

Interactions of bile salts with a dietary fibre, methylcellulose, and impact on lipolysis

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Pabois, O., Antoine-Michard, A., Zhao, X., Omar, J., Ahmed, F., Alexis, F., Harvey, R. D., Grillo, I., Gerelli, Y., Grundy, M. M.-L., Bajka, B., Wilde, P. J. and Dreiss, C. A. (2020) Interactions of bile salts with a dietary fibre, methylcellulose, and impact on lipolysis. *Carbohydrate Polymers*, 231. 115741. ISSN 0144-8617 doi: <https://doi.org/10.1016/j.carbpol.2019.115741> Available at <https://centaur.reading.ac.uk/88064/>

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To link to this article DOI: <http://dx.doi.org/10.1016/j.carbpol.2019.115741>

Publisher: Elsevier

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1 **Interactions of bile salts with a dietary fibre, methylcellulose, and impact**
2 **on lipolysis**

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30 **Highlights**

- 31 • BS, NaTC and NaTDC, impact the rheological properties and gelation of MC.
- 32 • NaTDC has a greater impact on the viscoelasticity of MC compared to NaTC.
- 33 • NaTDC desorbs from a MC-stabilised interface at lower concentrations than NaTC.
- 34 • Upon digestion, NaTDC destabilises more readily MC-stabilised emulsion droplets.
- 35 • During MC-stabilised emulsion digestion, NaTDC generates less FFA than NaTC.

36 **Abstract**

37 Methylcellulose (MC) has a demonstrated capacity to reduce fat absorption, hypothetically
38 through bile salt (BS) activity inhibition. We investigated MC cholesterol-lowering mechanism,
39 and compared the influence of two BS, sodium taurocholate (NaTC) and sodium
40 taurodeoxycholate (NaTDC), which differ slightly by their architecture and exhibit contrasting
41 functions during lipolysis.

42 BS/MC bulk interactions were investigated by rheology, and BS behaviour at the MC/water
43 interface studied with surface pressure and ellipsometry measurements. *In vitro* lipolysis
44 studies were performed to evaluate the effect of BS on MC-stabilised emulsion droplets
45 microstructure, with confocal microscopy, and free fatty acids release, with the pH-stat
46 method.

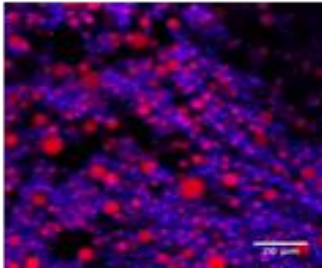
47 Our results demonstrate that BS structure dictates their interactions with MC, which, in turn,
48 impact lipolysis. Compared to NaTC, NaTDC alters MC viscoelasticity more significantly, which
49 may correlate with its weaker ability to promote lipolysis, and desorbs from the interface at
50 lower concentrations, which may explain its higher propensity to destabilise emulsions.

51 **Keywords**

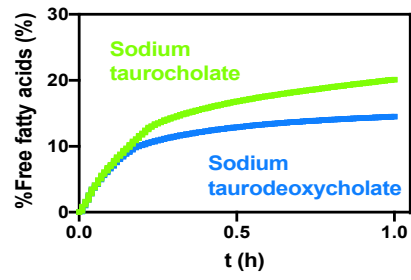
52 **Methylcellulose; bile salts; rheology; surface pressure measurements; *in vitro* duodenal**
53 **lipolysis**

54

55 **Graphical abstract**



Oil droplets stabilised by methylcellulose



Methylcellulose-stabilised emulsion *in vitro* digestion

56

57 1. Introduction

58 Obesity and associated health risks (such as chronic cardiovascular diseases and type-
59 2-diabetes mellitus) have become increasingly prevalent worldwide. In 2016, 39% of the
60 world's adult population were classified as overweight, and 13% as obese (World Health
61 Organization, 2019). Controlling the digestion of dietary lipids (fats) and optimising their
62 absorption are therefore crucial to addressing this ongoing health crisis (McClements & Li,
63 2010b; Mei, Lindqvist, Krabisch, Rehfeld, & Erlanson-Albertsson, 2006). With their
64 demonstrated capability to reduce food intake and aid weight loss, dietary fibres have shown
65 great potential against obesity (Slavin, 2005). Nonetheless, a better understanding of the
66 processes responsible for their ability to regulate calorie uptake still needs to be provided.
67 Due to its approved (Younes et al., 2018) and wide (The Dow Chemical Company, 2002) use in
68 the food industry, as well as its proven capacity to diminish blood cholesterol levels (without
69 inducing any adverse effect) (Agostoni et al., 2010), methylcellulose (MC) is an appropriate
70 model of dietary fibre for elucidating the mechanism by which dietary fibres reduce
71 hyperlipidaemia.

72 MC is a non-ionic polysaccharide belonging to the large family of cellulose ethers and
73 containing repeating anhydroglucose units, with methyl (hydrophobic) moieties substituting
74 hydroxyl (hydrophilic) groups (Nasatto et al., 2015b) (Figure 1). The capacity of this dietary
75 fibre to hinder lipolysis has been mainly attributed to its ability to induce loss of bile salts (BS)
76 and cholesterol in faeces by (i) increasing the viscosity of the small intestine content (Christos
77 Reppas, Meyer, Sirois, & Dressman, 1991), which slows down fat digestion and reduces
78 nutrients absorption (Bartley et al., 2010; Carr, Gallaher, Yang, & Hassel, 1996; Maki et al.,
79 2009; C. Reppas, Swidan, Tobey, Turowski, & Dressman, 2009; van der Gronde, Hartog, van
80 Hees, Pellikaan, & Pieters, 2016), and/or by (ii) trapping BS and/or cholesterol molecules in its
81 network, *via* hydrophobic interactions occurring both in the bulk aqueous phase and at the
82 fat droplet interface (Pilosof, 2017; Pizonos Ruiz-Henestrosa, Bellesi, Camino, & Pilosof, 2017;
83 Torcello-Gómez et al., 2015; Torcello-Gómez & Foster, 2014). BS are biosurfactants produced
84 in the liver and released into the small intestine (duodenum) (Hofmann & Mysels, 1987),
85 which play key roles in lipid digestion and absorption (Maldonado-Valderrama, Wilde,
86 Macierzanka, & Mackie, 2011; Wilde & Chu, 2011): on the one hand, they facilitate enzyme
87 adsorption to fat droplet interfaces, thus promoting enzyme-catalysed lipolysis (Borgström,

88 Erlanson-Albertsson, & Wieloch, 1979; Bourbon Freie, Ferrato, Carrière, & Lowe, 2006;
89 Erlanson-Albertsson, 1983; Labourdenne, Brass, Ivanova, Cagna, & Verger, 1997); on the
90 other, they remove the enzyme-inhibiting insoluble lipolysis products (diacylglycerols (DAG),
91 monoacylglycerols (MAG) and free fatty acids (FFA)) present at the interface, carrying them
92 to the gut mucosa for absorption (Hofmann & Mysels, 1987). In this work, we are focusing on
93 the interactions between MC and BS, which have been hypothesised to explain (i) MC
94 cholesterol-lowering effect, due to the reduction in BS re-absorption in the ileum and the
95 subsequent increased production of BS by the liver from cholesterol, and (ii) the early
96 signalling of satiation and lengthening of satiety feeling, by the accumulation of undigested
97 materials in the duodenum, due to BS being entrapped and prevented from fulfilling their
98 functions during lipolysis (Gunness & Gidley, 2010). Recent studies have demonstrated BS
99 inhibitory effect on MC thermally-induced structuring using microcalorimetry and rheology
100 (Torcello-Gómez et al., 2015; Torcello-Gómez & Foster, 2014), and the competition of BS with
101 MC for adsorption at the lipid droplet/water interface with tensiometry (Torcello-Gómez &
102 Foster, 2014). However, there is little structural evidence for the hypothesis of entrapment of
103 BS by MC, and a mechanistic understanding of the competitive processes leading to enzyme
104 inhibition, delayed fat digestion and the associated health benefits, is still lacking. Therefore,
105 further studies are required to clarify how MC interacts with BS during lipid digestion and how
106 this, in turn, correlates to BS molecular structure and their contrasting roles.

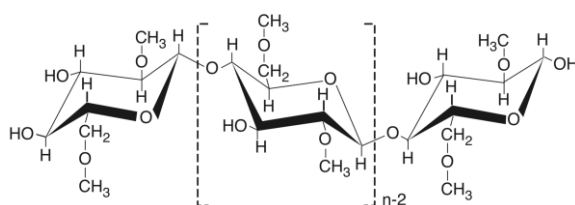
107 The work presented here increases our understanding of the mechanisms underlying
108 MC capacity to regulate fat digestion in the small intestine, with a particular focus on its ability
109 to compete with BS for adsorption at the lipid droplet/water interface. More specifically, by
110 combining bulk and interfacial experiments with *in vitro* lipolysis studies, we examined the
111 interactions between MC and BS in bulk water, at the MC/water interface, and at the oil/water
112 interface of fat droplets mimicking food colloids. It has been hypothesised that BS structural
113 diversity is responsible for the different functions they carry out in fat digestion; to explore
114 this postulate, two BS, sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC)
115 (Figure 2), were selected, as they display contrasting adsorption/desorption dynamics, which
116 are thought to reflect their different roles in the gut (Pabois et al., 2019; Parker, Rigby, Ridout,
117 Gunning, & Wilde, 2014). Since BS are expected to interact with MC both in the bulk aqueous
118 phase and at the surface of MC-stabilised emulsion droplets, we assessed the impact of BS on
119 MC rheological properties, using oscillatory shear rheology, and BS/MC interfacial behaviour

120 at the air/water interface, through surface pressure measurements in a Langmuir trough set-
121 up and ellipsometry. We then investigated how these interactions affect the lipolysis of an
122 MC-stabilised emulsion, by monitoring the structure of emulsion droplets after addition of BS
123 and enzymes, with different optical microscopy techniques, and by measuring the amount of
124 FFA released throughout *in vitro* lipid digestion, with the pH-stat method.
125

