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1 ***In vitro* digestibility and release of a mango peel extract encapsulated within water-in-**
2 **oil-in-water (W_1/O/W_2) emulsions containing sodium carboxymethyl cellulose**

3 Gustavo R. Velderrain-Rodríguez [a], Laura Salvia-Trujillo [c], Abraham Wall-Medrano [b],
4 Gustavo A. González-Aguilar [a]*, Olga Martín-Belloso [c]*

5 [a] Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en
6 Alimentación y Desarrollo, A.C. Carretera Gustavo Enrique Astiazarán Rosas No. 46 Col.
7 La Victoria, C.P. 83304, Hermosillo, Sonora, México; [b] Instituto de Ciencias Biomédicas,
8 Universidad Autónoma de Ciudad Juárez – Plutarco E. Calles 1210, C.P. 32310, Ciudad
9 Juárez, Chihuahua, México; [c] Department of Food Technology, University of Lleida –
10 Agrotecnio Center, Av. Alcalde Rovira Roure 191, 25198, Lleida, Spain.

11

12 *Corresponding authors: Olga Martín-Belloso (Tel.: +34 973702593. e-mail:
13 omartin@tecal.udl.es) and Gustavo A. González-Aguilar (Tel.: +52 (662) 289 2400 x 272,
14 Fax: +52 (662) 289 2400 x 272, e-mail: gustavo@ciad.mx).

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17 **Abstract**

18 Mango peel is a rich source of phenolic compounds (PC), which can be used for food
19 fortification. The use of water-in-oil-in-water ($W_1/O/W_2$) emulsions represent a potential
20 strategy to encapsulate, protect and incorporate PC from mango peel into food products.
21 Moreover, even though non-digestible biopolymers are usually incorporated into emulsions
22 to enhance stability, little is known about the effect on the digestibility and release of PC. In
23 this study, a mango peel extract (MPE) was encapsulated using $W_1/O/W_2$ emulsions
24 containing sodium carboxymethyl cellulose (CMC; 0, 0.5, 1.0% w/w) in W_2 and evaluated
25 their colloidal stability, lipid digestibility kinetics (free fatty acids release), and the release
26 (in terms of antioxidant activity) during *in vitro* digestion conditions. The presence of CMC
27 in emulsions caused the droplets flocculation, which remained unchanged during gastric
28 phase, suggesting that bridging flocculation occurred. Moreover, a slower lipid digestion rate
29 was observed in emulsions containing CMC, with *k*-values ranging between 0.21 and
30 0.25min^{-1}, compared to emulsions without CMC (around 0.14min^{-1}). However, despite CMC
31 may slow down the lipolysis reaction during the first 40min due to physical or steric
32 hindrance, at the end of the intestinal phase, emulsions with or without CMC had a similar
33 final FFA release. Moreover, the MPE release was triggered during gastric conditions,
34 probably by osmotic imbalance, showing a constant antioxidant activity value during the
35 intestinal phase only in emulsions containing CMC. This study provides relevant insights to
36 design double emulsions as delivery systems of water-soluble bioactive compounds with
37 antioxidant activity, such as PC.

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39 **1. Introduction**

40 Consumers' awareness of the relationship between diet and health has raised the interest from
41 both the industrial and the scientific community in developing new approaches for food
42 fortification with bioactive compounds. At the same time, the food industry generates high
43 amounts of residues such as peels, seeds and bagasse, which could be used as source of
44 bioactive compounds, such as phenolic compounds (PC), dietary fiber, and vitamins with
45 potential use for food fortification. Thus, this is being regarded as a valorization strategy of
46 waste streams from food industry and reducing its impact on the environment [1, 2]. In that
47 sense, large amounts of mango peel are usually discarded during the processing of juice or
48 peeled mango fruit pieces, as it represents from 15% to 20% of the total weight of the fruit.
49 However, it can be considered as a great source of by-products for mango industry, as it
50 contains significant concentrations of bioactive compounds, such as water-soluble phenolic
51 compound, vitamin C and fiber [3-5]. Recently, gallic acid-rich extracts from mango (cv.
52 'Ataulfo') peel have shown antiproliferative activity towards colon cancer cells (LS180),
53 suggesting that mango peel extracts may have an important role to protect or improve human
54 gut health [6].

55 Moreover, mango peel extracts may also contain other derivative forms of gallic acid,
56 mangiferin or quercetin, also related to potential human health benefits [7]. Hence, these
57 phenolic-rich extracts represent an excellent source of bioactive compounds that could be
58 incorporated into functional foods products. However, the incorporation of mango peel PC
59 into food products represents a challenge due to their susceptibility to oxidative reactions
60 during processing conditions (pH, time, temperature, oxygen availability) and unpleasant
61 taste (such as bitterness and astringency). In addition, a number of studies have shown that

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62 PC extracts are susceptible to oxidation at physiological intestinal pH (~ 6.0-7.5), limiting
63 their bioavailability and potential health benefits[8-11]. Therefore, the encapsulation of PC may
64 reduce these problems and enhance food products properties. In this regard, double emulsions
65 ($W_1/O/W_2$) arise as a potential encapsulation system to carry, protect and deliver PC extracts
66 into aqueous food systems.

67 Recently, it has been shown that phenolic-rich extracts from mango peel can be successfully
68 encapsulated using long-term stable double emulsions systems [12]. Double emulsions can
69 encapsulate PC from mango peel within the internal aqueous phase (W_1) of emulsions, being
70 surrounded by an oil droplet (O) that separates them from a second aqueous phase (W_2). This
71 compartment of mango peel PC may potentially show (*i*) protection of the encapsulated
72 bioactive compounds against degradation, (*ii*) masking of unpleasant flavors during ingestion
73 and oral processing and (*iii*) a targeted release of the encapsulated compounds in the small
74 intestine after digestion of oil droplet by intestinal lipases [13].

75 The formation of double emulsions typically consists of a two-step emulsification procedure
76 in which firstly a water-in-oil (W_1/O) emulsion is formed and subsequently this is re-
77 dispersed in a second aqueous phase (W_2) [14]. However, double emulsions are prone to suffer
78 gravitational destabilization leading to creaming phenomena due to their complexity and
79 large droplet size of the W_1/O phase, which is typically between 1 and 10 μm. Therefore,
80 hydrocolloids are often used as thickening agents in the external aqueous phase (W_2) in order
81 to increase the aqueous phase viscosity, reducing the movement and collision of particles and
82 ultimately retarding gravitational separation phenomena [15]. In this regard, carboxymethyl
83 cellulose (CMC) is a non-digestible polymer, which is commonly used as a thickening agent
84 and stabilizer in food emulsions. Specifically, CMC has been successfully used to form and

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85 stabilize double emulsions with a high encapsulation efficiency of bioactive compounds [16].
86 However, there is still scarce information about the effect of CMC, or other undigestible
87 polymers in the external aqueous phase (W_2), on the digestibility of double emulsions and
88 the subsequent release of the encapsulated bioactive compounds during human
89 gastrointestinal conditions.

90 The presence of biopolymers has shown to potentially affect the digestibility of simple oil-
91 in-water (O/W) due to a number of mechanisms [17, 18]. In this regard, lipases might have
92 limited access to oil/water interfaces possibly due to (*i*) an increased viscosity of the aqueous
93 medium, (*ii*) flocculated droplets or (*iii*) electrostatic interactions between the biopolymer
94 and intestinal lipases [19]. Nonetheless, the impact of polymers in the digestibility of double
95 emulsions might significantly differ from O/W emulsions as they have a higher complexity
96 and colloidal behavior.

97 Therefore, the aim of this work was to evaluate the formation of double emulsions ($W_1/O/W_2$)
98 as carriers of mango peel extracts (MPE) in the inner aqueous phase (W_1) and to evaluate the
99 role of the addition of CMC in the outer aqueous phase (W_2) at different concentrations (0,
100 0.5 or 1% *w/w*). The impact of CMC in double emulsions digestibility was investigated in
101 terms of the microstructural changes, the lipid digestibility kinetics, and the release of
102 antioxidants within MPE under simulated gastrointestinal conditions.

103 ## 2. Materials and methods

104 ### 2.1. Materials

105 Mangoes cv. 'Ataulfo' were selected at its commercial maturity stage according to [20], and
106 purchased from a local supermarket in Hermosillo, Sonora (Mexico). Corn oil was obtained
107 from a local supermarket in Lleida (Spain). Polyglycerol polyricinoleate (PGPR) was

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108 provided from Danisco (Denmark), whereas Tween 20, sodium carboxymethylcellulose
109 (CMC; $M_w \approx$ 700kDa), cellulose dialysis bags (molecular weight cut-off of 10kDa) and all
110 solvents used in this study were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).
111 The glycerol was acquired from Fischer Scientific (UK).

