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1 **Hydrophobically modified xanthan: thickening and surface active agent for**
2 **highly stable oil in water emulsions**

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7
8 Keywords: hydrophobically modified xanthan, surfactant free emulsion, thickener, surface
9 active, emulsion stability, partitioning

10
11 • Abstract

12 Oil-in-water (O/W) emulsions were prepared using exclusively a hydrophobically modified xanthan
13 (HMX) under ordered conformation without any surfactant. A series of HMX differing in grafting
14 density were utilized at different concentrations. It was demonstrated that HMX stabilizes O/W
15 emulsions where pristine xanthan do not. Indeed, in some cases, emulsions with HMX proved to be
16 homogeneous and stable over months. Emulsions microstructure and rheological properties were
17 investigated over time as a function of HMX concentration and grafting density in order to determine
18 the mechanisms governing this efficient stability. The higher the HMX concentration is, the better the
19 emulsions stability. However, unexpectedly, increasing the HMX grafting density leads to faster
20 destabilization. The whole set of results demonstrate that the stability of the emulsions against
21 coalescence and creaming is explained by partitioning of HMX molecules between bulk and interface,
22 thus split between viscosifying the aqueous phase and stabilizing the interface.

25 1. Introduction

26 Oil-in-water (O/W) emulsions are widely employed for industrial applications including foods,
27 cosmetics, pharmaceuticals or agrochemicals. Since the contact between oil and water molecules is
28 energetically unfavorable, emulsions are thermodynamically unstable(Tadros, 2009). Indeed, they
29 tend to undergo destabilization processes such as flocculation, coalescence, creaming or
30 sedimentation and Ostwald ripening(Robins, 2000). However, kinetically metastable emulsions can
31 be obtained by adding emulsifiers and thickening agent for instance(Dickinson, 2003). Emulsifiers
32 display surface activity and may adsorb at the O/W interface, thus favoring dispersion and
33 stabilization of the oil droplets(Dickinson & Golding, 1997). Among other thickening agents,
34 polysaccharides are widely used for their great ability to increase the aqueous phase viscosity,
35 leading to slow down droplets motion(Paraskevopoulou, 2005) ; nevertheless such molecules usually
36 own non-adsorbing character so they do not favor the interface stability. Among them, xanthan is
37 the most used due to its outstanding thickening properties(Hennock, Rahalkar, & Richmond, 1984;
38 Sun, Gunasekaran, & Richards, 2007). Xanthan is an anionic bacterial polysaccharide whose structure
39 is composed of a cellulosic backbone of β -D-glucose substituted on every two units with a charged
40 trisaccharide side chain that includes a D-glucuronic acid between two D-mannoses(Jansson, Kenne,
41 & Lindberg, 1975). Acetate and pyruvates groups can be present on inner and terminal mannoses of
42 the side chain respectively(Casas, Santos, & Garcia-Ochoa, 2000). Xanthan can adopt two different
43 conformations(Milas & Rinaudo, 1979) in an aqueous solution: an ordered, rigid double-helix strand
44 structure at low temperature and high ionic strength and a disordered, flexible coil state at high
45 temperature and low ionic strength. The impact of xanthan concentration on O/W emulsions
46 physicochemical properties has been intensively studied(Hemar, Tamehana, Munro, & Singh, 2001;
47 Sun et al., 2007; Ye, Hemar, & Singh, 2004). In all cases, due to xanthan molecules poor interfacial
48 properties, addition of low molecular emulsifiers remains necessary to reach O/W emulsions
49 stability. It has also been established that the presence of low amount of xanthan increases the rate

50 of emulsion creaming due to depletion flocculation effect(Cao, Dickinson, & Wedlock, 1990;
51 Dickinson, Ma, & Povey, 1994; McClements, 2000).

52 Nevertheless, the use of low molecular weight surfactants has been recently denounced due to
53 toxicological and environmental considerations(Makkar & Rockne, 2003; Olkowska, Polkowska, &
54 Namieśnik, 2011). On this basis, among other strategies the use of amphiphilic polysaccharides, such
55 as hydrophobically modified starch(Krstonošić, Dokić, Nikolić, & Milanović, 2015)or dextran(Rouzes,
56 Durand, Leonard, & Dellacherie, 2002), has been extensively studied during the last decades in order
57 to stabilize emulsions.

58 In a previous study, a series of amphiphilic xanthan derivatives was obtained by grafting alkyl
59 residues onto their backbones under their ordered conformations(A. Roy et al., 2014). The study of
60 the rheological properties of these derivatives at the O/W interface shows that the presence of
61 grafted moieties strongly favors the interfacial adsorption of amphiphilic chains(A. N. Roy, Benyahia,
62 Grisel, & Renou, 2016), hence confirming the potential stabilizing properties for O/W dispersions.
63 Based on these preliminary results, it was anticipated that such modified xanthan may combine both
64 powerful thickening and emulsifying properties, thus preventing the use of additional surfactants.

65 The present study focuses on the investigation of hydrophobically modified xanthan derivatives
66 (HMX) stabilizing properties of O/W emulsions. The strategy consists of reaching dispersions stability
67 by taking profit of both thickening and interfacial properties of HMX, without further any addition of
68 a low molecular weight surfactant. For this purpose, emulsions were prepared using amphiphilic
69 xanthans at different concentrations and with grafting densities comprised between 8 and 32 %. All
70 the emulsions properties and stability were studied over time with varying HMX content in order to
71 understand the mechanisms governing emulsions stability.

72

73 2. Materials and Methods

74 2.1. Materials

75 The xanthan was provided by Danisco (France). Depending on the fermentation process(Garcia-
76 Ochoa, Santos, Casas, & Gomez, 2000) or post fermentation treatment(Casas et al., 2000),
77 substitution degree of acetate and pyruvate on the trisaccharidic side chain varies and have an
78 impact on the polymer properties(Shatwell, Sutherland, & Ross-Murphy, 1990). These substitution
79 degrees can be determined by ¹H NMR (Rinaudo, Milas, & Lambert, 1983); the xanthan used in this
80 study owns substitution degrees of acetate and pyruvate are 0.87 and 0.49, respectively. In others
81 words, this xanthan owns in average 0.87 acetate and 0.49 pyruvate functions per repeating
82 unit(Fantou et al., 2017; A. Roy et al., 2014). The moisture content determined by thermo gravimetric
83 analysis and the protein content, determined by the Bradford procedure were 11.1 % and 0.1 %,
84 respectively. Ultra-pure grade water was used for all the preparations. Hydrochloric acid (HCl) and
85 sodium hydroxide (NaOH) were purchased from Acros Organics; sodium chloride (NaCl) and sodium
86 azide (NaN₃) used as preservative were from Sigma Aldrich. Isononyl isononanoate used as the oil
87 phase for all the emulsions was kindly given by Stearinerie Dubois (Boulogne Billancourt, France).

88 2.2. Synthesis and characterization of hydrophobically modified xanthan

89 HMX under ordered conformation were obtained based on a procedure described by A. Roy et al.,
90 2014. Indeed, octyl residues were grafted onto the carboxylic functions of the polysaccharide *via* a
91 peptidic coupling reaction between acidic moieties of xanthan and octylamine. On HMX NMR spectra
92 in comparison with the one of xanthan, additional peak corresponding to octyl grafted moieties are
93 detected and the grafting density was adjusted as previously described(A. Roy et al., 2014).