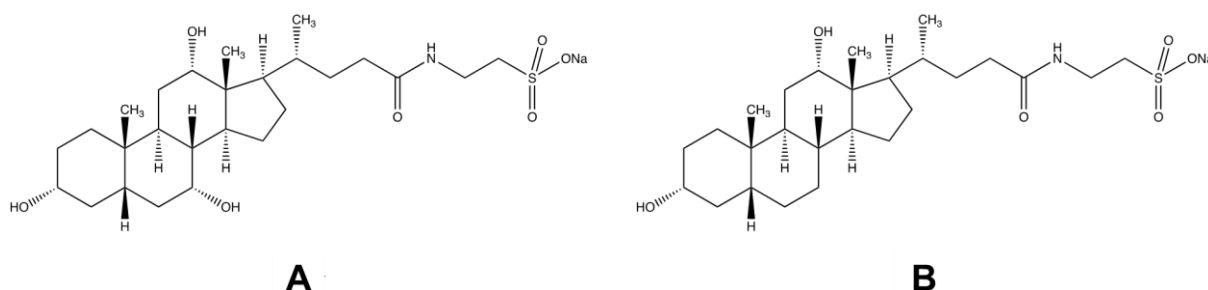
126 2. Experimental section

127 2.1 Materials

128 Methocel™ SG A7C (solution viscosity: 700 mPa.s at 2% w/w at 20°C; methoxyl degree of
129 substitution: 1.8; molecular weight: 400 - 500 kDa) (Figure 1) was kindly supplied by Dow Wolff
130 Cellulosics GmbH (Bomlitz, Germany). Chloroform (CHCl₃) was purchased from Fisher
131 Scientific (Loughborough, UK). NaTC (P97.0% TLC) (Figure 2A), NaTDC (P95.0% TLC) (Figure
132 2B), paraffin oil, ethanol (EtOH, P99.8% GC), orlistat (P98.0%), Nile red, fluorescent brightener
133 28 (calcofluor), dimethyl sulfoxide anhydrous (P99.9%), sunflower seed oil from *Helianthus*
134 *annuus*, pancreatin from porcine pancreas (or pancreatic lipase/co-lipase; activity: 40 U/mg
135 of solid, based on lipase activity using tributyrin as a substrate), sodium phosphate monobasic
136 dihydrate (NaH₂PO₄, P99.0% T), sodium phosphate dibasic dihydrate (Na₂HPO₄, P99.0% T),
137 sodium chloride (NaCl, P99.8%), calcium chloride dihydrate (CaCl₂, P99.0%) and sodium
138 hydroxide (NaOH, 0.1 M) were all obtained from Sigma-Aldrich (Gillingham, UK). Ultrapure
139 water, or MilliQ-grade water (H₂O, 18.2 MΩ·cm, Merck Millipore, Molsheim, France), was
140 used in all experiments. Phosphate buffer (10 mM, pH = 7.04 at 21°C) was prepared by mixing
141 0.01% wt NaH₂PO₄ with 0.01% wt Na₂HPO₄, in ultrapure water. All reagents were used as
142 supplied.



143
144 **Figure 1: Structure of methylcellulose (MC)**



145
146 **Figure 2: Structures of sodium taurocholate (NaTC) (A) and sodium taurodeoxycholate (NaTDC) (B)**

147 2.2 Methods

148 **2.2.1 Bulk and interfacial studies**

149 **2.2.1.1 Preparation of MC and MC/BS aqueous solutions**

150 MC aqueous solution was prepared using the “hot/cold” method (The Dow Chemical
151 Company, 2002, 2013). Solid MC was first dispersed into one third of the required mass of
152 ultrapure water heated to 80°C (for around 15 minutes), until complete wetting of particles;
153 then, the dispersion was transferred into an ice bath, and the remaining two thirds of cold
154 ultrapure water (4°C) were added progressively into the stirred solution, which was finally left
155 to stir overnight at 4°C, to ensure complete solubilisation. MC/BS solutions were prepared
156 simply by mixing both components together at the required concentrations.

157 **2.2.1.2 Rheology measurements**

158 Rheology experiments were performed with a strain-controlled rheometer (ARES, TA
159 instruments, Inc, Borehamwood, UK), fitted with a 25 mm diameter titanium parallel plate
160 and equipped with a temperature-controlled Peltier system (with a $\pm 0.1^\circ\text{C}$ temperature
161 stability at thermal equilibrium). Each sample was loaded onto the lower plate, and the upper
162 plate was adjusted to a gap size of 0.8 ± 0.3 mm. A thin layer of low viscosity paraffin oil was
163 deposited around the edges of the sample exposed to air to prevent sample drying and
164 evaporation throughout the measurement.

165 Dynamic temperature sweeps were performed at a fixed angular frequency of 6.28 rad/s and
166 strain of 1%, from 20°C to 80°C, with a heating rate of 2°C/min, to measure the evolution of
167 the storage (G') and loss (G'') moduli as a function of temperature, in the absence and
168 presence of BS. Dynamic frequency sweeps were performed over an angular frequency range
169 of 0.1 - 100 rad/s, at a fixed strain of 1%, and a fixed temperature of 60°C (above MC transition
170 temperature (T_t), which is the point where a break in the slope of G' is detected in the dynamic
171 temperature sweep curves). The strain of 1% was chosen within the linear viscoelastic regime,
172 which was established by performing dynamic strain amplitude sweeps on MC and MC/BS
173 solutions, over a strain range of 0.01 - 100%, at a constant angular frequency of 6.28 rad/s and
174 a temperature of 60°C. Each test was repeated at least twice to confirm reproducibility;
175 representative curves (rather than averages) are shown in the manuscript.

176 **2.2.1.3 Langmuir trough measurements**

177 Interfacial tension measurements were performed in a 50 mm diameter perfluoroalkoxy Petri
178 dish (19.6 cm² surface area and 20 mL volume of subphase), to study the adsorption of MC
179 and its interaction with BS at the air/water interface. All experiments were carried out under
180 constant stirring, at a fixed area, and at a temperature of 23 ± 2°C (room temperature). The
181 surface pressure (π) was measured by a Wilhelmy plate made of chromatographic paper
182 (Whatman International Ltd, Maidstone, UK) of 2.3 x 1.0 cm (length x width) and attached to
183 a calibrated Nima PS4 microbalance (Nima Technology Ltd, Coventry, UK). Prior to any
184 measurement, the trough was thoroughly cleaned with EtOH and CHCl₃ to remove organic
185 impurities, and then filled with ultrapure water (subphase). Surface-active contaminants, dust
186 and bubbles were all removed from the subphase by suction with a pump, and the subphase
187 was considered as clean when changes in surface pressure did not exceed ± 0.2 mN/m over
188 approximately two minutes.

189 **MC adsorption at the air/water interface.** Using a 1 mL syringe (Becton Dickinson, Madrid,
190 Spain) fitted with a 19 G x 1 ½ in. needle (Becton Dickinson, Madrid, Spain), a specific amount
191 of pure MC solution in ultrapure water was injected into the subphase, under constant stirring.
192 Surface pressure (π) was measured over time until it reached a plateau. Each experiment was
193 repeated at least twice; either a representative curve or an average measurement is shown.

194 **BS interaction with a MC layer at the air/water interface.** A MC layer was first formed at the
195 air/water interface, by addition of a specific amount of MC aqueous solution into the clean
196 and stirred water ($\pi_{MC} = 21 \pm 1$ mN/m with 0.5‰ w/w, and $\pi_{MC} = 18 \pm 2$ mN/m with 0.5×10⁻²
197 ‰ w/w). After film equilibration (ca. 1-2 hours), a specific amount of pure BS aqueous
198 solution was injected beneath the MC layer. The corresponding changes in surface pressure
199 (π) were recorded over time. Each experiment was repeated at least twice; either a
200 representative curve or the average measurement is shown.

201 **2.2.1.4 Ellipsometry**

202 MC adsorption and interaction with BS at the air/water interface was further investigated by
203 ellipsometry (Beaglehole Instruments, Wellington, New Zealand). Time-dependent
204 measurements were performed with a 632.8 nm-wavelength laser hitting the surface at an
205 incident angle of 50°. In this configuration, changes in the polarisation of light reflected by the

206 interface are measured over the 1 mm² area and ~1 μm depth probed by the laser beam;
207 these changes can be correlated to the amount of material adsorbed at this interface over
208 time. The polarisation state of the incident light is composed of an *s*- and *p*-component (where
209 the *s*-component is oscillating parallel to the sample surface, and the *p*-one parallel to the
210 plane of incidence). The ratio of the reflectivity of these two components (r_s for the *s*-
211 component and r_p for the *p*-component) characterises the polarisation change and is
212 expressed by the following equation:

$$213 \quad \frac{r_p}{r_s} = \tan(\psi) \cdot e^{i\Delta} \quad (1)$$

214 where ψ is the amplitude change and Δ the phase shift. In the thin film limit at the air/water
215 interface (i.e., film thickness \ll laser wavelength), Δ is found to be much more sensitive to
216 changes in the amount adsorbed at the interface than ψ (Motschmann & Teppner, 2001).
217 Therefore, time-dependent changes in phase shift ($\Delta\Delta$) were measured, with $\Delta\Delta(t) = \Delta(t) -$
218 $\Delta(t_0)$, where $\Delta(t_0)$ is the phase shift at the beginning of a given experiment, namely, the phase
219 shift of the bare air/water interface (Δ_0) for MC adsorption and interaction with BS, at the
220 air/water interface. Changes in the phase shift are directly proportional to the amount of
221 material adsorbed at the interface (Motschmann & Teppner, 2001). In order to measure
222 simultaneously the surface pressure and phase shift for the same surface, the instrument was
223 mounted on top of the Petri dish, used as a Langmuir trough. Data were acquired at a rate of
224 0.2 Hz, using the Igor Pro software.

