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Joana Corte-Real, Marie Bertucci, Christos Soukoulis, Charles Desmarchelier, Patrick Borel, et al.. Negative effects of divalent mineral cations on the bioaccessibility of carotenoids from plant food matrices and related physical properties of gastro-intestinal fluids. *Food and Function*, 2017, 8 (3), pp.1008-1019. 10.1039/C6FO01708H . inserm-01479975v2

HAL Id: inserm-01479975

<https://inserm.hal.science/inserm-01479975v2>

Submitted on 9 Apr 2018

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1 **Negative effects of divalent mineral cations on the bioaccessibility of**
2 **carotenoids from plant food matrices and related physical properties of gastro-**
3 **intestinal fluids**

4 Joana Corte-Real^{a, d}, Marie Bertucci^d, Christos Soukoulis^d, Charles Desmarchelier^b, Patrick
5 Borel^b, Elke Richling^c, Lucien Hoffmann^d, Torsten Bohn^{1, a*}

6

7 ^a Luxembourg Institute of Health (LIH), Population Health Department, 1 a-b, rue Thomas
8 Edison, L-1445 Strassen, Luxembourg

9 ^b NORT, INRA, INSERM, Aix-Marseille Université, 13005, Marseille, France

10 ^c Food Chemistry and Toxicology, Department of Chemistry, University of Kaiserslautern,
11 Erwin-Schroedinger-Strasse 52, D-67663 Kaiserslautern, Germany

12 ^d Luxembourg Institute of Science and Technology (LIST), Environmental Research and
13 Innovation (ERIN) Department, 41 rue du Brill, L-4422 Belvaux, Luxembourg

14

15 * To whom correspondence should be addressed:

16 Dr. Torsten Bohn

17 e-mail: torsten.bohn@gmx.ch

18 phone: +352-621-216-637

19 fax: +352-621-470-264

20

21 Running title: Divalent minerals affect carotenoid bioaccessibility and digesta rheology

22

23 Word count: 6280

24 **Abstract**

25 Carotenoid intake and tissue levels have been frequently associated with reduced risk of
26 chronic diseases. However, their bioavailability is low and influenced by many dietary related
27 parameters. Divalent mineral cations have been suggested to interfere with carotenoid digestion
28 and to hamper micellarization, a prerequisite for their uptake, via complexation of bile salts
29 and precipitation of fatty acids. In the present investigation, we have evaluated the effects of
30 increasing concentrations of magnesium (0 – 300 mg/L), calcium (0 – 1500 mg/L), zinc (0 –
31 200 mg/L), and sodium (0 – 1500 mg/L; control monovalent cation), on carotenoid
32 bioaccessibility from frequently consumed food items rich in carotenoids (tomato juice, carrot
33 juice, apricot nectar, spinach and field salad), following simulated gastro-intestinal digestion.
34 In addition, physicochemical parameters of digesta (macroviscosity, surface tension), micelle
35 size, and zeta-potential were evaluated. All divalent minerals (DM) reduced bioaccessibility of
36 total carotenoids ($P < 0.01$), as well as of individual carotenoids. Calcium and magnesium led
37 to reductions of up to 100% at the 2 highest concentrations. Curiously, sodium increased
38 ($P < 0.01$) carotenoid bioaccessibility of most investigated matrices. The absolute value of the
39 zeta-potential decreased with increasing concentrations of DM, suggesting a decreased stability
40 of the colloidal digesta dispersion. Viscosity decreased, except for apricot nectar samples,
41 while surface tension increased with DM concentration ($P < 0.05$). Thus, at physiological
42 ranges, calcium and magnesium could negatively impact carotenoid bioavailability, while for
43 zinc, negative effects were only seen at supplemental concentrations. The potential negative
44 effects of DM on carotenoid bioavailability should be further studied *in vivo*.

45

46 **Keywords:** phytoene; phytofluene; calcium; magnesium; digestion; micellization; surface
47 tension; zeta-potential; bile salts; soap formation.

48

49 INTRODUCTION

50 Carotenoids are secondary metabolites, found across all kingdoms of life. As pigments,
51 absorbing light in the visible spectrum, carotenoids colour many fruits and vegetables in
52 orange, red and yellow^{1,2}. Structurally, they are composed of a C-40 skeleton with a partly
53 conjugated double-bond system that is the key to many of the carotenoids' characteristics. One
54 important property is the ability to absorb excess energy from reactive oxygen species and to
55 quench singlet oxygen, meaning that they can act as anti-oxidants^{1,3,4}. This anti-oxidant
56 potential coupled with anti-inflammatory properties⁵ is of particular interest from a public
57 health perspective for the prevention and delayed progression of several chronic diseases⁶,
58 including eye related degenerative diseases⁷, cardiovascular diseases⁸, diabetes⁹ and prostate
59 cancer^{10,11}. In addition, certain carotenoids, including β -carotene, α -carotene and β -
60 cryptoxanthin, are also sources for vitamin A production by the human body.

61 Nevertheless, the bioactivity of carotenoids is firstly dependent on their bioavailability, i.e. the
62 fraction of ingested carotenoids that is available for physiological functions and/or storage in
63 organs and tissues¹². The first limiting factor of carotenoid bioavailability is their release from
64 the food matrix and their inclusion in bile acid-lipid mixed micelles^{13,14}, i.e. their
65 bioaccessibility. This step is mainly affected by 1) dietary factors (type and amount of fiber
66 and lipids, form of storage of carotenoids in the matrix, among other)¹⁵⁻²⁰, and 2) host-related
67 physiological factors (enzyme and bile acid concentrations secreted, gastric and intestinal pH,
68 etc.)^{21,22}.

69 The presence of divalent minerals during gastro-intestinal digestion, particularly calcium and
70 magnesium, has been hypothesized to negatively affect the bioaccessibility of carotenoids^{23,24}.

71 Divalent mineral cations are naturally present in the human gut and are part of our diet. The
72 effects of the interactions between divalent cations and lipids^{25,26}, and divalent cations and bile
73 acids^{27,28} have already been investigated. It has been hypothesized that during digestion, these

74 cations bind free fatty acids and bile acids to form fatty acid soaps and bile salts of generally
75 low solubility, respectively. Depending on the cation concentration, this could lead to
76 precipitations, removing lipids from the system and ultimately impairing the formation of bile
77 acid-lipid mixed micelles. Previously, we have scrutinized *in vitro* employing simulated gastro-
78 intestinal digestion the effects of the presence of divalent minerals, including calcium,
79 magnesium and zinc, on the bioaccessibility of carotenoids alone and from spinach^{23,24}. The
80 concentration-dependent effect of these minerals appeared obvious, with calcium and
81 magnesium reducing significantly, at increasing concentrations, carotenoid fractional
82 bioaccessibility. However, the effect of different food matrices including solid vs. liquid form
83 and thus different complexity on carotenoid bioaccessibility has so far not been studied.
84 Furthermore, we have previously observed an association between decreased viscosity and
85 increased surface tension and carotenoid bioaccessibility²³.

