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1 **Chemical modification of edible sodium caseinate: a new grafting method of oleic acid.**

2 **Characterization and thermal properties of the conjugate.**

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19  
20  
21 ***Highlights***

22  
23 ***A new synthesis method for grafting oleic acid onto sodium caseinate is described.***

24 ***A fine characterization of the conjugate is developed, especially by DOSY NMR.***

25 ***A high substitution degree (24 wt%) is obtained, measured by <sup>1</sup>H NMR.***

26 ***The thermal properties of the conjugate are evaluated and discussed.***

27

28

## 29 **Abstract**

30 Sodium caseinate is a well-known amphiphilic protein derived from natural products currently  
31 used for the preparation of edible films. To improve some properties, especially to decrease the  
32 hydrophilicity and water solubility of the caseinate, the covalent grafting of a hydrophobic  
33 edible fatty acid, namely oleic acid, onto caseinate, appears to be a solution. We describe a new  
34 synthesis method for the chemical modification of sodium caseinate involving the synthesis of  
35 an acid chloride derivative from oleic acid and a phase transfer catalysis reaction in a biphasic  
36 medium. Under these conditions, free amine and alcohol groups of the caseinate are likely to  
37 be grafted with a fairly high (>50%) substitution degree. The caseinate derivative is finely  
38 characterized, in particular by DOSY NMR, to assess the formation of a casein/oleic acid  
39 grafted compound as well as the absence of residual oleic acid.

40

41 *Keywords*

42

43 *Casein, sodium caseinate, oleic acid, grafting, covalent conjugate.*

44

## 45 **1. Introduction**

46

47 Sodium caseinate (SC) is a natural abundant and inexpensive amphiphilic protein widely used  
48 in food industry for its nutritional and functional properties (Atamer et al., 2017; Chen et al.,  
49 2019; Damin et al., 2009; Goff et al., 1989; Nascimento et al., 2020; Schou et al., 2005), as  
50 protector of compounds against thermal degradation (Sáiz-Abajo et al., 2013), as carrier for  
51 delivery of hydrophobic bioactive drugs (Livney, 2010; Rehan et al., 2019; Semo et al., 2007;  
52 Tavares et al., 2014) or to form packaging films (Khan et al., 2021; Wusigale et al., 2020).

53 Systems based on caseinate self-assemblies are non-toxic, biocompatible, and biodegradable  
54 (Gil et al., 2017; Sahu et al., 2008).

55 However, SC presents some weaknesses in terms of mechanical properties: it forms a hard and  
56 brittle film, which is unacceptable for many applications especially for packaging. To overcome  
57 these problems, many plasticizers such as fatty acids (Abu Diak et al., 2007; Aliheidari et al.,  
58 2013; Brzoska et al., 2018; Fabra et al., 2009; Jiménez et al., 2013a), polyols like glycerol or  
59 sorbitol (Brzoska et al., 2018; McHugh & Krochta, 1994) or starch and cellulose  
60 (Arvanitoyannis et al., 1996; Arvanitoyannis & Biliaderis, 1998; Jiménez et al., 2013a, 2013b;  
61 Pereda et al., 2011; Ranjbaryan et al., 2019) are used. Unfortunately, in many cases a partial  
62 demixing of these non-compatible compounds was observed.

63 Efforts were made to modify the caseinate structure by grafting hydrophilic or hydrophobic  
64 moieties, depending on the desired applications. To increase hydrophilicity, polysaccharides  
65 such as dextran are grafted using the Maillard reaction between the aldehyde chain end of the  
66 dextran and the  $\epsilon$ -NH<sub>2</sub> of lysyl residues on the caseinate backbone (Ajandouz et al., 2008;  
67 Cardoso et al., 2011; Corzo-Martínez et al., 2010; Landy et al., 1997; Pan et al., 2006;  
68 Semenova et al., 2016; Shepherd, 2000). For other applications, such as films to protect tablets  
69 used in aqueous media, hydrophilicity must be reduced, because caseinate is sensitive to  
70 moisture due to its inherent hydrophilicity and tends to disintegrate during storage, especially  
71 in a humid atmosphere. Accordingly, it should be of interest to increase the hydrophobicity of  
72 the caseinate while maintaining its water solubility and biodegradability. Mixtures of SC and  
73 hydrophobic fatty acids are often used to reduce the hydrophilicity of SC. However, simple  
74 mixtures of hydrophobic oleic acid and SC are inconclusive, as there is a phase separation and  
75 as release of oleic acid is observed with time (Fabra et al., 2010, 2012). They show that OA is  
76 in a separate phase and no notable interactions with the caseinate matrix take place. The  
77 hydrogen bonds between the carboxylic acid and the amine functions are not strong enough to

78 maintain the stability of the complex. In general, a mixture of 2 non-compatible compounds  
79 can be made compatible by adding a conjugate containing these 2 compounds, especially in the  
80 case of polymers (Fortelný & Jůza, 2021; Koning et al., 1998). To avoid this phenomenon, a  
81 covalent grafting of fatty acids on SC was carried out. For example, grafting of stearic acid onto  
82 casein to make nanocomposites with improved mechanical properties has been proposed, but  
83 the effectiveness of the grafting process has not been clearly demonstrated (Sophia A & Malar  
84 Retna, 2018). Zhang *et al.* grafted C8-C16 fatty acids by a two-step synthesis, leading to an  
85 exclusive grafting of the fatty acids on the  $\epsilon$ -NH<sub>2</sub> functions of the lysyl residues of the caseinate  
86 (Zhang, Yang, et al., 2018; Zhang, Yao, et al., 2018). The grafting degrees vary between 72.9%  
87 (C<sub>8</sub> chain) to 11.8% (C<sub>16</sub> chain), depending on the number of carbon atoms of the fatty acids.  
88 Finally, the material must be industrially transformable, in particular to give usable films.  
89 In this paper, we describe a new synthetic method for grafting oleic acid (OA) on SC and the  
90 corresponding characterizations of the SC-OA conjugate. This two-step method involves the  
91 formation of an acyl chloride derivative by reaction of the fatty acid with thionyl chloride,  
92 followed by coupling of the formed acyl chloride on SC in a biphasic medium at room  
93 temperature. In this method, the free amine and alcohol/phenol groups of the caseinate are  
94 susceptible to reaction, unlike with Zhang's method in which only the amine groups are  
95 involved (Zhang, Yao, et al., 2018). The advantage is a drastic increase in the amount of OA  
96 grafted on SC (grafting degree = 56%). Characterizations of the caseinate derivative were made  
97 by IRTF, <sup>1</sup>H and DOSY NMR, SEC, and thermal properties were studied by TGA and DSC.  
98 Special attention is paid to the actual ratio of OA grafting and verification of the absence of free  
99 OA, which has not been already clearly demonstrated in the literature.