112 **2.2. Mango Peel Extraction**

113 After washing mango fruits, the peel was carefully removed, freeze-dried and stored at -18
114 °C. Dried peel was powdered using a commercial blender. Subsequently, the extraction
115 procedure, PC profile and, the antioxidant activity of the MPE used in this study, was
116 described by Velderrain-Rodríguez, et al. (2018)[6]. The MPE extract (1mg/mL) was dissolved
117 using a Milli-Q water-NaCl (0.1 M) solution and filtered (at the day of use) through a 0.22
118 µm nylon membrane filter, as performed by Velderrain-Rodríguez, et al. (2019)[12].

119 **2.3. Preparation of MPE $W_1/O/W_2$ emulsions**

120 $W_1/O/W_2$ emulsions were prepared as performed by Teixé-Roig, et al. [21], with minor
121 modifications. The formation, colloidal stability and the MPE encapsulation efficiency of the
122 emulsions used in this study has been previously described by Velderrain-Rodríguez, et al.
123 (2019)[12]. The W_1/O emulsion consisted of 70 % (w/w) corn oil, 22 % (w/w) inner aqueous
124 phase (W_1), 3 % (w/w) glycerol and 5 % (w/w) PGPR as the lipophilic surfactant to stabilize
125 the interface between W_1 and O phases. Loaded W_1/O emulsions were prepared using the
126 filtered MPE solution as W_1, whereas non-loaded emulsions (without MPE) were prepared
127 using milli-Q water-NaCl (0.1 M) as W_1. Prior to emulsification, glycerol was dissolved in
128 W_1, whereas PGPR was dissolved in corn oil under magnetic stirring at 60 °C. Dispersion of
129 W_1 in corn oil was performed using an Ultra-Turrax T25 homogenizer (IKA® Works, Inc.
130 Wilmington, NC, USA) at 6,000 rpm, for 8 min. Afterwards, in order to reduce the water

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131 droplets particle size, W_1/O emulsions was sonicated with a UPS400S sonifier (Hielscher
132 USA, Inc., Ringwood, NJ, USA) equipped with a 22-mm sonotrode (Hielscher Ultrasound
133 Technology, Teltow, Germany), for 3 min at 400 W of nominal power, and a frequency of
134 24 kHz.

135 Subsequently, $W_1/O/W_2$ emulsions were prepared using 25 % (w/w) of W_1/O, 73 % (w/w)
136 of a second aqueous phase (W_2), and 2 % (w/w) of Tween 20 as hydrophilic surfactant to
137 stabilize the second interface between W_1/O and the W_2 phases. The W_2 of emulsions was
138 composed of milli-Q water-NaCl (0.1 M, and it was used to dissolve Tween 20 and CMC at
139 different concentrations (0.0, 0.5 or 1 % w/w). The final $W_1/O/W_2$ emulsion was obtained
140 using an Ultra-Turrax T25 homogenizer at 3,000 rpm for 4 min and then sonicated using a
141 UPS400S sonifier (Hielscher USA, Inc., Ringwood, NJ, USA) at 50 µm for 1.5 min.

142 2.4. Droplet size and droplet size distribution of $W_1/O/W_2$ emulsions

143 The particle size and particle size distribution of undigested and digested $W_1/O/W_2$ emulsions
144 were measured by static light scattering technique using a Mastersizer 3000 (Malvern
145 Instruments Ltd, Worcestershire, UK), as this equipment can measure particle sizes ranging
146 from 100 nm to 2 mm. The sample was dispersed in distilled water and using a refractive
147 index of 1.475 of the dispersant. Data was reported as volume-weighted average ($d_{4,3}$), based
148 on the Mie Scattering theory, and expressed in µm.

149 2.5. *In vitro* digestibility of $W_1/O/W_2$ emulsions

150 The *in vitro* digestibility of double emulsions, with or without CMC, was performed
151 according to [22], with minor modifications, consisting on a gastric and intestinal phase.
152 Simulated gastric fluid solution (SGF) was composed of a mixture of electrolytes [0.5 M
153 KCl, 0.5 M KH_2PO_4, 1 M NaCl, 2 M NaCl, 0.15 M $MgCl_2(H_2O)$, 1 M $(NH_4)_2CO_3$] dissolved

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154 in 20 mL of milli-Q water. Then, 18.2 mL of SGF was acidified adding 1.8 mL of 0.02 M
155 HCl and used to dissolve pepsin (8.8 mL/mL). Gastric digestion was performed by mixing
156 20 mL of emulsion with 20 mL of SGF solution. The mixture was incubated under subdued
157 light conditions for 2 h at 37 °C and continuous agitation using an orbital shaker working at
158 100 rpm. Afterward, 30 mL of the chyme was placed in a water bath at 37 °C. Finally, 3.5
159 mL of bile salts (54 mL/mL) and 1.5 mL of 0.1 M calcium chloride solutions were added to
160 the digested emulsions, adjusting pH to 7.0 prior addition of 2.5 mL of pancreatin (75
161 mL/mL) solution. The emulsions' digestibility during intestinal digestion was performed
162 using a pH-stat equipment (Metrohm USA Inc., Riverview, FL, USA), as reported by Gasa-
163 Falcon, et al. (2017)[18]. The free fatty acid (FFA) release in percentage was calculated
164 according to **Equation 1**:

165 $$FFA\,(\%) = \frac{V_{NaOH} x\, C_{NaOH}\, x\, M_{oil}}{2\, x\, m_{oil}}\, x\, 100 \qquad (1)$$

166 where V_{NaOH} represents NaOH volume needed to neutralize free fatty acids released during
167 intestinal digestion, whereas C_{NaOH} is the Molar concentration of NaOH (0.25 M, M_{oil} is the
168 molecular weight of corn oil (800 g/mol) and m_{oil} is the total oil weight within $W_1/O/W_2$
169 emulsions.

170 **2.6. Antioxidant activity of MPE double emulsions during *in vitro* digestion**

171 The antioxidant activity was measured as evidence of MPE release from emulsions, with or
172 without CMC, during digestion conditions. Cellulose dialysis bags were used in order to
173 separate the released antioxidant compounds from the chyme during *in vitro* digestion
174 conditions, as described by Aditya, et al. (2015) [23]. Gastric and intestinal phases were
175 performed as described previously in section 2.5, within a 10 cm-length cellulose acetate

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176 dialysis tubing (12 kDa cutoff). Dialysis bags were sealed and placed into a beaker containing
177 30 mL of milli-Q water (output solution). Aliquots of 1 mL of output solution were taken
178 every 30 minutes during gastric digestion, replacing the volume taken by adding 1 mL of
179 milli-Q water each time. Afterward, chyme samples were transferred from dialysis bags and
180 subsequently submitted to simulated intestinal conditions, as described in section 2.5.
181 Likewise, 1 mL-aliquots of output solution were taken every 30 minutes during simulated
182 intestinal conditions. The volume taken was replaced by adding 1mL of milli-Q water each
183 time. Aliquots obtained at several time moments during gastric and intestinal conditions were
184 collected and stored at -18 °C until the further determination of their antioxidant activity.

185 The antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric
186 reducing antioxidant power (FRAP) assays, which was adapted for small-volume microplate
187 reader spectrophotometer (Thermo Scientific™ Multiskan™ GO; Thermo Fisher Scientific,
188 Inc) described by Velderrain-Rodríguez, et al. (2015)[3]. The DPPH assay was used as an
189 overall method to evaluate the antioxidant activity of the released MPE, as this radical may
190 be stabilized either by hydrogen atom transfer mechanism (HAT) or single electron transfer
191 (SET). On the other hand, FRAP assay was used to evaluate only the SET mechanism shown
192 by MPE extract released during *in* vitro digestion. The DPPH and FRAP values of the MPE-
193 loaded emulsions during digestion were calculated by subtracting the absorbance value of
194 the non-loaded emulsions. Results were expressed as µmoles of Trolox equivalents (TE)/mL
195 of $W_1/O/W_2$ emulsion.