86 Here, we strived to obtain additional insights in this regard from real food matrices, also with
87 respect to micellar stability, as it could be speculated that an altered dynamic of the
88 micellarization process may impinge on micelle formation. The objective of the present study
89 was thus to evaluate the impact of various concentrations of the divalent minerals calcium,
90 magnesium and zinc, present during simulated digestion, on the bioaccessibility of individual
91 carotenoids from different frequently consumed food items rich in carotenoids, and to better
92 understand the mechanism of action of divalent minerals by scrutinizing viscosity and surface
93 tension changes during digestion.

94

95 **EXPERIMENTAL**

96 **Chemicals and standards**

97 Digestive enzymes, i.e., pepsin (porcine, ≥ 250 units/mg solid, measured as trichloroacetic acid-
98 soluble products using hemoglobin as substrate) and pancreatin (porcine, 4x US Pharmacopeia

99 specifications of amylase, lipase and protease), and porcine bile extract were purchased from
100 Sigma–Aldrich (Bornem, Belgium). The solvents hexane, diethyl ether, methanol (MeOH),
101 acetonitrile (ACN), and dichloromethane (DCM) were purchased from Carl Roth GmbH + Co.
102 KG (Karlsruhe, Germany), and all solvents were from Rotisol® HPLC grade. Acetone was
103 purchased from VWR (Leuven, Belgium), and methyl tert-butyl ether (MTBE) from Sigma–
104 Aldrich (Bornem, Belgium). Potassium hydroxide (KOH), sodium hydroxide (NaOH),
105 hydrochloric acid (HCl), calcium chloride anhydrous and zinc chloride anhydrous were
106 purchased from VWR (Leuven, Belgium). Sodium bicarbonate and sodium chloride were
107 purchased from Merck (Darmstadt, Germany), while magnesium chloride anhydrous was
108 acquired at Sigma–Aldrich, and ammonium acetate from VEL® (Leuven, Belgium). Lycopene,
109 β -carotene and β -apo-8'-carotenal standards were acquired from Sigma–Aldrich; violaxanthin,
110 neoxanthin, lutein, phytoene and phytofluene standards were purchased from CaroteNature
111 GmbH (Ostermundigen, Switzerland). Unless otherwise specified, all products were of
112 analytical grade or higher. 18 M Ω water was prepared with a purification system from
113 Millipore (Brussels, Belgium) and used throughout the study.

114

115 **Food matrices and test meals**

116 Five food items were investigated: Eden Organic 100% Carrot Juice (other ingredients: lemon
117 juice); Granini Apricot Nectar from concentrate (minimum fruit content: 40%) (other
118 ingredients: sugar, concentrate from lemon juice, ascorbic acid); Rauch 100% Tomato Juice
119 from concentrate (other ingredients: 0.3% salt, citric acid); frozen spinach leaves (*Spinachia*
120 *oleracea*) from CORA supermarket (Foetz, Luxembourg); and fresh field salad (*Valerianella*
121 *locusta*) from Delhaize (Belval, Luxembourg) supermarket. Matrices were chosen based on
122 their carotenoid profile, and in order to cover the range of several of the most common
123 carotenoids present in human diet. Fruit juices were aliquoted into 50 mL amber conical

124 propylene tubes, frozen in liquid nitrogen and stored at -80°C until the day of the experiments.
125 Spinach aliquots were prepared as follows: i) spinach was left to defreeze; ii) the excess water
126 was drained and the leaves were pat dry; iii) spinach was homogenized stepwise in a Grindomix
127 GM 200 (Retsch, Aartselaar, Belgium) at 3, 4 and 5 x 1000 rpm (5 seconds each); iv) the
128 homogenized spinach was weighted and aliquoted into polypropylene sample containers,
129 flushed with argon and stored at -80°C. Aliquots of field salad were prepared in a similar
130 fashion (step iii and iv).

131 To promote the solubilization of dietary carotenoids and micelle formation during gastro-
132 intestinal digestion, we have chosen to add coffee creamer (10% fat) to the tested matrices, as
133 a natural source of triglycerides and phospholipids, which are essential for the formation of
134 mixed bile-lipid micelles. Similar dairy products have been used previously in our lab in order
135 to foster carotenoid bioaccessibility^{24,29,30}. INEX coffee creamer (10% fat) was purchased at a
136 local supermarket (Delhaize).

137

138 **Simulation of gastro-intestinal digestion and factors investigated**

139 To investigate the effect of divalent minerals (calcium, magnesium and zinc), we tested the
140 following concentrations: calcium 0, 250, 500 and 1000 mg/L; magnesium 0, 100, 200 and 300
141 mg/L; zinc 0, 12.5, 25, 50, 100 and 200 mg/L; and sodium 0, 375, 750 and 1500 mg/L, which
142 was used as a control monovalent cation. Concentrations were chosen based on the dietary
143 reference intakes (RDA (recommended dietary allowance), or AI (acceptable intake) when no
144 RDA was available) and tolerable upper intake levels (UL)³¹. To determine the concentration
145 of the mineral per volume of digesta, we assumed a total volume of 2 L of intestinal fluids
146 during gastrointestinal (GI) digestion. For the purposes of this study, we have defined as
147 physiological a range of cation concentrations up to the daily RDA/AI (dissolved in 2 L), and
148 as supplemental concentrations above the RDA/AI per 2L. Standard solutions of divalent

149 minerals were prepared with a physiological saline solution. The *in vitro* gastro-intestinal
150 digestion protocol was adapted from Biehler *et al.* (2011)²² and is described below.

151

152 **Gastric phase.** The previously prepared frozen aliquots of the food matrices were thawed, and
153 4 g of matrix were weighed into 50 mL amber Falcon tubes. To improve carotenoid
154 bioaccessibility, 2 mL of coffee creamer (10% fat) were added to each test meal^{24,29}. To
155 simulate the gastric passage, varying volumes of physiological saline and of a standard solution
156 of the investigated mineral were added to equal final volumes of the test meal. The volumes of
157 the physiological saline and of the mineral solution were determined based on the desired
158 cation concentration at the intestinal phase. Test meal was acidified by adding 2 mL of pepsin
159 (40 mg/mL in HCl 0.01 M), and the pH was adjusted for each test meal at the beginning of the
160 gastric phase to a pH of 3. The tubes were sealed with Parafilm[®], and incubated during 1h at
161 37°C, in a shaking water bath (GFL 1083 from VEL[®], Leuven, Belgium) with reciprocating
162 motion at 100 strokes per minute.