100

## 101 **2. Materials and methods**

### 102 **2.1 Instrumentation:**

103 Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrophotometer  
104 using the attenuated total reflectance (ATR). Size exclusion chromatography (SEC) was  
105 performed on a Waters 515 (HPLC pump) equipment fitted with 2×300mm long 8 μm Polargel  
106 M columns coupled with a Waters 410 refractometric detector at 60 °C and a Waters 2996 PDA  
107 detector. The mobile phase is DMSO. Samples ( $\cong 5$  mg/mL) were filtrated through a 0.45 μm  
108 PTFE Millipore filter before injection.  $^1\text{H}$  NMR spectra were recorded at room temperature  
109 using an AMX300 Bruker spectrophotometer operating at 300 MHz. Deuterium oxide ( $\text{D}_2\text{O}$ )  
110 or deuterated dimethyl sulfoxide (DMSO- $d_6$ ) were used as solvents, and chemical shifts were  
111 expressed in ppm. Diffusion ordered spectroscopy (DOSY) experiments were performed on a  
112 Bruker Advance III spectrometer operating at 600 MHz using DMSO- $d_6$  as solvent. Sample  
113 concentrations were in the range 5-15 mg/mL. TGA thermograms were recorded on a TGA 2  
114 Star System (Mettler Toledo) with AL-crucibles (100 μL) under  $\text{N}_2$ . DSC thermograms were  
115 recorded on a DSC 3 Star System (Mettler Toledo) with AL-crucibles (100 μL) under  $\text{N}_2$ .

116

## 117 **2.2 Materials**

118 Thionyl chloride (reagent grade, 97%), tetrabutylammonium bromide (ACS reagent, >98.0%),  
119 dichloromethane (anhydrous, contains amylene as stabilizer,  $\geq 99.8\%$ ), chloroform ( $\geq 99\%$ ,  
120 anhydrous, contains amylene as stabilizer), NaOH (ACS reagent,  $\geq 97.0\%$ , pellets), HCl (ACS  
121 reagent, 37%), oleic acid (natural, FCC) and sodium caseinate (powder) were purchased from  
122 Sigma Aldrich.

123

## 124 **2.3 Methods**

### 125 ***2.3.1 Activation of oleic acid (OA)***

126 Thionyl chloride (10 mL, 0.14 mole) is poured under dry argon atmosphere into a round-  
127 bottomed flask and kept under mechanical stirring. OA (5.17 g,  $1.8 \cdot 10^{-2}$  mole) was added gently

128 *via* a pouring bulb. The mixture was then heated to 80°C for 30 min or held at ambient  
129 temperature for 1 hour. Thionyl chloride is then evaporated under reduced pressure. The oleyl  
130 chloride (OA-COCl) is characterized by FTIR and used without further purification.

131

### 132 ***2.3.2 Grafting of oleoyl chloride on sodium caseinate***

133 SC (10 g,  $4.2 \cdot 10^{-4}$  mol,  $M_n = 24\ 000$  g.mol<sup>-1</sup>) is dissolved in distilled water (250 mL) under  
134 stirring at 8000 RPM with an Ultra Turrax in a 2L beaker. Tetrabutylammonium bromide (4 g,  
135  $1.2 \cdot 10^{-2}$  mol) is added and pH is adjusted to 8 by adding a 2M NaOH solution. Dichloromethane  
136 (250mL) is then added, and the mixture is stirred at 13500 RPM with an Ultra Turrax for a few  
137 minutes. A solution of OA-COCl (10,34g,  $3.66 \cdot 10^{-2}$  mole) in dichloromethane (30mL) is then  
138 slowly added to the mixture for  $\cong$  30min under stirring at 13500 RPM. A 2M NaOH solution is  
139 simultaneously added to maintain the pH in the range 8-9. The entire mixture is then acidified  
140 to pH = 2-3 by the addition of a 2M HCl solution to precipitate the final compound. If a gel is  
141 formed, it could be broken by the addition of ethanol (150 mL). The suspension is then  
142 centrifuged at 5000 RPM for 5 minutes. The precipitate is filtrated, washed with methanol and  
143 then with THF to eliminate residual free OA, and dried under reduced pressure.

144

## 145 **3. Results and discussion**

### 146 **3.1 Syntheses**

#### 147 ***3.1.1 Activation of oleic acid***

148 Acyl chlorides are suitable reagents for the modification of amine and alcohol groups due to  
149 their high reactivity. For this reason, they are often preferred to the commonly used anhydrides,  
150 for example in the succinylation of caseinate (Santos & Tomasula, 2000). Ke *et al* described a  
151 synthesis of oleyl chloride by reaction of the carboxylic group of oleic acid with thionyl chloride  
152 (Ke *et al.*, 2011). In our case, we have adapted the protocol described by Ustariz-Peyret *et al.*

153 (Ustariz-Peyret et al., 2000) for poly lactic acid oligomers, in which an excess of thionyl  
154 chloride is used as solvent. When conducted at 80°C, the reaction is rapid (1/2 h), but a partial  
155 degradation of the double bond of OA was observed by <sup>1</sup>H NMR. Therefore, kinetics of the  
156 acylation at room temperature were studied by FTIR, thanks to the appearance of the acyl  
157 chloride band at 1800 cm<sup>-1</sup> and the disappearance of the carboxylic acid band at 1700 cm<sup>-1</sup>  
158 (Figure 1-SD). It is shown that the acylation is complete at room temperature after 1h. <sup>1</sup>H NMR  
159 spectrum of OA-COCl showed the total preservation of the double bond with the ratio:  
160 integration of ethylenic protons/integration of methyl protons  $\cong$  2/3 (Figure 2-SD).

