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► **To cite this version:**

Pascale Goupy, Claude Genot, Faiza Hammaz, Charlotte Halimi, Catherine Caris-Veyrat, et al.. Mechanisms Governing the Transfer of Pure and Plant Matrix Carotenoids Toward Emulsified Triglycerides. *Molecular Nutrition and Food Research*, 2020, 14 p. 10.1002/mnfr.201900911 . hal-02475159

HAL Id: hal-02475159

<https://hal.science/hal-02475159v1>

Submitted on 20 Apr 2021

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1 **MECHANISMS GOVERNING THE TRANSFER OF PURE AND PLANT MATRIX**
2 **CAROTENOIDS TOWARD EMULSIFIED TRIGLYCERIDES.**

3
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11

12 **Keywords:** β -carotene, lutein, lycopene, phospholipids, serum albumin.

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23 **Abbreviations:** β -carotene (β C), lycopene (LYC), lutein (LUT), phospholipids (PL), bovine
24 serum albumin (BSA), triglycerides (TG).

25 **Abstract**

26

27 **Scope:** to assess the role of factors assumed to be involved in the transfer of carotenoids
28 from plant matrices to dietary emulsions in the upper digestive tract. **Methods:** We first
29 measured transfer as a function of time of pure β -carotene (β C), lutein (LUT) and lycopene
30 (LYC) to triglyceride (TG) droplets dispersed in water. Then we measured the transfer to TG
31 droplets stabilized with either bovine serum albumin (BSA), phospholipids (PL), or both.
32 Finally, we measured transfer of tomato and spinach puree carotenoids to these emulsions.
33 **Main results:** the maximal transfer efficiency of the pure carotenoids to uncoated emulsions
34 was very efficient, ranging from 59 to 77%. However, it was dramatically impaired, ranging
35 from 0.5 to 31% ($p < 0.05$), when emulsions were stabilized by the emulsifiers. Conversely,
36 when LUT, and to a less extent β C, but not LYC, was provided by the vegetable purees, its
37 maximal transfer efficiency was significantly higher for the coated emulsions than for the
38 uncoated one. **Conclusions:** Emulsifiers can dramatically impair the transfer of pure
39 carotenoids to emulsion TG while they can facilitate the transfer of carotenoids from plant
40 matrices. This suggests that specific interactions between plant matrix compounds and
41 emulsifiers can enhance the transfer efficiency of carotenoids.

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50 **1) Introduction**

51

52 Carotenoids are hydrophobic plant pigments that belong to the lipid family. Although
53 all their biological effects on the human body are far from being elucidated, some effects are
54 acknowledged, e.g. the provitamin A activity of carotenoids that possess at least one β -ionone
55 ring in their chemical formulae, and other are more and more assumed, e.g. anti-inflammatory
56 properties^[1-4] and role of lutein (LUT) and zeaxanthin in visual function.^[5-7]

57 Carotenoid bioavailability is low and modulated by numerous factors.^[8-10] It is assumed
58 that this is mostly because they are both very hydrophobic and embedded in plant cells. It is
59 also assumed that carotenoids can only migrate out of plant cells when these cells are broken,
60 either through food processing, cooking or chewing. Indeed, carotenoids are deposited in
61 different forms within plant cells, e.g. chloroplasts, chromoplasts, lipid-dissolved forms,^[11] and
62 it has been suggested that cells walls and chromoplast substructure represent important barriers
63 for their bioaccessibility.^[12, 13] Thus, carotenoids hardly integrate in mixed micelles, which are
64 involved in their transport to their site of absorption.^[14, 15] In fact, although during digestion it
65 has been shown that a fraction of carotenoids directly transfers from plant matrices into
66 micelles,^[16] because of their high hydrophobicity most of the carotenoids present in plant food
67 matrices transfer first to the triglyceride (TG) phase of the food bolus,^[17] which is more or less
68 dispersed as a so-called 'dietary emulsion'.^[18, 19] Furthermore, because of their high
69 hydrophobicity, non-oxygenated carotenoids, i.e. carotenes, are mostly solubilized in the core
70 of the emulsion lipid droplets, i.e. in the TG phase,^[20, 21] while oxygenated carotenoids, i.e.
71 xanthophylls, are solubilized both in the core of the lipid droplets and in the phospholipid
72 monolayer located at the droplet surface.^[22] Indeed, dietary lipid droplets are coated by surface-
73 active molecules, namely emulsifiers, naturally present in foods or secreted in the intestinal
74 lumen during digestion.^[23, 24] The main dietary emulsifiers are phospholipids (PL) and the

75 proteins that possess hydrophilic and hydrophobic sequence(s) of amino acids that allow them
76 to adsorb at and stabilize the lipid droplet interface.^[25]

77 Although the transfer of carotenoids from plant matrices to dietary emulsions has been
78 suggested decade ago, and although several studies have studied the transfer of carotenoid to
79 oil in vitro,^[12, 26-30] in vivo experiments dedicated to measure the carotenoid transfer from plant
80 matrices to dietary emulsions are scarce. In fact, to our knowledge there is only one clinical
81 study dedicated to this topic.^[31] In that study it has been shown that the three main dietary
82 carotenoids, i.e. β -Carotene (β C), lycopene (LYC) and LUT, can be transferred from plant
83 matrices to dietary emulsions in the human stomach during digestion. More precisely, up to
84 about 60% carrot puree β -Carotene (β C) was transferred in the fat phase of the meal after 3 h
85 digestion. A second interesting observation of this study was that the transfer efficiency of
86 carotenoids can be very different depending on carotenoid species and on the nature of the food
87 matrix. For instance, the maximal transfer efficiency of tomato puree LYC was less than 10%
88 while that of carrot puree β C was about 60%.

89 Yet, factors that are involved in these very different transfer efficiencies have not been
90 completely understood so far and it is for example not known whether the differences between
91 the maximal transfer efficiency of LYC and β C were due to the different physicochemical
92 properties of the carotenoids, as observed for the transfer of carotenoids between TG and mixed
93 micelles,^[32] or to the effect of the food matrix,^[9, 11, 33-36] which can result both from physical
94 barriers or from interactions with molecules that are associated with carotenoids in plant
95 matrices.^[11] Furthermore, concerning the dietary emulsions, the effects of emulsifier(s) that
96 coat the lipid droplets, and which can also be present as isolated or as molecular assemblies in
97 the aqueous medium, on the transfer efficiency of carotenoids has never been investigated,
98 although a recent study has suggested a role of PL on the transfer efficiency of tomato LYC to
99 oil.^[29] Yet, we hypothesize that these compounds might impair the transfer of carotenoids to oil

100 droplets by creating a barrier hindering the direct diffusion of carotenoid to this hydrophobic
101 phase.^[37]

102 This project aimed at assessing the role of the three main factors that we assume to
103 modulate the transfer of carotenoids to dietary emulsions, i.e. carotenoid species, presence and
104 species of emulsifiers, and presence and nature of the plant matrix. To that aim, we first
105 compared the transfer of three pure solid carotenoids, with contrasted physicochemical
106 characteristics,^[10, 32] to uncoated sunflower oil droplets dispersed in a slightly acid aqueous
107 medium (pH 4.0), considered as representative of the physicochemical conditions found in the
108 first step of digestion, that is during bolus formation in the stomach. Then, we studied the effect
109 of the presence and the type of emulsifiers that coat these droplets by measuring the transfer of
110 the pure carotenoids to the TG droplets stabilized with either a model protein: bovine serum
111 albumin (BSA), well-known for its emulsifying properties, a mixture of PL, which was mainly
112 composed of L- α -phosphatidylcholine, or a mixture of the two emulsifiers, which best mimic
113 what happens in vivo. We finally assessed the role of the environment of carotenoids in plant
114 matrix by measuring the transfer of carotenoids from tomato and spinach purees to either the
115 uncoated TG droplets or to the TG droplets coated with the emulsifiers. The results obtained
116 have allowed us to suggest fundamental mechanisms that govern this first step of carotenoid
117 bioavailability.

118 2) Materials and methods

119

120 Chemicals and vegetable sources of carotenoids

121 Acetone, dichloromethane, methanol, *n*-hexane, ethyl-acetate and 2-propanol were
122 purchased from Fisher-Scientific (Illkirch, France). Sodium acetate, sodium chloride and formic
123 acid were purchased from VWR (Fontenay-sous-Bois, France). Ammonium formate, (all-*E*)- β -
124 carotene (β C) ($\geq 95\%$), bovine serum albumin (BSA) (fraction V A-9647, MW ca. 66500 g
125 mol⁻¹), and trans- β -Apo-8'-carotenal ($> 95\%$) were purchased from Sigma-Aldrich (St
126 Quentin-Fallavier, France). L- α -phosphatidylcholine from dried egg yolk ($\geq 50\%$ TLC) was
127 from Sigma-Aldrich (Buchs, Switzerland). Certificate of analysis of the lot used in this study
128 indicated that it contained 76% L- α -phosphatidylcholine. A previous analysis of another lot of
129 the same product by our lab (data not shown) showed that this product also contained some
130 triglycerides (TG) and a mixture of phosphatidylethanolamine, sphingomyelin,
131 lysophosphatidylcholine and phosphatidylinositol. It is therefore called the "PL mixture"
132 thereafter in the manuscript. (all-*E*)-Lycopene (LYC) ($>90\%$) was purified in the lab as
133 previously described^[38] from a tomato oleoresin provided by Conesa (Badajoz, Spain). (all-*E*)-
134 Lutein (LUT) ($>99\%$) was also purified in the lab,^[39] but from a Naturex (Montfavet, France)
135 oleoresin. Sunflower oil (Lesieur, France) was bought in a local supermarket. We chose this
136 food oil because it is a commonly consumed oil. However, we acknowledge that other results
137 could have been obtained with other oils. Indeed, it has been shown that the solubility of
138 carotenoids increased when the chain-length of the triglycerides' fatty acids decreased.^[22]

139 Two common dietary sources of carotenoids were used in this study: tomato, which is
140 rich in LYC and β C and also contains LUT, and spinach, which is rich in LUT and also
141 contains β C. Cold break tomato puree (cultivar Terradou®) was prepared according to previous
142 work^[40]. We chose this source of tomato because it was very well characterized and it allowed

143 us to compare data obtained in this study with data obtained with the same tomato matrix in a
144 previous study by our lab on the diffusivity of lycopene from tomato puree to oil.^[29] Canned
145 spinach leaves were bought in a local supermarket (Casino brand). We chose canned spinach
146 leaves in order to compare data obtained in this study with data obtained with the same spinach
147 matrix in a previous study by our lab on the effect of spinach matrix on lutein bioaccessibility
148 and bioavailability.^[33] To have a homogeneous matrix, spinach leaves were diluted four times
149 with deionized water, i.e. 100 g drained spinach leaves were dispersed in a total volume of 400
150 mL water, and transformed in puree using a rotor stator homogenizer Silentcrusher (Heidolph,
151 Grosseron, France) set at 16000 rpm for 2 min under dim light at room temperature.