163

164 **Intestinal Phase.** At the end of the incubation period, a volume of 9 mL of solution of porcine
165 bile extract (24 mg/mL) and pancreatin (4 mg/mL) in NaHCO₃ (0.1 M) was added to the
166 simulated digestive fluids, and the pH was adjusted to 7. The final volume of the samples was
167 brought to 50 mL with physiological saline. The tubes were once again sealed with Parafilm
168 and to simulate the intestinal passage, samples were incubated for 2 h in a shaking water bath
169 (100 rpm) at 37°C. At the end of the gastro-intestinal digestion, aliquots of 12 mL were
170 transferred into 15 mL Falcon tubes and centrifuged (Thermo Scientific Heraeus Multifuge
171 X3R) at 4700g for 1 h, at 4 °C. After centrifugation, a 6 mL aliquot was collected from the
172 middle aqueous fraction with a syringe and hypodermic needle, and filtered through a 0.2 µm

173 nylon membrane filter (Acrodisc® 13mm Syringe Filters, PALL Life Sciences, Ann Harbor,
174 MI) into a new 15mL Falcon tube. Four mL were then taken for extraction of carotenoids.

175

176 **Extraction of carotenoids**

177 **Extraction from food matrices.** Aliquots of 4 g of food material were weighted into 50 mL
178 falcon tubes. Spinach and field salad were wetted with 5 mL of methanol, and 1 mL of 30%
179 aqueous KOH was added for saponification of chlorophylls. Samples were then vortexed,
180 sonicated at 37 kHz for 10 min in an ultrasonic bath (Elmasonic Ultrasonic Bath, Elma,
181 Mägenwil, Switzerland), and further incubated in the dark for 20 min (still with KOH) at room
182 temperature. Samples were then centrifuged for 5 min at 1300g at 4°C. The supernatant was
183 collected into a second 50 mL Falcon tube. The following extraction steps were similar for
184 both green leafy matrices and fruits juices. Matrices were extracted once with 9 mL
185 hexane:acetone (1:1), vortexed, sonicated for 5 min, and centrifuged (5 min, 1300g, 4°C). The
186 supernatants were collected into a second 50 mL Falcon tube, while for saponified samples the
187 supernatant was combined with the methanol phase. Extraction was repeated once with 9 mL
188 of hexane and a second time with 9 mL hexane plus 4 mL of saturated NaCl. Supernatants were
189 combined in the second tube. For the extraction of the fruit juice matrices, only half of the
190 above mentioned volumes were used. All matrices were extracted once more with 4 mL of
191 diethyl ether, vortexed, sonicated and centrifuged, and the supernatant was combined with the
192 previously collected organic phases. To promote phase separation from water residues, samples
193 were spun down at 1300g, for 1 min, at 4°C. When water was present, the organic phase was
194 transferred into a third tube, and the total volume was written down prior to collecting a 10 mL
195 aliquot for evaporation. The aliquots of the combined extracts were dried under a stream of
196 nitrogen using a TurboVapLV (Biotage, Eke, Belgium) apparatus, for 45 min at 25°C. Dried
197 extracts were re-dissolved in 5 to 7 mL of MTBE:MeOH (3:7), filtered through a 0.2 µm PVDF
198 syringe filter, and the filter was rinsed with 1 mL of MTBE:MeOH (3:7).

199

200 **Extraction from the bioaccessible fraction.** A 4 mL aliquot of the bioaccessible fraction was
201 extracted with 6 mL of hexane:acetone (2:1) – in the case of green leafy matrices an additional
202 1 mL of aqueous KOH (30%) was added for saponification - vortexed and centrifuged for 2
203 min at 4000g, at 4°C. The supernatant was transferred to a new 15 mL falcon tube. The
204 bioaccessible fraction was re-extracted once with 5 mL of hexane, and a second time with 5
205 mL of diethyl ether. The combined and homogenized organic phases were centrifuged, during
206 2 min at 4000g, to separate the organic phase from any water carried over during extraction.
207 The totality of the organic phase was transferred into a new tube, and evaporated under a stream
208 of nitrogen on a TurboVap for 45 min at 25°C. The dried carotenoid extracts were spiked with
209 an appropriate amount of internal standard (IS), β -apo-8'-carotenal, of known concentration
210 (10 μ g/mL) to obtain a final concentration of 1 μ g/mL in the sample, and re-dissolved in 500
211 μ L of MTBE:MeOH (3:7). Samples were filtered through a 0.2 μ m PVDF syringe filter (PALL
212 Life Sciences, Ann Arbor, MI, USA), into an HPLC amber vial. The filter was then rinsed with
213 100 μ L of MTBE:MeOH, to reduce possible losses of carotenoids to the filter membrane,
214 which was combined with the previous filtered sample, to a total volume of 600 μ L.

215

216 **HPLC analysis**

217 Carotenoids were separated on an Agilent 1260 Infinity Preparative HPLC instrument (Agilent
218 Technologies, De Kleetlaan Belgium) by gradient elution with (A) water:MeOH (60:40) with
219 30 mM of ammonium acetate, and (B) ACN:DCM (85:15), passing through an Accucore™
220 C30 column (2.6 μ m particle size, 100 mm length, 3 mm diameter, from Thermo Fisher
221 Scientific) at 30°C, 10 μ L injection volume. Elution gradient was as follows: 0 min, 48% B; 4
222 min, 48% B; 5 min, 52% B; 11 min, 52% B; 13 min, 75% B; 18 min, 90% B; 35 min, 90% B;
223 36min, 42% B. Carotenoids were detected with a UV/VIS photodiode array detector, and
224 identified according to their retention times and spectral data, based on the comparison to the

225 corresponding individual standard. All peaks were integrated manually at 286 nm (phytoene),
226 350 nm (phytofluene), 440 nm (neoxanthin and violaxanthin), 450 nm (lutein and α -carotene),
227 455 nm (β -carotene, β -cryptoxanthin and IS), and at 470 nm (lycopene), according to each
228 carotenoid's absorption maxima. Quantification was done using the internal standard method³².

229

230 **Surface tension and macroviscosity analysis**

231 To investigate if the addition of divalent minerals affected the physico-chemical characteristics
232 of the digesta, we measured the surface tension and macroviscosity of the aqueous micellar
233 fractions of the digesta. Macroviscosity was determined by measuring the steady state shear
234 flow of the samples in an Anton-Paar rheometer (MCR 302, WESP, Graz, Austria), using a
235 double gap concentric cylinder geometry (DG 26.7). Measurements were carried out by
236 applying an upward–downward ramp shear stress range from 0.1 to 200 s⁻¹, with a 60 s
237 maintenance shear rate step (at 200⁻¹). Shear stress (τ) – shear rate data ($\dot{\gamma}$) data were fitted
238 according to the Ostwald–de Waele model:

$$239 \quad (1) \quad \tau = K\dot{\gamma}^n$$

240 where: τ_0 = the yield stress (Pa), K = consistency coefficient (mPa * s⁻ⁿ) and n = rheological
241 behavior index (dimensionless). All measurements were performed at 25 \pm 0.03 °C. Surface
242 tension of digesta samples, pre-conditioned at 25 \pm 0.1 °C, were determined via the weight-
243 drop method as previously described³³ by Permprasert & Devahastin (2005). The air–water
244 interfacial properties of digesta were calculated as follows³⁴:

$$245 \quad (2) \quad \sigma_{digesta} = \frac{m_{digesta}}{m_{H2O}} \times \sigma_{H2O}$$

246 where σ_{H2O} = 71.99 dyn/cm is the surface tension of pure water (Pallas & Harrison, 1990).