225 **2.2.2 *In vitro* lipolysis studies**

226 **2.2.2.1 Preparation of MC-stabilised emulsion**

227 MC (0.5% w/w) was dispersed into sunflower oil (15% w/w). Cold phosphate buffer (84.5%
228 w/w, at $T < T_{\text{dissolution}} = 10^\circ\text{C}$) was added to the oil phase and the mixture stirred for a few
229 minutes. The dispersion was then pre-emulsified at 11,000 rpm for 1 minute, using a high-
230 shear mixer (T25 digital Ultra-Turrax, IKA®-Werke GmbH & Co. KG, Staufen, Germany). This
231 pre-emulsion was transferred into a 10 mL volume beaker in an ice bath and was sonicated at
232 a frequency of 20 kHz and amplitude of 70% for 5 minutes with a tip sonicator (SONOPULS HD
233 3100 ultrasonic homogeniser, microtip model: MS 73, BANDELIN electronic GmbH & Co. KG,
234 Berlin, Germany).

235

2.2.2.2 Simulation of the duodenal lipolysis environment

236 For each *in vitro* lipolysis experiment, the following model (Grundy, Wilde, Butterworth, Gray,
237 & Ellis, 2015) was employed to simulate the duodenum (small intestine) environment: 19 mL
238 of MC-stabilised emulsion was added to a thermostatically-controlled and mechanically-
239 stirred reaction vessel at 37°C, followed by 15 mL of a BS aqueous solution (NaTC, NaTDC; 2.5,
240 25, 125 mM, in phosphate buffer). Then, 1 mL of NaCl (4.9 M, in ultrapure water) and 1 mL of
241 CaCl₂ (0.37 M, in ultrapure water) were added to the mixture, under continuous stirring.
242 Finally, 1.5 mL of either phosphate buffer (for the blank assay, used as a control) or freshly
243 prepared pancreatic lipase/co-lipase suspension (17 mg/mL, in phosphate buffer) (for the
244 lipolysis assay) were added. The final system was made up of 7.6% w/w lipid, 1, 10, or 50 mM
245 BS, 130 mM NaCl, 10 mM CaCl₂, and 0.68 mg/mL pancreatic lipase/co-lipase.

246

2.2.2.3 Optical microscopy

247 The structural changes induced on an MC-stabilised emulsion upon duodenal digestion, were
248 monitored over time by brightfield optical (Olympus BX61 microscope, Olympus France S.A.S.,
249 Rungis, France) and confocal (Leica TCS SP2, DMIRE2 inverted, Leica Microsystems UK Ltd,
250 Milton Keynes, UK) microscopy. Prior to *in vitro* lipolysis studies, the pure emulsion was
251 characterised; then, the mixture modelling the duodenal environment was added to the
252 emulsion and samples measured at different time points (t = 5, 15, 30 and 60 min), to analyse
253 the evolution of emulsion droplet microstructure from the beginning to the end of duodenal
254 lipolysis. The influence of each component (NaTC, NaTDC, NaCl and CaCl₂) used individually
255 and together was assessed to better understand their impact on duodenal lipolysis. A blank
256 assay was also measured as a control to monitor changes over time in the absence of enzymes.

257 For confocal microscopy, prior to visualisation, samples were mixed with 1 mg/mL orlistat
258 (prepared in dimethyl sulfoxide) to stop lipolysis, and then stained with 10 µg/mL Nile red
259 (prepared in dimethyl sulfoxide) and 20 µg/mL calcofluor (prepared in ultrapure water), to
260 detect lipids (red fluorescence) and MC (blue fluorescence), respectively. Samples were
261 excited at 488 nm (for Nile red) and 405 nm (for calcofluor), and the fluorescence emitted by
262 the samples was detected between 510 - 650 nm (for Nile red) and 410 - 480 nm (for
263 calcofluor). Images were captured using objective lenses of 10×, 20× or 63×, and micrographs

264 were compiled with the Olympus image analysis software (for optical microscopy, Olympus
265 France S.A.S., Rungis, France) and Fiji software (“Fiji,” 2019) (for confocal microscopy).

266 **2.2.2.4 pH-stat measurements**

267 The rate and extent of lipolysis were evaluated by titrating the amount of FFA released from
268 an MC-stabilised emulsion with 0.1 M NaOH, at 37°C and pH 7.0, in conditions mimicking the
269 duodenal (small intestine) environment. Each assay was carried out over 1 hour of digestion,
270 using a pH-stat titration unit (848 Titrino plus, Metrohm AG, Herisau, Switzerland). The blank
271 experiment was performed as a control, to measure pH fluctuation in the absence of enzymes;
272 the volume of NaOH released during this assay was then subtracted from the data recorded
273 in the presence of pancreatic lipase/co-lipase (lipolysis assay). Each blank and lipolysis
274 experiment was repeated at least six times.

275 The volume of NaOH released during MC-stabilised emulsion digestion was converted into the
276 percentage of FFA produced, using this equation:

$$277 \quad \%FFA(t) = 100 \times \frac{V_{NaOH}(t) \cdot [NaOH] \cdot M_{Lipid}}{2 \cdot m_{Lipid}} \quad (2)$$

278 where V_{NaOH} is the volume of NaOH required to neutralise the FFA produced over time, $[NaOH]$
279 the concentration of the NaOH solution used, M_{Lipid} the molecular weight of the oil employed
280 in this experiment (in our case, $M_{Sunflower\ oil} = 876\text{ g/mol}$ (Sánchez, Maceiras, Cancela, &
281 Rodríguez, 2012)), and m_{Lipid} the mass of triacylglycerol (TAG) initially present in the digestion
282 vessel. This equation has been established considering the ideal case where the hydrolysis of
283 one molecule of TAG leads to the formation of one molecule of MAG and two molecules of
284 FFA. The results are shown as the proportion of FFA release as a function of time.

285 The pH-stat data were analysed with the GraphPad Prism software (“GraphPad Prism,” 2019);
286 statistical analysis was carried out using the two-way analysis of variance (ANOVA), followed
287 by the Tukey post-test, with a 95% confidence level, meaning that differences were
288 considered as statistically significant when $P < 0.05$.

289 **3. Results**

290 **3.1 BS interaction with MC in the bulk**

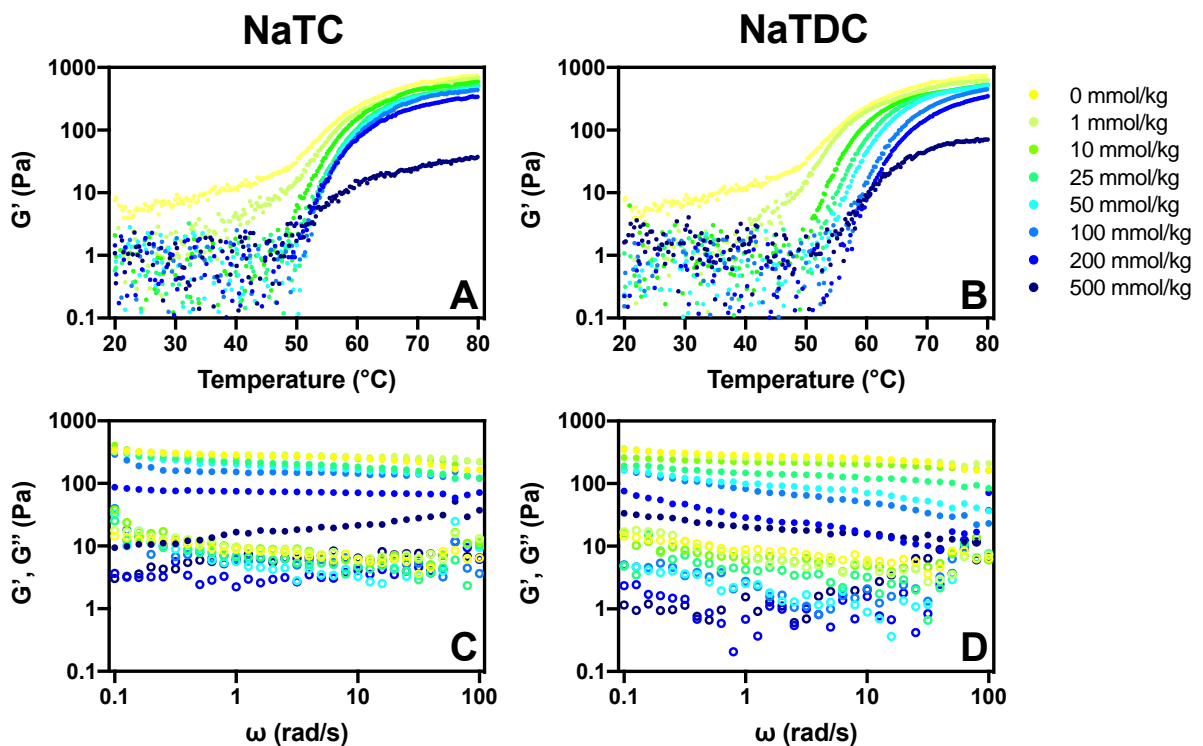
291 **MC viscoelastic behaviour.** The temperature-dependence of MC rheological properties was
292 investigated by performing dynamic temperature sweep measurements on MC solutions
293 prepared at concentrations ranging between 0.1 and 2.0% w/w (Figure S1).

294 At all the MC concentrations studied, a relatively flat region is observed for the storage
295 modulus (G') in the lower temperature range (ca. 20 - 40°C), followed by a steep increase
296 beyond a transition temperature (T_t) and a final plateau at high temperatures. As MC
297 concentration increases, the transition temperature from which G' starts to level off shifts
298 towards lower values (from 55°C at 0.1% w/w, to 37°C at 2.0% w/w). Below and above T_t , MC
299 behaves as a predominantly solid-like material over the whole range of temperatures studied
300 (G' dominates over G'' over the range of frequencies measured), and above T_t , both moduli
301 increase and are still independent of frequency (data not shown) (Funami et al., 2007; L. Li et
302 al., 2001; Lin Li, 2002); the transition temperature thereby corresponds to a weak-to-strong
303 gel transition. The increase in MC concentration also induces a relatively weak change in MC
304 elastic properties (G') at low temperatures, and a much more significant one in the high
305 temperature region, in agreement with previous studies (Nasatto et al., 2015a).