152

153 Phospholipid purification

154 In order to measure the association of carotenoids with pure PL we eliminated residual
155 neutral lipids (mainly triglycerides) from the egg yolk product rich in L- α -phosphatidylcholine
156 according to the method modified from Gladkowski et al.^[41] In summary, absolute ethanol (10
157 mL) was added to 2 g of the egg yolk product in a 50 ml flask placed on a magnetic stirrer.
158 After solubilization, the solution was placed at -20°C for 4 h and then centrifuged at 3000 x g at
159 4°C for 5 min. The supernatant was recovered, placed again at -20°C for 12 h and centrifuged
160 at 3000 xg at 4°C for 5 min. The new supernatant was recovered and evaporated under vacuum.
161 Cold acetone (20 mL) was finally added to solubilize contaminating TG and the mixture was
162 shake in ice. PL, which are insoluble in acetone, were precipitated, collected and washed with
163 cold acetone to remove remaining TG still present. Purified PL were stored at -20 ° C until
164 used.

165

166 Standard solutions of carotenoids

167 Standard solutions of carotenoids ($\sim 50 \mu\text{mol.L}^{-1}$) were prepared by dissolving the dry
168 standards in dichloromethane. Apo-8'-carotenal was dissolved in absolute ethanol. The
169 integrity of the carotenoid standards and their concentrations in the stock solutions were
170 checked and calculated by using a Specord S-600 diode-array UV-vis spectrophotometer
171 (Analytical Jena, Saint-Aubin, France). The following molar absorption coefficients were used:
172 $128000 \text{ L.mol}^{-1}.\text{cm}^{-1}$ at 460 nm for βC in dichloromethane, $178000 \text{ L.mol}^{-1}.\text{cm}^{-1}$ at 482 nm
173 for LYC in dichloromethane, $140516 \text{ L.mol}^{-1}.\text{cm}^{-1}$ at 444 nm for LUT in methanol containing
174 1% dichloromethane, $108900 \text{ L.mol}^{-1}.\text{cm}^{-1}$ at 457 nm for apo-8'-carotenal in absolute ethanol.

175

176 CAR extraction from the vegetable sources of carotenoids

177 Tomato and spinach puree carotenoids were extracted using a method described by
178 Serino et al.^[42] with few modifications. More precisely, 500 mg of spinach puree or 250 mg of
179 tomato puree were put in 2 ml Eppendorf tubes containing around 100 mg of 0.1 mm Zirconia/
180 Silica Beads (Biospec products, Bartlesville, USA) and 50 μL of a stock solution of apo-8'-
181 carotenal (33 μM in ethanol) used as internal standard. Then, 100 μL of saturated aqueous
182 NaCl solution, 100 μL of n-hexane, 200 μL of dichloromethane and 800 μL of ethyl-acetate
183 were successively added, each time followed by a 60 s full-speed high-energy shaking, using a
184 FastPrep[®] homogenizer (Thermo scientific, Waltham, USA). Then, the Eppendorf tubes were
185 centrifugated (5 min at $10,000 \times g$ at 4°C) and the upper phase was collected. Then, the pellet
186 was re-extracted with the same successive solvents and the second upper phase was pooled
187 with the first one and evaporated under argon to obtain the extracted lipids containing the
188 carotenoids. Carotenoids were quantified by HPLC as described in the dedicated paragraph.
189 Tomato puree contained $7.88 \pm 0.53 \text{ mg}/100 \text{ g}$ fresh weight LYC, $0.26 \pm 0.015 \text{ mg}/100 \text{ g}$ βC , and

190 0.05±0.01 mg/100 g LUT. Spinach puree contained 2.34±0.02 mg/100 g fresh weight LUT and
191 0.36±0.02 mg/100 g βC.

192

193

194

195 Preparation of sunflower oil-in-water emulsions

196 Four different emulsions were prepared: sunflower oil droplets dispersed in an aqueous
197 medium (uncoated emulsion), oil droplets stabilized by BSA (BSA-emulsion), oil droplets
198 stabilized by PL (PL-emulsion) or oil droplets stabilized by both emulsifiers (BSA/PL-
199 emulsion). We studied the effect of the combination of the two emulsifiers because in most
200 meals the two types of emulsifiers are simultaneously present and partition at the droplet
201 interface according to their own interfacial properties. All systems were adjusted to pH 4.0
202 because we aimed to study the transfer of carotenoids from vegetable food matrices to dietary
203 emulsions that occurs in the human stomach,^[31] and pH 4.0 is the mean pH observed in the
204 human stomach after ingestion of vegetable-rich meals that contained tomato and spinach
205 purees.^[31] The proportions of the different component families, i.e. TG, PL and proteins, were
206 representative of those occurring in the human gastrointestinal tract during digestion of a
207 typical western meal.^[31] Sunflower oil was chosen as the source of long chain TG because it is
208 one of the most consumed one in Western countries. For the preparation of the uncoated-
209 emulsions, 4 g of sunflower oil were added to 32 mL 10 mM acetate buffer at pH 4.0 in a 60
210 mL bottle. Then, the mixture was vigorously agitated by using a magnetic barrel stirrer for 1
211 min at room temperature. Obviously, this mixture was unstable, and the TG droplets coalesced
212 very quickly. Thus, it was used extemporaneously in the transfer experiments described in the
213 dedicated paragraph. The PL, BSA and BSA/PL-emulsions were prepared as follows: the
214 emulsifiers were first dispersed in 32 mL 10 mM pH 4.0 acetate buffer (BSA: 4.44 g/L; PL-rich

215 mixture: 3.13 g/L; BSA 4.44 g/L + PL-rich mixture 3.13 g/L). Then the emulsifiers were
216 dispersed using a rotor stator homogenizer (Heidolph Silent Crusher M) at 24,000 rpm for 2
217 min at room temperature. Then, 4 g of sunflower oil was added to the emulsifier dispersions.
218 The mixtures were then sonicated (Qsonica sonicator and a 3/4" probe) to obtain normal
219 distributions with mean particle sizes ranging between 2 and 4 μm , which was close to the size
220 of lipid droplets observed in the human gastrointestinal tract during digestion.^[19] The
221 sonication conditions were empirically adjusted to obtain emulsions that were stable during the
222 transfer experiments. Concerning the BSA and BSA/PL-emulsions the power was set at 20-
223 25W, the amplitude at 40, and the sonication was performed in ice for 4 periods of 30 s with
224 rest intervals of 30 s. Concerning the PL-emulsions, the power was set at 40 W, the amplitude
225 at 80, and the sonication was performed in ice for 4 periods of 60 s with rest intervals of 30 s.
226 The final pH of the mixtures was adjusted to 4.0 with either HCl or NaOH depending on the
227 initial pH.

228

229 Measurement of the size distribution of the emulsion lipid droplets

230 The size distribution of the stabilized emulsions was measured using a Mastersizer 2000
231 laser diffraction instrument (Malvern Panalytical SARL, Orsay, France). Each emulsion was
232 prepared 3 times independently as described above and 2 measures were performed on each
233 sample. Thus, means were obtained from 6 values. The parameters modes: D [4,3], D [3,2],
234 Dv10, Dv50 and Dv90 were calculated using the instrument's software. Calculations were
235 performed using the Mie theory with the following refractive indexes: 1.475 for oil, 1.333 for
236 the dispersant, and an absorbance value of 0.01 for the emulsion particles. The specific surface
237 area (m^2/g) was calculated by the software (total area of the particles divided by the total
238 weight of oil). This calculation was based on the assumption that the particles were both
239 spherical and non-porous.^[43]

240

241 Measurement of the zeta potential of the emulsion lipid droplets and of the tomato and spinach
242 particles

243 The zeta potential was measured on stabilized emulsions or vegetable puree particles
244 dispersed and diluted with 10 mM acetate buffer at pH 4.0 (dilution ratio between 50 and
245 5000). Measurements were performed in triplicate at 25°C using the Wallis zeta potential
246 analyser (Cordouan Technologies, Pessac, France). Ten sequential runs were averaged to obtain
247 one value. Zeta potentials were calculated using Henry's equation ^[44] and the Smoluchowski
248 approximation. A mean zeta-potential measurement was obtained from three independent
249 samples. Main settled parameters were: laser power 70%, low resolution (5 Hz), dielectric
250 constant (relative permittivity) 78.06, applied field 21.47 mV, solvent (water) viscosity
251 0.888cP, carrier frequency 8223 Hz.