94 The nomenclature applied for the corresponding polysaccharides is X^AC₈, where A is the grafting
95 density and 8 corresponds to the number of carbon atoms in the alkyl chain.

96

97

98 2.3. Protocol for emulsion preparation

99 Aqueous solutions of xanthan (pristine xanthan PX or hydrophobic derivative HMX) at concentration
100 ranging from 0.0125 to 1.125 % w/w in water were prepared and stirred using a magnetic stirrer at
101 room temperature (20 to 25°C) overnight. The pH was adjusted to 4.3 with 0.1 M HCl and the ionic
102 strength to 1000 $\mu\text{S}/\text{cm}$ with NaCl. 300 ppm of NaN_3 was added to the aqueous solution to prevent
103 bacterial growth. All emulsions were O/W containing 20% oil (w/w) as presented in Table 1. This
104 percentage was fixed since it corresponds to a common emulsions composition.
105 The overall content (100 % w/w) is adjusted by the quantity of water (Quantum Satis).

106 **Table 1:** Composition of the formulated system

| | Content % (w/w) | Phase |
|------------------------------|-----------------|---------------|
| Isononyl isononanoate | 20 | Oil phase |
| Xanthan (PX or HMX) | 0.01 to 0.9 | Aqueous phase |
| Preservative | 0.025 | |
| Ultrapure water | QS | |

107

108 Due to the limited quantities of xanthan derivatives available, a formulation protocol adapted for
109 emulsifying small quantity (20g) was developed based on both the literature (Kuhn & Cunha, 2012;
110 Saharudi et al., 2016) combined with a preliminary study. Before mixing, each phase was pre-heated
111 separately at 30 °C for 30 min. Phases were then mixed together and homogenized at 20 000 rpm for
112 3 min using a T25 digital ultra-turrax (IKA, Freiburg, Germany) equipped with the dispersing head
113 S25N-10G. Each emulsion was prepared in duplicate to check preparation process reliability.
114 Emulsion with non-modified xanthan was prepared following the same procedure and used as
115 reference.

116 Once prepared, all emulsions were stored at room temperature. Ageing was assessed by visual
117 observations, optical microscopy and particle size distribution through granulometry measurements.

118 In addition, rheological properties were analyzed on both solutions and emulsions using flow and
119 oscillatory modes.

120 2.4. Methods

121 2.4.1. Visual observations

122 The macroscopic aspect of the emulsions was evaluated visually by means of photographs just after
123 formulation and over time. Thus, it is possible to observe whether the entire oil phase was initially
124 emulsified but also to follow the macroscopic destabilization phenomena during aging.

125 2.4.2. Droplet size distribution

126 Emulsion droplets size was measured by static light scattering using a laser diffraction particle size
127 analyzer SALD–7500 nano (Shimadzu Co., Ltd, Japan) equipped with a violet semi-conductor laser
128 (405 nm) and a reverse Fourier optical system. Emulsions were first diluted in deionized water prior
129 to measurement in order to achieve absorption parameter equal to 0.15. Then, the dispersion
130 introduced in the batch cell (7 cm³) and stirred during the measurement to assure dispersion
131 homogeneity. A refractive index of 1.45, corresponding to the oil dispersed phase, was used. Each
132 measurement was performed twice. Data were analyzed using software Wing SALD II.

133 In order to highlight creaming process a specific test protocol was developed; it consists in sampling
134 separately 0.2 mL of the top and the bottom of the emulsion container at specific height of the tube
135 using a graduated pipette, directly after emulsification and then over time (after 1, 2, 3, 7, 14 and 30
136 days). The total emulsion height was 40 mm. The analysis of the emulsion top refers to a sampling at
137 5 mm below the upper surface, in order to avoid sampling of the oil phase if any. For the bottom,
138 sampling was performed 5 mm from the bottom of the container.

139 Characterizations after one day will be referred as D+1; the same designation applies to other
140 periods. Additionally, most stable emulsions were monitored over months.

141

142

143 2.4.3. Optical Microscopy

144 Emulsions microstructure (top and bottom, according to the sampling procedure described in the
145 previous paragraph) was observed, at room temperature, by means of an optical microscope at a
146 magnification of x20 (DMLP/DC 300, Leica Microsystems, Wetzlar, Germany) equipped with a camera
147 and controlled by the Leica Application Suite software. Leica IM 1000 software was used to analyze
148 the micrograph.

149 2.4.4. Rheology

150 Oscillatory shear studies were carried out with a stress controlled rheometer HR3 (TA Instruments)
151 with a Peltier temperature control device using a cone-plate aluminium geometry (diameter, 60 mm;
152 cone angle, 2°00'43''; gap, 59 μm) for the pristine xanthan and xanthan derivatives solutions. Solvent
153 evaporation was prevented by covering the geometry with low viscosity silicon oil. The storage (G')
154 and loss (G'') moduli were measured within the linear viscoelasticity domain at different temperature
155 from 5 to 80 °C in order to build the viscosity master curve(Choppe, Puaud, Nicolai, & Benyahia,
156 2010; Pelletier, Viebke, Meadows, & Williams, 2001). Polymer solutions from 0.0125 to 0.4 % w/w
157 were prepared as mentioned above and centrifuged for 3 min to get rid of air bubbles. Aluminum
158 parallel plate geometry (diameter, 60 mm; gap, 200 μm) was used to study emulsions.

159 Emulsions flow properties were obtained as a function of shear rate during a decreasing ramp from
160 100 to 0.01s⁻¹ during 300s at 20°C. The procedure included a conditioning step that consisted in
161 applying a pre-shear at 50s⁻¹ for 60s, and then samples were left relaxing for 2 min prior to
162 measurement. Emulsions were measured immediately after formulation in order to avoid the
163 potential effects of emulsion's destabilization. Indeed, no destabilization was observed within the
164 first few hours after formulation. Therefore, there no significant evolution of the system within the
165 measurement time scales; hence, the rheological data do correspond to undisturbed emulsions.

166

167

168 2.4.5. Thermogravimetric analysis

169 In order to determine the xanthan concentration in the aqueous phase, emulsions were first
170 centrifuged at 8 000 g for 20 min. Then the aqueous phase was analyzed by thermogravimetry. These
171 measurement were performed using a Setsys apparatus (Setaram Instrumentation, Caluire-et-Cuire,
172 France), equipped with the Calisto software (version 1.54a, Setaram Instrumentation, Caluire-et-
173 Curie, France). About 80 mg of xanthan solution are weighted in an alumina crucible. Heating
174 procedure is performed under air as follow:

- 175 - Equilibration at 110°C during 45 min
- 176 - Temperature ramp from 110°C to 550°C at a rate of 20°C/min
- 177 - Isotherm at 550°C during 10 min

178 2.4.6. Homogeneity and Stability criteria for emulsions

179 The emulsions were characterized with considering two criteria, namely homogeneity and stability.

180 On the one hand, emulsions were considered as homogeneous as far as both droplet size and
181 distribution were identical over the entire volume (from top to bottom) of the sample. This criterion
182 was evaluated from initial state (24h after emulsion preparation) over the whole period of ageing.

183 On the other hand, the different phenomenon involved in emulsions ageing, namely creaming,
184 flocculation and coalescence, were carefully considered by mean of particle size analyses in order to
185 understand the mechanisms implicated in the stabilization / destabilization phenomenon.