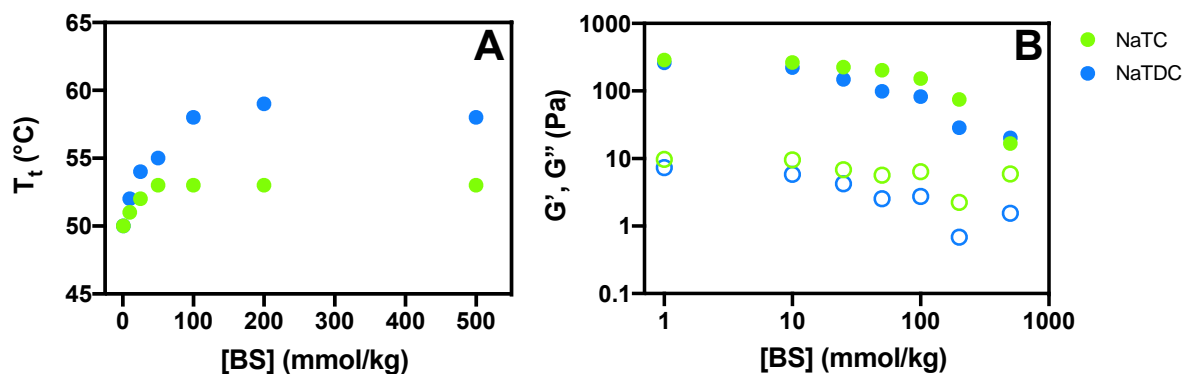
306 The gelation of MC – whose chains are arranged as ‘bundles’ at room temperature (or packed
307 ‘strands’ held together by packing of unsubstituted regions and the hydrophobically-driven
308 aggregation of methyl groups in regions of denser substitution) – has been postulated to
309 follow two steps (Haque & Morris, 1993; Sarkar, 1995; Desbrières, Hirrien, & Rinaudo, 1998;
310 Hirrien, Chevillard, Desbrières, Axelos, & Rinaudo, 1998; Kobayashi, Huang, & Lodge, 1999; L.
311 Li et al., 2001, 2002; Lin Li, 2002; Lin Li, Wang, & Xu, 2003; Funami et al., 2007; Torcello-Gómez
312 & Foster, 2014; Torcello-Gómez et al., 2015; Nasatto et al., 2015a; Isa Ziembowicz et al., 2019):
313 upon heating, MC strands separate, allowing intermolecular associations to form between MC
314 hydrophobic (methyl) groups, therefore inducing the formation of a strong, physical gel
315 network; at low temperatures, these hydrophobic polymer-polymer interactions take place to
316 a much lower extent because of water molecules surrounding MC methyl moieties (*via*
317 hydrogen bonds), thus resulting in the swelling of ‘bundles’ and the formation of a softer,
318 weaker gel. The effect of MC concentration on its rheological properties is therefore

319 attributed to the increase in the number of methyl groups in solution, resulting in a larger
320 number of hydrophobic interactions from lower temperatures.

321 **Effect of BS on MC viscoelastic behaviour.** The impact of the two BS on MC rheological
322 properties was assessed by following the dynamic moduli (G' , G'') of a 1.0% w/w MC solution
323 over a range of temperatures and frequencies (Figure 3). The evolution of the transition
324 temperature (T_t , from which the increase in G' becomes steeper) and of both dynamic moduli
325 (G' , G'') are shown as a function of BS concentration in Figures 4A and 4B, respectively.



326
327 Figure 3: (A, B) Temperature-dependent evolution of the storage modulus (G') obtained from dynamic temperature
328 sweeps, and (C, D) angular frequency-dependent evolution of the dynamic moduli: (\bullet) G' , the storage modulus, (\circ) G'' , the
329 loss modulus, obtained from dynamic frequency sweeps performed at a constant temperature of 60°C, on a 1.0% w/w MC
330 aqueous solution containing increasing amounts (1, 10, 25, 50, 100, 200, 500 mmol/kg) of BS: (A, C) NaTC, (B, D) NaTDC.
331 The curves obtained in the absence of BS are also shown for comparison.



332
 333 **Figure 4:** Evolution of MC transition temperature (T_t) (A) and dynamic moduli: (●) G' , the storage modulus, (○) G'' , the loss
 334 modulus, obtained at an angular frequency of 1 rad/s (B), as a function of the concentration in BS: NaTC, NaTDC. The
 335 transition temperature (T_t) is the temperature from which G' starts changing. These data are extracted from, respectively,
 336 (A) dynamic temperature sweeps performed over a temperature range of 20 - 80°C (Figures 3, A, B), and (B) dynamic
 337 frequency sweeps performed over an angular frequency range of 0.1 - 100 rad/s, at a constant temperature of 60°C (Figures
 338 3, C, D).

339 In the presence of BS, the dynamic temperature sweeps of MC solutions show a similar profile
 340 as the pure MC solution, namely, a moderate increase in G' followed by a sharp rise (Figures
 341 3, A, B). However, BS have a significant impact on MC rheological properties, leading to a
 342 notable, and gradual increase in the transition temperature from around 50°C, in the absence
 343 of BS, to 53°C with 500 mmol/kg NaTC and 58°C with 500 mmol/kg NaTDC (Figure 4A). In
 344 addition, both BS (from the lowest concentration studied of 1 mmol/kg) induce a drop in MC
 345 viscoelasticity (G') at all temperatures studied, most visibly at high temperatures (Figures 3,
 346 A, B). At physiological temperature (37°C), a decrease from $G' = 10$ Pa in the absence of BS, to
 347 $G' = 5$ and 2 Pa in the presence of 1 mmol/kg of, respectively, NaTC and NaTDC, is observed.
 348 Dynamic frequency sweeps performed at 60°C, where MC forms a strong gel and changes
 349 caused by BS are most visible (Figures 3, C, D), reveal a 10-fold decrease in G' , from ca. 280 Pa
 350 in the absence of BS, to ca. 20 Pa with the highest concentration of BS studied, at a frequency
 351 of 1 rad/s (Figure 4B). In addition, G' shows an increasing dependence on frequency with the
 352 addition of BS, more notably so with NaTDC. Overall therefore, the presence of the BS
 353 converts MC gel into a less solid-like material. Comparing the two BS, it is clear that NaTDC
 354 has a much stronger impact; for instance, only 10 mmol/kg of NaTDC are needed to
 355 significantly reduce the value of the storage modulus (G') (Figures 3D and 4B), while 25
 356 mmol/kg of NaTC are required to induce the same effect (Figures 3C and 4B). Similar
 357 observations have been reported elsewhere (Torcello-Gómez et al., 2015).

358 Overall, over the whole temperature range studied, MC behaves as a gel whose strength
359 increases with temperature. The addition of BS induces a transition to a softer material (lower
360 elastic modulus (G')), both above and below MC transition temperature (T_t); in addition, this
361 transition occurs at lower concentrations of NaTDC, compared to NaTC.

362 **3.2 BS interfacial properties in the presence of MC**

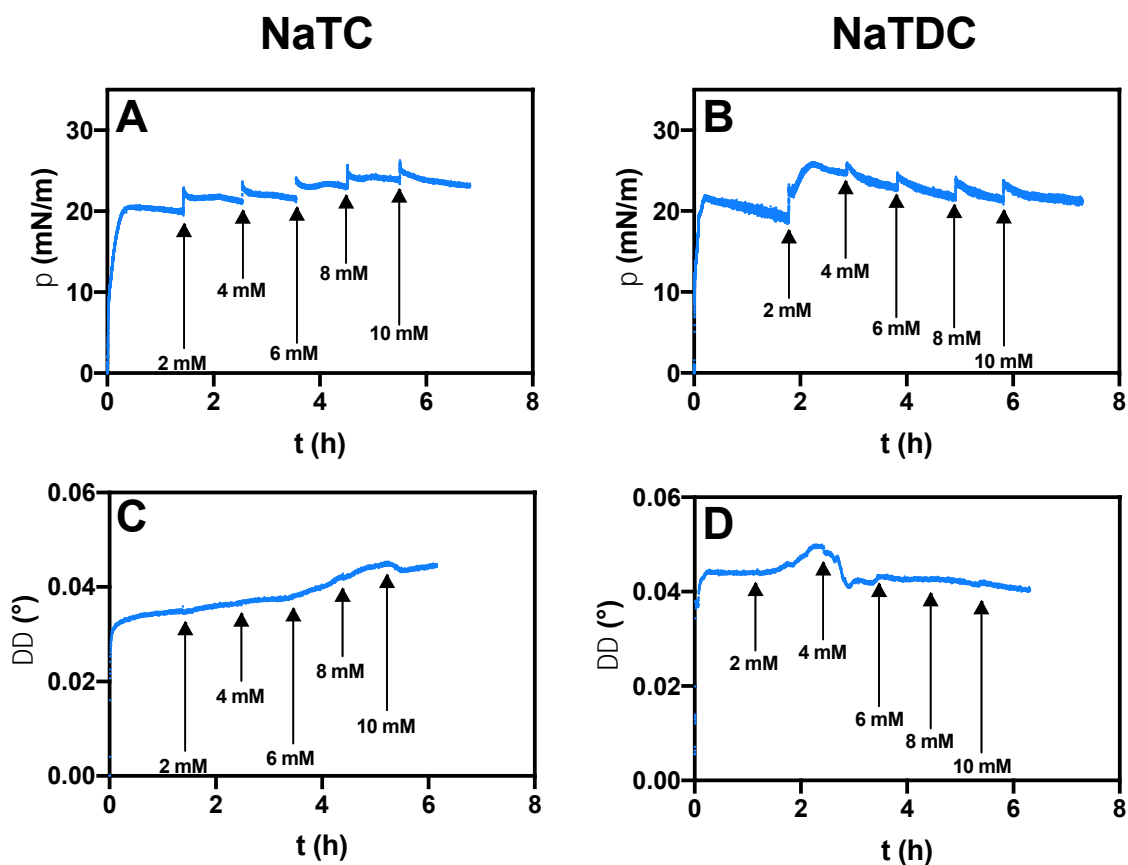
363 **MC adsorption dynamics at the air/water interface.** MC behaviour at the bare air/water
364 interface was studied using both a Langmuir trough and ellipsometer, by monitoring the time-
365 dependent evolution of the surface pressure (π) and phase shift ($\Delta\Delta$), respectively, upon
366 injection into the water subphase of either successive quantities of MC (0.5×10^{-1} , 0.25 and
367 0.5% w/w (Figure S2); 0.5×10^{-2} , 0.25×10^{-1} and $0.5 \times 10^{-1}\%$ w/w (Figure S3)), or fixed amounts
368 over a longer period of time (0.5×10^{-3} , 0.5×10^{-2} , 0.5×10^{-1} or 0.5% w/w) (Figure S4).