252

253 Determination of the apparent solubility of carotenoids in aqueous solutions containing the
254 emulsifiers

255 We deposited about 50 µg of each pure carotenoid solubilized in dichloromethane in 8
256 mL glass tubes that possessed plugs and we dry evaporated them at room temperature under
257 argon and gentle agitation (Vibrax Orbital movement shaker model VXR basic set at 500 rpm).
258 The gentle agitation was performed to obtain a homogeneous film of carotenoids glued at the
259 bottom of to the glass tube. Then, we added 2 mL 0.1 mM pH 4.0 acetate buffer containing
260 either BSA, PL-rich mixture, purified PL, or a 50/50 mixture of BSA and PL-rich mixture, or
261 purified PL. Emulsifier concentrations were the same than those used to make the emulsions,
262 i.e. 4.44 g/L for BSA and 3.125 g/L for PL-rich mixture and purified PL. After 4 h incubation
263 at 37°C under gentle agitation (same shaker and setting than above), the solutions were
264 centrifugated (1000 x g for 5 min at 4 °C) to discard potential carotenoid crystals remaining in

265 suspension. Carotenoids remaining in the aqueous medium were theoretically associated with
266 the emulsifiers because of their very low solubility in water, were quantified by UPLC.

267

268 Measurement of the transfer of carotenoids from either pure carotenoids or vegetable puree
269 carotenoids to either uncoated or coated emulsions

270 To study the transfer of pure carotenoids to the TG phase of the different emulsions we
271 dry evaporated about 50 µg of each carotenoid in 8 mL glass tubes as described above. Then,
272 we added 2 mL of the different emulsions. Note that in the case of uncoated emulsions we first
273 added 2 mL 10 mM pH 4.0 acetate buffer then we added 0.25 mL sunflower oil in order to
274 avoid direct contact of the oil with the carotenoid film. We then closed the tubes with their
275 plugs, and we incubated them at 37°C under constant stirring for 240 min (same shaker and
276 setting than above). After 0.5, 15, 30, 60, 90, 120, 150 and 240 min, 1.5 mL of the mixtures
277 were sampled (we used one glass tube per incubation time), and TG, which was theoretically
278 rid of emulsifiers,^[20, 21] was recovered as described below.

279 To study the transfer of vegetable puree carotenoids to the TG phase of the different
280 emulsions we first diluted the vegetable purees with ultrapure H₂O (MilliQ system) to obtain
281 liquid mixtures that could be readily blended with the emulsions and that provided a quantity of
282 carotenoids nearly equivalent to that of pure carotenoids. Indeed, these dilutions provided
283 around 20 and 50 µg of total carotenoids per incubation condition for spinach and tomato
284 purees, respectively. Two mL of the emulsions were then added, and the mixtures were
285 incubated at 37°C under constant stirring (same apparatus and speed than above) for 240 min.
286 Samples of the mixtures were collected at the same interval times than above. The TG phase,
287 free of emulsifiers, was recovered as described below.

288 To break the emulsions and recover the TG phase free of emulsifiers and of vegetable
289 puree particles, samples that came from the transfer experiments were centrifuged for 15 min at

290 5,000 x g, frozen overnight at -20°C, thawed at room temperature and centrifuged again for 30
291 min at 5,000 x g. This allowed to obtain a floating layer of pure TG at the top of the tubes.
292 Furthermore, to avoid any contamination of the TG samples with emulsifiers that are located at
293 the interface between the TG and the aqueous phase,^[20, 21] only a fraction of the TG layer
294 floating at the top of the centrifuge tube, about 0.1 g, was collected. Each experiment was
295 performed in triplicate.

296

297 CAR quantification by UPLC

298 Carotenoids extracted from the vegetable puree matrices or incorporated in the emulsion
299 TG phases recovered after the transfer experiments were analyzed by HPLC as described
300 thereafter. The dried extracts of carotenoids obtained after tomato and spinach puree extraction
301 were first dissolved in 1 mL of dichloromethane/methanol (50/50; v/v) that contained 3.3 µM
302 apo-8'-carotenal as internal standard. The recovered emulsion TG phases containing the
303 transferred carotenoids were weighed and dissolved in 200 µL of 2-propanol and 800 µL of
304 dichloromethane/methanol (50/50; v/v), which also contained 3.3 µM apo-8'-carotenal as
305 internal standard. The analyses were performed using an ACQUITY UPLC® system (Waters
306 Corp., Milford, USA) linked to a diode array detector 190–800 nm. Empower™ 2 software
307 (Waters Corp.) was used for instrument control and chromatogram integrations. The separation
308 was carried out using an ACQUITY UPLC® HSS T3 C18 column (150 mm × 2.1 mm, internal
309 diameter 1.8 µm; Waters Corp.), which temperature was set at 50 °C. Solvents used were
310 methanol/water (80:20, v/v) (solvent A) and ethyl acetate (solvent B) containing 0.2% formic
311 acid. Gradient conditions were: A, 100%, 0–2 min; A, 100–30%, 2–20 min; A, 30–20%, 20–22
312 min; A, 20–100%, 22–23 min; and returned to the initial conditions (solvent A, 100%) for a 4
313 min re-equilibration period^[45]. The system operating pressure was 800 bars at initial gradient
314 conditions and injection volume was 7.5 µL with partial-loop with needle overfill injection.

315 The peaks were detected at 440 and 470 nm for LUT and the carotenes (LYC and β C),
316 respectively. The limit of quantification was 2 ng for LYC, 3 ng for β C and 0.2 ng for LUT.

317

318 Calculations and statistics

319 The transfer efficiency was defined as the percentage of carotenoids recovered in the
320 TG phase of the emulsions (measured mg CAR/g TG x 0.2 g TG, which was the total amount
321 of TG provided by the 2 mL emulsion used in each experiment) relative to the amount of
322 carotenoids, either pure or provided by the vegetable purees, that was incubated with the
323 emulsions. The maximal transfer efficiency was the highest measured transfer efficiency that
324 was mostly obtained after 240 min. incubation.

325 Results were expressed as means \pm SEM. Differences between means were assessed
326 using ANOVA followed by *post hoc* Tukey-Kramer tests. All *p* values under 0.05 were
327 considered significant. Statistical comparisons were performed using StatView software,
328 version 5.0 (SAS Institute Inc., Cary, NC).

329

330 Dosage information / Dosage regimen

331 The doses of pure carotenoids used in this study were non
332 supraphysiological/pharmacological. Indeed, we incubated 50 μ g pure carotenoids per tube
333 while the average intake of each of these phytochemicals is higher than 1 mg/person/d.^[46]

334 **3) Results**

335

336 *Main characteristics of the different emulsions*

337 Because we hypothesized that differences in transfer efficiency, and/or transfer rate, of
338 carotenoids between the different emulsions could be due to different characteristics of the
339 emulsions we measured both their lipid droplet size distribution and their electric charge.
340 Indeed, for a given amount of oil, the lower the size of the droplets the higher the emulsion
341 specific surface area (m^2/g oil) and thus the surface of the lipid droplets that can get in contact
342 with the carotenoids. Note that the characteristics of the uncoated emulsion were not measured
343 because, as stated in the material and method section, it was composed of coarse non-
344 homogeneous TG droplets, apparently in the mm range, which coalesced very quickly. As
345 expected, all coated emulsions exhibited monomodal particle size distributions with droplet
346 sizes in the range 0.5-4 μm (supporting information Fig. S4). Several parameters characterizing
347 the lipid droplet size distributions of the different coated emulsions are shown in table 1. All of
348 them, except the D3,2, were significantly ($p < 0.05$) different between the 3 emulsions. The
349 specific surface area of the PL-emulsion was about 60% higher than that of the BSA-emulsion
350 and about two times higher than that of the BSA/PL-emulsion. We calculated, from the mass
351 charge of BSA at the interface (theoretical surface load) given by the literature and the
352 emulsion's specific surface area, that in the BSA-emulsion about 25% of the BSA was
353 adsorbed at the oil-water interface, meaning that around 75% of the BSA remained in the
354 aqueous phase.^[47] Concerning the PL-emulsion, 65 % of PC were adsorbed at the interface.
355 Finally, concerning the BSA/PL-emulsion, about 30 % PL present in the system were enough
356 to cover the TG droplets and we assumed that all BSA remained in the aqueous phase. Indeed,
357 it is acknowledged that there is a preferential adsorption of low-molecular weight emulsifiers as
358 compared to high molecular weight emulsifiers such as proteins.^[47-49]

359 Concerning the zeta potential, as for the lipid droplet size distribution it was not
360 possible to measure it for the uncoated emulsion because this emulsion was too unstable. The
361 zeta potential of the BSA-emulsion was 24.6 ± 0.4 mV, that of the PL-emulsion was -9.3 ± 0.4
362 mV, while that of the BSA/PL-emulsion was intermediate, i.e. 8.3 ± 0.6 mV. These zeta
363 potentials were significantly different ($p < 0.05$). Concerning the vegetable puree particles, they
364 had close negative charges, i.e. -11.5 ± 0.2 and -10.2 ± 0.2 mV for the tomato and spinach
365 puree particles, respectively.