196

197 **2.7. Confocal laser scanning microscopy (CLSM)**

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198 An Olympus FV1000 confocal laser microscope (CLSM) was used to visualize the
199 microstructure of double emulsions before and after simulated gastric and intestinal phases,
200 as described by Aditya, et al. (2015)[24]. Images were captured using a 100x magnification
201 lens. The oil phase was dyed with Nile red (1 mL/mL) dissolved in polyethylene glycol,
202 adding 12 µL of dye/mL of emulsion. The oil phase of emulsions was properly dyed by
203 allowing it to stand for 15 min prior to its visualization. The Nile red fluorochrome was
204 excited at 488 nm, detecting its fluorescence spectra between 523-650 nm.

205 **2.8. Statistical analysis and kinetic modeling**

206 All experiments were performed in duplicate, using at least three measurements of each
207 analysis. Statistical differences in the particle size and antioxidant capacity of emulsions with
208 different formulations were determined by one-way ANOVA and Tukey-Kramer multiple
209 comparison test ($p < 0.05$) using the statistical software NCSS 2007.

210 The kinetics of FFA release during the first 40 min of simulated intestinal digestion was
211 assessed with a Gompertz sigmoidal model (**Equation 2**):

212 $$FFA\,(t) = C_f x\, e^{e^{-k(t-\mu)}} \qquad (2)$$

213 where C_f is the maximum FFA release value (%), k is the rate of the FFA release at the
214 inflection point (min^{-1}) and μ is the lag-phase time (min).

215 Estimated parameters were determined by non-linear regression using the JMP statistical
216 software (version 11). The average FFA release data of two different replicates of each
217 sample was used for modelling purposes. The fit of the model was assessed by calculating
218 R^2 and visually analyzing the residue plots. Significant differences between the estimated

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219 parameters of different samples were determined by calculating the confidence intervals
220 (95%).
221

222 3. Results and discussion

223 3.1. Initial particle size and particle size distribution of $W_1/O/W_2$ emulsions

224 The double emulsions without CMC showed a bimodal particle size distribution with a minor
225 peak of submicron particles and a major peak around 4µm (**Figure 1**). As discussed by
226 Kowalska and Krzton-Maziopa (2015)[25], the particle size of conventional $W_1/O/W_2$
227 emulsions is above 1 µm, and usually between 1 and 20 µm depending on the processing
228 conditions and the main emulsion components (surfactants, encapsulated compounds,
229 thickening agents, among others) [26]. In this study, the results have shown that the presence
230 of CMC has a stronger influence than the MPE on the emulsions particle size and distribution.

231 On the one hand, the presence of MPE in the inner aqueous phase caused a slight aggregation
232 of the oil droplets in emulsions without CMC, as seen in the confocal images of initial double
233 emulsions (**Figure 2**). Despite this, double emulsions with MPE presented a similar particle
234 size and distribution concerning the non-loaded emulsions, suggesting that this aggregates
235 did not lead to coalescence and were disrupted during particle size measurements. In fact, it
236 has been reported that PC may present interfacial activity in emulsion-based systems favoring
237 emulsions formation and stabilization [27, 28]. In the current work, the lipophilic surfactant
238 (PGPR) used might have covered the internal W_1/O interface, thus, explaining the weak
239 effect of the presence of MPE in the particle size of double emulsions.

240 On the other hand, the presence of CMC in the external aqueous phase (W_2) had a significant
241 impact on the double emulsions particle size and distribution, leading to the formation of

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242 emulsions with smaller W_1/O droplet sizes. For instance, the initial particle size diameter
243 ($d_{4,3}$) of the W_1/O droplets significantly decreased from 4 µm in the non-loaded double
244 emulsions, down to 2 µm in those with a CMC concentration up to 1% (*w/w*), as shown in
245 **Figure 3**. In agreement, [29] reported that only the addition of CMC or chitosan to the external
246 aqueous phase led to a significant particle size decrease of emulsions, compared to other
247 macromolecules such as pectins and gums.

248 Moreover, the particle size distribution (**Figure 1**) of double emulsions with CMC in the W_2
249 phase at 0.5 or 1 % (*w/w*) shifted towards smaller particle sizes with an increase in the
250 intensity peak corresponding to submicron particles yet showing a wider particle size
251 distribution in comparison with those without CMC. Overall, the significantly smaller
252 particle size presented by double emulsions containing CMC may be attributed to the
253 contribution of this biopolymer to the emulsification of the oil droplets. These results are in
254 agreement with previous publications where the interfacial activity of cellulose-based
255 biopolymers is reported, thus, contributing in rendering emulsions with smaller oil droplet
256 sizes [30].

257 Nonetheless, in the current study, confocal images of initial double emulsions (**Figure 2**)
258 evidenced the presence of increasing oil droplets (W_1/O) flocculation at increasing the CMC
259 concentration from 0.5 to 1 % (*w/w*), regardless the presence of MPE in the inner W_1 phase.
260 This might be attributed to either bridging or depletion flocculation phenomena. In this
261 regard, it is not clear whether if CMC molecules are being adsorbed or not at the oil/water
262 interface. In case that CMC is adsorbed, due to its anionic nature, it might lead to electrostatic
263 interactions between other CMC molecules adsorbed to lipid droplets, thus, causing bridging
264 flocculation [31]. Conversely, if CMC is not adsorbed at the oil/water and is rather solubilized

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265 in the external aqueous phase, it may generate a biopolymer-depleted zone around the oil
266 droplets which subsequently leads to an osmotic driving force that causes droplet flocculation
267 [32].

268

269 **3.2. Particle size, particle size distribution, and microstructure during *in vitro***
270 **digestion conditions**

271 The colloidal stability of $W_1/O/W_2$ emulsions during *in vitro* digestive conditions as affected
272 by the presence of MPE or CMC was assessed by determining the changes in oil droplets
273 diameter (**Figure 3**), particle size distribution (**Figure 4**) and microstructure (**Figure 2**) of
274 the emulsions after simulated gastric and intestinal phases.

275 The results have shown that double emulsions without CMC had similar colloidal
276 characteristics after gastric digestion conditions compared to undigested emulsions. Thus,
277 there were no significant changes in their particle size (**Figure 3**) or distribution (**Figure 4**)
278 with respect the undigested emulsions, regardless the presence of MPE. This could also be
279 confirmed by confocal microscopy (**Figure 2**), where oil droplets remained unchanged after
280 the gastric phase even in the presence of MPE within the W_1 phase. Therefore, the initial
281 droplet size of emulsions remained practically stable after simulated gastric conditions, with
282 values around 4 μm of particle size. Other authors have reported that oil-in-water (O/W)
283 emulsions stabilized with small molecule surfactants, such as Tweens, show high stability
284 during gastric conditions [33, 34]. [35] attributed the stability during gastric digestion of emulsions
285 stabilized by Tween to the non-ionic nature of these surfactants, which explains the
286 insensitivity of these molecules to pH changes.

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287 Similarly, the presence of CMC did not cause a significant impact of the oil droplets size
288 (**Figure 3**) or particle size distribution (**Figure 4**) with regards the initial double emulsions,
289 regardless the CMC concentration used, during simulated gastric conditions. Moreover,
290 double emulsions containing CMC remained flocculated after gastric conditions as seen in
291 the confocal images (**Figure 2**). This indicates that despite that double emulsions are diluted
292 with gastric fluids to mimic stomach conditions, the oil droplets remained flocculated in the
293 presence of CMC, suggesting that CMC is strongly bound at the oil/water interface.
294 Therefore, it can be postulated that CMC is causing bridging flocculation in double emulsions
295 rather than depletion flocculation because otherwise the flocs would have been disrupted by
296 the dilution of the external water phase during the simulation of gastric conditions.

297 After simulated small intestine conditions, double emulsions experienced a tremendous
298 increase in the oil droplet size (**Figure 3**) regardless of the presence of MPE or CMC in their
299 formulation, reaching values around 50 μm. This was also observed in the particle size
300 distribution of double emulsions (**Figure 4**), showing an increase in the intensity peaks
301 corresponding to particles larger than 10 μm. Additionally, confocal images (**Figure 2**)
302 revealed the presence of large droplets of bulk oil after the small intestinal phase. This
303 suggests that double emulsions are prone to suffer intense coalescence in the presence of
304 surface-active molecules, such as pancreatic lipases or bile salts, which may destabilize the
305 oil/water interface. In agreement, [36] observed that only large oil droplets remained after the
306 intestinal digestion, as a product of a surfactant displacement by bile salts and a rapid
307 lipolysis process affecting Tween 20-stabilized emulsions.