369 Upon addition of $0.5 \times 10^{-1}\%$ w/w MC into the aqueous subphase, the surface pressure
370 increases until reaching a near-plateau at $\pi = 19 \pm 1$ mN/m, which stays relatively constant
371 with following injections ($\pi = 19 \pm 1$ mN/m at 0.25% w/w, and $\pi = 18 \pm 3$ mN/m at 0.5% w/w)
372 (Figure S2A). With the same injection sequence, the ellipsometry phase shift, which is
373 measured at the same time as the surface pressure and relates to the amount of material
374 adsorbed at the interface (Motschmann & Teppner, 2001), exhibits the same trend as the
375 surface pressure (Figure S2B): it reaches a value of $\Delta\Delta = 0.033^\circ$, which then slightly increases
376 to $\Delta\Delta = 0.035^\circ$ at 0.25% w/w and $\Delta\Delta = 0.036$ at 0.5% w/w. Both measurements thus show
377 that MC adsorbs at the air/water interface up to a saturation point, independently of its
378 concentration in the bulk. The two experiments differ, nevertheless, by the presence of peaks
379 of surface pressure visible straight after MC injection, not detected in the phase shifts, which
380 could be explained by an initial strong adsorption, followed by a relaxation process as the
381 polymer rearranges at the air/water interface, changing conformation (Graham & Phillips,
382 1979). These transient surface pressure peaks were also observed in a previous study with BS
383 injected under the air/water interface (Pabois et al., 2019). The trends in surface pressure
384 (Figure S3A) and phase shift (Figure S3B) are reproduced with lower amounts of MC (0.5×10^{-2} ,
385 0.25×10^{-1} , and $0.5 \times 10^{-1}\%$ w/w) injected into water.

386 In order to study the kinetics of adsorption of MC molecules at the air/water interface, surface
387 pressure measurements were performed over longer periods of time (Figure S4). Results show
388 that, above $0.5 \times 10^{-2}\%$ w/w, the same equilibrium surface pressure ($\pi = 17 \pm 1$ mN/m) is

389 always reached, irrespective of MC concentration, whereas a much lower value is obtained at
390 the lowest concentration studied of $0.5 \times 10^{-3} \text{‰ w/w}$ ($\pi = 10 \pm 0.4 \text{ mN/m}$). Arboleya and Wilde
391 (Arboleya & Wilde, 2005) also observed a saturation point from a similar MC concentration
392 (i.e., $1 \times 10^{-2} \text{‰ w/w}$), and obtained comparable interfacial tension values. Furthermore, as MC
393 concentration decreases, the surface pressure rises at a slower rate: a change in surface
394 pressure is immediately observed after injection of both 0.5×10^{-1} and 0.5‰ w/w , while a lag
395 period of about 3 and 40 min is seen with solutions containing 0.5×10^{-2} and $0.5 \times 10^{-3} \text{‰ w/w}$
396 MC, respectively. The amount injected into the aqueous subphase thus affects MC adsorption
397 rate and extent, such that the lower the concentration, the slower the adsorption process and
398 the lower the quantity of material adsorbed, therefore indicating a diffusion-controlled
399 adsorption mechanism, as already observed elsewhere with hydroxypropyl MC (Avranas &
400 Tasopoulos, 2000; Camino, Pérez, Sanchez, Rodriguez Patino, & Pilosof, 2009; Pérez, Sánchez,
401 Pilosof, & Rodríguez Patino, 2008; Wollenweber, Makievski, Miller, & Daniels, 2000). In the
402 literature, MC adsorption has been suggested to occur in three stages: MC first slowly diffuses
403 from the bulk phase to the sub-surface region and then adsorbs at the air/water interface,
404 while undergoing conformational changes (Arboleya & Wilde, 2005).
405 All these results are consistent with data reported elsewhere (Nasatto et al., 2014; Pizones
406 Ruiz-Henestrosa et al., 2017).

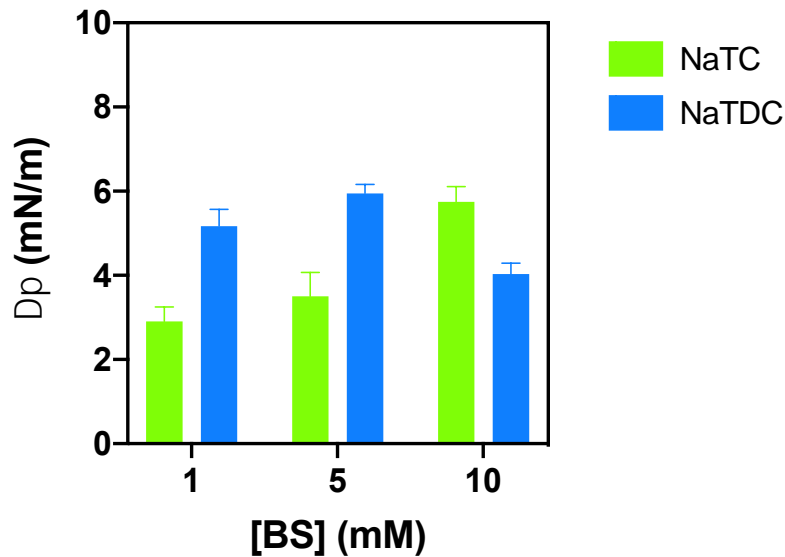
407 **BS interaction with a MC layer at the air/water interface.** The interfacial behaviour of the
408 two selected BS (NaTC and NaTDC) in the presence of a MC film at the air/water interface was
409 then evaluated, by injecting BS below the polysaccharide layer. Measurements were carried
410 out either by adding increasing amounts of BS every hour (2, 4, 6, 8 and 10 mM) (Figures 5
411 and S5) or by injecting fixed concentrations and measuring over longer times (1, 5 or 10 mM)
412 (Figures 6 and S6). These BS concentrations were selected to be below, around, and above
413 their critical micelle concentration (CMC), which is 4 – 7 mM for NaTC (gradual micellisation
414 process) and 2 mM for NaTDC in ultrapure water (data not shown) (Matsuoka, Maeda, &
415 Moroi, 2003). Prior to BS injection, a saturated film of MC at the interface was formed by
416 injecting it into the water subphase, at either 0.5‰ w/w (Figures 5, 6 and S6) or $0.5 \cdot 10^{-2} \text{‰ w/w}$
417 w/w (Figure S5).



418
 419 **Figure 5:** Time-dependent evolution of (A, B) the surface pressure (π) measured in a Langmuir trough, and (C, D) phase
 420 shift ($\Delta\Delta(t) = \Delta(t) - \Delta_0$) measured by ellipsometry, upon successive injections of either (A, C) NaTC or (B, D) NaTDC into the
 421 aqueous subphase (at $23 \pm 2^\circ\text{C}$). The first increase in surface pressure corresponds to the adsorption of MC at the air/water
 422 interface, which was added into water at a concentration of 0.5% w/w ($\pi_{\text{MC}} = 21 \pm 1 \text{ mN/m}$, $\Delta\Delta_{\text{MC}} = 0.039 \pm 0.005^\circ$). Each
 423 addition of BS is shown by an arrow, together with the corresponding BS concentration achieved in the subphase. Each
 424 experiment was reproduced twice, and a representative measurement was selected for each experiment.

425 The evolution of the surface pressure is quite different for the two BS (Figures 5, A, B): while
 426 the successive injections of NaTC lead to a continuous increase in surface pressure (up to $\pi =$
 427 $23 \pm 0.5 \text{ mN/m}$ at 10 mM) (Figure 5A), with NaTDC, a steep rise to $\pi = 25 \pm 1 \text{ mN/m}$ (at 2 mM),
 428 followed by a gradual drop to $\pi = 22 \pm 1 \text{ mN/m}$ (at 10 mM), is observed (Figure 5B). These
 429 trends are also obtained with a lower amount of MC at the air/water interface (Figure S5). The
 430 ellipsometry phase shift obtained in parallel follows the same trends (Figures 5, C, D): with
 431 NaTC, it gradually increases up to $\Delta\Delta = 0.045 \pm 0.003^\circ$ upon successive additions of BS into the
 432 subphase (Figure 5C); instead, the injection of 2 mM NaTDC into the water induces a sharp
 433 increase to $\Delta\Delta = 0.047 \pm 0.003^\circ$, followed by a decrease to $\Delta\Delta = 0.042 \pm 0.001^\circ$ from 4 mM
 434 (Figure 5D). As observed with successive injections of MC, temporary surface pressure peaks
 435 are also present after each addition of BS; here again, these peaks could be attributed to MC

436 film compression and subsequent relaxation, induced by BS adsorption (Graham & Phillips,
437 1979).