366

367 *Stability of carotenoids in the emulsions*

368 To accurately measure the transfer efficiency of carotenoids toward the TG phase of the
369 studied emulsions, and because it has been shown that Z-isomerization of LYC can enhance the
370 transfer efficiency,^[30, 50] we checked whether carotenoids were stable in these emulsions during
371 the experiment. To that purpose we quantified carotenoid concentrations as well as possible
372 formation of carotenoid (Z)- isomers, at regular time intervals from up to 240 min, in the TG
373 phase of BSA/PL-emulsions (data not shown). Neither the total amount of each carotenoid
374 incorporated in TG nor their (Z) to (all-E)-isomer ratios significantly changed ($p > 0.05$) over
375 time, i.e. all along the 240 min. This shows that the studied carotenoids were relatively stable in
376 the studied emulsions, at least in the conditions of our experiments, i.e. 240 min at pH 4.0 at
377 37°C, which agrees with recent results showing that β C is not significantly degraded in the
378 upper human gastrointestinal tract lumen during digestion.^[51]

379

380 *Apparent solubility of carotenoids in emulsifiers dispersed in aqueous medium*

381 Results reported in table 2 show that the apparent solubility of carotenoids in the
382 emulsifiers dispersed in an aqueous medium depended both on carotenoid and emulsifier
383 species. For example, while about 11% of LUT was recovered in PL-rich mixture dispersion,

384 only about 0.5% of β C was. Concerning LYC it was not detected in the aqueous medium
385 whatever the emulsifier(s) used. PL were better than BSA to solubilize β C, 0.52 vs 0.03%
386 ($p < 0.05$), while it was the opposite for LUT, 19.54 vs 11.12% ($p < 0.05$). The apparent solubility
387 of both carotenoids were higher when BSA and PL were mixed than when they were used
388 alone ($p < 0.05$). Finally, when purified PL were used instead of the PL mixture, which was a
389 mixture of PL, lysophosphatidylcholine, TG and sphingomyelin (see material and methods), the
390 apparent solubility of β C was non significantly different, 0.77 vs 0.52%, while that of LUT was
391 significantly decreased, 5.82 vs 11.12%. Also, the mixture of BSA plus purified PL led to a
392 lower apparent solubility of LUT than the mixture of BSA plus PL mixture.

393

394 *Transfer of carotenoids toward uncoated sunflower oil droplets*

395 Figure 1 shows the time-dependence of carotenoid incorporation (wt % of initial
396 amount of each carotenoid added in the system) in uncoated sunflower oil dispersed in acetate
397 buffer at pH 4.0. As shown in Fig. 1A the transfer of pure solid carotenoids, which initially
398 glued to the wall of the glass tubes, to the uncoated TG droplets, increased as a function of time
399 to nearly reach a plateau. The maximal transfer efficiency was about 59% for LUT and about
400 72 and 77% for LYC and β C, respectively (Fig. 5). When carotenoids were provided in the
401 tomato puree matrix the curves of transfer showed very different shapes (Fig. 1B) and the
402 maximal transfer efficiencies of all the carotenoids were significantly ($p < 0.05$) lower than that
403 of the pure carotenoids (supporting information Fig. S1A, S2A and S3A). The maximal transfer
404 efficiency for LYC was about 12% and that for β C and LUT were about 27 and 28%,
405 respectively (supporting information Fig. S3A, S2A and S1A). Finally, when carotenoids were
406 in spinach puree (Fig. 1C) the maximal transfer efficiencies of carotenoids to uncoated TG
407 droplets were the lowest with values of about 8% for LUT and about 5% for β C (supporting

408 information Fig. S1A and S2A). Note that there is no data on the transfer of LYC from spinach
409 puree because this carotenoid is not significantly present in this vegetable.

410

411 *Transfer of carotenoids toward sunflower oil droplets emulsified with BSA*

412 The only difference between Fig. 2 and Fig. 1 is that the carotenoids were incubated
413 with TG droplets stabilized with BSA instead of uncoated TG droplets. Fig. 2A shows that the
414 presence of BSA dramatically impaired the transfer of pure carotenoids to TG located in the
415 core of these droplets. Indeed, the maximal transfer efficiencies of the solid pure carotenoids
416 ranged between 0.5 and 4.4% as compared to values higher than 59% for pure solid carotenoids
417 to the uncoated emulsion (Fig. 5). In contrast, when the carotenoids were provided by tomato
418 puree (Fig. 2B), their maximal transfer efficiencies for the BSA-emulsion (7.6% for LYC,
419 26.8% for β C, and 51.6% for LUT; Fig. 6B) were higher than those observed when they were
420 provided as pure solids (0.5% for LYC, 0.7% for β C, and 4.4% for LUT; Fig. 5). The same
421 observation could be made for the maximal transfer efficiencies of carotenoids provided in
422 spinach puree. They were higher for the BSA-emulsion than for the uncoated emulsion.

423

424 *Transfer of carotenoids toward sunflower oil droplets emulsified with PL*

425 Fig. 3A and 5 show that the maximal transfer efficiencies of the pure carotenoids to
426 these emulsions ranged between 1.5% for LYC to 30.9% for LUT. When the carotenoids were
427 provided by tomato puree (Fig. 3B), the maximal transfer efficiency dramatically increased for
428 LUT, reaching 72.5% (Fig 6A). The maximal transfer efficiency of LYC also increased, from
429 1.5 to 8.5% (Fig. 5C and 6C). Finally, when the carotenoids were provided by the spinach
430 puree (Fig. 3C), their maximal transfer efficiencies were about 41% and 10% for LUT and β C
431 (Fig. 7A and 7B), respectively.

432

433 *Transfer of carotenoids toward sunflower oil droplets stabilized with both BSA and PL*

434 Fig. 4A shows that the maximal transfer efficiency of pure carotenoids to TG located in
435 BSA/PL-emulsions was very low. Indeed, it ranged between 0.9% for LYC to 8% for β C (Fig.
436 5C, A and B). As observed for the two other emulsions, when LUT was provided in tomato
437 puree its maximal transfer efficiency to the TG of the BSA/PL-emulsion dramatically increased
438 ($p<0.05$) reaching about 81% (Fig. 6A) as compared to pure LUT (7.0%, Fig. 5A). The
439 maximal transfer efficiencies of the two other carotenoids also significantly ($p<0.05$) increased
440 reaching about 10 and 32% for tomato puree LYC and β C (Fig. 6C and B) as compared to pure
441 carotenoids, 0.9 and 8.0%, respectively (Fig. 5C and B). The curves of transfer of spinach
442 carotenoids to the BSA/PL-emulsion are shown in Fig. 4C and the corresponding maximal
443 transfer efficiencies are shown in Fig. 7. In summary, the spinach- β C maximal transfer
444 efficiency was about 14% and that of spinach-LUT was about 50%.

445

446 *Comparison of the maximal transfer efficiencies of pure carotenoids toward sunflower oil when*
447 *this oil was either uncoated or emulsified with PL, BSA or both*

448 Fig. 5 to 7 give a summary of the maximal transfer efficiencies measured in the transfer
449 experiments shown in Fig. 1 to 4. They allow us to compare the maximal transfer efficiency
450 measured for each CAR, either pure or incorporated in the two different vegetable purees, with
451 each different species of emulsion.

452 Concerning the transfer of pure carotenoids, two general observations can be made.
453 First, the transfer was dramatically impaired when the TG were coated with the emulsifiers
454 (Fig. 5). Second, the lowest maximal transfer efficiencies were obtained when BSA was used
455 as the only emulsifier. Regarding the effect of carotenoid species, it is noteworthy that LUT
456 had the lowest maximal transfer efficiency to uncoated TG, i.e. about 59%, while LYC and β C
457 exhibited close maximal transfer efficiencies for this emulsion, i.e. about 72 and 77%

458 respectively. This ranking markedly changed in the presence of emulsifiers with LYC having
459 the lowest maximal transfer efficiency, regardless the emulsifiers.

460

461 *Comparison of the maximal transfer efficiencies of tomato puree carotenoids toward sunflower*
462 *oil when this oil was either uncoated or emulsified with PL, BSA or both*

463 The maximal transfer efficiencies of tomato puree LUT to the different emulsion
464 species were always higher than those of the two other tomato carotenoids (Fig. 6). The
465 maximal transfer efficiencies of the three tomato carotenoids were always higher when PL and
466 BSA were both present, i.e. in the BSA/PL-emulsions, than when they were used alone.

467

468 *Comparison of the maximal transfer efficiencies of spinach puree carotenoids toward*
469 *sunflower oil when this oil was either uncoated or emulsified with PL, BSA or both*

470 The first observation that arises from Fig. 7 is that, conversely to what was observed
471 with pure carotenoids (Fig. 5), the lowest maximal transfer efficiencies of both spinach
472 carotenoids were observed with the uncoated emulsion. Moreover, the effect of the emulsifiers
473 on the maximal transfer efficiencies of the spinach carotenoids depended on carotenoid species.
474 Indeed, concerning spinach LUT, PL led to a significantly ($p < 0.05$) higher maximal transfer
475 efficiency than BSA while it was the opposite for β C.

476

477 *Comparison of the maximal transfer efficiency of LUT toward the different emulsions when*
478 *LUT was provided either as a pure solid or incorporated in tomato or spinach puree*

479 Results presented in supporting information Fig. S1 are not commented because they
480 are presented, in another way, in Fig. 5 to 7. However, we believe that showing these results
481 this way can help the reader to better see another interesting result of this study, that is the
482 effect of the food matrix on the transfer of LUT to the different emulsion species.

483

484 *Comparison of the maximal transfer efficiency of β C toward the different emulsions when β C*
485 *was provided either as a pure solid or incorporated in tomato or spinach puree*

486 Same comment for supporting information Fig. S2 than for supporting information Fig.
487 S1, but concerning β C.

488

489 *Comparison of the maximal transfer efficiency of LYC toward the different emulsions when*
490 *LYC was provided either as a pure solid or incorporated in tomato puree*

491 Same comment for supporting information Fig. S3 than for supporting information Fig.
492 S1 and S2 but concerning LYC. Obviously, there is no data for spinach LYC because this
493 carotenoid is not present in this vegetable.