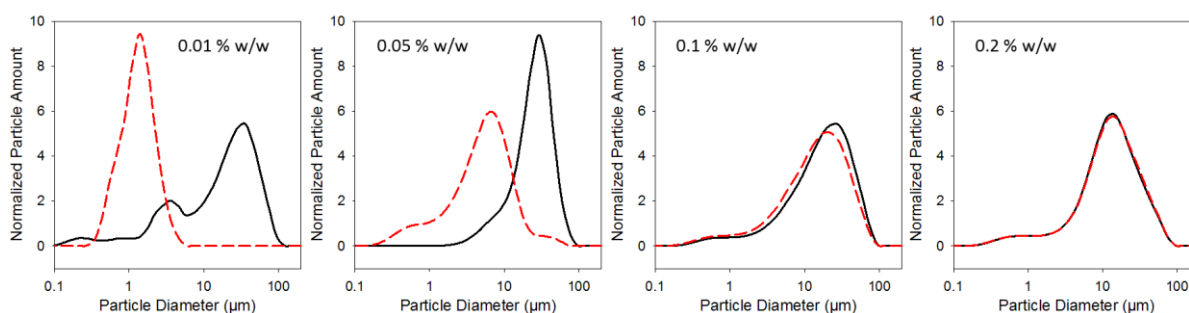
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188 3. Results and Discussion

189 Without any surfactant, preliminary results demonstrated that PX, as expected, does not stabilize
190 emulsion at all as phase separation occurred immediately or within only few hours after emulsion
191 preparation for PX concentration up to 0.9 % w/w despite its very good suspending ability. These
192 results clearly confirmed that native xanthan is a non-adsorbing polysaccharide and therefore does
193 not exhibit any interfacial stabilizing properties(Cao et al., 1990; Gunning, Hibberd, Howe, & Robins,
194 1988). Therefore, the first part of this paper focuses on the utilization of HMX in emulsion to evaluate
195 its stabilizing properties compared to PX. At this stage, it is important pointing out that both PX and
196 its amphiphilic derivatives were studied within the same concentration regime. Indeed, both PX and
197 HMX polymers own an overlap concentration C^* around 0.2 g/L as determined by rheology. This is
198 consistent with the values were earlier reported in the literature for native xanthan
199 polysaccharide(Cuvelier & Launay, 1986; Milas, Rinaudo, Knipper, & Schuppiser, 1990).

200 First of all, the impact of the polymer concentration on emulsion stabilization has been investigated,
201 using X^8C_8 at five concentrations ranging from 0.01 to 0.32 % w/w. Droplet size distributions of these
202 systems after 24h are presented in Figure 1, with superimposing both bottom and top samplings.
203 Thus, the closer the bottom and top particle size distributions the more homogeneous the mixture.
204 For clarity, results obtained for emulsion with 0.32 % w/w of X^8C_8 are not presented as the
205 corresponding data are similar to the one with 0.2 %w/w.

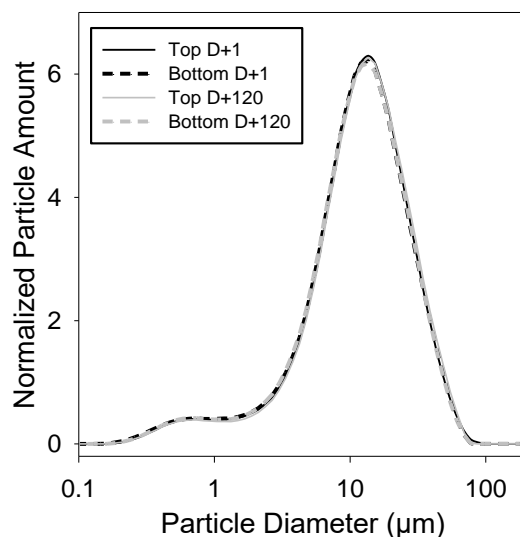


206
207 **Figure 1:** Emulsion particle size distribution at different X^8C_8 concentrations after 24 h. Solid and dashed lines
208 refer to sampling at the top and the bottom of the emulsion respectively.

209 For the lower HMX contents, it is obvious that destabilization occurs quickly after formulation.
210 Indeed, as shown in Figure 1, the emulsion bottom is only composed of small droplets when the top
211 part presents a huge distribution of larger droplets. This can be explained by the oil droplets
212 migration from the bottom to the top of the emulsion corresponding to an inhomogeneity thought
213 creaming phenomenon. Additionally, an oil layer on the emulsion top was observed which was
214 attributed either to a non-emulsified oil portion due to insufficient HMX content and/or to the
215 occurrence of coalescence, proving that the emulsion is not stable at all. For emulsions with 0.05 and
216 0.1 % w/w of X⁸C₈, the emulsion bottom still shows droplets smaller than the top. However, the
217 difference in the droplet size between the bottom and the top is strongly reduced with increasing the
218 polymer concentration. The oil layer on the top is no longer observed, thus confirming that
219 emulsifying character is improved as HMX concentration increases. Finally, when the HMX
220 concentration reaches 0.2 % w/w, the emulsion initially appears homogeneous as the droplet size
221 distribution is identical on the whole sample height as visible on Figure 1.

222 In addition, the latter emulsion was monitored over time either from microstructure and
223 macroscopic aspects in order to investigate the ability for HMX to get long term stable emulsions
224 without adding any low molecular weight surfactant. As presented in Figure 2, both top and bottom
225 droplet distribution after 1 and 120 days superimpose perfectly. Indeed, neither creaming nor
226 coalescence was observed within 4 months. Thus, such an emulsion with 0.2 % w/w HMX can be
227 considered as very stable. As stipulated above, similar results were observed at higher polymer
228 concentrations. In fact a HMX concentration of 0.2 % w/w clearly appears efficient enough to get
229 stable emulsion in spite of fairly low amphiphilic polymer content. Thus, when compared to PX and
230 as far as it is used at a sufficient concentration (above C*), amphiphilic X⁸C₈ undoubtedly allows
231 getting homogeneous and stable O/W dispersions over months. It is important to point out that the
232 HMX content necessary for reaching such stable emulsion remains quite low, at most similar to the
233 level usually employed when utilizing unmodified xanthan as a thickener combined with
234 surfactants(Krstonošić et al., 2015). Indeed, it is important to stress that HMX appears much more

235 efficient than any combination of xanthan with classical surfactants. In this latter case the level of
236 surfactant necessary for reaching emulsion stability is above several % as described for Tween
237 20(Krstonošić et al., 2015; Vélez, Fernández, Muñoz, Williams, & English, 2003) or whey protein
238 isolate(Sun et al., 2007), thus much more than the lonely HMX content in the present study.