247

248 **Micelle size and zeta potential analysis**

249 An aliquot of the aqueous micellar fraction was taken for the analysis of the micelle size and
250 zeta potential (assumed to modulate the stability of the particles in solution). Aliquots were
251 filtered beforehand, through a 0.2 μm syringe filter. The intensity-weighted mean
252 hydrodynamic radius and zeta-potential were determined by dynamic light scattering, and
253 Laser Doppler Micro-electrophoresis respectively. Measurements were done at room
254 temperature with a Zetasizer Nano Zs (Malvern Instruments, Malvern, UK).

255

256 **Statistical analysis**

257 Unless described otherwise, all values are given as mean \pm SD. Normality of distribution and
258 equality of variance of the data were tested by normality plots and box plots, respectively. If
259 required, log-transformation was conducted. All bioaccessibility data was normalised to a
260 respective control which was run for every set of analyses. Bioaccessibility itself was
261 calculated as the amount recovered in the final micellar phase compared to the original matrix
262 content. A general linear model was developed to test the effect of minerals and their
263 concentration on bioaccessibility, with concentration, matrix, type of mineral and carotenoid
264 (total carotenoids for comparison across matrices, individual carotenoids to compare within-
265 matrix effects) as fixed factors, and fractional bioaccessibility as the dependent (observed)
266 parameter. A P-value below 0.05 (2-sided) was considered statistically significant. Following
267 significant Fisher F-values, Bonferroni's post hoc-tests were conducted.

268

269 **RESULTS**

270 **Carotenoid profile of the food matrices**

271 In terms of total carotenoid content ($\mu\text{g/g}$ wet weight of matrix), frozen spinach contained the
272 highest amount, followed by tomato juice, carrot juice, field salad, and finally, apricot nectar
273 (Table I).

274 In the green leafy matrices (spinach and field salad), the xanthophylls (lutein, violaxanthin and
275 neoxanthin) were the major carotenoids, representing 94% and 88% of the determined total
276 carotenoid content, respectively, which is rather high compared to earlier studies³⁵, due to the
277 rather low β -carotene content found in the samples. In the case of the carrot and tomato juices
278 and apricot nectar, carotenes (β - and α -carotene, and lycopene) were the major carotenoids,
279 with only a very small contribution from lutein. Also, all the three latter matrices were rich in
280 the colourless carotenoids phytoene and phytofluene, which were not detected in green leafy
281 varieties. The contribution of phytofluene and phytoene to the total quantified carotenoid
282 content was 34% in carrot juice, 46% in tomato juice and 83% in apricot nectar.

283

284 **Bioaccessibility of total carotenoids across matrices**

285 The bioaccessibility of total carotenoids was significantly different ($p < 0.001$) between most
286 matrices, except for apricot nectar and tomato juice. Bioaccessibility of total carotenoids was
287 higher from the juices than it was from the green leafy varieties (Figure 1) and followed the
288 order: apricot nectar (21.4%) > tomato juice (20.1%) > carrot juice (17.1%) > field salad (9.7%)
289 > spinach (5.2%).

290 Another aspect we observed was the remarkable bioaccessibility of phytofluene and phytoene
291 of 18.5 – 46.9% and 21.1 – 47.6%, respectively (Supplementary Table I), and their large
292 contribution to average total carotenoid bioaccessibility in the juices and nectar (Fig. 1). This
293 was especially evident in the case of tomato juice, where phytofluene and phytoene together
294 represented 91.5% of the total bioaccessible fraction, while in the original matrix they made up
295 46.2% of the total carotenoid content.

296

297 **Bioaccessibility of β -carotene: effect of divalent minerals across different matrices**

298 Beta-carotene was the only carotenoid detectable in all of the 5 investigated matrices, thus it
299 served as a reference carotenoid to compare the effect of the minerals across all tested matrices.

300 Bioaccessibility of β -carotene was significantly affected ($p < 0.001$) by the divalent minerals
301 in a concentration-dependent fashion (Figure 2). Addition of magnesium led to a significant
302 decrease of its bioaccessibility in all matrices. The addition of 200 mg/L magnesium to the test
303 meals reduced the bioaccessibility of β -carotene by more than half (compared to the control),
304 while at 300 mg of magnesium, no more β -carotene was found in the micellar fraction (except
305 for spinach). Similarly, a higher concentration of calcium, i.e. 1000 mg/L, also reduced the
306 bioaccessibility of β -carotene up to 100% for all tested matrices.

307 Interestingly, the addition of sodium had an overall significant positive effect on the
308 bioaccessibility, especially in the case of β -carotene from the juices and apricot nectar, while
309 for the green leafy varieties the effect was less pronounced (field salad) or inexistent (spinach).

310 Regarding zinc, its presence significantly improved β -carotene bioaccessibility from field salad
311 and spinach for all concentrations tested, and in carrot juice for a concentration range between
312 12.5 and 50 mg/L. On the other hand, at higher concentrations (≥ 100 mg/L), the presence of
313 zinc significantly decreased β -carotene bioaccessibility from apricot nectar and tomato juice
314 (up to 43%), and up to 100% in carrot juice.

315

316 **Comparison of the bioaccessibility of carotenes, xanthophylls, and colourless** 317 **carotenoids**

318 **Differences between carotenes and xanthophylls.** The relative variations of the
319 bioaccessibility of the carotene β -carotene, as a function of varying concentrations of the
320 investigated minerals, were compared to those of the xanthophylls lutein and neoxanthin, from
321 either spinach or field salad (Figure 3, supplementary Table X for bioaccessibility values). The
322 statistical interaction “matrix * mineral” had a significant ($P < 0.001$) effect on the
323 bioaccessibility of carotenoids.

324 Carotenoids from spinach were overall less affected by the addition of minerals, than those
325 from field salad. Sodium and zinc had no statistically significant effect on the bioaccessibility
326 of xanthophylls measured in spinach test meals, while β -carotene bioaccessibility increased
327 significantly in the presence of zinc (> 12.5 mg/L, Figure 2). As for field salad, zinc improved
328 significantly ($P < 0.001$) the bioaccessibility of both lutein, neoxanthin and β -carotene, by up
329 to 39%, 31%, and 63%, respectively.