494 **4) Discussion**

495

496 Before discussing the results in details it should be mentioned that the transfer values
497 that we observed for the vegetable puree carotenoids to the BSA/PL-emulsion, which is the
498 emulsion assumed to best mimic the dietary emulsions that exist *in vivo*, showed similar trends
499 than what was observed *in vivo*.^[31] Indeed, we measured about 10% maximal transfer
500 efficiency for tomato LYC that can be compared to about 6% recovered in the fat phase of a
501 meal observed *in vivo* after 3 h digestion,^[31] and about 50% for spinach LUT as compared to
502 about 30% measured *in vivo*.^[31]

503 In the first part of this study we aimed to obtain basic data on the transfer efficiency of
504 carotenoids to TG. We thus measured the transfer efficiency of pure carotenoids toward
505 uncoated TG droplets dispersed in an aqueous medium. Results showed that this transfer was
506 very efficient. Indeed, the maximal transfer efficiency ranged between 59 and 77% depending
507 on carotenoid species. To explain this observation, we hypothesize that the transfer efficiency
508 depends both on the collisions between the agitated TG droplets and the film of carotenoids
509 stuck to the glass tubes, and on the hydrophobic attraction between carotenoids and TG
510 molecules when TG droplets enter in contact with the solid pure carotenoid film. Indeed, the
511 transfer of nonpolar molecules from a polar environment to a nonpolar environment is
512 driven both by the free energy gain^[52] and the hydrophobic force.^[53] The involvement of the
513 hydrophobic force is further supported by previous finding^[28] and by the fact that the maximal
514 transfer efficiency of LUT was lower than that of β C and LYC, i.e. 59 vs 77 and 72%
515 respectively, which can be related to their respective hydrophobicity schematically reflected by
516 their Log *P*, i.e. 11.78 vs 15.51 and 15.19, respectively (www.chemspider.com). Nevertheless,
517 the use of Log *P*, which measures the partition of a molecule between octanol and water,
518 appears not to be accurate to predict the solubility of carotenoids in oil because (Z)-isomers of

519 lycopene, which have the same Log P than the (all-E)-isomer, are more soluble in oil than the
520 (all-E)-isomer.^[30, 50] Nevertheless, because carotenoid hydrophobicity has also been correlated
521 with transfer of carotenoids from tomato and carrot based matrices to oil,^[28] as well as with
522 their transfer efficiency from lipid droplets toward mixed micelles,^[32, 54] these results suggest
523 that the hydrophobic force is a key player in carotenoid bioaccessibility.

524 Because dietary fats are coated with amphiphilic dietary molecules in the upper
525 gastrointestinal tract,^[18] the second part of this study was dedicated to assessing the effect of
526 the main dietary emulsifiers, i.e. phospholipids and proteins, on the transfer of pure solid
527 carotenoids to the TG located in the core of lipid droplets.^[20-22] When TG droplets were
528 emulsified in the presence of the emulsifiers, the maximal transfer efficiencies of pure
529 carotenoids were dramatically impaired (Fig. 5). Furthermore, the ranking of carotenoids
530 regarding their maximal transfer efficiency markedly changed with pure LYC having the
531 lowest maximal transfer efficiency, regardless the emulsifier, meaning that the inhibitory effect
532 of the emulsifiers on the transfer of carotenoids was higher for LYC than for β C and LUT. This
533 inhibition was *a priori* surprising because, as expected, when the TG were stabilized by the
534 emulsifiers the size of the emulsion lipid droplets decreased and the specific surface area, and
535 thus the available surface of exchange between carotenoids and TG, dramatically increased
536 (table 1). Thus, the mechanism(s) that lead(s) to the inhibitory effect of the emulsifiers on the
537 transfer of carotenoids far outweigh(s) the hypothesized potential of the emulsifiers in
538 increasing transfer by increasing the surface of transfer. Several inhibitory mechanisms can be
539 proposed. First, it can be hypothesized that, when the emulsions stabilized with the emulsifiers
540 were incubated with the solid films of pure carotenoids, a fraction of the carotenoids could have
541 been incorporated/associated to the fraction of the emulsifiers that remained dispersed in the
542 aqueous medium,^[55, 56] becoming not available for the transfer to the lipid droplets. Indeed, the
543 results obtained in this study on the apparent solubility of carotenoids in the emulsifiers

544 confirm that these compounds can associate with PL ^[52, 57, 58] and BSA. ^[59-61] They further show
545 that the apparent solubility in the emulsifiers is mainly determined by carotenoid species, the
546 carotenoid with two polar groups, LUT, being better associated with the emulsifiers than the
547 two other carotenoids, likely because it associates more easily with amphiphilic molecules.
548 Nevertheless, such a mechanism is not sufficient to explain the inhibitory effect of the
549 emulsifiers on the transfer of carotenoids because the transfer of LUT, which was by far the
550 carotenoid that showed the greatest apparent solubility in PL and BSA (table 2), was less
551 affected by the presence of the emulsifiers than LYC, which was not significantly associated
552 with the emulsifiers (Fig. 5). We thus hypothesize that the emulsifiers adsorbed at the droplet
553 interface could also create a physical barrier (or an energy barrier) that impairs the contact of
554 carotenoids with TG and thus impairs their transfer into the lipid droplets. Furthermore, we
555 hypothesize that this barrier is most important for the carotenoids that are not soluble in the
556 emulsifiers than for those who are. Indeed, we assume that carotenoids solubilized in the
557 emulsifier located at the droplet interface ^[22], and thus in contact with the TG core of the
558 droplets, can easily transfer to the TG phase, while carotenoids not solubilized in the interface
559 emulsifiers can hardly transfer. This hypothesis explains why the transfer of pure LYC, which
560 was not soluble in the emulsifiers, was more impaired by the emulsifiers than that of the two
561 other pure carotenoids (Fig. 5). Thus, when TG are coated with these emulsifiers, the transfer
562 efficiency of pure carotenoids to TG results from opposite effects: negative effects due to both
563 the quenching of the hydrophobic force by the emulsifier barrier between carotenoids and TG
564 and to the trapping of a fraction of carotenoids by the fraction of emulsifiers that remain in the
565 aqueous phase, ^[22, 55, 62] and positive effects due to both the facilitated transfer of carotenoids
566 that are incorporated in the fraction of emulsifiers located at the lipid droplet interface, and to
567 the higher surface of exchange induced by the emulsifiers. Obviously, these hypotheses should
568 be verified by dedicated experiments and we cannot extrapolate these observations to the effect

569 of other dietary emulsifiers on the transfer of other carotenoid species. Nevertheless, our results
570 and their interpretations allow us to hypothesize that the transfer efficiency of any carotenoid
571 species to the TG phase of an emulsion stabilized with amphiphilic molecules will mainly
572 depend on the ability of these carotenoids to associate with the amphiphilic molecules located
573 at the interface and to those solubilized in the aqueous phase.

574 The third part of this study aimed at assessing whether plant matrices, which contain
575 physical barriers that can impair carotenoid diffusion,^[13, 26] and which also contain carotenoids
576 that can be in different physical states and that can be associated with different matrix
577 molecules,^[11] can significantly affect the transfer efficiency. To that aim we chose two
578 vegetables that are usual dietary sources of carotenoids, i.e. tomato^[63] and spinach,^[33] and we
579 turned them into puree to mimic the size of their particles in the stomach during digestion.
580 After having studied the transfer of carotenoids in the same conditions than those used for the
581 pure carotenoids, we first observed that the transfer efficiency of the carotenoids incorporated
582 in these matrices to the uncoated TG droplets was markedly lower than that of the pure
583 carotenoids (supporting information Fig. S1A, S2A and S3A). To explain this observation, we
584 first hypothesize that this is because carotenoids in plant cells have to cross different barriers
585 that impair their release from the cells.^[12, 13] Nevertheless, this hypothesis disagrees with the
586 observation that the vegetable puree carotenoids were, in most cases, better transferred to the
587 coated emulsions than the pure carotenoids (supporting information Fig. S1 to S3). Therefore,
588 we suggest that some compounds in the vegetable purees are able to improve the transfer by a
589 mechanism that far outweighs the inhibitory effect of the cell barriers. A second hypothesis to
590 explain the lower transfer of vegetable puree carotenoids to uncoated TG droplets, as compared
591 to pure carotenoids, is that plant cells contain amphiphilic molecules, e.g. proteins and PL,^[64]
592 that adsorb at the uncoated TG droplet interface and that create a barrier that impairs the
593 transfer. A second, and very intriguing, observation was that the transfer of both spinach and

594 tomato puree LUT was significantly improved by the presence of the emulsifiers (supporting
595 information Fig. S1B, S1C and S1D vs supporting information Fig. S1A). This phenomenon
596 was not observed for tomato β C and LYC (supporting information Fig. S2 and supporting
597 information Fig. S3) and it was of less amplitude for spinach β C (supporting information Fig.
598 S2). To explain this phenomenon, we suggest that the complexes of molecules that were
599 associated with LUT in spinach and tomato purees and with β C in spinach puree, or which had
600 been associated with these carotenoids after the transformation of these matrices in purees,
601 associate very efficiently to the TG droplets coated with emulsifiers. The electric charges of the
602 coated or uncoated TG droplets and of the vegetable purees (Table 2) cannot explain why the
603 plant complexes of molecules apparently readily associate with coated TG droplets, while they
604 do not to uncoated TG droplets. Thus, we hypothesize that spinach and tomato LUT, as well as
605 spinach β C, were associated with molecules that facilitate anchoring of these complexes to
606 interfaces coated with emulsifiers. Furthermore, the fact that the transfer efficiency of tomato
607 LYC was not enhanced by the presence of emulsifiers (supporting information Fig. S3) was
608 perhaps because LYC in tomatoes is present as crystals in chromoplasts ^[11] and these crystals
609 are not coated by the molecules that, we hypothesized to, have a high affinity for interfaces
610 coated with emulsifiers. A third observation on the transfer efficiency of the vegetable puree
611 carotenoids to emulsion TG was that this transfer was always more efficient for tomato LUT
612 and β C than for spinach LUT and β C (supporting information Fig. S1 and supporting
613 information Fig. S2). We hypothesis, but this should be verified in a dedicated study, that
614 carotenoids located in these two different plant matrices could have to cross different biological
615 barriers, ^[12, 13] to go out of these matrices. Another possibility is that the size of the cells or the
616 percentage of remaining intact cells was different in the two purees. Whatever the mechanism,
617 the fact that the transfer is different in the two different plant matrices is in agreement with the
618 very variable effect of different plant matrices on carotenoid bioaccessibility.^[13, 36]