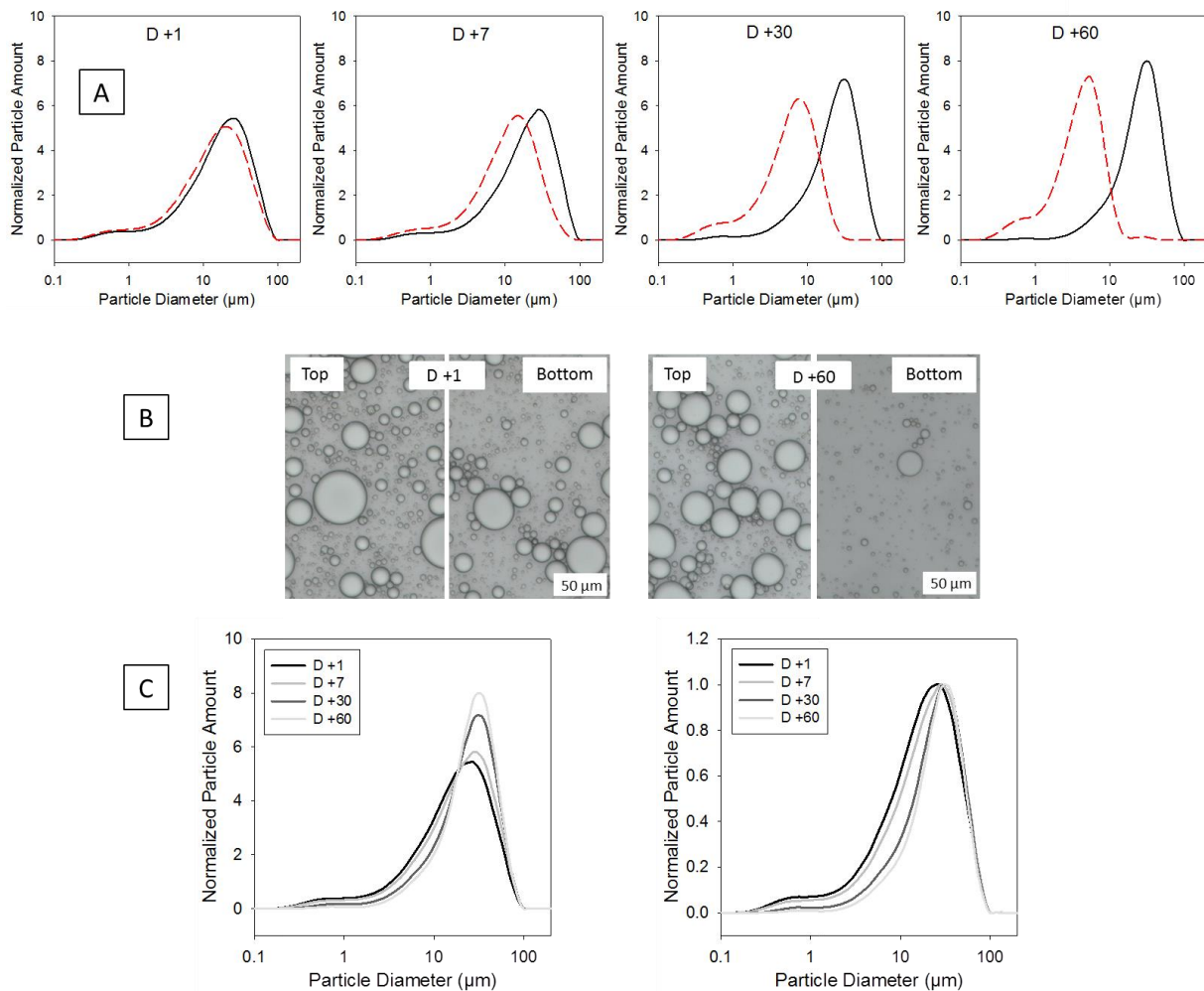


239
240 **Figure 2:** Emulsion particle size distribution at 0.2 % w/w in modified X^8C_8 polymer. Solid line and dash line refer
241 to the top and the bottom of the emulsion, respectively.

242 Based on these first promising results and in order to better understand the mechanisms responsible
243 for the stabilizing ability of HMX, a series of further investigations were performed with considering
244 “fairly but not too stable” O/W emulsions in order to follow the evolution of the dispersions within a
245 reasonable time scale. Different parameters will then be simultaneously investigated such as the
246 effect of HMX concentration and the influence of HMX alkyl grafting density.

247 In a first step, emulsions’ ageing was investigated for a system prepared with 0.1 % w/w X^8C_8 HMX.
248 Figure 3 shows the evolution of this emulsion over 2 months through both droplet size measurement
249 and optical microscopy. Results presented in Figure 3 A clearly show that the top and the bottom
250 emulsions microstructure, which appears initially fairly similar, becomes more and more different
251 with time.

252



253

254 **Figure 3:** (A) Emulsion particle size distribution at 0.1 % w/w X^8C_8 at different time after preparation. Solid line
 255 and dash line refer to the top and the bottom of the emulsion, respectively. (B) Optical microscopy image of the
 256 corresponding emulsion at D +1 and D+60, respectively. (C) Particle size distribution of the top of the emulsion
 257 (left: non normalized; right: normalized) at 0.1% w/w X^8C_8 at different time after preparation.

258 First, one can notice that the bottom droplet size distribution is progressively shifted to lower values
 259 with time due to the impoverishment in larger droplets. These results issued from granulometric
 260 data are obviously confirmed by microscopic observations as visible in Figure 3 B.

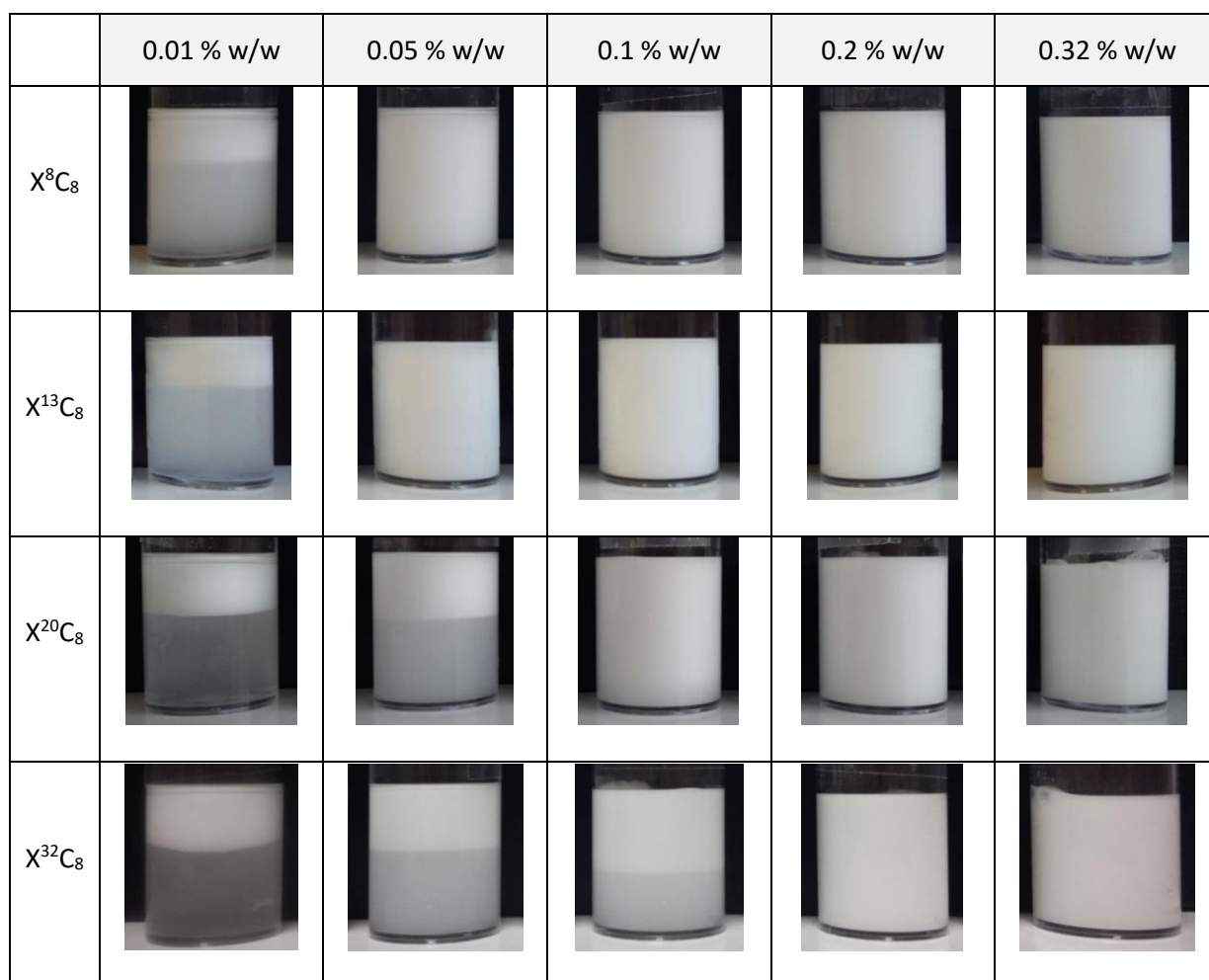
261 Initially no significant difference can be perceived between the emulsion top and bottom parts. On
 262 the contrary after two months, the emulsion bottom only presents few small droplets while more
 263 numerous large droplets are observed on the top as a consequence of creaming. Nevertheless, it is
 264 noticeable that once migrated to the top part of the emulsion, the droplets size does not evolve