308

309 **3.3. Lipid digestibility of $W_1/O/W_2$ emulsions**

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310 The influence of MPE or CMC on the *in vitro* lipid digestibility of double emulsions was
311 assessed in terms of the FFA release (%) during small intestine conditions (**Figure 5 DEF**).
312 Additionally, to study the lipid digestion kinetics, the FFA release at the first 40 min of
313 intestinal digestion was modeled by a sigmoidal-shape equation (Eq.2) (**Figure 5 ABC**). All
314 the studied double emulsions showed a short delay in the FFA release immediately after the
315 beginning of the intestinal phase, followed by a steep increase on the FFA release.
316 Nonetheless, the overall FFA release was significantly different depending on the double
317 emulsion formulation.

318 On the one hand, the encapsulation of MPE did not cause a significant impact on the
319 digestibility of double emulsions showing similar lipid digestion patterns (**Figure 5 A and**
320 **D**) than non-loaded emulsions. Double emulsions with or without MPE presented an
321 estimated delay phase (μ) of around 10 min and k-values of 0.14 min^{-1} during the exponential
322 release phase (Table 1), reaching final FFA values of 40 % at the end of the small intestinal
323 phase (120 min). Similar results were reported by Bellesi, et al. (2016)[37] in O/W emulsions
324 stabilized with soy isolate proteins and hydroxypropylmethylcellulose (HPMC), as they
325 observed final FFA values of 42.53 ± 0.36 and 44.36 ± 0.68 %, respectively. As the lipid
326 digestion is considered as an interfacial process of the lipase–colipase complex binding onto
327 the surface of emulsified dropletts, the results of this study suggest that the presence of MPE
328 does not cause an interfacial impediment to alter the lipid digestion process of emulsions.

329 On the other hand, the presence of CMC caused a significant change in the lipid digestibility
330 kinetics of double emulsions. In this case, it is possible that the presence of CMC at 0.5 or 1
331 % modified the interfacial structure of double emulsions, as they showed a slightly shorter
332 delay phase (μ-values between 6 and 8 min) at the beginning of the intestinal phase in

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333 comparison with emulsions without CMC (**Table 1**). Nevertheless, the presence of CMC
334 showed significantly slower lipid digestion rate at increasing the CMC concentration in the
335 W_2 phase from 0.5 to 1 % (*w/w*), with *k*-values ranging between 0.21 and 0.25 min^{-1} (**Table
336 1**). The slower digestion rate in these emulsions may related to a resistance of the interfacial
337 network formed by CMC to be displaced by bile salts, as suggested by Sarkar, et al. (2016)[38].
338 Thus, this CMC interfacial network may hinder the adsorption of intestinal lipases at the
339 oil/water interface showing a slower lipid digestibility. This is in agreement with previous
340 studies, which report a similar behavior of double emulsions in the presence of CMC during
341 *in vitro* digestion [21]. In fact, it has been postulated that polysaccharides in the aqueous phase
342 may limit lipid digestibility due to a number of reasons. First, the increase in the micro-
343 viscosity around the oil droplets may retard the movement of lipases at the interface, thus,
344 slowing down the lipolysis reaction. Second, the flocculation observed in double emulsions
345 may cause a physical impediment for lipid digestion. And third, CMC may be adsorbed at
346 the oil/water interface thus causing a steric hindrance for lipases to reach the substrate [17, 39,
347 40].

348 Nonetheless, besides the delayed lipolysis during the first 40 min of intestinal digestion, our
349 results show that double emulsions with CMC in the W_2 phase at 0.5 or 1 % (*w/w*) presented
350 a delay after the first exponential FFA release followed by a further abrupt increase after
351 approximately 60 min of the intestinal phase. This means that while double emulsions
352 without CMC in the W_2 phase showed a gradual increase in the FFA release from 60 to 120
353 min (**Figure 5 D**), double emulsions with CMC presented a double sigmoidal curve shape,
354 with a fast increase in the FFA release from 60 to 120 min (**Figure 5 EF**). In fact, at the end
355 of the intestinal phase, double emulsions with CMC showed the similar final extent of FFA

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356 release in comparison with the non-loaded emulsions, being around 40 % of FFA release.
357 This suggests that despite CMC may slow down the lipolysis reaction during the first 40 min
358 due to physical or steric hindrance, it may eventually desorb due to the dilution in intestinal
359 juices or be displaced by surface-active compounds such as bile salts and therefore lipase
360 may be eventually able to access the substrate and reactivate the lipid digestion reaction.

361

362 **3.4. Antioxidant activity of MPE $W_1/O/W_2$ emulsions *during vitro* digestion**
363 **conditions**

364 Double emulsions ($W_1/O/W_2$) containing MPE without or with CMC at different
365 concentrations (0.5 or 1 % *w/w*) were subjected to simulated gastrointestinal conditions
366 within dialysis bags. The aqueous phase outside the dialysis bags was analyzed by
367 determining the antioxidant activity against two radical assays (DPPH and FRAP) every 30
368 min of the simulated gastric and intestinal phases (**Figure 6**). The antioxidant activity
369 observed in MPE-loaded emulsions was considered as an indicator of the release of
370 compounds with antioxidant activity from the inner aqueous phase (W_1) without or with the
371 presence of CMC in the outer aqueous phase (W_2).

372 During the gastric phase, a gradual increase in the antioxidant activity was observed at
373 increasing gastric digestion times for all the studied double emulsions by both FRAP and
374 DPPH assays (**Figure 6**). On the one hand, the highest antioxidant increase during gastric
375 conditions was observed in the double emulsions without CMC, in which it increased from
376 0 up to a maximum of 0.016 μmoles TE/mL of $W_1/O/W_2$ emulsion after 90 min of gastric
377 conditions (**Figure 6 A**). The increase of antioxidant activity during gastric conditions might
378 be attributed to the diffusion of the encapsulated antioxidants within MPE, from the W_1 phase

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379 to the W_2 phase and ultimately through the dialysis bag due to an osmotic effect. The salts
380 within simulated gastric juices might have caused an osmotic imbalance between the W_1 and
381 W_2 phases, causing the release of the antioxidant compounds within MPE. On the other hand,
382 double emulsions containing CMC (**Figure 6 C and E**) showed a similar increase in the
383 antioxidant activity values, yet up slightly lower levels in comparison with double emulsions
384 without CMC (**Figure 6 A**). This suggests that CMC may interact with the released
385 antioxidants by either hindering their diffusion from the W_1 to the W_2 phase or binding them
386 and avoiding their transfer through the dialysis bag.

387 With regards to the antioxidant activity during the small intestinal phase, a different behavior
388 was observed depending on the assay used to conduct the antioxidant measurements. The
389 DPPH assay showed a rapid decrease in the antioxidant activity of double emulsions at
390 increasing the small intestinal time regardless of the presence of CMC (**Figure 6 B, D, and**
391 **F**). In this regard, the antioxidant activity of double emulsions without CMC was found to
392 be undetectable after 120 min of intestinal phase (**Figure 6 A**). The antioxidant activity of
393 the MPE used in this study has been attributed to their monomeric PC (gallic acid,
394 mangiferin, quercetin, catechin, among others) showing different antioxidant mechanisms [6].
395 Specifically, the antioxidant activity of MPE is attributed to the high concentration of gallic
396 acid (23.81 ± 0.28 mg/g of dried peel), which is highly soluble in water and it has the highest
397 antioxidant activity when measured by FRAP assay, as compared to the other PC in MPE.
398 The decrease in antioxidant activity during small intestinal conditions may be due to a
399 number of reasons. First, after exposure of PC to gastrointestinal conditions, their antioxidant
400 activity may be compromised mainly due to exposure of intestinal enzymes, such as lipase
401 [41]. Second, the pH conditions of the different simulated gastrointestinal steps might have

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402 determined their antioxidant capacity. In this regard, gallic acid has four potential acidic
403 protons having pKa values of 4.0 (carboxylic acid), 8.7, 11.4, and >13 (phenolic OHs) [42],
404 thus, being partially deprotonated under intestinal conditions. Therefore, deprotonated gallic
405 acid molecules are less likely to follow the HAT antioxidant mechanism during intestinal
406 digestion, explaining the decrease in the antioxidant activity observed with the DPPH assay.
407 Third, the presence of free fatty acids in the intestinal phase, which might have pro-oxidant
408 activity, can diminish the reducing capacity of the antioxidants within MPE measured by the
409 DPPH assay. Oppositely, the FRAP assay evidenced that the antioxidant activity of double
410 emulsions containing CMC in the W_2 phase was maintained constant from the end of the
411 gastric phase until the small intestinal phased finished (**Figure 6 D and F**), with values
412 around 0.010 and 0.005 µmoles TE/mL of $W_1/O/W_2$ emulsion for double emulsions
413 containing 0.5 or 1 % CMC in the W_2 aqueous phase, respectively. Hence, those partially
414 deprotonated gallic acid molecules may be maintaining the ability to follow SET mechanism
415 during intestinal digestive conditions.