619 In summary, this study has allowed us to suggest fundamental mechanisms governing
620 the transfer of carotenoids to the TG phase of dietary emulsions. We have first suggested that
621 this transfer is basically governed by the hydrophobic force between carotenoids and TG. Then
622 we have shown that amphiphilic molecules that coat TG droplets strongly impair the transfer of
623 pure carotenoids, likely by creating a barrier between them and TG. Furthermore, the transfer
624 of carotenoids that are significantly solubilized in the amphiphilic molecules is less affected.
625 We then found that plant carotenoids hardly transfer to uncoated TG droplets, and we have
626 suggested that this is because they have to cross structural barriers but it is also likely that
627 amphiphilic molecules present in plant matrices coat the interface and hinder the transfer.
628 Conversely, we found that spinach and tomato puree LUT, and to a less extent spinach β C,
629 were better transferred to TG droplets coated with emulsifiers than to uncoated TG droplets and
630 we hypothesized that this is because lipid/protein complexes that are present with these
631 carotenoids in the studied vegetable matrices facilitate anchoring of these complexes to coated
632 lipid droplets. Further experiments are needed to complete this pioneering study. Indeed, it is
633 likely that digestive enzymes that hydrolyze lipids and proteins in the gastric and intestinal
634 lumen, as well as bile salts that adsorb at the lipid droplet interface during digestion, modify the
635 transfer rate and/or the transfer efficiency of carotenoids. It is also likely that different results
636 would have been obtained with other types of food oil. Indeed, carotenoid solubility in TG
637 depends on the type of TG fatty acids.^[22] Nevertheless, we believe that a full understanding of
638 the mechanisms that govern the transfer of plant carotenoids to food emulsions will allow
639 researchers to propose new strategies to improve the bioavailability of these phytochemicals.

640 **Acknowledgments:**

641 The authors thank Conesa and Naturex for supplying tomato puree and CAR-rich oleoresins,
642 respectively. Thanks also to Stéphanie Oriol for photographing the lycopene crystals shown in
643 Fig 1 to 3.

644

645 **Author contributions:**

646 The authors' responsibilities were as follows---PB and CC-V: designed the research and have
647 primary responsibility for final manuscript content. PG and FH: conducted research. PB and
648 CG: analysed data and interpreted them. PB: wrote the paper; and all authors: read and
649 approved the final manuscript.

650

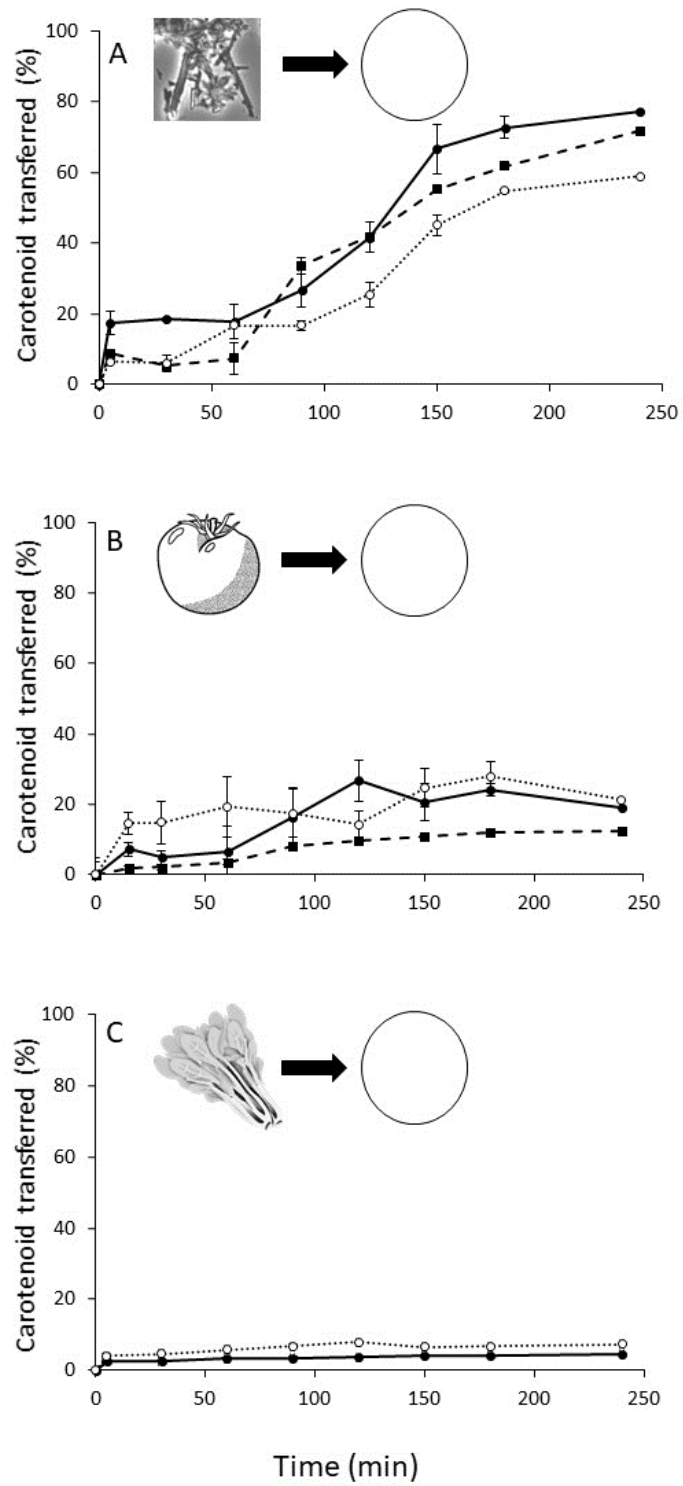
651 **Conflicts of interest:**

652 None of the authors reported a potential conflict of interest.

653

654 **Funding:**

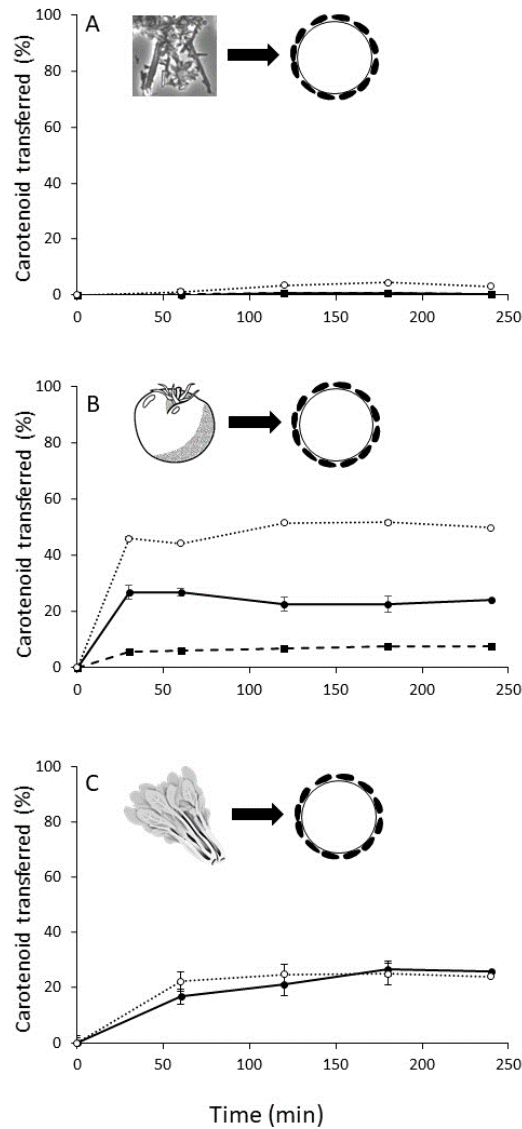
655 This project received funding from two incentive programs of the French National Institute for
656 Agricultural Research (INRA): AIC Cepia "Factors limiting the bioaccessibility of carotenoids
657 during digestion" and Action Prioritaire AlimH "Bioavailability of micronutrients".



659

660 **Figure 1.** Transfer of carotenoids to uncoated sunflower TG.

