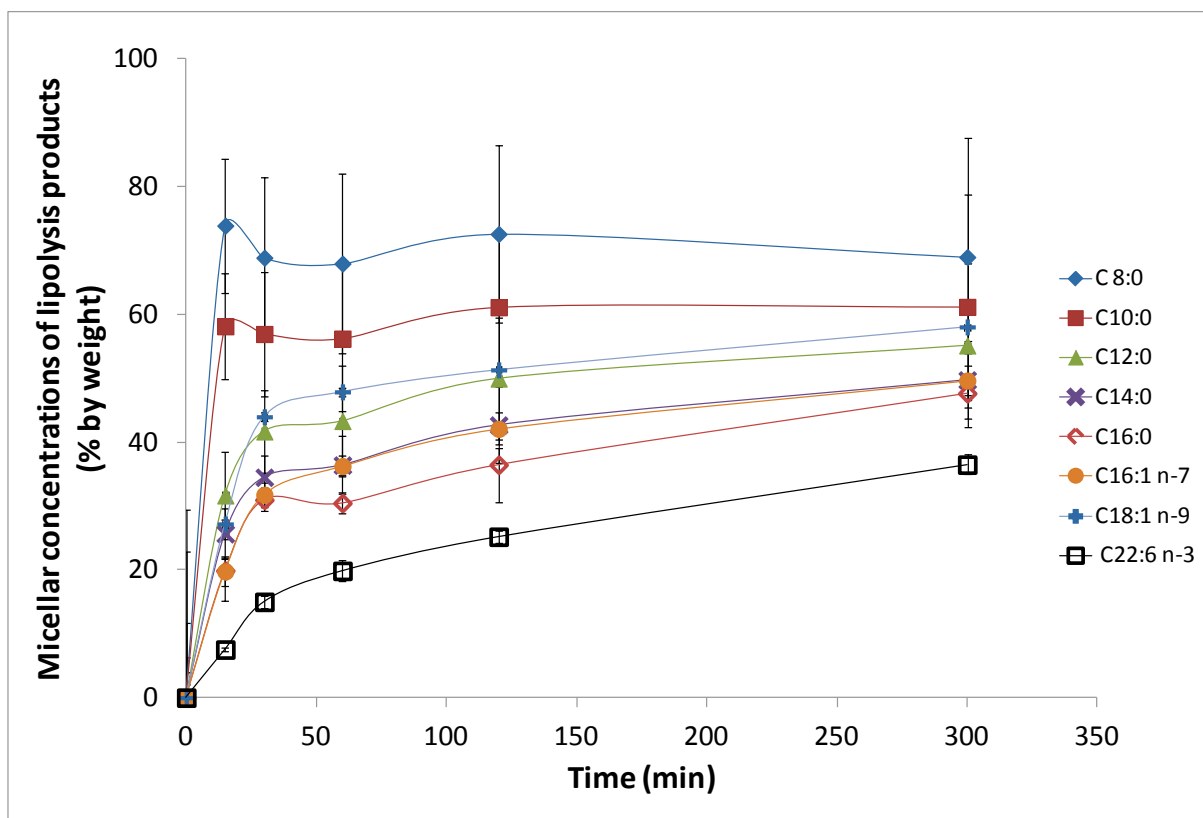


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538 **Figure 2**

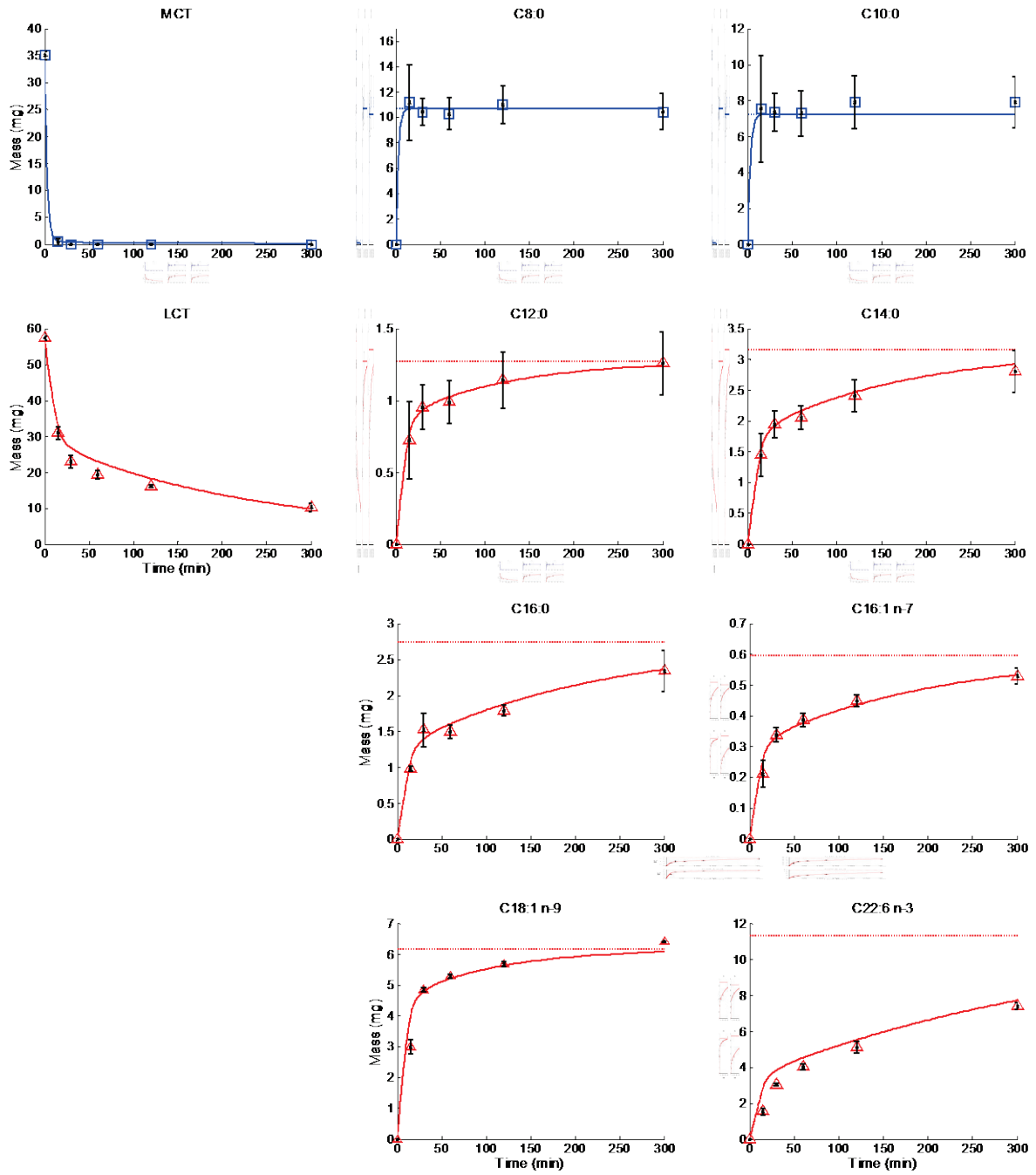