265 significantly with time. In order to confirm these observations, the emulsion particle size distribution
266 corresponding to the emulsion top were superimposed as illustrated in Figure 3 C (left). Over time,
267 result shows that the size distribution becomes thinner and higher. Two hypotheses are possible to
268 explain this increase: either the small droplets remain at the bottom since they cream more slowly,
269 or there is coalescence. In the Figure 3 C (right), droplet size distribution have been normalized by
270 the curve maximum height. One can clearly observe that the maximum position does not evolve
271 much with storage time thus proving that no significant coalescence occurred within a period of 60
272 days.

273 Based on these observations, it appears that the main phenomenon responsible for emulsions
274 destabilization is creaming, coalescence remaining quite limited. This also clearly allows establishing
275 that the HMX molecules are extremely efficient for stabilizing the O/W interface. The droplets are
276 mainly subject to creaming but highly resistant to coalescence over months.

277 To summarize, emulsion with 0.1 % w/w of X^8C_8 undergoes a destabilization process through
278 creaming that actually intervenes quite rapidly as it starts no later than the day after emulsion
279 preparation but no coalescence was observed. Nevertheless, creaming can be strongly slowed down
280 or even stopped for periods of months by increasing X^8C_8 concentration as exposed earlier in the
281 paper (See Figure 2).

282 The same approach was applied to investigate the impact of the grafting density on emulsion
283 stability. Emulsions at different concentrations were prepared using HMX owning an octyl grafting
284 density comprised between 8 % and 32 %. The photographs of the corresponding emulsions 24 h
285 after preparation are presented in Figure 4. One day after preparation, all the emulsions at a low
286 concentration (0.01 % w/w) present three phases: a thin oil layer on the top, then a creamed layer of
287 oil droplet and finally the continuous phase at the bottom. As visible in Figure 4, when increasing the
288 HMX concentration to 0.05 % w/w and 0.1 % w/w, the emulsions with X^8C_8 and $X^{13}C_8$ appear stable
289 since they look homogeneous from a macroscopic point of view.



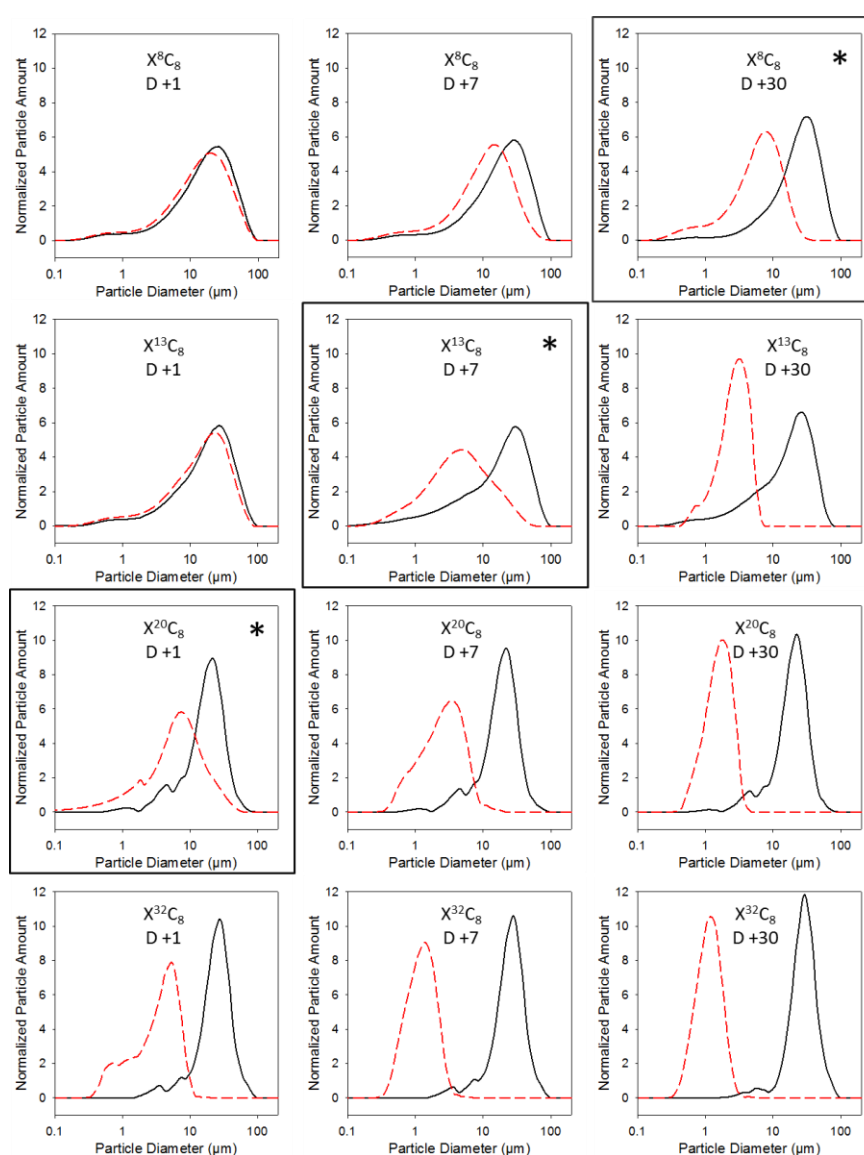
290 **Figure 4:** Photographs of the different emulsions after 24h as a function for HMX grafting density and
 291 concentration in the emulsion.

292 However, still for the same polymer content, emulsions prepared with HMX of higher grafting
 293 densities show a marked creaming, which seems to be more and more pronounced as the grafting
 294 density rises. This clearly evidences that the extent of the phase separation (or instability) is strongly
 295 influenced by the HMX concentration as well as the octyl grafting density of HMX. For 0.2 % w/w and
 296 0.32 % w/w, emulsions appear macroscopically stable whatever the grafting density. Thus, in
 297 accordance with the previous section, whatever the amphiphilic xanthan grade used, emulsion
 298 homogeneity can be improved by increasing the HMX concentration.