161

### 162 *3.1.2 Grafting of oleoyl chloride on sodium caseinate*

163 A phase transfer catalysis (PTC) reaction was used for the grafting OA-COCl on SC, because  
164 this method is known to be rapid, even at room temperature when an acyl chloride is used (Van  
165 Den Berghe, H. et al., 2011). To our knowledge, this method has never been used for grafting  
166 reactions on SC. The biphasic medium is a mixture of methylene chloride (used to dissolve the  
167 OA-COCl) and water (used to dissolve the SC). The pH of the aqueous phase is maintained at  
168 8-9 by the addition of a saturated solution of NaOH because a basic pH is required to maintain  
169 the amine functions unprotonated during grafting. A phase transfer catalyzer, tetrabutyl  
170 ammonium chloride, is added in the aqueous phase. Rapid stirring (15000 rpm), necessary to  
171 obtain an important interface between the organic and aqueous phases, is obtained with an Ultra  
172 Turrax<sup>®</sup>. PTC is usually used for reaction of acyl chloride on amine groups, but alcohol groups  
173 are also able to react. A SC molecule contains about 30 free alcohol/phenol groups (from the  
174 threonine, tyrosine and serine units) and 14 free amine groups (from lysine) that are capable of  
175 reacting with the acyl chloride. In comparison with Zhang's method, where only amine groups  
176 can react, the number of reactive sites is therefore  $\cong$  3 times higher. The grafted caseinate (SC-

177 OA) is recovered at the interface water/dichloromethane. Residual free OA is eliminated by  
178 washings with THF.

179

## 180 **3.2 Characterization of SC-OA**

181 The characterization of SC-OA was assessed by FTIR, <sup>1</sup>H, <sup>13</sup>C and DOSY NMR (in deuterated  
182 DMSO), TGA, DSC and SEC.

### 183 **3.2.1 FTIR**

184 The progress of the reaction is followed by FTIR by the decrease of the acyl chloride band at  
185 1800cm<sup>-1</sup>. The FTIR spectrum of SC-OA (Figure 3-SD) shows the presence of peaks at 2920  
186 and 2854 cm<sup>-1</sup> (aliphatic C-H bonds) in good agreement with the presence of OA on the  
187 copolymer. If the newly formed amide functions cannot be identified due to the numerous  
188 amide functions of casein, a shoulder is observed around 1730 cm<sup>-1</sup>, characteristic of the  
189 presence of some ester functions absent on SC and OA. New amide functional groups cannot  
190 be observed because they are masked by the large number of amide groups in the caseinate.

191

### 192 **3.2.2 <sup>1</sup>H NMR and grafting degree.**

193 The FTIR results are confirmed by <sup>1</sup>H, <sup>13</sup>C and DOSY NMR analyses. The <sup>1</sup>H NMR spectra  
194 (Figure 1) show the presence of OA moiety in SC-OA, thanks to the peaks at 5.5 ppm (ethylenic  
195 protons of OA) and 1.2 ppm (methylene protons of OA), framed in blue in figure 1. The grafting  
196 ratio and the weight proportion of grafted OA were determined by <sup>1</sup>H NMR using the ratio:

197

198  $\alpha = \text{integration of peak at 5.5ppm (ethylenic protons of OA)}/\text{integration of peak at 0.8ppm}$   
199  $\text{(methyl protons of caseinate and OA)}$

200

201 Taking into account the presence of 2 ethylenic and 3 methyl hydrogen atoms in each molecule  
202 of OA, and of  $\cong 276$  hydrogen atoms from  $\cong 92$  methyl groups (from alanine, valine, leucine,  
203 isoleucine and threonine units) in a caseinate chain,  $\alpha = 2x/(3x+276)$ , x standing for the number  
204 of oleic segments on a caseinate chain.

205 Consequently, 
$$x = 276\alpha / (2 - 3\alpha)$$

206 The molar masses of OA and SC being respectively 282g and 22000g, the weight proportion of  
207 OA (y) is:

208 
$$y \cong 282x / (282x + 22000)$$

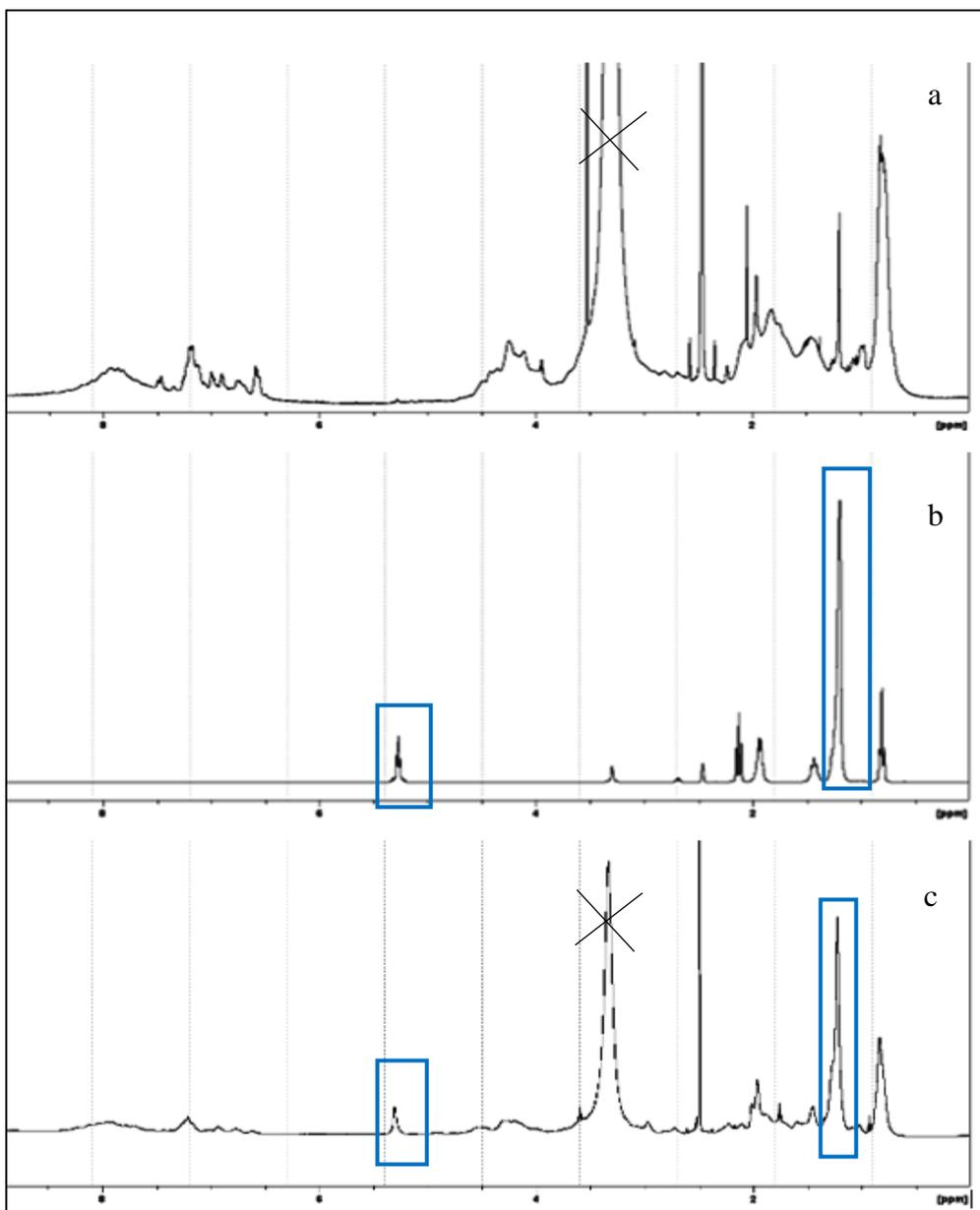
209 Taking into account the presence of the 14 amine groups and 30 alcohol/phenol groups in the  
210 caseinate, the grafting degree (GD) is:

211 
$$GD = x/44$$

212 The different parameters of the grafting reaction are summarized in

213 Table 1.

214



215

216 *Figure 1:  $^1\text{H}$  NMR spectra of a) SC ( $\text{D}_2\text{O}$ ), b) OA ( $\text{D}_6$  DMSO) and c) SC-OA( $\text{D}_2\text{O}$ ). Peaks at*  
 217 *5.5ppm (ethylenic protons of OA moiety) and 1.2 ppm (methylene protons of OA moiety) are*  
 218 *framed in blue*

219

220

*Table 1: Main parameters of the grafting reaction*

$\alpha$ (NMR integration ratio 5.5ppm/0.8ppm)	x (Number of OA segments in SC-OA)	y (Weight % of OA in SC-OA)	GD (Grafting degree of OA in SC-OA)
0.14	24.5	24	0.56

221

222 The value of GD (0.56) is particularly high compared to that obtained by the Zhang's method  
223 (11.8% grafting with a C16 fatty acid). In addition, Zhang's calculation only considers the 14  
224 amine functions of lysine, not the alcohols functions, which corresponds to a weight proportion  
225 of the oleic moiety of 2%.

226

### 227 **3.2.3 <sup>13</sup>C NMR**

228 <sup>13</sup>C NMR spectrum in DMSO (Figure 4-SD) confirmed the results of <sup>1</sup>H NMR: Characteristic  
229 peaks of OA, typically the ethylenic carbon atoms at 130 ppm, the methylene carbon atoms at  
230 22-35 ppm and the methyl protons at 13 ppm are found in SC-OA. (Given the rather low  
231 solubility of SC in DMSO, the spectrum of SC was expanded).

232

### 233 **3.2.4 DOSY NMR**

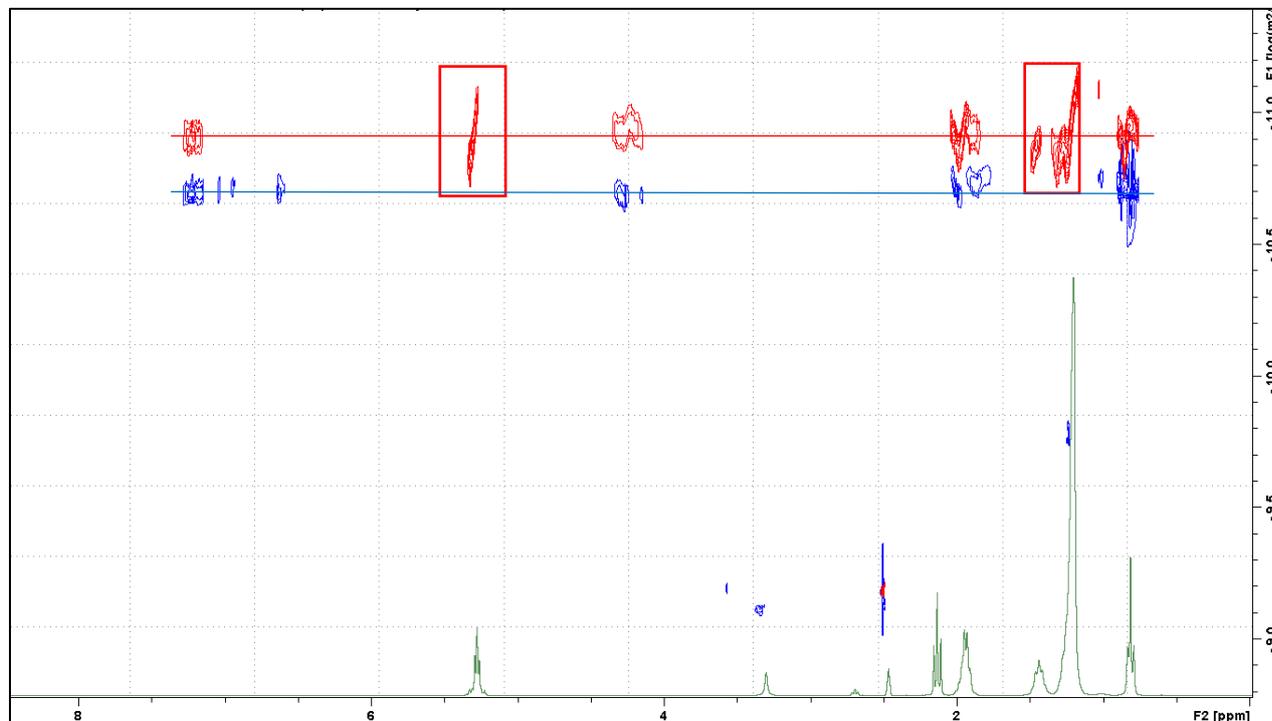
234 However, while FTIR, <sup>1</sup>H and <sup>13</sup>C NMR showed the presence of both SC and OA moieties in  
235 SC-OA, this cannot prove the actual grafting of OA onto SC, nor can it prove the presence of  
236 free OA or free SC in the SC-OA product, which may skew the results. To our knowledge, there  
237 is no method in the literature to discriminate between grafted and free OA in the final conjugate.  
238 The authors simply measured the number of residual amine functions by reacting the remaining  
239 lysine residues with OPA (O-phthalaldehyde) (Markman & Livney, 2012). For this purpose,  
240 we used DOSY NMR, which can distinguish SC (blue spectrum) and SC-OA (red spectrum)  
241 according to their different <sup>1</sup>H NMR spectra and viscosity diffusion coefficients (Figure 2).