330 Addition of sodium to the field salad test meals led to a significant ($P < 0.05$) increase in lutein
331 and β -carotene bioaccessibility, by ca. 4% and 31%, respectively, at a concentration of 350
332 mg/L. At highest concentrations (750 mg/L and 1500 mg/L), bioaccessibility of xanthophylls,
333 but not that of β -carotene, was significantly reduced (up to 35%).

334 Also for magnesium, the bioaccessibility of carotenoids from spinach appeared less strongly
335 affected than from field salad. The significant effects were seen > 300 mg/L for spinach samples
336 and > 100 mg/L in field salad samples. Addition of calcium at a concentration of 250 mg/L
337 improved significantly the amount of lutein (ca. 13%) and neoxanthin (ca. 24%) recovered in
338 the micellar fraction from field salad test meals, followed by a significant ($P < 0.001$) reduction
339 (> 500 mg/L).

340

341 **Differences between lycopene and phytoene and phytofluene.** Despite their similar
342 molecular structure, the bioaccessibility of lycopene was significantly lower ($P < 0.001$) than
343 that of phytoene and phytofluene, from tomato juice samples. Under control conditions (i.e. no
344 additional mineral), the average bioaccessibility of lycopene from tomato juice was of
345 $2.9 \pm 1.8\%$, compared to $49.0 \pm 14.5\%$ and $53.6 \pm 24.4\%$ for phytoene and phytofluene,
346 respectively.

347 Concerning the response of these carotenoids to the added minerals (Figure 4), the patterns of
348 bioaccessibility variations between these three carotenoids were similar in the presence of

349 additional sodium and magnesium. Adding magnesium at >100 mg/L to the tomato juice
350 digesta decreased ($P<0.001$) bioaccessibility of all three carotenoids, and at 300 mg/L the
351 bioaccessibility dropped close to zero. Sodium, at 750 mg/L significantly ($P<0.001$) enhanced
352 the bioaccessibility of phytoene and phytofluene; at 1500 mg/L the bioaccessibility of all three
353 carotenoids significantly improved by 87% for lycopene, 118% for phytoene and 148% for
354 phytofluene. Adding 250 mg/L of calcium increased the recovery of phytoene and phytofluene
355 in the micellar fraction by 18% and 22%, while it had no significant effect on lycopene.
356 Similarly to other carotenoids, 500 mg/L calcium reduced the bioaccessibility by more than
357 50%, and at 1000 mg/L no more carotenoids were detectable in the micellar fraction. Zinc, at
358 concentrations <50 mg/L had no effect on the bioaccessibility of lycopene, phytoene and
359 phytoene, while higher concentrations reduced lycopene and phytofluene bioaccessibility
360 (Figure 4).

361

362 **Effect of divalent minerals on the physico-chemical properties of the digesta**

363 **Effect of mineral addition on the zeta-potential of the digesta.** To verify the presence of
364 mixed micelles in the micellar fraction of digesta and to evaluate the stability of the particles
365 in solution, we attempted to measure micelle size as well as the zeta potential. However, due
366 to time and cost reasons, measurements were performed only for spinach and carrot juice
367 samples. Unfortunately, micelle size results were not informative. As measurements were not
368 done *in situ* and had to be shipped, micellar fractions had to be previously frozen and thawed
369 on the day of the analysis. The temperature changes might have caused particle size variations,
370 making it impossible to accurately measure the micelle size distribution of the micellar fraction
371 of the digesta.

372 The average zeta-potential value of the micellar fraction of the spinach and carrot juice digesta,
373 under control conditions (i.e. no added mineral, $C = 0$ mg/L) was of -30.9 ± 2.4 mV and -30.6

374 ± 9.6 mV, respectively. Variations of the zeta potential of the micellar fractions, depended on
375 the type of mineral and concentration (Figure 5 and 6). While zinc and sodium did not cause
376 visible variations in the zeta-potential, a more obvious variation was seen for calcium and
377 magnesium. As the concentrations of the divalent minerals increased, the zeta-potential tended
378 towards zero (Fig 5 and Fig 6), especially in the case of calcium and magnesium, suggesting
379 that the colloid became unstable. Lowest zeta-potential values (-2.53 mV) were observed for
380 spinach samples digested with calcium (at 1000 mg/L).

381

382 **Effect of divalent minerals on the macroviscosity and surface tension of the digesta.** In
383 general, the digesta were prevalently characterised by a Newtonian fluid behaviour, with
384 digesta from tomato, spinach and field salad exerting the lowest macroviscosities (0.97-1.02
385 mPa·s). Significantly ($P < 0.01$) higher macroviscosity values were recorded in the case of carrot
386 juice (ca. 1.08 mPa·s) and apricot nectar (2.16 mPa·s). It should be noted that in the latter case,
387 the digesta exhibited a rather pseudoplastic (shear thinning) behaviour, suggesting the presence
388 of a loosely structured/thickened bulk phase. Regarding sodium addition, the digesta
389 macroviscosities exerted a slight increase (up to 4%), as a result of its buffering role to the
390 biopolymers' hydrodynamic radii. In the presence of divalent minerals, a significant decrease
391 of the digesta macroviscosity was detected. This was steeper for the high pectin content systems
392 i.e. apricot, tomato and carrot juice. Contrary to calcium containing digesta, the apricot nectar
393 – magnesium or zinc digesta exerted a distinct macroviscosity inflection point at low mineral
394 concentrations, followed by a steep decrease upon further mineral content increase. In the case
395 of leafy vegetable matrices, macroviscosity was also adversely affected by the presence of
396 divalent minerals, but to a much lesser extent.

397 As for surface tension (Fig. 7e-h), the cationic mineral species present in the intestinal chymes,
398 and to a lesser extent, the type of the food matrix, substantially impacted the air-water

399 interfacial properties. Surface tension for control digesta systems ranged from ca. 36.2 (for
400 apricot nectar and carrot juice) to 37.5 dyn/cm (for tomato, spinach and field salad) showing a
401 moderate negative ($r=-0.74$, $P<0.05$) correlation to the macroviscosity data. In the presence of
402 the divalent cationic species, surface tension increased by 5 to 30%, depending on the digested
403 food matrix, with field salad and spinach based digesta exerting the highest depletion in surface
404 active compounds. Apricot nectar and carrot juice obtained digesta experienced a rather
405 moderate increase of surface tension.