299 Similarly to the previous section focusing on X^8C_8 , the droplet size distribution was monitored over
 300 time for series of emulsions prepared with HMX of different grafting densities.

301 Firstly, emulsions were characterized as homogeneous and stable over months whatever the HMX
 302 grafting density as far as the polymer content was above 0.2% (results not shown); this being in good
 303 accordance with previous observations. Thus, experiments for investigating stability focused on
 304 emulsions prepared with a total HMX content of 0.1% (w/w) as corresponding to emulsions with
 305 apparent homogeneity and sufficient stability for monitoring over time, for the four grafting densities
 306 investigated.

307 Figure 5 reports the evolution of the droplet size distributions for both the bottom and the top part
 308 of the emulsions, for a total period of 1 month.



309
 310 **Figure 5:** Emulsion particle size distribution at 0.1 % w/w in HMX with different grafting densities. Solid line and
 311 dash refer to the top and the bottom of the emulsion respectively.

312 Only three sets of data are represented, corresponding to 1, 7 and 30 days of aging to keep the figure
313 clear. Firstly, after one-day emulsions with X^8C_8 and $X^{13}C_8$ behave similarly, both emulsion top and
314 bottom part droplet distributions being nearly superimposed. However, emulsions with $X^{20}C_8$ clearly
315 behave differently as the emulsion bottom part initially presents a smaller droplet size distribution
316 compared to the top. This difference is even more pronounced for $X^{32}C_8$. Indeed, for the emulsion
317 prepared with $X^{32}C_8$, two very distinct droplet size distributions are initially observed, the bottom part
318 containing small droplets, and the top owning larger ones. These results are consistent with creaming
319 process as evidenced through macroscopic observations. After one month, the emulsion top and
320 bottom presented a completely different profile, especially for the highest grafting density.
321 Nevertheless, the droplet size distribution does not evolve with time on the top of the emulsion for
322 all the grafting density. Thus, all the emulsions could be considered as stable as no coalescence
323 occurred (no phase separation) but non homogeneous since presenting markedly different
324 microstructures if comparing top and bottom parts (creaming).

325 To sum up, the way the emulsions evolved over time remains the same whatever the polymer
326 grafting density, only the kinetic being accelerated with increasing grafting density. As an illustration
327 the emulsions profiles for the one with X^8C_8 after 1 month, the one with $X^{13}C_8$ after 7 days and,
328 finally, to the one with $X^{20}C_8$ after one day look quite similarly (the corresponding graphs are marked
329 with * on Figure 5).

330 Such a result is of primary importance as increasing the polymer grafting density unexpectedly leads
331 to a faster emulsion destabilization at 0.1%. This surprising behavior has been investigated and
332 discussed in the next section.

333 To better understand the phenomenon involved in creaming processes of emulsions prepared with
334 HMX, viscosity of the emulsions and the corresponding aqueous phases were investigated.

335 Figure 6A shows the master curves of dynamic viscosity (η') for HMX solutions at 0.125 % (w/w), for
336 different grafting densities. This polymer concentration was chosen as corresponding to the actual
337 xanthan concentration in the aqueous phase for emulsions prepared at 0.1 % w/w. As already

338 reported for amphiphilic xanthan by A. Roy et al., 2014, at high shear rates, all modified xanthans
 339 behave similarly whatever the grafting density. Indeed, whatever the grafting density HMX exhibits a
 340 shear-thinning behavior and flow curves perfectly superimpose for shear rates higher than 1 s^{-1} .
 341 However, at low frequencies, the different HMX tend to a Newtonian plateau (e.g. $\eta_0 \approx 1.5 \text{ Pa}\cdot\text{s}$ for $\omega = 0.01 \text{ rad/s}$ for X^8C_8). The higher the grafting density the higher the plateau viscosity becomes.

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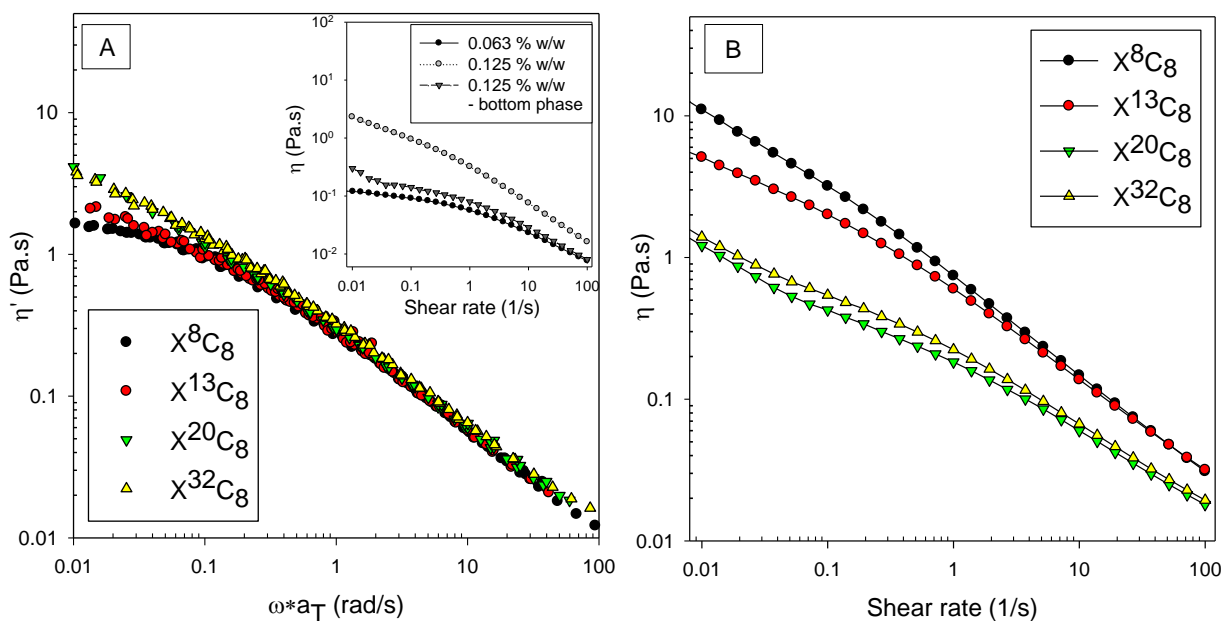
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351 **Figure 6:** (A) Master curves of the dynamic viscosity (η') of the solutions as a function of $\omega \cdot a_T$ with $T_{ref} = 20 \text{ }^\circ\text{C}$ at
 352 0.125 % w/w. Inset: Flow curves of the solutions for $X^{20}C_8$ at 20°C at 0.125% w/w and 0.063% w/w and the
 353 bottom phase of the emulsion prepared with 0.125% w/w of $X^{20}C_8$. (B) Viscosity of the emulsions with 0.1 %
 354 w/w of modified polymer as a function of shear rate at 20°C .

355

356 Thus, consistently with the works done by A. Roy et al., 2014, the presence of grafted moieties onto
 357 the xanthan backbone affects only the flow behavior of the solutions at low shear rate by slowing
 358 down the chains relaxation due to sticky relaxation (Aubry & Moan, 1994; Choppe et al., 2010).