416 Therefore, these results evidenced the release of encapsulated water-soluble bioactive
417 compounds with antioxidant activity from double ($W_1/O/W_2$) emulsions is triggered during
418 gastric conditions as a result of an osmotic imbalance between the W_1 and W_2 phases.
419 However, the addition of CMC to emulsions slightly reduced the water diffusion between
420 phases and kept a controlled release during the hydrolysis of the lipid phase during small
421 intestine conditions.

422 4. Conclusions

423 The findings of this study have shown that the presence CMC significantly affects the
424 colloidal stability of double ($W_1/O/W_2$) emulsions and in turn, this determines their behavior

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425 during gastrointestinal conditions. Even when no differences were observed in emulsions at
426 the different CMC concentrations, it contributed to the emulsification of oil droplets into the
427 W_2 phase, thus presenting smaller particle sizes than double emulsions without CMC.
428 Nevertheless, the presence of CMC causes strong flocculation of the oil droplets, which
429 persist during simulated gastric conditions subsequently causing a delayed digestibility of oil
430 droplets during small intestine conditions. On the other hand, the release of mango peel
431 antioxidants encapsulated using double emulsions is triggered by osmotic imbalance caused
432 during gastric conditions rather than due to the digestion of the lipids by pancreatic lipases
433 during the small intestinal phase. Nevertheless, the results observed in this work may differ
434 from *in vivo* digestive conditions, since both the lipid digestion kinetics and the release of
435 encapsulated antioxidant compounds may be affected by bile salts and lipase concentration.
436 Hence, this work reveals important information regarding the use of thickening agents, such
437 as CMC, in the formulation of double ($W_1/O/W_2$) emulsions as carriers of bioactives and its
438 implications in their digestibility and release during digestive conditions. Nevertheless, the
439 findings observed in static digestion models, like the one performed in this study, should be
440 validated by performing further studies in complex and more representative digestion
441 models, such as cell lines cultures or animal models.

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View Article Online
DOI: 10.1039/C9FO01266D

Published on 03 September 2019. Downloaded on 9/4/2019 2:34:09 AM.

Food & Function Accepted Manuscript

451 References

452 1. J. F. Ayala-Zavala, V. Vega-Vega, C. Rosas-Domínguez, H. Palafox-Carlos, J. A. Villa-
453 Rodriguez, M. W. Siddiqui, J. E. Dávila-Aviña and G. A. González-Aguilar, Agro-industrial
454 potential of exotic fruit byproducts as a source of food additives, *Food Research*
455 *International*, 2011, **44**, 1866-1874.
456 2. F. Girotto, L. Alibardi and R. Cossu, Food waste generation and industrial uses: A review,
457 *Waste Management*, 2015, **45**, 32-41.
458 3. G. R. Velderrain-Rodríguez, M. Ovando-Martínez, M. Villegas-Ochoa, J. F. Ayala-Zavala, A.
459 Wall-Medrano, E. Álvarez-Parrilla, T. J. Madera-Santana, H. Astiazarán-García, O.
460 Tortoledo-Ortiz and G. A. González-Aguilar, Antioxidant Capacity and Bioaccessibility of
461 Synergic Mango (cv. Ataulfo) Peel Phenolic Compounds in Edible Coatings Applied to
462 Fresh-Cut Papaya, *Food and Nutrition Sciences*, 2015, **6**, 365.
463 4. S. G. Sáyago-Ayerdi, C. L. Moreno-Hernández, E. Montalvo-González, M. L. García-Magaña,
464 M. M.-M. de Oca, J. L. Torres and J. Pérez-Jiménez, Mexican 'Ataulfo'mango (Mangifera
465 indica L) as a source of hydrolyzable tannins. Analysis by MALDI-TOF/TOF MS, *Food*
466 *Research International*, 2013, **51**, 188-194.
467 5. A. Wall-Medrano, F. J. Olivas-Aguirre, G. R. Velderrain-Rodriguez, A. Gonzalez-Aguilar, L. A.
468 de la Rosa, J. A. Lopez-Diaz and E. Alvarez-Parrilla, MANGO: AGROINDUSTRIAL ASPECTS,
469 NUTRITIONAL/FUNCTIONAL VALUE AND HEALTH EFFECTS, *Nutricion Hospitalaria*, 2015,
470 **31**, 67-75.
471 6. G. Velderrain-Rodríguez, H. Torres-Moreno, M. Villegas-Ochoa, J. Ayala-Zavala, R. Robles-
472 Zepeda, A. Wall-Medrano and G. A. González-Aguilar, Gallic Acid Content and an
473 Antioxidant Mechanism Are Responsible for the Antiproliferative Activity of 'Ataulfo'
474 Mango Peel on LS180 Cells, *Molecules*, 2018, **23**, 695.
475 7. R. Pacheco-Ordaz, M. Antunes-Ricardo, J. Gutiérrez-Uribe and G. González-Aguilar,
476 Intestinal Permeability and Cellular Antioxidant Activity of Phenolic Compounds from
477 Mango (Mangifera indica cv. Ataulfo) Peels, *International Journal of Molecular Sciences*,
478 2018, **19**, 514.
479 8. Y.-T. Chen, W.-T. Kao and K.-W. Lin, Effects of pH on the total phenolic compound,
480 antioxidative ability and the stability of dioscorin of various yam cultivars, *Food Chemistry*,
481 2008, **107**, 250-257.
482 9. M. A. Krook and A. E. Hagerman, Stability of polyphenols epigallocatechin gallate and
483 pentagalloyl glucose in a simulated digestive system, *Food Research International*, 2012,
484 **49**, 112-116.
485 10. P. Yang, C. Yuan, H. Wang, F. Han, Y. Liu, L. Wang and Y. Liu, Stability of Anthocyanins and
486 Their Degradation Products from Cabernet Sauvignon Red Wine under Gastrointestinal pH
487 and Temperature Conditions, *Molecules*, 2018, **23**, 354.
488 11. S. Honda, R. Ishida, K. Hidaka and T. Masuda, Stability of Polyphenols under Alkaline
489 Conditions and the Formation of a Xanthine Oxidase Inhibitor from Gallic Acid in a Solution
490 at pH 7.4, *Food Science and Technology Research*, 2019, **25**, 123-129.
491 12. G. R. Velderrain-Rodríguez, A. Acevedo-Fani, G. A. González-Aguilar and O. Martín-Belloso,
492 Encapsulation and stability of a phenolic-rich extract from mango peel within water-in-oil-
493 in-water emulsions, *Journal of Functional Foods*, 2019, **56**, 65-73.
494 13. G. Muschiolik and E. Dickinson, Double Emulsions Relevant to Food Systems: Preparation,
495 Stability, and Applications, *Comprehensive Reviews in Food Science and Food Safety*, 2017,
496 **16**, 532-555.