406

407 **DISCUSSION**

408 In the present study, we investigated the interaction of divalent minerals with carotenoids from
409 various food matrices during simulated gastro-intestinal digestion. Previously, we found that
410 the presence of calcium and magnesium, at varying concentrations, had the ability to
411 significantly decrease the amount of isolated carotenoids (in absence of a food matrix), present
412 in the micellar fraction of the digesta²³. The different food matrices chosen in the present study
413 represented regularly consumed fruits and vegetables, providing the most common dietary
414 carotenoids³⁵: β -carotene and α -carotene, lycopene, and lutein. Two additional regularly
415 consumed carotenoids investigated were the colourless carotenoids phytoene and phytofluene.
416 Although they are less commonly reported, they are present at relatively high concentrations
417 in a broad spectrum of fruits and vegetables, including apricot, carrot and tomato³⁵.

418 Carotenoid bioaccessibility was reduced significantly in all matrices by the highest
419 concentrations of magnesium and calcium studied, to almost zero. This is similar to what has
420 been reported earlier by Biehler et al (2011)²⁴, investigating micellarization of spinach-borne
421 carotenoids digested with calcium, magnesium and iron. However, the influence of the matrix
422 was apparent when evaluating the effects of divalent minerals on the bioaccessibility of
423 carotenoids. Responses to divalent minerals appeared to differ between green leafy matrices

424 and the juices. Increasing concentrations of calcium and magnesium generally resulted in
425 reduced bioaccessibility of carotenoids following digestion. However, at lower concentrations
426 (250 mg/L calcium, 100 mg/L magnesium), the bioaccessibility of carotenes, specifically β -
427 carotene, was enhanced in juices and nectar, though neither in spinach nor field salad.
428 Similarly, though employed as a control monovalent ion, sodium improved bioaccessibility in
429 juices and apricot nectar, especially at higher concentrations (≥ 750 mg/L), while lutein and
430 neoxanthin bioaccessibility were significantly ($P < 0.05$) reduced in field salad. It is possible
431 that the more rigid cellular matrix of solid constituents, with carotenoids not yet present in lipid
432 droplets at earlier phases of digestion, is less prone to interactions with divalent cations, but
433 this remains speculative.

434 The negative effects of the divalent cations at higher concentrations were correlated with
435 increased surface tension, suggesting the depletion of surfactants (bile salts) from the system,
436 similar as observed earlier for carotenoids in systems without food matrix²³. The fact that
437 macroviscosity in parallel decreased, towards values similar to that of pure water, also
438 suggested removal of soluble compounds from the system by flocculation, as observed also
439 visually. Depletion of carotenoids is likely to occur as a consequence of the binding of divalent
440 cations to unconjugated bile acids³⁶ and free fatty acids, forming bile salts of poor solubility
441 and insoluble fatty acids soaps^{25,37,38}, resulting in precipitation. This precipitation was visible
442 at the end of the digestion, when compared to control conditions (no minerals added). Without
443 the presence of bile acids, or free fatty acids, stimulating the formation of mixed-micelles,
444 bioaccessibility of carotenoids is expected to be drastically reduced. Similarly, sodium can also
445 interact with bile acids, resulting in higher solubility of the bile salts, and lower critical micelle
446 concentrations, promoting mixed-micelle formation²⁸ and thus bioaccessibility of carotenoids,
447 explaining the higher recovery from the aqueous micellar fraction of the digesta with added
448 sodium.

449 The observed positive effects of divalent minerals at lower concentrations on bioaccessibility
450 are more challenging to explain, but could include lipolysis. Lipolysis occurs via the adsorption
451 of pancreatic lipase to the surface of lipid droplets. As lipid digestion proceeds and the products
452 of lipolysis (e.g. free fatty acids) build up at the interface, lipase activity is inhibited. In order
453 for lipolysis to proceed, fatty acids and other molecules adsorbed to the surface of lipids (e.g.
454 proteins and soluble fibres) need to be displaced³⁹. This would occur with increasing cation
455 concentrations, precipitating the products, allowing for continuous lipase activity, aiding in the
456 transferral of lipid droplets to mixed micelles and fostering carotenoid release from lipid
457 droplets. Calcium and other divalent cations also have the ability to cross-link proteins⁴⁰ and
458 soluble fibres^{41,42}, perhaps removing some factors that could negatively affect carotenoid
459 bioaccessibility.

460 Concerning differences in carotenoid bioaccessibility between matrices, spinach contained the
461 highest amount of total carotenoids, followed by tomato juice, carrot juice, field salad, and
462 finally apricot nectar. Nevertheless, all tested matrices had a unique carotenoid profile, and
463 apart from β -carotene, no other carotenoid was commonly measured across all the five
464 matrices. The investigated spinach and field salad had similar carotenoid composition as
465 previously reported for other green leafy vegetables, containing β -carotene, lutein, violaxanthin
466 and neoxanthin⁴³, with 90% of total carotenoids constituted by xanthophylls (lutein,
467 violaxanthin and neoxanthin). In contrast, xanthophylls accounted for less than 1% of the total
468 carotenoid content determined for the juices and apricot nectar.

469 Despite the fact that spinach and field salad contained mainly xanthophylls, reported as being
470 more bioaccessible than carotenes^{23,44,45}, their average total carotenoid bioaccessibility was
471 significantly lower than that of the juices and nectar. This could have been the result of different
472 storage form of carotenoids in plant tissues⁴³ - chromoplasts (for carrot and tomato) vs.
473 chloroplast pigment-protein complexes (spinach and field salad)^{19,46,47} and other matrix

474 constituents such as dietary fiber⁴⁸. Furthermore, food processing¹³ and different methods of
475 disruption of the cell matrix also impact the release of carotenoids. Juicing of carrots has been
476 shown to improve the bioaccessibility of carotenoids from the food matrix⁴⁶ compared to raw
477 carrots, as a result of cell rupture, making carotenoids more accessible.

478 Phytoene and phytofluene were present in the tested juices and apricot nectar, where they
479 contributed at least to a third to the total carotenoid content. Despite their high contribution to
480 total carotenoid content of many different fruits and vegetables³⁵, their appearance in blood
481 plasma⁴⁹, and reported bioactive aspects such as anti-oxidant activity⁵⁰, not much information
482 is available on bioavailability aspects, including bioaccessibility. In the present investigation,
483 phytoene and phytofluene fractional bioaccessibilities were surprisingly high, between 27%
484 and 70%. Bioaccessibility nonetheless depended on the type of matrix. Carrot juice showed a
485 significantly lower bioaccessibility of these colourless carotenoids than tomato juice and
486 apricot nectar, while apricot nectar digestion resulted in significantly higher phytoene and
487 phytofluene bioaccessibility, compared to the other two matrices. These results are in line with
488 those found in a previous study⁵¹. Despite being similar to lycopene in its molecular structure,
489 very different bioaccessibilities were encountered. Meléndez-Martínez *et al.* (2015) suggested
490 that the number of conjugated double bonds (cDB) influences the shape of the molecule. Its
491 high number in lycopene (11 cDB) would prevent molecule folding, while phytoene (3 cDB)
492 and phytofluene (5 cDB) bonds are able to rotate more freely, assuming less rigid shapes. This
493 could facilitate the incorporation of the colourless carotenoids into mixed micelles, explaining
494 their higher bioaccessibility. Similarly, another study reported higher bioaccessibility of
495 phytoene, from different food matrices, compared to β -carotene and even lutein, including in
496 carrots and tomatoes¹⁹. Contrarily, phytoene bioaccessibility was comparable to that of β -
497 carotene from orange pulp and juice, perhaps due to the low amount of β -carotene present in
498 those matrices, facilitating its solubility⁵².