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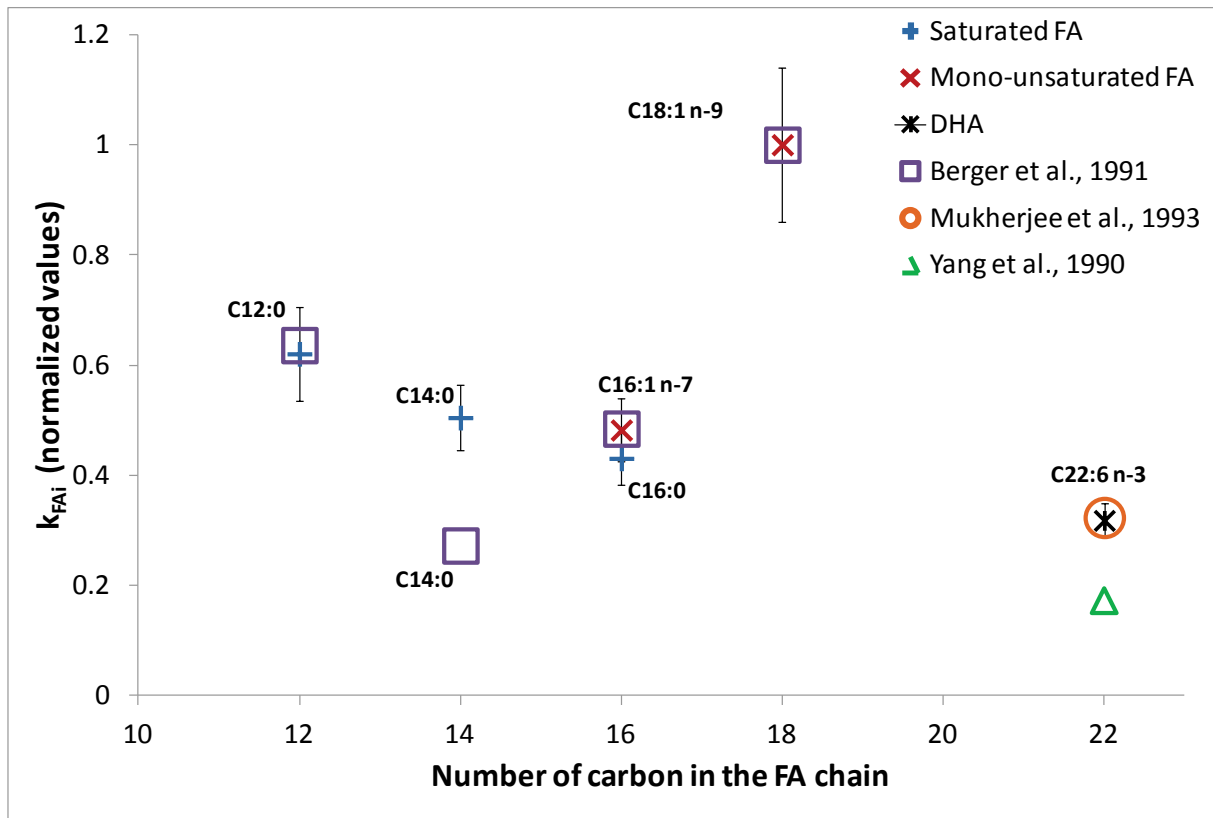
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543 **Figure 3**