View Article Online
DOI: 10.1039/C9FO01266D

497 14. M. Artiga-Artigas, A. Molet-Rodríguez, L. Salvia-Trujillo and O. Martín-Belloso, Formation
498 of Double (W1/O/W2) Emulsions as Carriers of Hydrophilic and Lipophilic Active
499 Compounds, *Food and Bioprocess Technology*, 2018, DOI: 10.1007/s11947-018-2221-3.
500 15. N. Mollakhalili Meybodi, M. A. Mohammadifar, M. Farhoodi, J. L. Skytte and K.
501 Abdolmaleki, Physical stability of oil-in-water emulsions in the presence of gamma
502 irradiated gum tragacanth, *Journal of Dispersion Science and Technology*, 2017, **38**, 909-
503 916.
504 16. A. Schuch, C. Helfenritter, M. Funck and H. P. Schuchmann, Observations on the influence
505 of different biopolymers on coalescence of inner water droplets in W/O/W (water-in-oil-
506 in-water) double emulsions, *Colloids and Surfaces A: Physicochemical and Engineering*
507 *Aspects*, 2015, **475**, 2-8.
508 17. M. Espinal-Ruiz, F. Parada-Alfonso, L.-P. Restrepo-Sánchez, C.-E. Narváez-Cuenca and D. J.
509 McClements, Impact of dietary fibers [methyl cellulose, chitosan, and pectin] on digestion
510 of lipids under simulated gastrointestinal conditions, *Food & Function*, 2014, **5**, 3083-3095.
511 18. A. Gasa-Falcon, I. Odriozola-Serrano, G. Oms-Oliu and O. Martín-Belloso, Influence of
512 mandarin fiber addition on physico-chemical properties of nanoemulsions containing β-
513 carotene under simulated gastrointestinal digestion conditions, *LWT - Food Science and*
514 *Technology*, 2017, **84**, 331-337.
515 19. M. Espinal-Ruiz, F. Parada-Alfonso, L.-P. Restrepo-Sánchez, C.-E. Narváez-Cuenca and D. J.
516 McClements, Interaction of a Dietary Fiber (Pectin) with Gastrointestinal Components (Bile
517 Salts, Calcium, and Lipase): A Calorimetry, Electrophoresis, and Turbidity Study, *Journal of*
518 *Agricultural and Food Chemistry*, 2014, **62**, 12620-12630.
519 20. H. Palafox-Carlos, E. Yahia, M. A. Islas-Osuna, P. Gutierrez-Martinez, M. Robles-Sánchez
520 and G. A. González-Aguilar, Effect of ripeness stage of mango fruit (Mangifera indica L., cv.
521 Ataulfo) on physiological parameters and antioxidant activity, *Scientia Horticulturae*, 2012,
522 **135**, 7-13.
523 21. J. Teixé-Roig, G. Oms-Oliu, G. R. Velderrain-Rodríguez, I. Odriozola-Serrano and O. Martín-
524 Belloso, The Effect of Sodium Carboxymethylcellulose on the Stability and Bioaccessibility
525 of Anthocyanin Water-in-Oil-in-Water Emulsions, *Food and Bioprocess Technology*, 2018,
526 DOI: 10.1007/s11947-018-2181-7.
527 22. M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carriere, R.
528 Boutrou, M. Corredig and D. Dupont, A standardised static in vitro digestion method
529 suitable for food–an international consensus, *Food & function*, 2014, **5**, 1113-1124.
530 23. N. P. Aditya, S. Aditya, H. Yang, H. W. Kim, S. O. Park and S. Ko, Co-delivery of hydrophobic
531 curcumin and hydrophilic catechin by a water-in-oil-in-water double emulsion, *Food*
532 *Chemistry*, 2015, **173**, 7-13.
533 24. N. Aditya, S. Aditya, H.-J. Yang, H. W. Kim, S. O. Park, J. Lee and S. Ko, Curcumin and
534 catechin co-loaded water-in-oil-in-water emulsion and its beverage application, *Journal of*
535 *Functional Foods*, 2015, **15**, 35-43.
536 25. M. Kowalska and A. Krzton-Maziopa, Viscoelastic effects in carrot oil emulsions thickened
537 with carboxymethylcellulose, *Colloids and Surfaces A: Physicochemical and Engineering*
538 *Aspects*, 2015, **464**, 121-128.
539 26. T. S. H. Leong, M. Zhou, D. Zhou, M. Ashokkumar and G. J. O. Martin, The formation of
540 double emulsions in skim milk using minimal food-grade emulsifiers – A comparison
541 between ultrasonic and high pressure homogenisation efficiencies, *Journal of Food*
542 *Engineering*, 2018, **219**, 81-92.

Published on 03 September 2019. Downloaded on 9/4/2019 2:34:09 AM.

Food & Function Accepted Manuscript

View Article Online
DOI: 10.1039/C9FO01266D

543 27. M. Katsouli, V. Polychniatou and C. Tzia, Influence of surface-active phenolic acids and
544 aqueous phase ratio on w/o nano-emulsions properties; model fitting and prediction of
545 nano-emulsions oxidation stability, *Journal of Food Engineering*, 2017, **214**, 40-46.
546 28. M. Estévez, C. Güell, S. De Lamo-Castellví and M. Ferrando, Encapsulation of grape seed
547 phenolic-rich extract within W/O/W emulsions stabilized with complexed biopolymers:
548 Evaluation of their stability and release, *Food Chemistry*, 2019, **272**, 478-487.
549 29. J. Li, Y. Shi, Y. Zhu, C. Teng and X. Li, Effects of Several Natural Macromolecules on the
550 Stability and Controlled Release Properties of Water-in-Oil-in-Water Emulsions, *Journal of*
551 *Agricultural and Food Chemistry*, 2016, **64**, 3873-3880.
552 30. P. Paximada, A. A. Koutinas, E. Scholten and I. G. Mandala, Effect of bacterial cellulose
553 addition on physical properties of WPI emulsions. Comparison with common thickeners,
554 *Food Hydrocolloids*, 2016, **54**, 245-254.
555 31. Y. Huan, S. Zhang and B. Vardhanabhuti, Influence of the molecular weight of
556 carboxymethylcellulose on properties and stability of whey protein-stabilized oil-in-water
557 emulsions, *Journal of Dairy Science*, 2016, **99**, 3305-3315.
558 32. L. Salvia-Trujillo, E. A. Decker and D. J. McClements, Influence of an anionic polysaccharide
559 on the physical and oxidative stability of omega-3 nanoemulsions: Antioxidant effects of
560 alginate, *Food Hydrocolloids*, 2016, **52**, 690-698.
561 33. L. Salvia-Trujillo, S. H. E. Verkempinck, L. Sun, A. M. Van Loey, T. Grauwet and M. E.
562 Hendrickx, Lipid digestion, micelle formation and carotenoid bioaccessibility kinetics:
563 Influence of emulsion droplet size, *Food Chemistry*, 2017, **229**, 653-662.
564 34. S. H. E. Verkempinck, L. Salvia-Trujillo, L. G. Moens, L. Charleer, A. M. Van Loey, M. E.
565 Hendrickx and T. Grauwet, Emulsion stability during gastrointestinal conditions effects
566 lipid digestion kinetics, *Food Chemistry*, 2018, **246**, 179-191.
567 35. E. Koukoura, M. Panagiotopoulou, A. Pavlou, V. Karageorgiou, D. G. Fatouros, C. Vasiliadou
568 and C. Ritzoulis, In Vitro Digestion of caseinate and Tween 20 Emulsions, *Food Biophysics*,
569 2019, **14**, 60-68.
570 36. S. Lamothe, V. Desroches and M. Britten, Effect of milk proteins and food-grade
571 surfactants on oxidation of linseed oil-in-water emulsions during in vitro digestion, *Food*
572 *Chemistry*, 2019, **294**, 130-137.
573 37. F. A. Bellesi, M. J. Martinez, V. M. Pizones Ruiz-Henestrosa and A. M. R. Pilosof,
574 Comparative behavior of protein or polysaccharide stabilized emulsion under in vitro
575 gastrointestinal conditions, *Food Hydrocolloids*, 2016, **52**, 47-56.
576 38. A. Sarkar, B. Murray, M. Holmes, R. Ettelaie, A. Abdalla and X. Yang, In vitro digestion of
577 Pickering emulsions stabilized by soft whey protein microgel particles: influence of
578 thermal treatment, *Soft Matter*, 2016, **12**, 3558-3569.
579 39. E. Malinauskytė, J. Ramanauskaitė, D. Leskauskaitė, T. G. Devold, R. B. Schüller and G. E.
580 Vegarud, Effect of human and simulated gastric juices on the digestion of whey proteins
581 and carboxymethylcellulose-stabilised O/W emulsions, *Food Chemistry*, 2014, **165**, 104-
582 112.
583 40. E. Malinauskytė, J. Ramanauskaitė, M. Keršienė, I. Jasutienė, D. Leskauskaitė, T. G. Devold
584 and G. E. Vegarud, Impact of Interfacial Composition on Emulsion Digestion Using In Vitro
585 and In Vivo Models, *Journal of Food Science*, 2018, **83**, 2850-2857.
586 41. G. R. Velderrain-Rodriguez, H. Palafox-Carlos, A. Wall-Medrano, J. F. Ayala-Zavala, C. Y. O.
587 Chen, M. Robles-Sanchez, H. Astiazaran-Garcia, E. Alvarez-Parrilla and G. A. Gonzalez-
588 Aguilar, Phenolic compounds: their journey after intake, *Food & Function*, 2014, **5**, 189-
589 197.