499 Regarding physicochemical properties, the higher macroviscosity of the apricot juice was
500 likely due to the high content of pectin and/or due to higher total solids (>14% w/w) and sugar
501 content (>13% w/w), promoting viscosity via decreasing the hydrodynamic free volume
502 between the biopolymer molecules, triggering hydrophobic interaction or hydrogen bonding of
503 side chain segments. In case of leafy vegetable matrices, their very low viscosimetric
504 responsiveness is likely to be attributed to their higher content in insoluble fibre, compared to
505 juices⁵³. Regardless of the food matrix, it is assumed that the observed macroviscosity
506 reduction with increasing divalent mineral concentration was mainly induced by the
507 precipitation of bile salt matter, depleting total solids. The rheological behaviour of the digesta
508 were diverse, with apricot nectar – magnesium or zinc binary systems undergoing structure
509 conformational changes as suggested by the observed rheological behaviour transitions, e.g.
510 pseudoplastic to Newtonian. The inflection point for apricot digesta containing magnesium or
511 zinc is also likely explained by its pectin content. It is well established that pectins (depending
512 on their methyl ester content) may undergo electrostatic interactions (via the egg-box structure
513 conformation) with divalent cations, exerting a selective chemical affinity (for
514 polygalacturonic segments) following the order: calcium > zinc > magnesium⁵⁴. This implies
515 that electrostatic bridging of calcium with pectins can be triggered at much lower
516 concentrations compared to the other two cations. This would explain the absence of the
517 inflection point in the case of apricot nectar – calcium digesta samples. Upon increasing the
518 cation concentration, an antagonistic ion binding action between bile salts and polygalacturonic
519 segment occurs, with the latter being disfavoured, inducing a strong precipitation. As for
520 surface tension, it is hypothesised that the bile salt binding capacity antagonism between
521 divalent cations and pectins played a buffering role on aggregative depletion phenomena. The
522 amount of soluble pectin in the bulk phase appeared to be well corroborating the average

523 depletion of surfactants i.e. 9% (magnesium) to 16% (calcium) for carrot juice and apricot
524 nectar and 20% (magnesium) to 26% (calcium) for tomato juice, spinach and field salad.

525

526 **CONCLUSIONS**

527 Bioaccessibility of different carotenoids, from different matrices, was affected negatively by
528 divalent mineral cations, and this was mirrored by decreased macroviscosity, increased surface
529 tension of the digesta, as well as by the decrease of the absolute zeta-potential of the mixed
530 micelles. Interestingly, the colourless carotenoids phytoene and phytofluene, displayed a
531 bioaccessibility higher than those obtained for other carotenes. However, the outcomes of the
532 influence of divalent cations on bioaccessibility appear as a result of a complex interaction
533 between matrix, type of carotenoids and minerals and their concentration. Interestingly,
534 concentrations of divalent minerals, much below the equivalent to the RDA for calcium and
535 magnesium, appeared to improve bioaccessibility of carotenoids, likely by acting
536 synergistically with bile acids, possibly promoting lipolysis. The results presented could be
537 relevant regarding the intake and dosage of food supplements, specifically when combining
538 carotenoids with high divalent mineral doses. Further investigations in vivo, possibly also
539 including other liposoluble dietary constituents are warranted to attest these observations.

540

541 **ACKNOWLEDGEMENTS**

542 The authors are grateful for the support of the Fonds National de la Recherche Luxembourg
543 (Grant No. C11/SR/1268260). The help of Boris Untereiner with extractions and digestions,
544 and of Cedric Guignard and Emmanuelle Coco for their help and advice regarding HPLC
545 analyses, is greatly appreciated.

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547 **REFERENCES**

- 548 1 C. Stange and C. Flores, in *Advances in Photosynthesis - Fundamental Aspects*, ed. M. Najafpour, InTech, 2010, pp.
549 77–96.
- 550 2 P. D. Fraser and P. M. Bramley, *Prog. Lipid Res.*, 2004, **43**, 228–65.
- 551 3 B. Demmig-Adams, A. M. Gilmore and W. W. Adams III, *FASEB J.*, 1996, **10**, 403–412.
- 552 4 G. Britton, *FASEB J.*, 1995, **9**, 1551–1558.
- 553 5 A. Kaulmann and T. Bohn, *Nutr. Res.*, 2014, **34**, 907–29.
- 554 6 N. I. Krinsky and E. J. Johnson, *Mol. Aspects Med.*, 2005, **26**, 459–516.
- 555 7 N. K. Sripsema, D. Hu and R. B. Rosen, *J. Ophthalmol.*, 2015, **2015**, 1–13.
- 556 8 S. Voutilainen, T. Nurmi, J. Mursu and T. H. Rissanen, *Am. J. Clin. Nutr.*, 2006, **83**, 1265–71.
- 557 9 L. Brazionis, K. Rowley, C. Itsiopoulos and K. O’Dea, *Br. J. Nutr.*, 2009, **101**, 270–7.
- 558 10 O. Kucuk, F. H. Sarkar, Z. Djuric, W. Sakr, M. N. Pollak, F. Khachik, M. Banerjee, J. S. Bertram and D. P. Wood,
559 *Exp. Biol. Med. (Maywood)*, 2002, **227**, 881–5.
- 560 11 G. a Sonn, W. Aronson and M. S. Litwin, *Prostate Cancer Prostatic Dis.*, 2005, **8**, 304–10.
- 561 12 J. J. Castenmiller and C. E. West, *Annu. Rev. Nutr.*, 1998, **18**, 19–38.
- 562 13 K. van het Hof and C. West, *J. Nutr.*, 2000, **103**, 503–506.
- 563 14 W. Stahl, H. Van Den Berg, J. Arthur, A. Bast, J. Dainty, R. M. Faulks, C. Gärtner, G. Haenen, P. Hollman, B.
564 Holst, F. J. Kelly, M. Cristina Polidori, C. Rice-Evans, S. Southon, T. Van Vliet, J. Viña-Ribes, G. Williamson and
565 S. B. Astley, *Mol. Aspects Med.*, 2002, **23**, 39–100.
- 566 15 O. O’Connell, L. Ryan, L. O’Sullivan, S. A. Aherne-Bruce and N. M. O’Brien, *Int. J. Vitam. Nutr. Res.*, 2008, **78**,
567 238–246.
- 568 16 H. Palafox-Carlos, J. F. Ayala-Zavala and G. a. González-Aguilar, *J. Food Sci.*, 2011, **76**, 6–15.
- 569 17 S. R. Goltz, W. W. Campbell, C. Chitchumroonchokchai, M. L. Failla and M. G. Ferruzzi, *Mol. Nutr. Food Res.*,
570 2012, **56**, 866–77.
- 571 18 T. Huo, M. G. Ferruzzi, S. J. Schwartz and M. L. Failla, *J. Agric. Food Chem.*, 2007, **55**, 8950–7.
- 572 19 J. L. Jeffery, N. D. Turner and S. R. King, *J. Sci. Food Agric.*, 2012, **92**, 2603–10.
- 573 20 J. Jeffery, A. Holzenburg and S. King, *J. Sci. Food Agric.*, 2012, **92**, 2594–602.
- 574 21 V. Tyssandier, B. Lyan and P. Borel, *Biochim. Biophys. Acta*, 2001, **1533**, 285–92.
- 575 22 E. Biehler, A. Kaulmann, L. Hoffmann, E. Krause and T. Bohn, *Food Chem.*, 2011, **125**, 1328–1334.
- 576 23 J. Corte-Real, M. Iddir, C. Soukoulis, E. Richling, L. Hoffmann and T. Bohn, *Food Chem.*, 2016, **197**, 546–553.
- 577 24 E. Biehler, L. Hoffmann, E. Krause and T. Bohn, *J. Nutr.*, 2011, **17**, 1769–1776.
- 578 25 B. Tadayyon and L. Lutwak, *J. Nutr.*, 1969, **248**, 246–254.
- 579 26 G. Gacs and D. Barltrop, *Gut*, 1977, **18**, 64–8.