242 DOSY spectra (D<sub>6</sub> DMSO) show that:

243 -Viscosity diffusion coefficient (VDC) of SC-OA (= -10.9 log m<sup>2</sup>/s) is lower than the  
244 one of SC (= -10.7 log m<sup>2</sup>/s), due to the higher molar mass of SC-OA.

245 -OA is clearly grafted onto the SC, as shown by the characteristic peaks of OA at 5.5,  
246 1.4 and 1.2 ppm (red frames) at the same VDC as SC-OA (-10.9 log m<sup>2</sup>/s).

247 -There is no detectable presence of free OA ( $VDC = -9.55 \log m^2/s$ ) in the copolymer.  
248 Therefore, values of grafting ratios calculated by  $^1H$  NMR are not distorted by the presence of  
249 any residual OA.

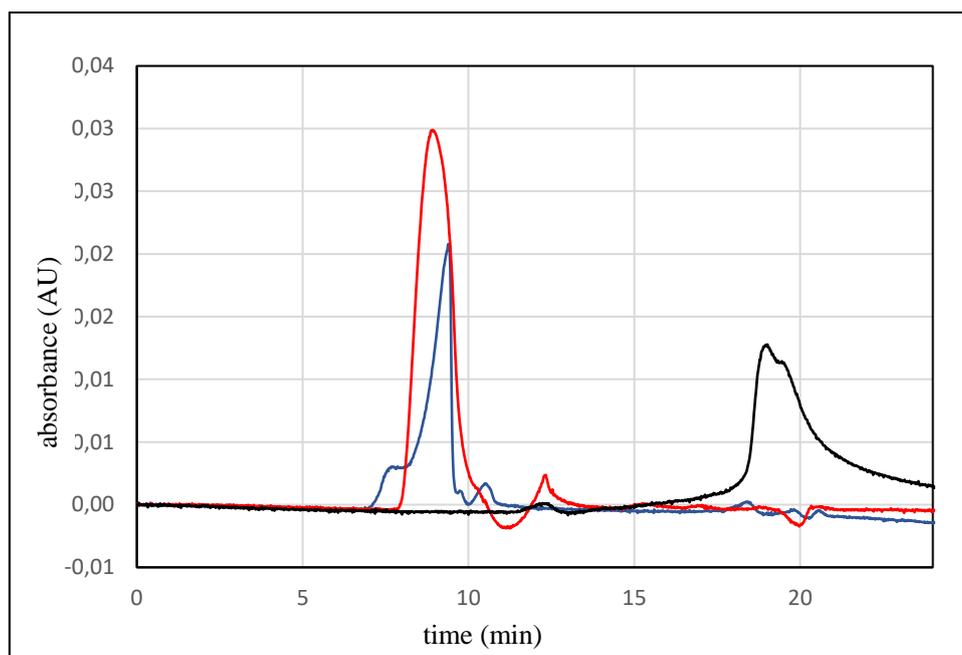


250  
251 *Figure 2: DOSY spectra of SC (blue), SC-OA (red) and the  $^1H$  NMR spectrum of OA (green).*  
252 *Characteristic peaks of OA part of SC-OA are framed in red*

253  
254 As a conclusion,  $^1H$  NMR,  $^{13}C$  NMR, and DOSY NMR spectra show that a OA-grafted  
255 caseinate is obtained, with a grafting ratio around 56 % and no detectable residual free OA.

### 256 257 **3.2.5 SEC**

258 SEC analyses were performed in DMSO where SC, OA and SC-OA are sufficiently soluble, at  
259 least at low concentrations. Figure 3 shows the traces of chromatograms of SC, SC-OA and OA  
260 with a UV detection at 280 nm (refractive index detection gave no traces in DMSO because of  
261 the low solubility). Retention time of SC-OA (9.11 min) is lower than the one of SC (9.47 min)  
262 due to its higher molar mass. Moreover, these chromatograms, as well as DOSY analyses, show  
263 that there is no residual OA (no peak at 19 min) in SC-OA.



264

265 *Figure 3: SEC chromatograms in DMSO (UV detection at 280nm) of SC (blue,) SC-OA (red)*  
 266 *and OA (black)*

267

268

### 3.3 Thermal properties

269

#### 3.3.1 TGA

270 TGA thermograms (Figure 4) were carried out from 20°C to 550°C at 20°C/min heating rate

271 under N<sub>2</sub>. They show that OA is fully degraded at 300°C whereas SC and SC-OA have the same

272 behavior, with only a partial degradation at 550°C. The percentages of residual weights at

273 550°C are 0% (OA), 25.7% (SC), 23.5% (SC-OA), and 19.1% (mixture SC 80% + 20% OA

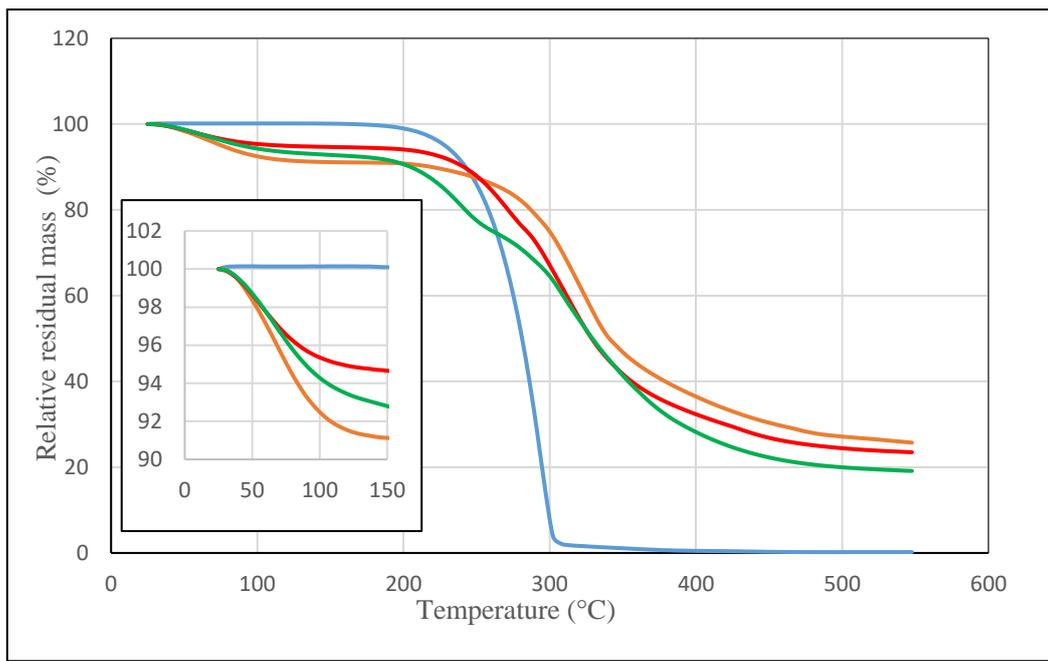
274 w/w). These values suggest that the grafted OA degraded less than free OA. The main reason