Published on 03 September 2019. Downloaded on 9/4/2019 2:34:09 AM.
Food & Function Accepted Manuscript

View Article Online
DOI: 10.1039/C9FO01266D

590 42. A. F. Campos, P. H. S. Santos, S. B. Santos, C. M. F. Soares and Á. S. Lima, Potassium
591 Phosphate Salts-Based Aqueous Two-Phase Systems Applied in the Extraction of Gallic
592 Acid from Guava AU - Reis, Igor A. O, *Separation Science and Technology*, 2015, **50**, 520-
593 528.

594

595

Published on 03 September 2019. Downloaded on 9/4/2019 2:34:09 AM.

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View Article Online
DOI: 10.1039/C9FO01266D

596 **Table 1**. Kinetic parameters (C_f, k and μ) and correlation coefficients (R^2) of the sigmoidal
597 Gompertz model (Eq. 2) fitted to experimental data of Free Fatty Acid (FFA) release (%)
598 during small intestine phase time (min) of double emulsions ($W_1/O/W_2$) with or without
599 mango peel extract (MPE) in presence of carboxymethylcellulose (CMC) in the external
600 aqueous phase (W_2) at different concentrations (0, 0.5 and 1 %).

	C_f(%)	k (min^{-1})	μ (min)	R^2
$W_1/O/W_2$	$27,68 \pm 0,48^{A}$	$0,1451 \pm 0,0097^{B}$	$10,49 \pm 0,31^{A}$	0,9988
$W_1/O/W_2$-MPE	$27,36 \pm 0,47^{A}$	$0,1434 \pm 0,0095^{B}$	$10,69 \pm 0,31^{A}$	0,9978
$W_1/O/W_2$– 0.5% CMC	$21,07 \pm 0,37^{B}$	$0,2162 \pm 0,0209^{BA}$	$8,54 \pm 0,34^{B}$	0,9952
$W_1/O/W_2$-MPE – 0.5% CMC	$22,38 \pm 0,46^{B}$	$0,2265 \pm 0,0285^{BA}$	$6,67 \pm 0,41^{C}$	0,9916
$W_1/O/W_2$– 1% CMC	$19,44 \pm 0,19^{C}$	$0,2148 \pm 0,0121^{A}$	$8,35 \pm 0,19^{B}$	0,9984
$W_1/O/W_2$-MPE – 1% CMC	$18,34 \pm 0,27^{C}$	$0,2582 \pm 0,0241^{A}$	$7,15 \pm 0,29^{C}$	0,9955

601 Different upper case letter indicate significant differences between each estimated kinetic parameter between
602 double emulsions (WOW) without or with mango extract (ME) and different carboxymethylcellulose (CMC)
603 concentrations.
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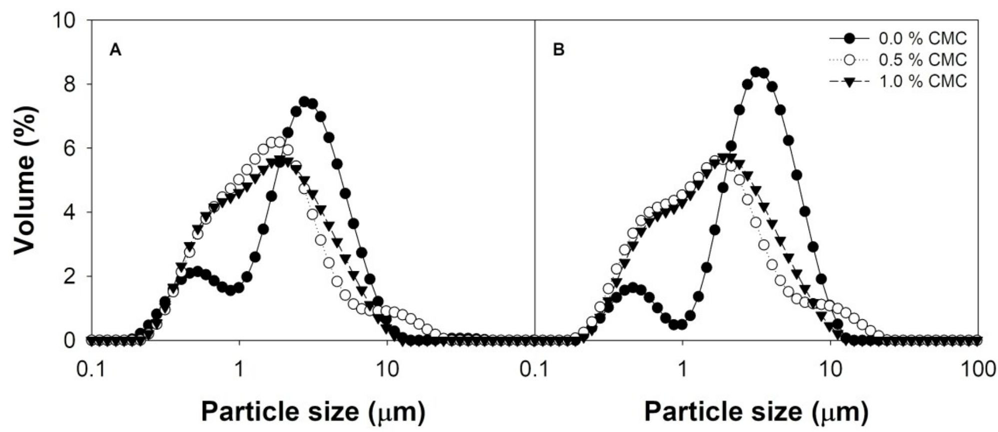

Figure 1. Particle size distribution of $W_1/O/W_2$ emulsions mixed with sodium carboxymethyl cellulose (CMC). A = Non-loaded $W_1/O/W_2$ emulsions; B = MPE-loaded $W_1/O/W_2$ emulsions.

281x131mm (300 x 300 DPI)

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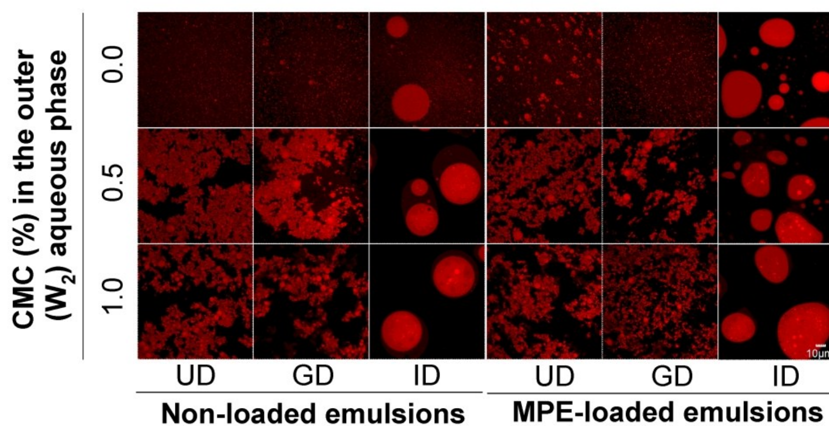

Figure 2. Confocal laser scanning microscopy (CLSM) images of double emulsions' stability after simulated digestion conditions, added with different concentrations of carboxymethyl cellulose (CMC). UD=Undigested, GD=Gastric digestion, ID=Intestinal digestion.

1236x875mm (72 x 72 DPI)

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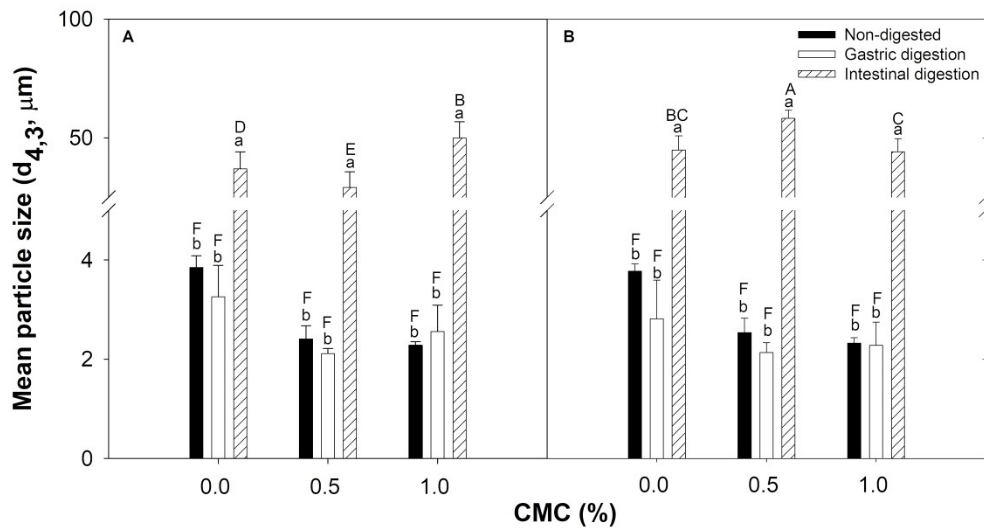

Figure 3. Effect of carboxymethyl cellulose (CMC) on the particle size of $W_1/O/W_2$ emulsions during simulated gastric and intestinal digestion. A = Non-loaded $W_1/O/W_2$ emulsions; B = Mango peel extract (MPE)-loaded $W_1/O/W_2$ emulsions. Values are expressed as the mean ± standard deviation (n=3): Lower-case letters indicate differences between CMC concentration, whereas upper-case letters indicate significant differences between control and MPE emulsions.