438

439 **Figure 6:** Evolution of the surface pressure ($\Delta\pi = \pi_{Equilibrium} - \pi_{MC}$) as a function of BS concentration, measured in a Langmuir
440 trough, upon injection of fixed concentrations (1, 5, 10 mM) of BS (NaTC, NaTDC) into the aqueous subphase (at $23 \pm 2^\circ\text{C}$).
441 0.5% w/w MC were injected into water to form a layer at the air/water interface, at $\pi_{MC} = 21 \pm 1$ mN/m. These data were
442 extracted from individual BS injections measurements (Figure S6). Each experiment was reproduced at least twice, and the
443 average measurement was selected for each BS at each concentration.

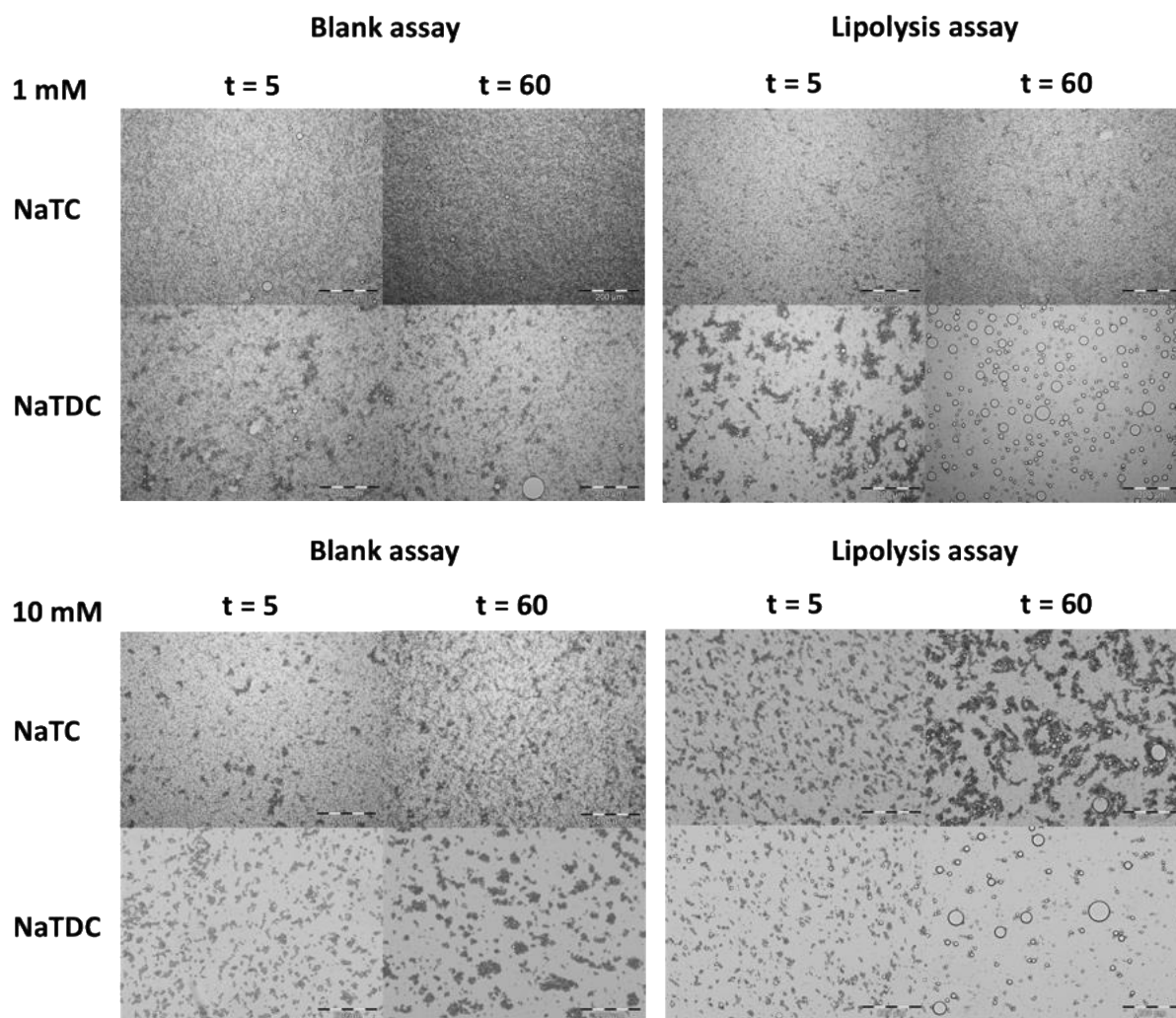
444 Upon injection of fixed BS concentrations, the surface pressure increases sharply over time
445 until reaching a plateau value, independently of the BS type and concentration (Figure S6).
446 The surface pressure values achieved at equilibrium are summarised in Figure 6, showing $\Delta\pi$
447 = $\pi_{Equilibrium} - \pi_{MC}$, where π_{MC} is the initial MC layer surface pressure ($\pi_{MC} = 21 \pm 1$ mN/m). The
448 surface pressure changes induced by the two BS are relatively small, in agreement with
449 previous studies performed on the interaction of a hydroxypropyl MC layer with bile extract
450 (Pizones Ruiz-Henestrosa et al., 2017). At 1 and 5 mM, NaTDC induces a higher increase in
451 surface pressure ($\Delta\pi = 5 \pm 0.4$ mN/m at 1 mM, and $\Delta\pi = 6 \pm 0.2$ mN/m at 5 mM), compared to
452 NaTC ($\Delta\pi = 3 \pm 0.3$ mN/m at 1 mM, and $\Delta\pi = 4 \pm 1$ mN/m at 5 mM); at high BS concentration
453 (10 mM), the opposite trend is observed ($\Delta\pi = 6 \pm 0.4$ mN/m for NaTC, and $\Delta\pi = 4 \pm 0.3$ mN/m
454 for NaTDC).

455 **3.3 Effect of BS structure and concentration on the duodenal digestion of an MC-stabilised**
456 **emulsion**

457 A range of *in vitro* duodenal lipolysis studies was carried out on a sunflower oil emulsion
458 stabilised by MC. Before reaching the small intestine, ingested fat droplets pass through
459 simulated oral and gastric digestion, where their physicochemical and structural properties
460 are significantly affected; however, because our main aim is to understand BS roles during
461 lipolysis, the work performed here focuses on the duodenum part of the lipolysis process,
462 where BS are acting.

463 **Evolution of emulsion droplets microstructure.** The structure of the pure MC-stabilised
464 emulsion droplets was first characterised using both optical and confocal microscopy (Figure
465 S7). Optical microscopy demonstrates that emulsion droplets are uniformly dispersed with a
466 size ranging between 2 and 5 μm , and with a small number of larger droplets around 10 μm
467 (Figure S7A). Confocal microscopy highlights the presence of a MC network (stained in blue
468 with calcofluor) in the bulk and at the interface of emulsion droplets (stained in red with Nile
469 red) (Figure S7B).

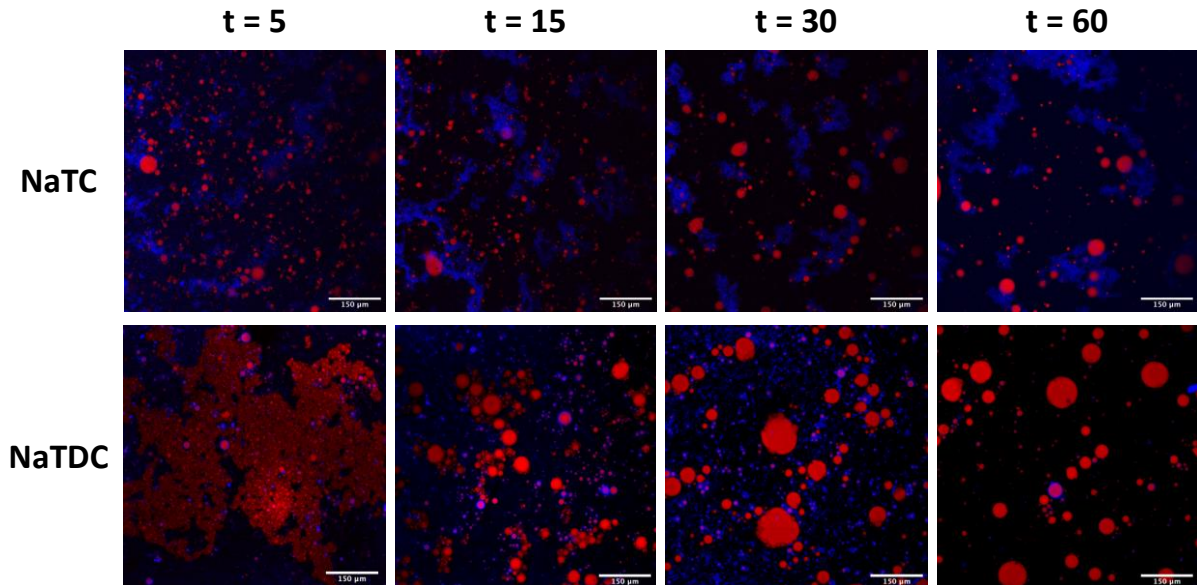
470 *In vitro* lipolysis studies were performed on the emulsion by adding the digestive medium and
471 monitoring the structural changes of the emulsion droplets by microscopy (Figures 7, S8 and
472 S9). Using brightfield optical microscopy, the influence of both BS type and concentration on
473 the structure of MC-stabilised emulsion droplets was assessed in control assays (no enzyme),
474 as well as the effect of enzymes (lipolysis assays) (Figure 7). In the absence of enzymes (blank
475 assays), the emulsion droplets microstructure is affected by the digestive fluid, as revealed by
476 the occurrence of droplets flocculation, and some – limited – coalescence, which is more
477 visible with NaTDC, and particularly evident for both BS at high concentration (10 mM). Upon
478 the addition of enzymes (lipolysis assays), flocculation occurs to a higher extent, and droplet
479 coalescence (size increase) is observed in all samples, to a larger extent, again, with NaTDC.
480 To further elucidate the mechanism of digestion of an MC-stabilised emulsion, the influence
481 of the different components of the digestive fluid (NaCl, CaCl_2 and BS) on droplet stability was
482 also evaluated (Figures S8 and S9). Brightfield optical micrographs show that extensive
483 flocculation occurs when both BS and salts are present, which suggests that the association of
484 BS with the different salts (NaCl, CaCl_2) is responsible for the droplet aggregation observed in
485 Figure 7.