544  
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546 **Figure 4**

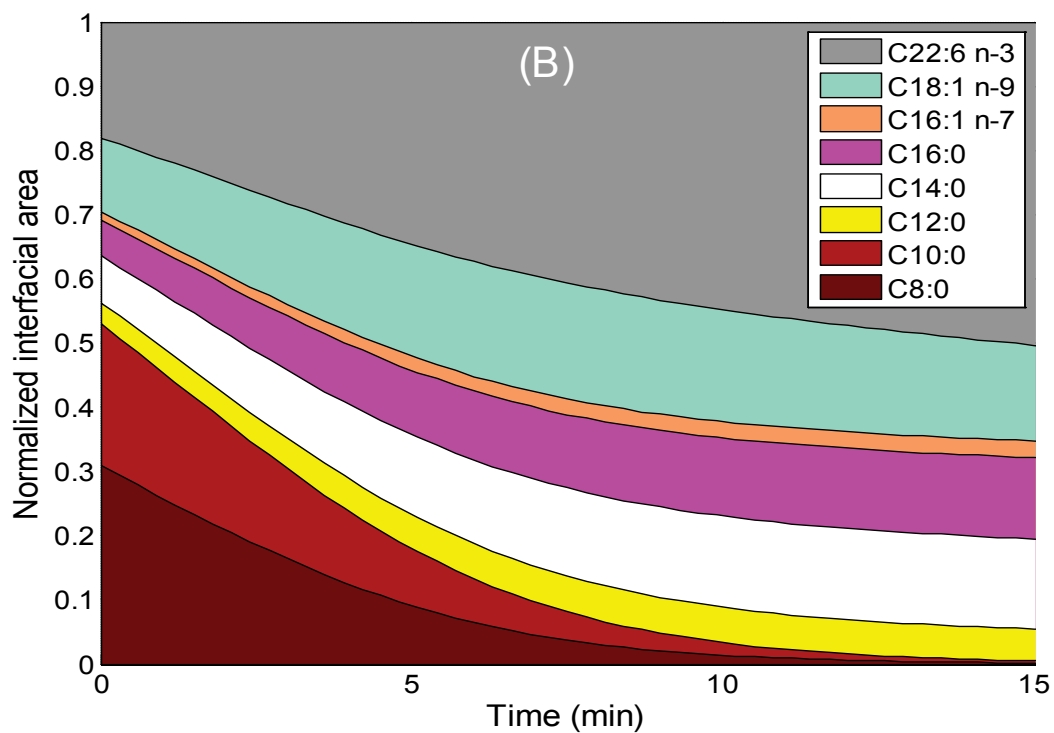
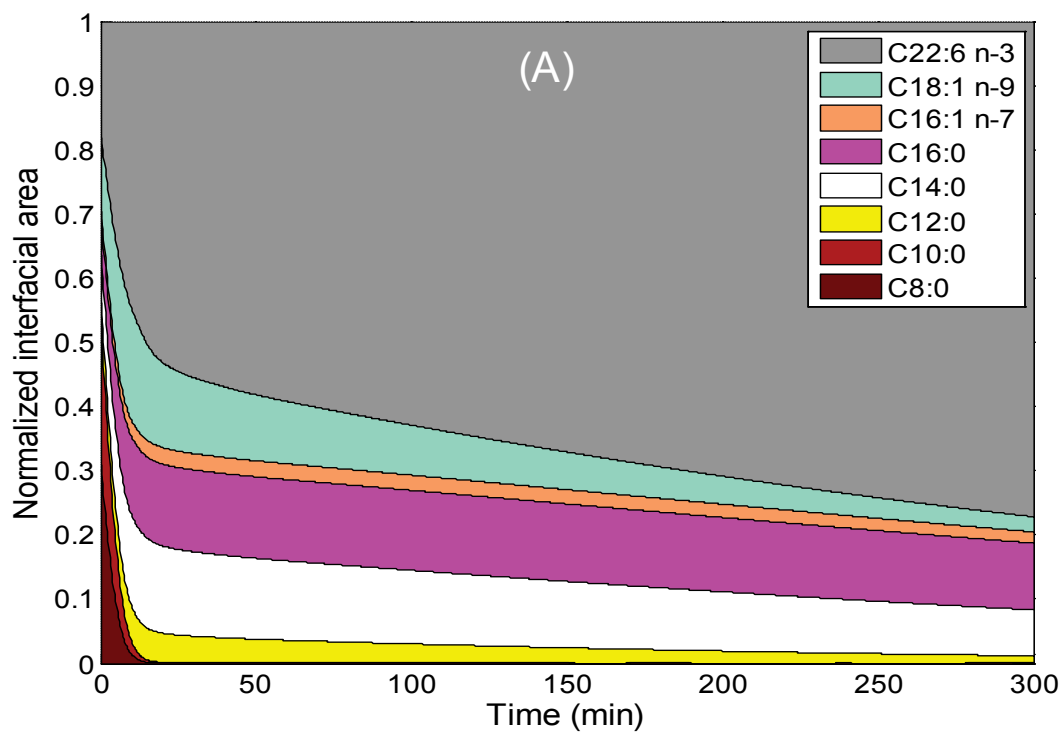


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549

550 **Figure 5**



551

552 **Table 1.** Fatty acid composition of the native emulsion which was made of a mixture of MCT  
 553 and LCT (37.5 and 62.5 % by weight, respectively).

Fatty acid	TAG composition		Origin of each FA	
	Mean of 3 replicates (% by weight)	STD of 3 replicates (% by weight)	Fraction coming from LCT $\alpha_{FA_i}$	Fraction coming from MCT $1-\alpha_{FA_i}$
C8:0	19.76	0.71	0.02	0.98
C10:0	16.89	0.33	0.05	0.95
C12:0	2.98	0.20	0.93	0.07
C14:0	7.36	0.09	0.97	0.03
C16:0	6.40	0.13	0.92	0.08
C16:1 n-7	1.39	0.07	0.99	0.01
C18:1 n-9	14.41	0.14	0.98	0.02
C22:6 n-3	26.47	0.10	1.00	0.00
Other FA	4.31	1.72	0.89	0.11

554

555

556

557 **Table 2.** Results of the parameter estimation for the lipolysis rate  $k_{FA_i}$  and the micellar  
 558 fraction  $f_{FA_i}^{mic}$  for each fatty acid.

Fatty acid	$k_{FA_i}$ ( $\text{mg min}^{-1} \text{m}^{-2}$ )		$f_{FA_i}^{mic}$	
	Value	STD	Value	STD
C8:0	10*		0.70	0.01
C10:0	10*		0.56	0.01
C12:0	1.93	0.16		
C14:0	1.57	0.11		
C16:0	1.34	0.10		
C16:1 n-7	1.50	0.11		
C18:1 n-9	3.11	0.25		
C22:6 n-3	0.99	0.06		

559 (\*) minimum value providing a complete hydrolysis of MCT in 15 min.

560