275 is probably the lack of carboxylic acid function in the grafted OA. It can also be observed (insert

276 in Figure 4) that the water loss at 100°C is lower in SC-OA (5%) than in SC (7.5%), which

277 shows the higher hydrophobicity of SC-OA due to the grafting of hydrophobic OA.

278



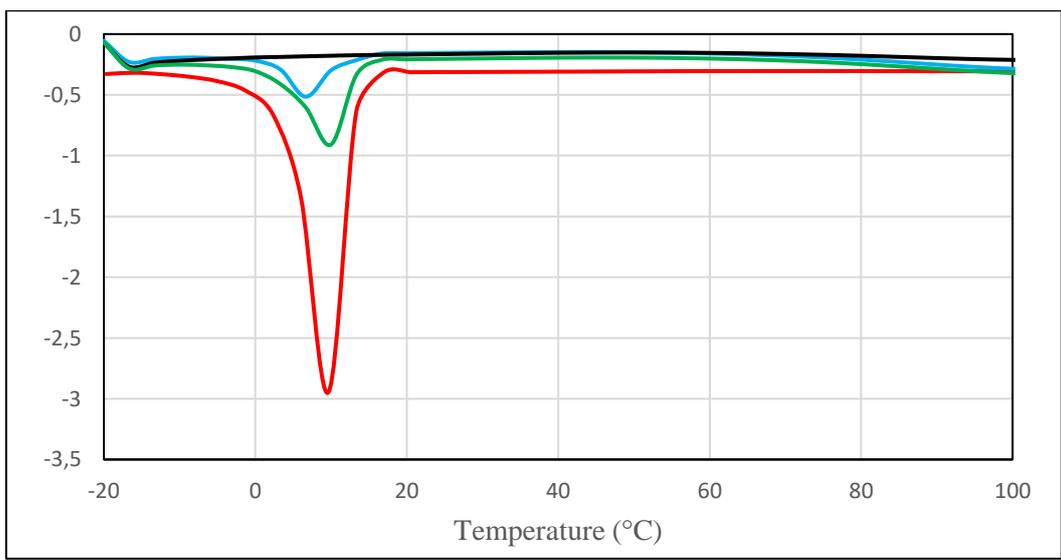
279  
280  
281  
282  
283

Figure 4: TGA thermograms of OA (blue), SC (brown), SC-OA (red) and a mixture SC + OA (20%) (green). Insert: enlargement of the zone 0-150°C

**3.3.2 DSC**

284  
285  
286  
287  
288

DSC thermograms of OA (Mp = 16°C), SC-OA (containing 24% OA w/w) and mixtures of SC and OA (20% and 30% OA w/w) were carried out from -20°C to 100°C at 10°C/min heating rate to observe the behavior of SC-OA in comparison with mixtures of SC and OA (Figure 5).



289  
290  
291

Figure 5: DSC thermograms of OA (8.9 mg, red) and mixtures of SC and OA at 20% OA (5.4 mg, blue) and 30% OA (4.64 mg, green) and SC-OA (4.6mg, % OA w/w = 24%), black)

292

293 Melting temperature and  $\Delta H_f$  fusion ( $\Delta H_f$ ) of OA were measured. The melting temperature (if  
294 any) varies slightly from 7 to 11°C with the proportion of oleic acid in the mixture. There is no  
295 melting temperature for SC-OA and the blend SC+OA at 10%OA (data not shown).  
296 “Normalized  $\Delta H_f$ ” is considering the proportion of OA in the mixtures: for a 20% proportion  
297 of OA, “normalized  $\Delta H_f$ ” is  $\Delta H_f / 0.2$ . If there is no compatibilization, normalized  $\Delta H_f$  should  
298 stay at 111J/g in all blends. Normalized  $\Delta H_f$  increased with the proportion of OA in the mixture  
299 SC+OA (97.6 J/g in pure OA, 82.7 J/g in SC+OA 30%, 62.9 J/g in SC+OA 20%, 0 J/g in  
300 SC+OA 10%), showing a very partial compatibility of the mixtures, only part of OA being in a  
301 crystalline form. There is no detectable melting point of OA in the blend SC+OA 10%, showing  
302 a compatible blend when there is a low proportion of OA. In contrast, no melting temperature  
303 or  $\Delta H_f$  is observed in SC-OA that contains 24% w/w of OA, showing the absence of crystalline  
304 form of OA in SC-OA, which confirms the formation of a conjugate (Table 2).

305

306

307

308 *Table 2: normalized  $\Delta H_f$  and proportion of crystallized OA in blends SC+OA*

Blend	OA proportion	Melting temperature Tm (°C)	Normalized $\Delta H_f$ (J/g)	crystallized OA proportion
SC+OA 10%	0,1	-	0	0
SC+OA 20%	0,2	7	-62,9	0,13
SC-OA	0.24	-	0	0
SC+OA 30%	0,3	10	-82,7	0,25
OA	1	11	-97,6	1,00

309

310 Furthermore, the curve relating the proportion of crystallized OA in the mixture as a function  
311 of the proportion of OA in the mixture is a straight line. Using the trend line equation, it can be  
312 seen that in a mixture containing less than 10% OA no crystallization of OA is observed. (Figure  
313 5-SD)

314

### 315 **3.4 Water solubility**

316 Considering the grafting of a hydrophobic OA moiety, the behavior of SC and SC-OA in water  
317 was evaluated. Films of SC and SC-OA (with a high OA substitution degree) obtained by hot  
318 compression were placed in a vial containing water. While the SC film was rapidly dissolved  
319 in water, the SC-OA film swelled by water uptake and formed a gel within minutes, due to  
320 hydrophobic interactions of the oleic moiety of SC-OA in water (Figure 6-SD).