359 On the other hand, Figure 6B reports the flow curves of emulsions prepared with the series of HMX
 360 corresponding to one studied on Figure 6A at 0.1 % w/w (thus the same polymer concentration as in
 361 the aqueous phase). All emulsions clearly display a shear thinning behavior at a shear rate between
 362 100 and 0.01 s^{-1} . Surprisingly at first glance, inversely to the corresponding solutions, emulsions

363 viscosity sharply decreases with increasing grafting density. In addition, it is interesting to point out
364 that emulsions viscosity is higher than the one of the corresponding solutions for low grafting density
365 HMX while the opposite is observed when grafting density increase.

366 Such a bewildering behavior may be explained by two distinct characters of xanthan amphiphilic
367 derivatives: on the one hand the octyl moieties tend to adsorb at the O/W interface thus allows
368 forming and stabilizing the oil droplets. On the other hand, xanthan acts as a viscosifier that
369 increases the suspending properties of the aqueous phase, hence slows down creaming. In other
370 words, with increasing grafting density, more moieties tend to adsorb at the O/W interface;
371 nevertheless, alkyl group being grafted onto the polymer backbone, the latter is also pulled away
372 from bulk to the interface, thus, leading to impoverish the aqueous phase in xanthan molecules.
373 Consequently, the aqueous phase viscosity is lowered thus favoring droplets creaming. This
374 phenomenon is reinforced when the grafting density increases as HMX – oil affinity increases when
375 grafting density rises.

376 In order to confirm these hypotheses, the different emulsions with 0.1 % (w/w) of HMX were
377 centrifuged in order to accelerate creaming and to remove all droplets from the aqueous phase. The
378 viscosity of the bottom phase was then analyzed (as illustrated for X²⁰C₈ in the inset of Figure 6). As
379 expected, the corresponding viscosity is much lower (about 1 decade) than the HMX solution at
380 0.125 % (w/w). For comparison, the viscosity of the same HMX at half the concentration (0.063 %) is
381 also plotted. One can observe that the viscosity of the latter is pretty close to the one of the bottom
382 phase. Hence the HMX concentration in the bottom phase is close to half its initial value
383 ($C_{ini}=0.125\%$). Therefore, the rest of the HMX has likely migrated to the interface.

384 In order to emphasize this observation, the actual HMX concentration present in the bulk was
385 determined as follow: first emulsions were centrifuged according to the procedure detailed in the
386 experimental section. Then the HMX content in the aqueous lower phase (C_{bulk}) was estimated by
387 TGA (see experimental section). Corresponding results are reported in Table 2, the estimated ratio of
388 HMX concentration between bulk and interface is also mentioned. One can observe that the

389 concentration in the bottom phase is less than its initial concentration whatever the HMX grafting
 390 density. For instance, the bulk concentration for $\chi^{32}\text{C}_8$ is only 35% of its initial value (thus almost
 391 divided by 3). Hence this clearly demonstrates that HMX is actually portioned between bulk of the
 392 aqueous phase and droplet interface. Moreover, it is striking that the higher the grafting density the
 393 more the HMX is adsorbed at the oil/water interfaces. These data undoubtedly confirmed our
 394 hypothesis on the partitioning of HMX between bulk and interface. These results are crucial in
 395 explaining the way emulsions behave initially and age depending on the HMX grafting density.

396
 397 **Table 2:** HMX concentration in the emulsion bulk phase and estimation of the HMX distribution between the
 398 Bulk and the Interface. Reference value is $C_{ini} = 0.125\%$ (w/w).

| Sample | C_{bulk} (% w/w) | Estimated ratio Bulk vs Interface |
|-----------------------|---------------------------|-----------------------------------|
| $\chi^8\text{C}_8$ | 0.11 | 88/12 |
| $\chi^{13}\text{C}_8$ | 0.099 | 79/21 |
| $\chi^{20}\text{C}_8$ | 0.072 | 58/42 |
| $\chi^{32}\text{C}_8$ | 0.044 | 35/65 |

399
 400
 401 It is important reminding that whatever the HMX grafting density, the total amount of polymer used
 402 to prepare the emulsions here (0.1% w/w) is not sufficient to reach high stability. Based on the
 403 results for the HMX partition between both the bulk and the interface, the emulsions aqueous
 404 continuous phase viscosity appears too low to prevent efficiently creaming. Indeed, as suggested by
 405 our hypothesis, when increasing the polymer grafting density, HMX molecules being more and more
 406 adsorbed at the oil/water interface so that oil droplets are more stabilized while the aqueous phase
 407 viscosity decreases thus favoring creaming but without any coalescence.

408 Noteworthy, concerning emulsions with HMX at 0.2 % (w/w), the same phenomenon may occur but
 409 the polymer concentration is high enough to both stabilize the interface and to maintain the viscosity

410 sufficiently high to limit creaming. Thus, this explains why emulsions prepared with at least 0.2 %
411 (w/w) of modified polymer are homogeneous and stable over months whatever the grafting density.

412 To sum up, all these observations confirm the essential role of HMX concentration on emulsion
413 stabilization. A compromise exists between thickening properties and interface stabilization by
414 amphiphilic xanthan derivative that governs either stabilization or destabilization of the emulsions
415 due to HMX chains partitioning between bulk and interface that depends on the grafting density.

416

417 4. Conclusion

418 In this work, the potential of HMX to stabilize O/W emulsions was investigated. Xanthan was
419 chemically modified under its ordered conformation by grafting octyl moieties from 8% up to 32%.
420 The effects of both the polymer concentration and grafting density were investigated in such a way
421 that it was possible to determine the mechanisms responsible for the ability of HMX to get stable
422 emulsions.

423 First, it was demonstrated that HMX alone, without additional surfactant, is a highly efficient
424 emulsion stabilizer since it allows getting homogeneous emulsions with long term stability without
425 any creaming, coalescence or phase separation where PX does not produce any emulsion. Such a
426 remarkable ability for HMX to stabilize O/W emulsions was observed whatever the grafting density
427 (ranging from 8 to 32%) as far as the polymer content was above a polymer concentration close to
428 the critical overlap concentration (0.2% w/w).

429 In addition, "fairly but not too stable" O/W emulsions obtained at low HMX concentration with
430 various grafting density were studied. Both the homogeneity and microscopic properties evaluated
431 at initial state and during ageing over several weeks allowed understanding the mechanisms involved
432 in the emulsions formation and the stabilization/destabilization phenomena. Indeed, it was
433 unambiguously established that the HMX molecules are actually partitioned between the bulk (water
434 continuous phase) and the O/W interface. The lower the HMX grafting density the higher the HMX

435 portion dispersed within the bulk, while the higher the HMX grafting density the more polymer
436 molecules condensed at the oil-water interface. Obviously, such a result is explained by the
437 difference of polymer hydrophilic / lipophilic character, this balance depending on the polymer
438 grafting density. So the homogeneity evolution over time for the corresponding emulsions is
439 governed by the HMX partitioning between both bulk and the interface. On the one hand,
440 amphiphilic molecules do adsorb at the oil droplet interface, therefore allowing its formation, its
441 stabilization and preventing coalescence. On the other hand, the polymer molecules dispersed in the
442 aqueous continuous phase induce viscosity enhancement, the latter limits droplets creaming. Here it
443 was demonstrated that very stable O/W emulsions can be obtained in the presence of
444 hydrophobically modified xanthan without any addition of conventional surfactant. This finding may
445 open up promising applications in formulations that require little or no surfactant in order to limit
446 their toxicological and environmental impact. Finally, a controlled modification protocol was
447 developed and allowed the formulation of stable emulsions whose stability can be tuned by both
448 HMX concentration and grafting density.