486
 487 **Figure 7:** Time-dependent evolution of the microstructure of MC-stabilised emulsion droplets in the presence of BS: NaTC,
 488 NaTDC, used at 1 and 10 mM, under duodenal digestion conditions (at 37°C). MC-stabilised emulsion was made up of 0.5%
 489 MC and 15% sunflower oil. Both blank (without enzymes) and lipolysis (with enzymes) assays were performed to assess,
 490 respectively, the effect of BS type and concentration on the droplets stability, and of enzymes on the droplets
 491 microstructure. Microscopy observations were made at t = 5 and 60 minutes. The scale bar is 200 μm.

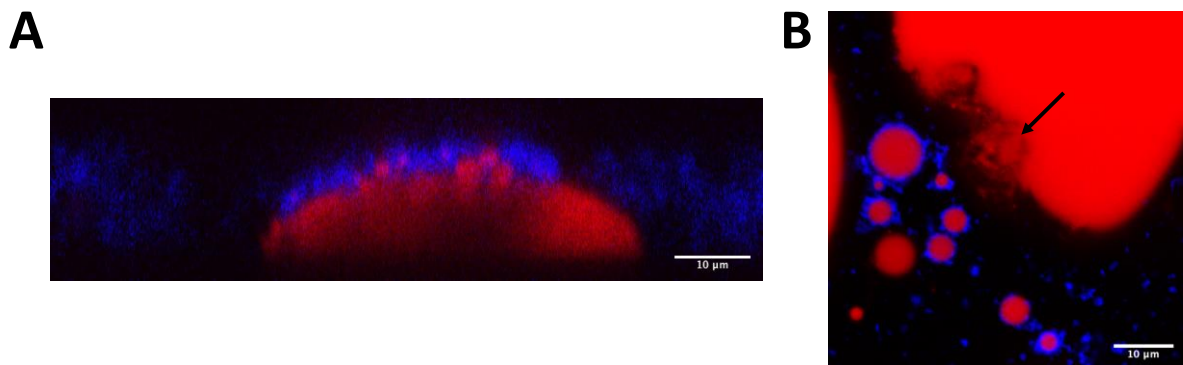
492 This *in vitro* lipolysis study was complemented with micro-structural assessment of the
 493 emulsion droplets with confocal microscopy, to determine the localisation of MC throughout
 494 the emulsion and its evolution during lipid digestion (Figures 8 and 9). Based on our pH-stat
 495 results (see the following section), 50 mM BS was used here, as it shows the higher extent of
 496 FFA release. The images obtained suggest that the addition of digestive fluid not only breaks
 497 down the network of MC, but also displaces it from the lipid/water interface (Figure 8);
 498 interestingly, MC bulk network is disrupted to a higher extent in the presence of NaTDC,
 499 compared to NaTC. Additionally, the lipid droplets become non-spherical with “rough”
 500 surfaces, compared to the initial emulsion (Figures 8 and 9). This demonstrates coalescence

501 and may be an indication of fats being digested by enzymes; in particular, small oil droplets
502 were seen to flocculate or coalesce onto the surface of larger droplets (Figure 9A) and areas
503 with an undefined oil/water interface suggest the presence of digestion products (Figure 9B).



504

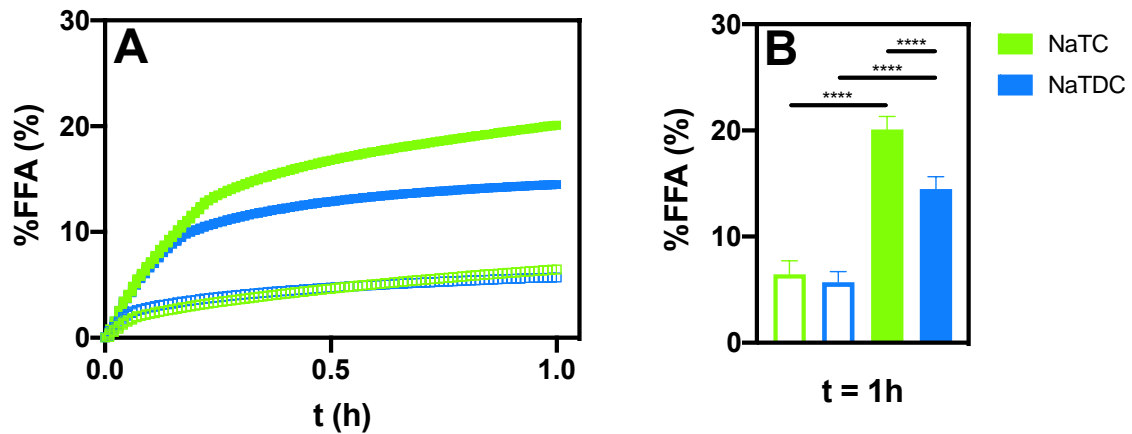
505 **Figure 8:** Time-dependent evolution of the microstructure of MC-stabilised emulsion droplets in the presence of 50 mM
506 BS: NaTC, NaTDC, under duodenal digestion conditions (at 37°C). MC-stabilised emulsion was made up of 0.5% MC and
507 15% sunflower oil. The lipid droplets are stained in red (with Nile red), while MC is stained in blue (with calcofluor).
508 Microscopy observations were made at t = 5, 15, 30 and 60 minutes, to compare the structural changes occurring during
509 digestion; at each time point, orlistat was used to inhibit lipolysis. The scale bar is 150 µm.



510

511 **Figure 9:** (A) Cross-section confocal image of MC-stabilised emulsion droplets in the presence of 50 mM NaTC, under
512 duodenal digestion conditions (at 37°C). The microscopy observation was made at t = 15 minutes. (B) MC-stabilised
513 emulsion droplets in the presence of 50 mM NaTDC, under duodenal digestion conditions (at 37°C). Insoluble lipolysis
514 products seem to be presumably present at the fat droplet interface (see the arrow). MC-stabilised emulsion was made
515 up of 0.5% MC and 15% sunflower oil. The lipid droplets are stained in red (with Nile red), while MC is stained in blue (with
516 calcofluor). The scale bar is 10 µm.

517 **Quantification of FFA release from the MC-stabilised emulsion.** The ability of NaTC and
 518 NaTDC to promote or inhibit the duodenal digestion of an MC-stabilised emulsion was
 519 compared by monitoring the release of FFA (%FFA) over time with the pH-stat method (Y. Li,
 520 Hu, & McClements, 2011) (Figure 10). The effect of BS concentration on the rate of lipolysis
 521 and its extent was also evaluated using the two BS at both 10 and 50 mM.



522
 523 **Figure 10: (A) Proportion of FFA released (%FFA) over time from an MC-stabilised emulsion, using two different BS: NaTC,**
 524 **NaTDC, at two different concentrations: (□) 10 and (■) 50 mM, under duodenal digestion conditions (at 37°C). (B)**
 525 **Proportion of FFA released (%FFA) after 1 hour of digestion of an MC-stabilised emulsion, using the two BS, at 10 and 50**
 526 **mM, under duodenal digestion conditions (at 37°C). Statistical significance was determined using the two-way ANOVA,**
 527 **followed by the Tukey post-test (**** indicates P < 0.0001, i.e., differences are extremely significant). MC-stabilised**
 528 **emulsion was made up of 0.5% MC and 15% sunflower oil.**

529 Independently of the BS type and concentration, the proportion of FFA generated during
 530 lipolysis increases steeply after the addition of enzymes (Figure 10A); this rapid initial rate of
 531 lipolysis, already observed elsewhere (Bellesi, Martinez, Pizones Ruiz-Henestrosa, & Pílosof,
 532 2016; McClements & Li, 2010a), can be attributed to the immediate adsorption of lipase/co-
 533 lipase onto fat droplets surfaces, which then triggers TAG break-down and thus lipid digestion.
 534 After a certain time (t = 0.07 h with both BS at 10 mM, t = 0.18 h with 50 mM NaTDC and t =
 535 0.24 h with 50 mM NaTC), the release of FFA starts slowing down, until it reaches a near-
 536 plateau. This decrease in the rate of lipolysis can be explained by the accumulation of lipolysis
 537 products at the oil/water interface during the process of fat digestion (Patton & Carey, 1979;
 538 P. Reis, Holmberg, Watzke, Leser, & Miller, 2009; P. Reis et al., 2008; P. M. Reis et al., 2008),
 539 which then leads to the inhibition of enzymes binding to the substrate, as previously
 540 demonstrated (Bellesi et al., 2016; Borel et al., 1994). Increasing BS concentration from 10 to
 541 50 mM leads to a significant increase in the percentage of FFA produced, for both BS ($P_{NaTC} <$

542 0.0001 and $P_{\text{NaTDC}} < 0.0001$) (Figure 10B): more specifically, a 14% and 9% increase is obtained
543 with, respectively, NaTC and NaTDC. This can be attributed to the larger amount of BS
544 micelles, which can solubilise a larger amount of FFA released, thereby preventing droplets
545 surface saturation by these products (Wilde & Chu, 2011). While no significant differences are
546 observed between the two BS at the lowest concentration (10 mM) ($\% \text{FFA}_{t=1h} = 6 \pm 1 \%$ for
547 both NaTC and NaTDC; $P_{10 \text{ mM}} = 0.4$), a significant difference is seen at high concentration (50
548 mM), with NaTC inducing a higher extent of lipolysis ($\% \text{FFA}_{t=1h} = 20 \pm 1 \%$ and $14 \pm 1 \%$ for,
549 respectively, NaTC and NaTDC; $P_{50 \text{ mM}} < 0.0001$).