321

### 322 **4. Conclusion**

323 Oleoyl-caseinate (SC-OA), a fatty acid-based casein derivative, is prepared by a novel method  
324 of chemical modification of SC, involving the grafting, in a biphasic medium and *via* a phase  
325 transfer catalysis reaction under mild conditions at room temperature, of an activated oleic acid.  
326 This synthesis method is gentle, fast, respects the integrity of the reagents and allows to obtain  
327 much higher substitution rates than other methods in the literature (Zhang, Yang, et al., 2018;  
328 Zhang, Yao, et al., 2018). The amine, alcohol/phenol groups of the SC are likely to react with  
329 the oleoyl chloride previously formed. A high grafting degree (56%) is obtained, corresponding  
330 to a 24% w/w OA proportion in the conjugate. DOSY NMR allowed a precise characterization  
331 of SC-OA. For low substitution degrees of OA, SC-OA remained water-soluble while it forms  
332 a gel for a higher substitution degree. DSC thermograms show that, unlike the simple mixtures  
333 of OA and caseinate, there is no crystalline form of the OA moiety in the conjugate SC-OA.  
334 This method could obviously be extended to various acid chlorides of fatty acids and more

335 generally to other acid chlorides. Mechanical properties, compatibilization properties, and  
336 plasticization properties of mixtures of SC and OA by the conjugate in an industrial process  
337 will be addressed in a further article.

338

339 **References**

340

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## Supplementary data (SD)

494

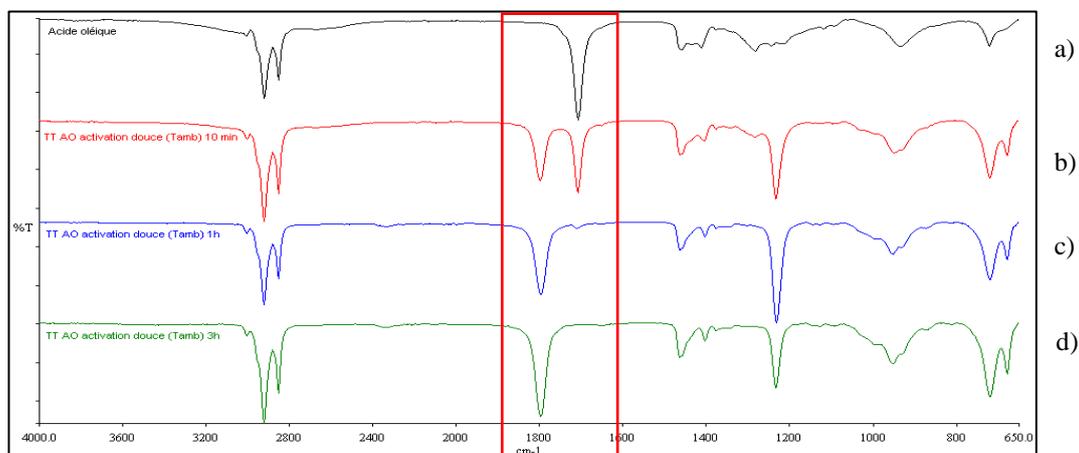


Figure 1-SD: FTIR spectra of a) Oleic acid; acylation reaction b) after 10 min c) after 1h d) after 3 h

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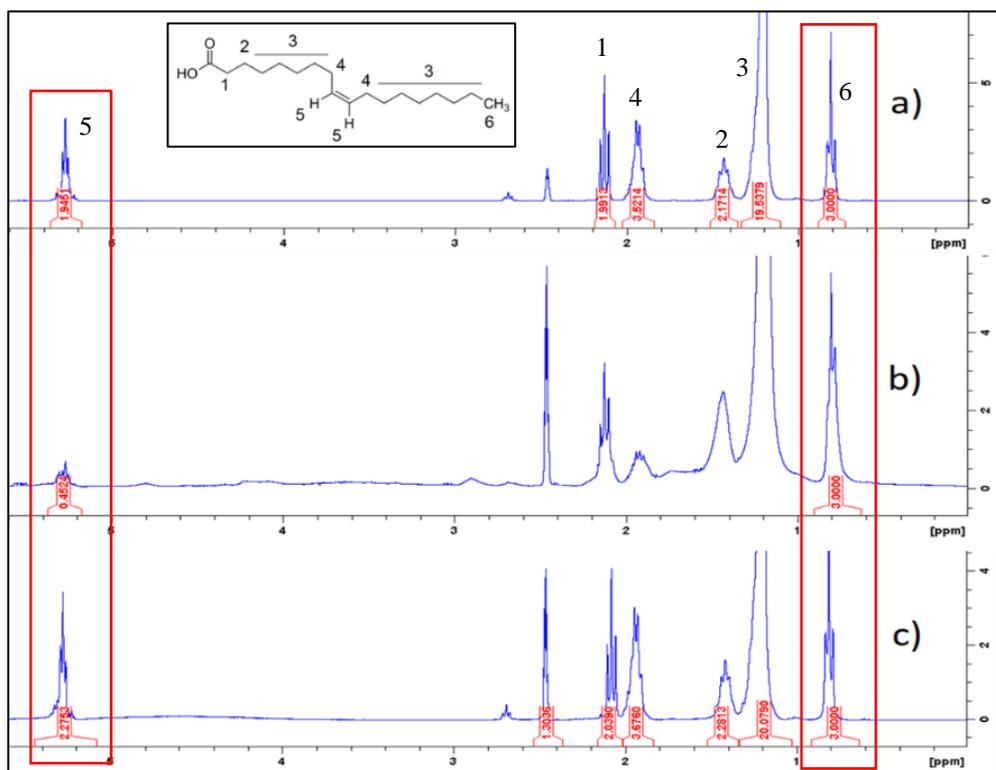


Figure 2-SD  $^1\text{H}$  NMR spectra (D6 DMSO) of a) oleic acid, b) oleyl chloride prepared at  $80^\circ\text{C}$ , c) oleyl chloride prepared at ambient temperature. Red frames emphasize ethylenic and methyl protons of the various OA derivatives. (integration values in red)