387x210mm (300 x 300 DPI)

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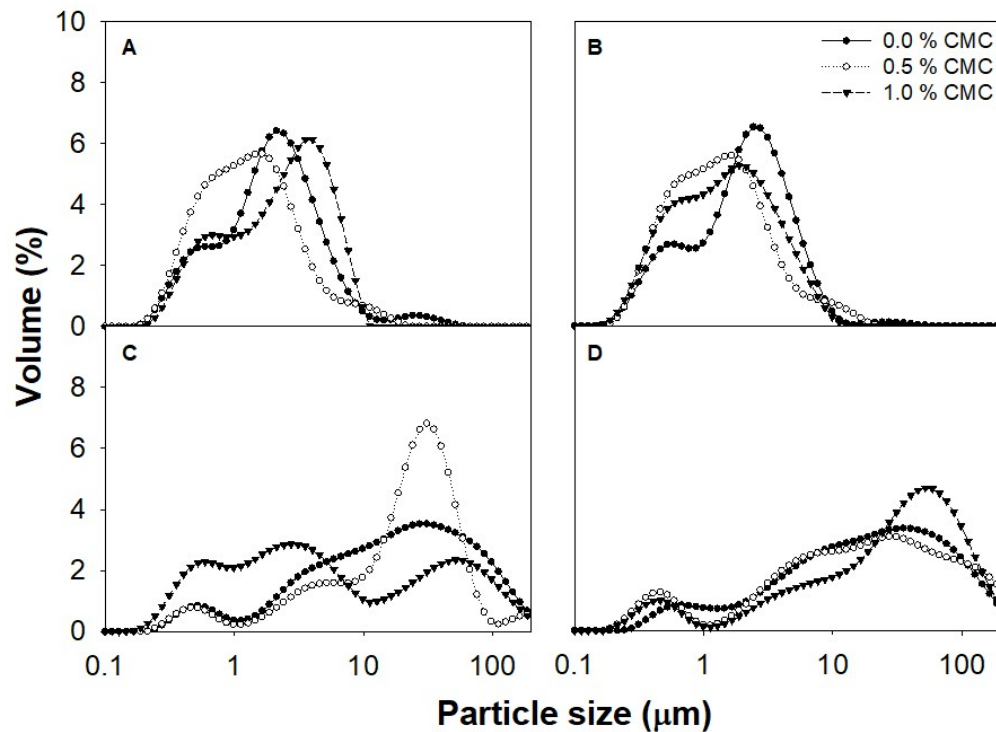

Figure 4. Particle size distribution of MPE non-loaded (A, C) and MPE-loaded (B, D) double emulsions after being subjected to gastric (A, B) and small intestinal (C, D) simulated digestion conditions, without or with carboxymethyl cellulose (CMC) at different concentrations.

150x110mm (150 x 150 DPI)

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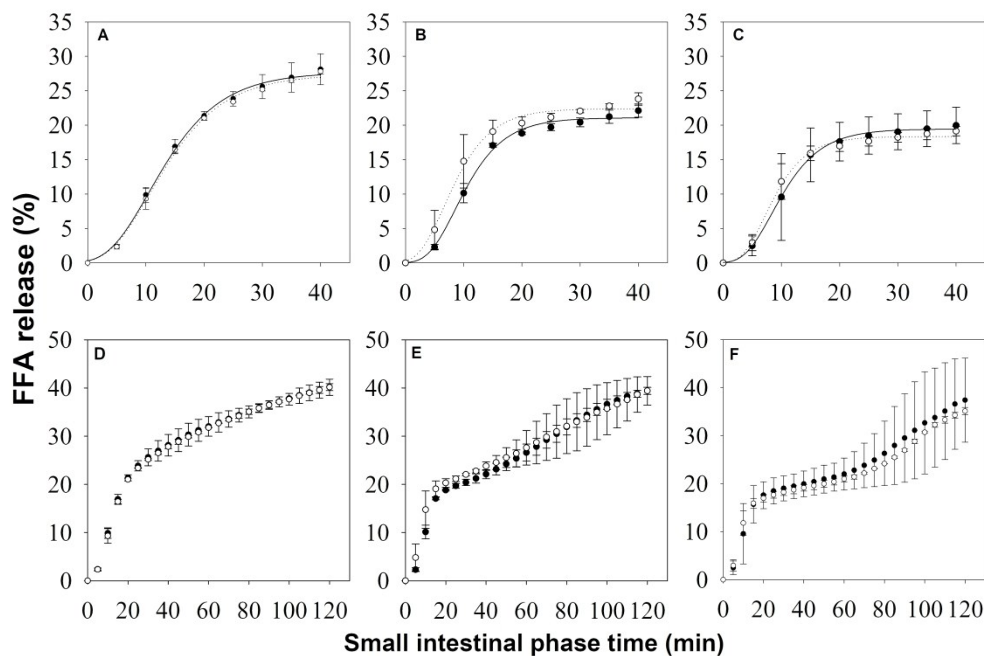

Figure 5. Lipid digestibility during *in vitro* small intestinal conditions of $W_1/O/W_2$ emulsions being non-loaded (filled circles) or loaded (empty circles) with mango peel extract (MPE) in the W_1 phase, and without carboxymethyl cellulose (CMC) (A, D) or with CMC at 0.5 (B, E) or 1% (C, F) w/w in the W_2 phase. Full lines represent the early lipid digestion kinetics modeled with a sigmoidal curve up to 40 min (A, B, C). Values are expressed as the mean ± standard deviation (n=3)

345x227mm (300 x 300 DPI)

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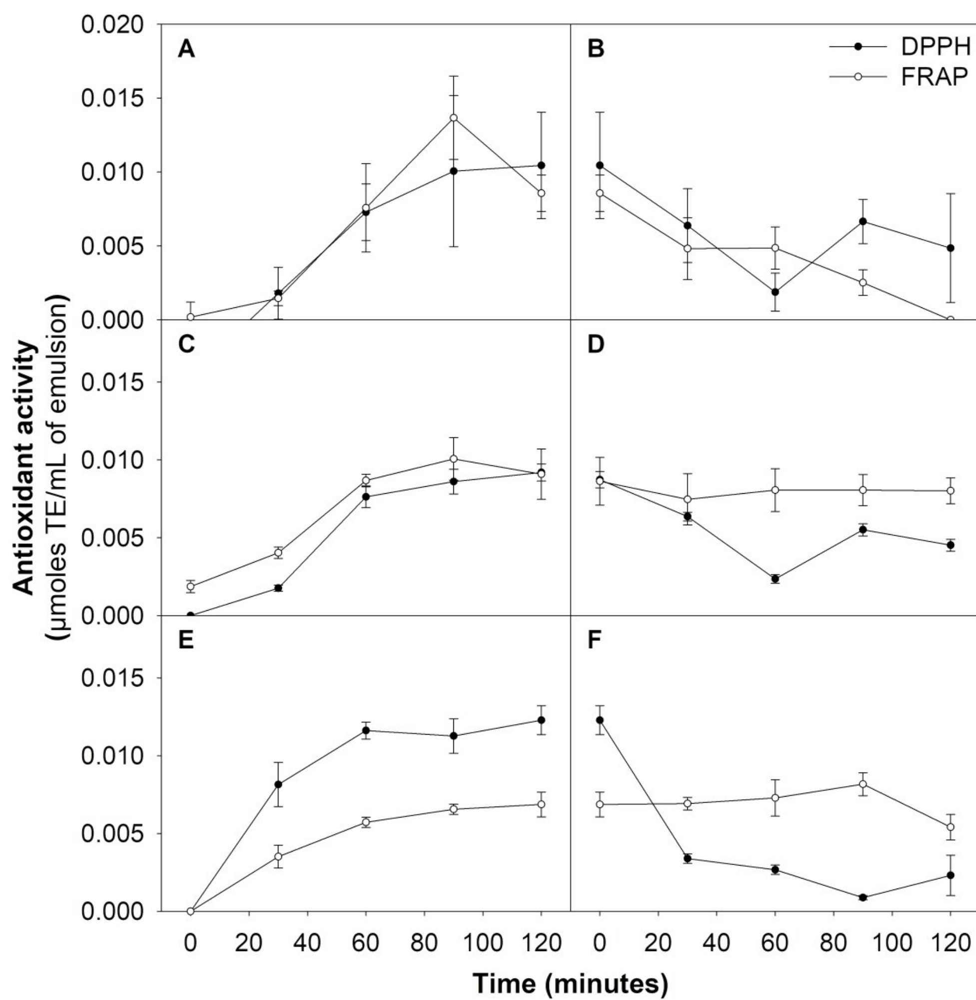

Figure 6. Antioxidant activity (µmoles TE/mL of emulsion) of mango peel extract (MPE)-loaded $W_1/O/W_2$ emulsions added with 0.0 % (A, B), 0.5 % (C, D) or 1.0 % (E, F) of carboxymethyl cellulose (CMC) during gastric (A, C, E) and intestinal (B, D, F) *in vitro* digestive conditions. Values are expressed as the mean ± standard deviation (n=3).

304x311mm (300 x 300 DPI)

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