449

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453 • References

454 Aubry, T., & Moan, M. (1994). Rheological behavior of a hydrophobically associating water soluble
455 polymer. *Journal of Rheology*, 38(6), 1681–1692.

456 Cao, Y., Dickinson, E., & Wedlock, D. J. (1990). Creaming and flocculation in emulsions containing
457 polysaccharide. *Food Hydrocolloids*, 4(3), 185–195.

458 Casas, J. A., Santos, V. E., & Garcia-Ochoa, F. (2000). Xanthan gum production under several
459 operationnal conditions : molecular structure and rheological properties. *Enzyme and*
460 *Microbial Technology*, 26, 282–291.

461 Choppe, E., Puaud, F., Nicolai, T., & Benyahia, L. (2010). Rheology of xanthan solutions as a function
462 of temperature, concentration and ionic strength. *Carbohydrate Polymers*, 82(4), 1228–1235.

463 Cuvelier, G., & Launay, B. (1986). Concentration regimes in xanthan gum solutions deduced from flow
464 and viscoelastic properties. *Carbohydrate Polymers*, 6(5), 321–333.

465 Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed
466 systems. *Food Hydrocolloids*, 17(1), 25–39.

467 Dickinson, E., & Golding, M. (1997). Rheology of sodium caseinate stabilized oil-in-water emulsions.
468 *Journal of Colloid and Interface Science*, 191(1), 166–176.

469 Dickinson, E., Ma, J., & Povey, M. J. W. (1994). Creaming of concentrated oil-in-water emulsions
470 containing xanthan. *Food Hydrocolloids*, 8(5), 481–497.

471 Fantou, C., Roy, A. N., Dé, E., Comesse, S., Grisel, M., & Renou, F. (2017). Chemical modification of
472 xanthan in the ordered and disordered states: an open route for tuning the physico-chemical
473 properties. *Carbohydrate Polymers*, 178, 115–122.

474 Garcia-Ochoa, F., Santos, V. E., Casas, J. A., & Gomez, E. (2000). Xanthan gum: production, recovery,
475 and properties. *Biotechnology Advances*, 18(7), 549–579.

476 Gunning, P. A., Hibberd, D. J., Howe, A. M., & Robins, M. M. (1988). Gravitational destabilization of
477 emulsions flocculated by non-adsorbed xanthan. *Food Hydrocolloids*, 2(2), 119–129.

478 Hemar, Y., Tamehana, M., Munro, P. A., & Singh, H. (2001). Influence of xanthan gum on the
479 formation and stability of sodium caseinate oil-in-water emulsions. *Food Hydrocolloids*, 15,
480 513–519.

481 Hennock, M., Rahalkar, R. R., & Richmond, P. (1984). Effect of xanthan gum upon the rheology and
482 stability of oil-water emulsions. *Journal of Food Science*, 49(5), 1271–1274.

483 Jansson, P., Kenne, L., & Lindberg, B. (1975). Structure of the extracellular polysaccharide from
484 *Xanthomonas campestris*. *Carbohydrate Research*, 45(1), 275–282.

485 Kuhn, K.R., Cunha, R.L. (2012) Flaxseed oil – Whey protein isolate emulsions: Effect of high pressure
486 homogenization. *Journal of Food Engineering*, 111(2) 449-457.

487 Krstonošić, V., Dokić, L., Nikolić, I., & Milanović, M. (2015). Influence of xanthan gum on oil-in-water
488 emulsion characteristics stabilized by OSA starch. *Food Hydrocolloids*, 45, 9–17.

489 Makkar, R. S., & Rockne, K. J. (2003). Comparison of synthtic surfactants and biourfactants in
490 enhancing biodegradation of polycyclic aromatic hydrocarbons. *Environmental Toxicology*
491 *and Chemistry*, 22(10), 2280–2292.

492 McClements, D. . (2000). Comments on viscosity enhancement and depletion flocculation by
493 polysaccharides. *Food Hydrocolloids*, 14(2), 173–177.

494 Milas, M., & Rinaudo, M. (1979). Conformational investigation on the bacterial polysaccharide
495 xanthan. *Carbohydrate Research*, 76, 189–196.

496 Milas, M., Rinaudo, M., Knipper, M., & Schuppiser, J. L. (1990). Flow and viscoelastic properties of
497 xanthan gum solutions. *Macromolecules*, 23(9), 2506–2511.

498 Olkowska, E., Polkowska, Ż., & Namieśnik, J. (2011). Analytics of surfactants in the environment:
499 problems and challenges. *Chemical Reviews*, 111(9), 5667–5700.

500 Paraskevopoulou, A. (2005). Stabilization of olive oil - lemon juice emulsion with polysaccharides.
501 *Food Chemistry*, 90(4), 627–634.

502 Pelletier, E., Viebke, C., Meadows, J., & Williams, P. A. (2001). A rheological study of the order–
503 disorder conformational transition of xanthan gum. *Biopolymers*, 59(5), 339–346.

504 Rinaudo, M., Milas, M., & Lambert, F. (1983). 1H and 13C NMR investigation of xanthan gum.
505 *Macromolecules*, 16(5), 816–819.

506 Robins, M. M. (2000). Emulsions — creaming phenomena. *Current Opinion in Colloid & Interface*
507 *Science*, 5(5–6), 265–272.

508 Rouzes, C., Durand, A., Leonard, M., & Dellacherie, E. (2002). Surface activity and emulsification
509 properties of hydrophobically modified dextrans. *Journal of Colloid and Interface Science*,
510 253(1), 217–223.

511 Roy, A., Comesse, S., Grisel, M., Hucher, N., Souguir, Z., & Renou, F. (2014). Hydrophobically modified
512 xanthan: an amphiphilic but not associative polymer. *Biomacromolecules*, 15(4), 1160–1170.

513 Roy, A. N., Benyahia, L., Grisel, M., & Renou, F. (2016). Shear interfacial viscoelasticity of native and
514 hydrophobically modified xanthan at oil/water interface. *Food Hydrocolloids*, 61, 887–
515 894Saharudin, S. H., Ahmad, Z. and Basri, M. (2016). Role of xanthan gum on physicochemical
516 and rheological properties of rice bran oil emulsion. *International Food Research Journal*
517 23(4) 1361-1366.

518 Shatwell, K. P., Sutherland, I. W., & Ross-Murphy, S. B. (1990). Influence of acetyl and pyruvate
519 substituents on the solution properties of xanthan polysaccharide. *International Journal of*
520 *Biological Macromolecules*, 12(2), 71–78.

521 Sun, C., Gunasekaran, S., & Richards, M. P. (2007). Effect of xanthan gum on physicochemical
522 properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocolloids*,
523 21(4), 555–564.

524 Tadros, T. F. (2009). Emulsion science and technology: a general introduction. *In Emulsion Science*
525 *and Technology (T. F. Tadros)*, 1–56.

526 Vélez, G., Fernández, M. A., Muñoz, J., Williams, P. A., & English, R. J. (2003). Role of hydrocolloids in
527 the creaming of oil in water emulsions. *Journal of Agricultural and Food Chemistry*, 51(1),
528 265–269.

529 Ye, A., Hemar, Y., & Singh, H. (2004). Enhancement of coalescence by xanthan addition to oil-in-water
530 emulsions formed with extensively hydrolysed whey proteins. *Food Hydrocolloids*, 18(5),
531 737–746.

532