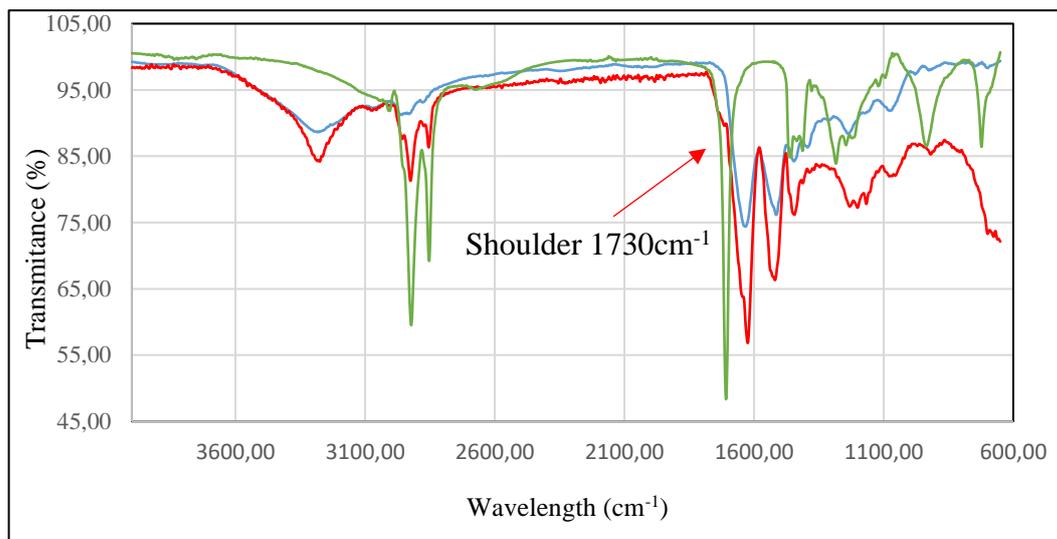


Figure 3-SD: FTIR spectra of SC (blue), SC-OA (red) and OA (green)

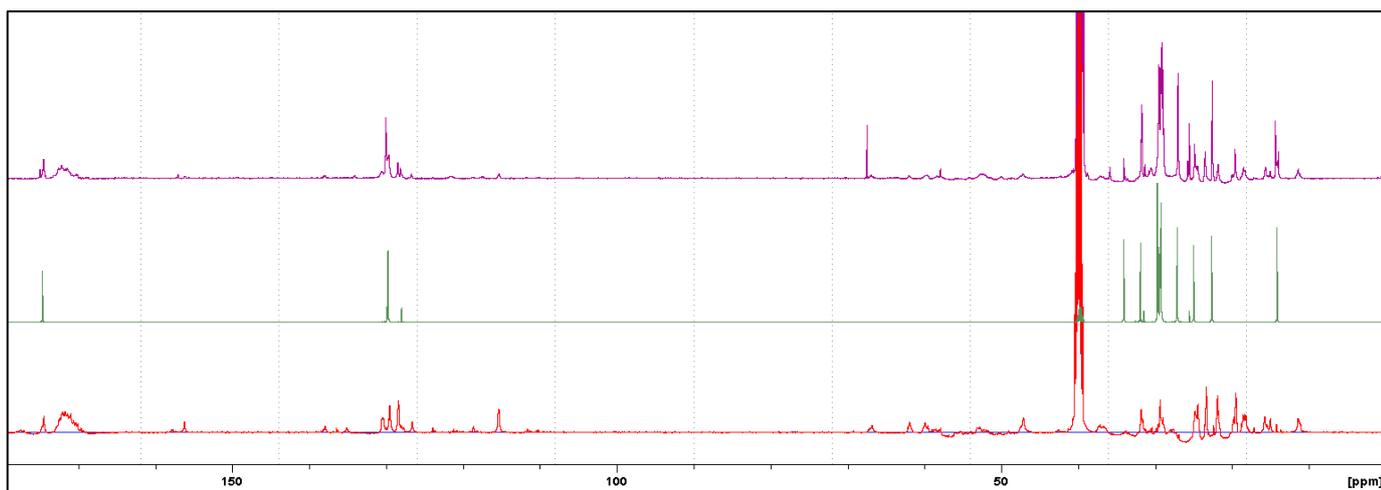
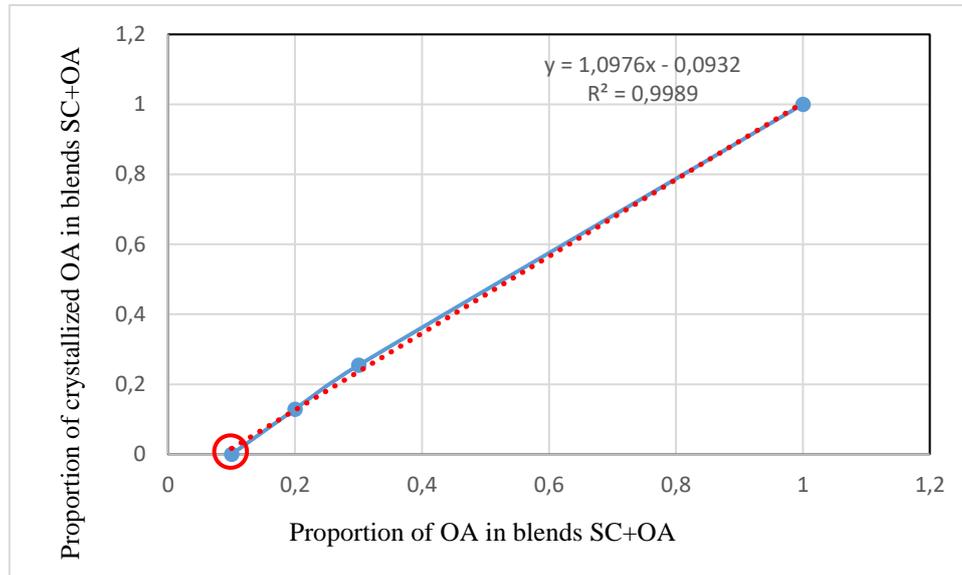


Figure 4-SD: <sup>13</sup>C NMR (D<sub>6</sub> DMSO) of SC (red), OA (green) and SC-OA (purple)

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*Figure 5-SD: variations of the proportions of crystallized OA in blends SC+OA.  
(red dotted line represents the trend line)*



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*Figure 6-SD: gel of SC-OA in water.*

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518

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