661 The graph shows the percent of carotenoid transferred as a function of incubation time (min). **A:** the photography
 662 of lycopene crystals (magnification 100x) and the circle helps to see that this is the transfer of pure solid
 663 carotenoids to uncoated TG droplets in aqueous medium. **B:** the drawing of tomato helps to see that this is the
 664 transfer of tomato puree carotenoids, i.e. lycopene (LYC), β -carotene (β C) and lutein (LUT), to uncoated TG
 665 droplets. **C:** the drawing of spinach leaves helps to see that this is the transfer of spinach puree carotenoids, i.e. β -

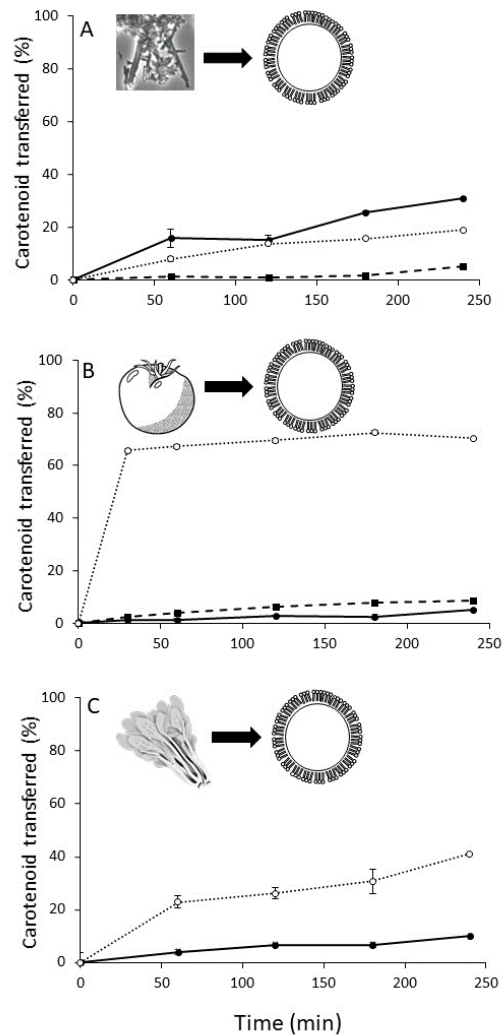


666 carotene (β C) and lutein (LUT), to uncoated TG droplets. ■ LYC, ● β C, ○ LUT. Dots represent mean \pm SEM of 4
 667 independent experiments.

668

669 **Figure 2.** Transfer of carotenoids to sunflower TG located in emulsions stabilised by BSA.

670 The graph shows the percent of carotenoid transferred as a function of incubation time (min). **A:** the photography
 671 of lycopene crystals (magnification 100x) and the circle coated with black forms helps to see that this shows the
 672 transfer of pure solid carotenoids to BSA-emulsions. **B:** the drawing of tomato helps to see that this is the transfer
 673 of tomato puree carotenoids, i.e. lycopene (LYC), β -carotene (β C) and lutein (LUT), to BSA-emulsions. **C:** the
 674 drawing of spinach leaves helps to see that this is the transfer of spinach puree carotenoids, i.e. β -carotene (β C)

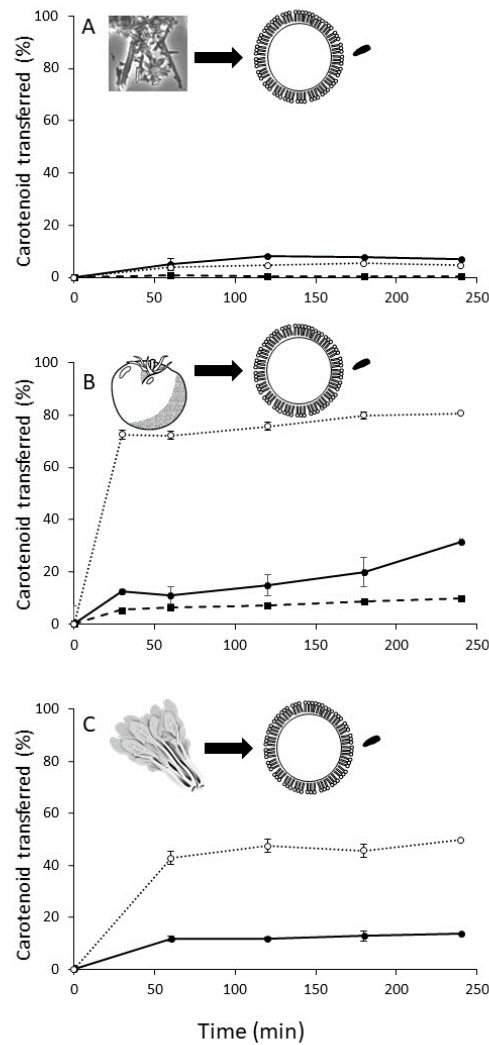


675 and lutein (LUT), to BSA-emulsions. ■ LYC, ● β C, ○ LUT. Dots represent mean \pm SEM of 4 independent
 676 experiments.

677

678 **Figure 3.** Transfer of carotenoids to sunflower TG located in emulsions stabilised by PL-rich mixture.

679 The graph shows the percent of carotenoid transferred as a function of incubation time (min). **A:** the photography
 680 of lycopene crystals (magnification 100x) and the circle coated with two arm pins helps to see that this shows the
 681 transfer of pure solid carotenoids to PL-emulsions. **B:** the drawing of tomato helps to see that this is the transfer of
 682 tomato puree carotenoids, i.e. lycopene (LYC), β -carotene (β C) and lutein (LUT), to PL-emulsions. **C:** the
 683 drawing of spinach leaves helps to see that this is the transfer of spinach puree carotenoids, i.e. β -carotene (β C)

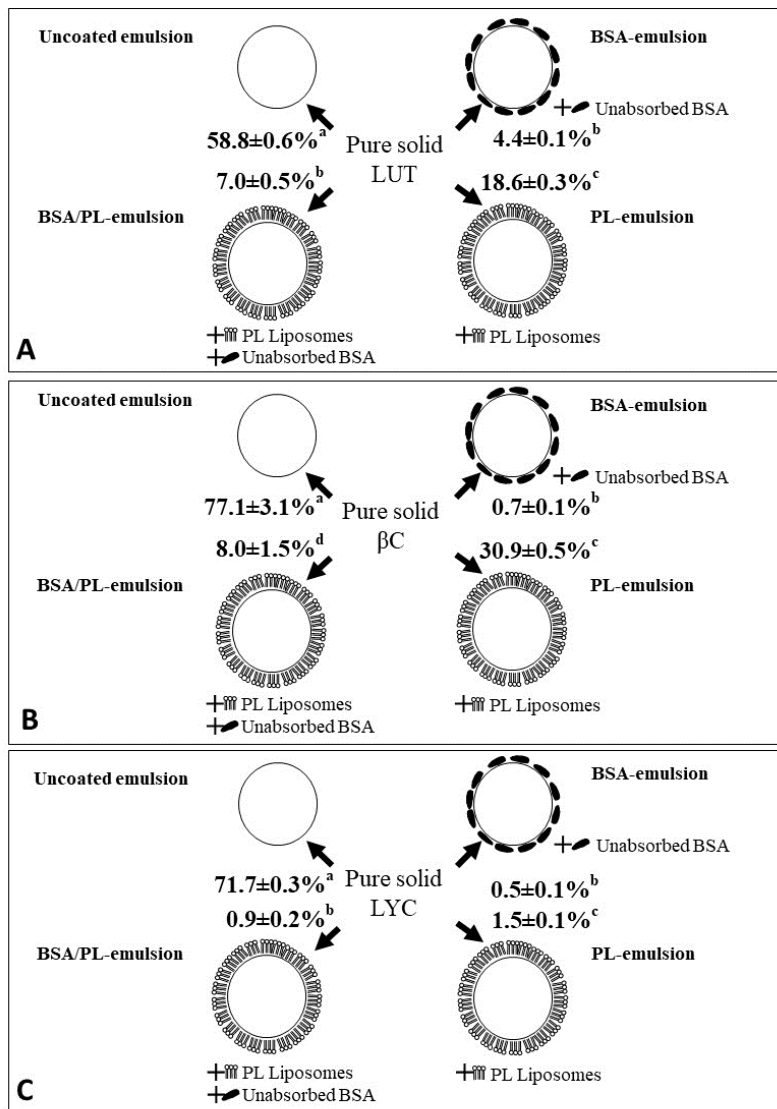


684 and lutein (LUT), to PL-emulsions. ■ LYC, ● β C, ○ LUT. Dots represent mean \pm SEM of 4 independent
 685 experiments.

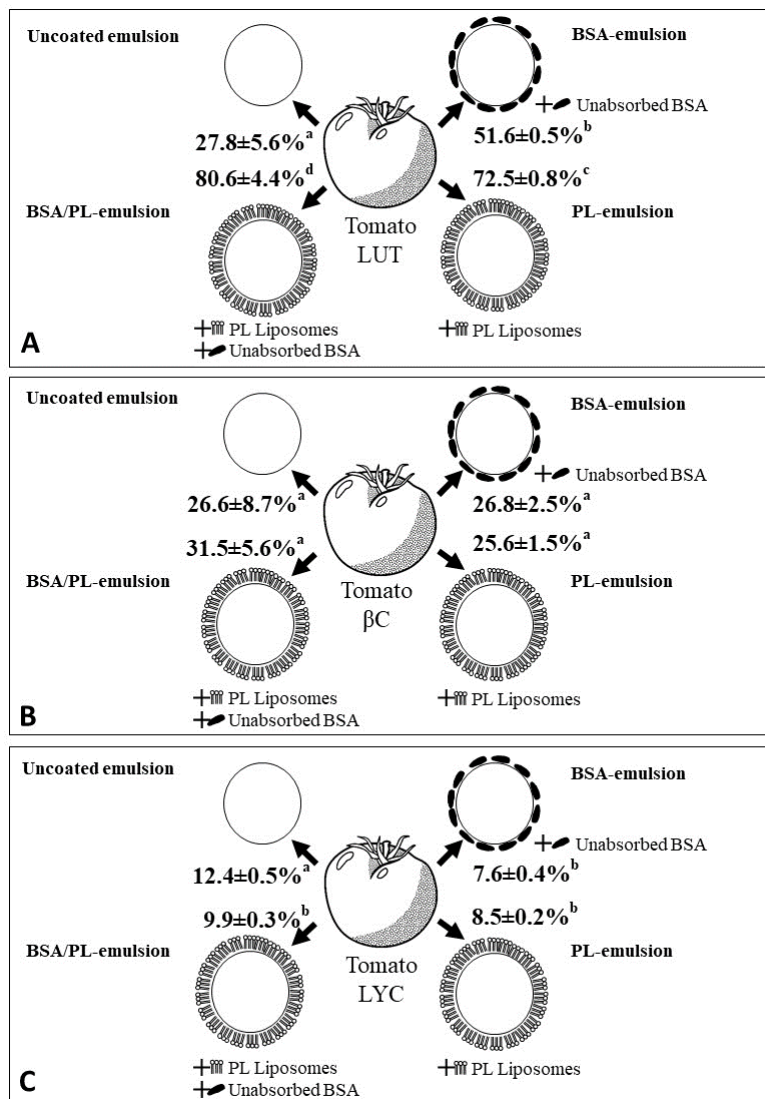
686

687 **Figure 4.** Transfer of carotenoids to sunflower TG located in emulsions stabilised by a mixture of BSA and PL as
 688 emulsifiers.