550 **4. Discussion**

551 The objective of this study was to investigate the interactions of MC with BS, in
552 particular the ability of MC to inhibit BS activity, and thus to shed light on the mechanism of
553 lipid digestion regulation by MC – a dietary fibre with a proven potential to lower cholesterol
554 levels (Agostoni et al., 2010). Bulk (rheology) and interfacial (surface pressure measurements
555 and ellipsometry) studies were carried out to characterise the interactions between these two
556 components in the bulk and at the interface, while *in vitro* lipolysis (microscopy, pH-stat)
557 experiments were performed to link these interactions to the lipid digestion of an MC-
558 stabilised emulsion. The two BS, which differ by the presence (NaTC) or absence (NaTDC) of a
559 hydroxyl group on their steroid skeleton (Figure 2) and constitute 20% of human bile (Staggers,
560 Hernell, Stafford, & Carey, 1990), were chosen for this study, as they have been reported to
561 exhibit different interfacial behaviours, hypothesised to explain the contrasting roles they play
562 during the process of lipolysis (Pabois et al., 2019; Parker et al., 2014).

563 **4.1 Interaction between MC and BS in the bulk and at the interface**

564 The impact of BS on MC rheological properties was investigated to explore the
565 interaction of BS with MC in the bulk, where MC is present in excess. Increasing the amount
566 of BS in solution led to a notable shift in the transition temperature (T_t) to higher values, as
567 well as a gradual drop in viscoelastic properties, which were more substantial with NaTDC
568 (Figures 3 and 4). In particular, MC – which presents predominantly solid-like properties in the
569 absence of BS – turned into a softer gel above a threshold concentration of BS (25 mmol/kg
570 for NaTC vs. 10 mmol/kg for NaTDC, at 60°C) (Figures 3, C, D and 4B). MC gelation occurs *via*
571 the association of the hydrophobic (methyl) moieties (Haque & Morris, 1993; Sarkar, 1995;
572 Desbrières, Hirrien, & Rinaudo, 1998; Hirrien, Chevillard, Desbrières, Axelos, & Rinaudo, 1998;
573 Kobayashi, Huang, & Lodge, 1999; L. Li et al., 2001, 2002; Lin Li, 2002; Lin Li, Wang, & Xu, 2003;
574 Funami et al., 2007; Torcello-Gómez & Foster, 2014; Torcello-Gómez et al., 2015; Nasatto et
575 al., 2015a; Isa Ziembowicz et al., 2019). The presence of BS and its association with MC may
576 thus prevent hydrophobic groups from assembling with each other, thus weakening the gels
577 or hindering gelation altogether. The stronger effect observed with NaTDC may be attributed
578 to its higher hydrophobicity (Armstrong & Carey, 1982), which may result in a more efficient
579 connection between BS and MC hydrophobic regions (Torcello-Gómez et al., 2015). Overall,

580 these rheological measurements reveal the presence of strong interactions between BS and
581 the dietary fibre, which have a substantial impact on MC viscosity; the presence (NaTC) or
582 absence (NaTDC) of a hydroxyl group on BS steroid backbone impacts this behaviour
583 considerably.

584 The interfacial properties of BS in the presence of a MC layer formed at the air/water
585 interface were then studied to determine the interactions occurring when a BS molecule
586 approaches a fat droplet stabilised by MC. Studies with a Langmuir trough set-up (Figures 5,
587 A, B, S5, 6 and S6) combined to ellipsometry (Figures 5, C, D) demonstrate that the two BS
588 behave quite differently when injected beneath an almost-saturated MC film: NaTC was
589 shown to gradually adsorb at the interface with increasing concentration, whereas NaTDC first
590 adsorbed at low concentrations (up to 2 – 3 mM) and then desorbed above 4 – 5 mM. This
591 contrasting interfacial behaviour correlates with their micellisation behaviour, which occurs
592 over 4 – 7 mM for NaTC and at 2 mM for NaTDC. Similar differences have been observed when
593 BS were injected below a phospholipid monolayer (Pabois et al., 2019). Nevertheless, BS were
594 found to adsorb and/or desorb to a much lower extent in the presence of a MC film, compared
595 to the phospholipid monolayer (surface pressures changes as high as 30 mN/m were
596 monitored in the presence of the lipid film, whereas an increase of up to 10 mN/m was
597 observed with the MC layer). This may in part be explained by the likely presence of MC excess
598 in the bulk, which could interact with BS and therefore limit their adsorption at the interface.

599 **4.2 Impact of BS/MC interactions on fat digestion**

600 Next, we performed *in vitro* lipolysis studies by following the evolution of the structure
601 of an MC-stabilised emulsion with optical and confocal microscopy, to compare the effect of
602 the two BS on the droplets (Figures S7A and 7) and shed light on the behaviour of MC during
603 emulsion digestion (Figures S7B, 8 and 9). The characterisation of the MC-stabilised emulsion
604 by confocal microscopy clearly demonstrates that fat droplets are entrapped in a network of
605 MC present in excess in the bulk, which may be responsible for the stabilisation of the
606 emulsion against droplets flocculation or coalescence (Figure S7B). Optical microscopy images
607 (Figure 7) demonstrate that, even in the absence of digestive enzymes, the presence of both
608 BS destabilises the emulsion, inducing some flocculation; upon the addition of lipases,
609 droplets destabilisation (namely, flocculation and coalescence) was found to occur to a large

610 extent, and more markedly with NaTDC, compared to NaTC. Confocal microscopy images
611 (Figure 8) suggest that flocculation and coalescence observed during lipolysis are due to the
612 MC network being broken down and removed from the lipid/water interface. The better
613 ability of NaTDC to induce coalescence could therefore be explained by its higher capacity to
614 disturb MC bulk network (as observed by confocal microscopy observations), which, in turn,
615 could be attributed to its stronger interactions with MC (as seen from rheology
616 measurements) and higher propensity to desorb from the interface at lower concentrations
617 (as detected by interfacial measurements). While the displacement of MC from the interface
618 by BS may facilitate the access of BS and enzymes to the lipid droplets surface, the network of
619 MC remaining in the bulk may also trap BS (*via* hydrophobic interactions) and thus prevent
620 them from removing insoluble lipolysis products, which could explain how MC hinders lipase
621 activity. Emulsion droplets coalescence (and thus the decrease in droplets surface area), which
622 occurs under duodenal digestion conditions, could also explain the slowing down of lipolysis.

623 The capacity of the two BS to promote or inhibit MC-stabilised emulsion digestion was
624 then explored with the pH-stat method; results revealed that NaTC favoured FFA release to a
625 higher extent than NaTDC (at 50 mM) (Figure 10). The lower proportion of FFA release
626 obtained with NaTDC can be explained by its higher efficiency at binding to MC network (as
627 suggested by rheology measurements), which may result in this BS becoming trapped in the
628 bulk and therefore not contributing to the lipolysis process.

629 5. Conclusion

630 The demonstrated potential of MC, a dietary fibre, to regulate lipolysis is thought to
631 be due to its ability to reduce BS activity by sequestration; the objective of this work was to
632 compare the interactions of two structurally different BS, NaTC and NaTDC, with MC, and to
633 determine their impact on the digestion of an emulsion stabilised by this polysaccharide.
634 These findings are key to establishing a molecular-level, mechanistic understanding of the
635 ability of MC to lower fat absorption.

636 Both BS were found to decrease the elasticity of MC gels, and to shift the transition
637 temperature (T_t) to stiffer gels to higher temperatures, to a higher extent with NaTDC. When
638 injected below a MC film at the air/water interface, NaTC remained adsorbed at the interface
639 over a wider concentration range, compared to NaTDC, which desorbed at a lower
640 concentration, correlating with the onset of micellisation in the bulk (between 4 – 7 mM for
641 NaTC and at 2 mM for NaTDC). The small difference in the two BS molecular structure,
642 specifically their bile acid portion, is responsible for their contrasting behaviour, and explains
643 the different results obtained during *in vitro* lipid digestion: (i) NaTDC has a higher propensity
644 to disrupt MC network in the bulk and interfacial layer, and thus induces more extensive
645 emulsion destabilisation (as seen from optical and confocal microscopy); (ii) the release of FFA
646 is lower with NaTDC, which can be linked to its higher capacity to bind MC in the bulk, resulting
647 in BS being unable to access the oil/water interface. Overall, it is clear that BS architectural
648 diversity – whose importance is often neglected – plays a key role in their functionalities
649 during fat digestion.

650 This work is a first step towards unlocking the mechanism of lipid digestion regulation
651 by MC. Additional structural studies, in particular with techniques such as small-angle neutron
652 scattering and neutron reflectometry, should bring significant knowledge to the area, in
653 particular to examine the structure of MC in the presence of BS and the evolution of the fat
654 droplet interface during digestion; this is the focus of current work. Building upon these
655 results, the next challenge will be to engineer MC-stabilised lipid emulsions with appetite-
656 suppressing or satiety-enhancing properties and evaluate their effect on cholesterol levels.

657

658 **Acknowledgements**

659 The Institut Laue-Langevin (ILL, Grenoble, France) is acknowledged for the provision of a PhD
660 studentship (OP). The authors acknowledge the Partnership for Soft Condensed Matter
661 (PSCM) for access to sample preparation facilities and the use of the Langmuir trough,
662 ellipsometer and optical microscope. Prof Peter Ellis is thanked for many useful discussions
663 and access to laboratory facilities. OP also thanks Dr Angélique Pabois for her kind help with
664 the statistical analysis of pH-stat data, and Taniya Akhtar for her contribution to the project.
665 PJW and MM-LG gratefully acknowledge the support of the Biotechnology and Biological
666 Sciences Research Council (BBSRC) through the BBSRC Institute Strategic Programme Food
667 Innovation and Health BB/R012512/1 and its constituent project BBS/E/F/000PR10345. Dr
668 Isabelle Grillo, one of our co-authors and OP's PhD co-supervisor, sadly passed away during
669 the writing-up of this manuscript; we dedicate this to her memory.

670 **Declarations of interest**

671 None

672

673 **References**

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