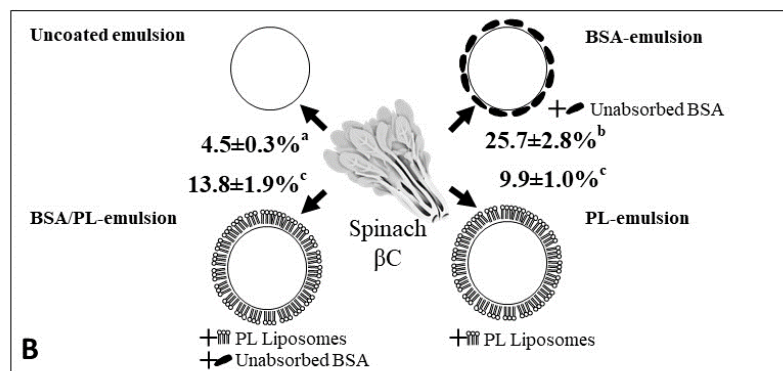
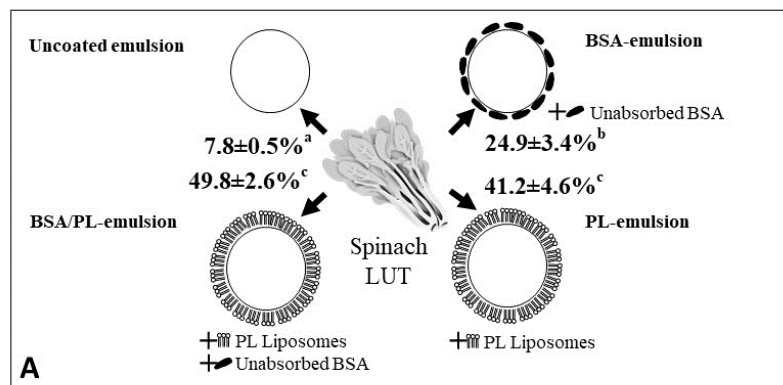
689 The graph shows the percent of carotenoid transferred as a function of incubation time (min). **A:** the photography
 690 of lycopene crystals (magnification 100x) and the circle coated with two arm pins, together with a black form
 691 close to it, helps to see that this shows the transfer of pure solid carotenoids to BSA/PL-emulsions. **B:** the drawing
 692 of tomato helps to see that this is the transfer of tomato puree carotenoids, i.e. lycopene (LYC), β -carotene (β C)
 693 and lutein (LUT), to BSA/PL-emulsions. **C:** the drawing of spinach leaves helps to see that this is the transfer of
 694 spinach puree carotenoids, i.e. β -carotene (β C) and lutein (LUT), to BSA/PL-emulsions. ■ LYC, ● β C, ○ LUT.
 695 Dots represent mean \pm SEM of 4 independent experiments.



696 **Figure 5.** Summary of the maximal transfer efficiencies measured for each pure solid carotenoid in each type of
 697 emulsion. The maximal transfer efficiencies were obtained from the curves presented in figures 1 to 4. A:
 698 Maximal transfer efficiency of pure lutein (LUT) to the different emulsions, B: Maximal transfer efficiency of
 699 pure β -carotene (β C), C: Maximal transfer efficiency of pure lycopene (LYC). The theoretical localisation of the
 700 emulsifiers is presented. For example, it has been calculated (see result section) that 65% of PL were located at the
 701 lipid droplet interface and that the remaining fraction was dispersed in the aqueous phase. Values represent mean \pm
 702 SEM of 4 independent experiments. In each figure, means that bear different superscript letters are significantly
 703 different ($p < 0.05$), ANOVA followed by Tukey-Kramer post-hoc test.



704 **Figure 6.** Summary of the maximal transfer efficiencies measured for each tomato puree carotenoid in each type
 705 of emulsion. The maximal transfer efficiencies were obtained from the curves presented in figures 1 to 4. A:
 706 Maximal transfer efficiency of pure lutein (LUT) to the different emulsions, B: Maximal transfer efficiency of
 707 pure β -carotene (β C), C: Maximal transfer efficiency of pure lycopene (LYC). The theoretical localisation of the
 708 emulsifiers is presented. For example, it has been calculated (see result section) that 65% of PL were located at the
 709 lipid droplet interface and that the remaining fraction was dispersed in the aqueous phase. Values represent mean \pm
 710 SEM of 4 independent experiments. In each figure, means that bear different superscript letters are significantly
 711 different ($p < 0.05$), ANOVA followed by Tukey-Kramer post-hoc test.



712

713 **Figure 7.** Summary of the maximal transfer efficiencies measured for each spinach puree carotenoid in each type
714 of emulsion.

715 The maximal transfer efficiencies were obtained from the curves presented in figures 1 to 4. A: Maximal transfer
716 efficiency of pure lutein (LUT) to the different emulsions, B: Maximal transfer efficiency of pure β -carotene (β C).

717 The theoretical localisation of the emulsifiers is presented. For example, it has been calculated (see result section)
718 that 65% of PL were located at the lipid droplet interface and that the remaining fraction was dispersed in the
719 aqueous phase. Values represent mean \pm SEM of 4 independent experiments. In each figure, means that bear
720 different superscript letters are significantly different ($p < 0.05$), ANOVA followed by Tukey-Kramer post-hoc test.

721 **Table 1**

722

723 Main characteristics of the emulsions stabilised by BSA, PL mixture or both*.

	BSA-emulsions	PL-emulsions	BSA/PL-emulsions
Dv10 (μm)	0.81 ± 0.05^a	0.43 ± 0.02^b	1.55 ± 0.06^c
Dv50 (μm)	1.81 ± 0.12^a	1.42 ± 0.09^b	2.30 ± 0.05^c
Dv90 (μm)	3.84 ± 0.25^a	2.75 ± 0.13^b	4.60 ± 0.20^c
D3,2 (μm)	1.52 ± 0.15^a	1.48 ± 0.04^a	2.46 ± 0.11^b
D4,3 (μm)	2.11 ± 0.19^a	0.95 ± 0.04^b	1.18 ± 0.12^c
Specific Surface area ($\text{m}^2/\text{g oil}$)	3.99 ± 0.17^a	6.50 ± 0.12^b	3.04 ± 0.05^c
Non-adsorbed emulsifier**			
g/L in aqueous phase	3.4	0.9	1.7 (PL); 4.44 (BSA)
% in aqueous phase	77.6	35.1	69.7 (PL); 100*** (BSA)
Zeta potential (mV)	24.6 ± 0.4^a	-9.3 ± 0.4^b	8.3 ± 0.6^c

724 Values are means \pm SEM of 6 measurements (3 different emulsions measured 2 times each).

725 Dv10 is the droplet diameter below which 10% of the total lipid volume of the sample is

726 contained. Dv50 is the median size. Dv90 is the droplet diameter below which 90% of the total

727 lipid volume of the sample is contained. D3,2 is the surface mean diameter. D4,3 is the volume

728 mean diameter. In each line, means bearing different letters are significantly different (ANOVA

729 followed by Tukey-Kramer post-hoc test, $p < 0.05$).

730 *: it was not possible to measure these characteristics for the uncoated emulsion, i.e. pure TG

731 suspended in aqueous medium, because the TG droplets were very unstable and coalesced very

732 quickly. **: amounts of emulsifiers not absorbed at the droplet interface have been estimated

733 using 2 mg/mL as theoretical surface load for both BSA and PL,^[47] ***: in the presence of PL
734 in excess, BSA is estimated not to be adsorbed at the interface.

735 **Table 2**

736

737 Apparent solubility of pure carotenoids in aqueous dispersions of BSA, PL mixture, Purified

738 PL or mixtures of the emulsifiers.

	BSA	PL mixture	Purified PL	BSA+PL mixture	BSA+Purified PL
β C	$0.03 \pm 0.02^{*a}$	0.52 ± 0.11^b	0.77 ± 0.11^b	1.36 ± 0.21^c	0.65 ± 0.02^b
LUT	19.54 ± 0.61^c	11.12 ± 1.46^b	5.82 ± 3.44^a	43.19 ± 5.91^d	11.42 ± 1.53^b
LYC	ND	ND	ND	ND	ND

739 Pure carotenoids were incubated in aqueous solutions of either BSA, PL mixture, purified PL,

740 or a 50/50 mixture of both emulsifiers, and carotenoids recovered in the aqueous dispersions of

741 emulsifiers were quantified by UPLC. *Maximal percentage of carotenoid recovered in

742 dispersions of emulsifiers. Values are mean \pm SEM of 3 to 9 measurements. ND: not detected,743 i.e. $< 0.01\%$. In each line, means bearing different letters were significantly different (ANOVA744 followed by Tukey-Kramer post-hoc test, $p < 0.05$).

745 **5) References**

746

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