580 27 E. Baruch, D. Lichtenberg, P. Barak and S. Nir, *Chem. Phys. Lipids*, 1991, **57**, 17–27.

581 28 C. a Jones, A. F. Hofmann, K. J. Mysels and A. Roda, *J. Colloid Interface Sci.*, 1986, **114**, 452–470.

582 29 A. Kaulmann, C. M. André, Y. Schneider, L. Hoffmann and T. Bohn, *Food Chem.*, 2016, **197**, 325–332.

583 30 J. Corte-Real, E. Richling, L. Hoffmann and T. Bohn, *Nutr. Res.*, 2014, **34**, 1101–10.

584 31 Food and Nutrition Board, *Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate*

585 *Intakes , Vitamins Food and Nutrition Board , Institute of Medicine , National Academies*, 2011.

586 32 K. Rome and A. Mcintyre, *Chromatogr. Today*, 2012, 52–56.

587 33 J. Permprasert and S. Devahastin, *J. Food Eng.*, 2005, **70**, 219–226.

588 34 N. R. Pallas and Y. Harrison, *Colloids and Surfaces*, 1990, 43, 169–94.

589 35 E. Biehler, A. Alkerwi, L. Hoffmann, E. Krause, M. Guillaume, M.-L. Lair and T. Bohn, *J. Food Compos. Anal.*,

590 2012, **25**, 56–65.

591 36 A. Hofmann and K. Mysels, *J. Lipid Res.*, 1992, **33**, 617–626.

592 37 G. V Appleton, R. W. Owen, E. E. Wheeler, D. N. Challacombe and R. C. Williamson, *Gut*, 1991, **32**, 1374–1377.

593 38 J. K. Lorenzen, S. Nielsen, J. J. Holst, I. Tetens, J. F. Rehfeld and A. Astrup, *Am. J. Clin. Nutr.*, 2007, **85**, 678–87.

594 39 P. J. Wilde and B. S. Chu, *Adv. Colloid Interface Sci.*, 2011, **165**, 14–22.

595 40 P. Etcheverry, M. a Grusak and L. E. Fleige, *Front. Physiol.*, 2012, **3**, 317.

596 41 G. T. Grant, E. R. Morris, D. A. Rees, P. J. C. Smith and D. Thom, *FEBS Lett.*, 1973, **32**, 195–198.

597 42 S. J. J. Debon and R. F. Tester, *Food Chem.*, 2001, **73**, 401–410.

598 43 G. Britton, S. Liaaen-Jensen and H. Pfander, Eds., *Carotenoids Volume 5: Nutrition and Health*, Birkhäuser Verlag,

599 Basel, Switzerland, 2009.

600 44 R. M. Schweiggert, D. Mezger, F. Schimpf, C. B. Steingass and R. Carle, *Food Chem.*, 2012, **135**, 2736–42.

601 45 C. Sy, B. Gleize, O. Dangles, J.-F. Landrier, C. C. Veyrat and P. Borel, *Mol. Nutr. Food Res.*, 2012, **56**, 1385–97.

602 46 G. T. Rich, R. M. Faulks, M. S. J. Wickham and A. Fillery-Travis, *Lipids*, 2003, **38**, 947–56.

603 47 N. Ljubescic, M. Wrischer and Z. Devide, *Int. J. Dev. Biol.*, 1991, **35**, 251–258.

604 48 T. Bohn, *Curr. Nutr. Food Sci.*, 2008, **4**, 240–258.

605 49 A. J. Meléndez-Martínez, P. Mapelli-Brahm, A. Benítez-González and C. M. Stinco, *Arch. Biochem. Biophys.*,

606 2015.

607 50 N. Engelmann, S. K. Clinton and J. W. Erdman Jr, *Adv. Nutr.*, 2011, **2**, 51–61.

608 51 P. Mappeli-Brahm, J. Corte-Real, A. J. Meléndez-Martínez and T. Bohn, *Under Revis.*, 2017.

609 52 M. J. Rodrigo, A. Cilla, R. Barbera and L. Zacarias, *Food Funct.*, 2015, **6**, 1950–1959.

610 53 S. W. Souci, W. Fachmann and H. Kraut, *Food Composition and Nutrition Tables*, Medpharm Scientific Publishers,

611 Stuttgart, 6th edn., 2000.

612 54 D. C. Bassett, A. G. Håti, T. B. Melø, B. T. Stokke and P. Sikorski, *J. Mater. Chem. B*, 2016, **4**, 6175–6182.

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614 Table I – Carotenoid content ($\mu\text{g/g}$ food wet weight) for each of the tested food matrices.

Food matrix	Latin name	α -Car	β -Car	Lyc	Lut	Vio	Neo	PTE	PTF	Total
Apricot nectar	<i>Prunus armeniaca</i>	nd	2.87	nd	nd	nd	nd	11.27	2.79	16.87
Tomato juice	<i>Solanum lycopersicum</i>	nd	2.98	85.04	0.31	nd	nd	65.09	8.20	158.6
Carrot juice	<i>Daucus carota</i>	20.52	66.67	nd	1.24	nd	nd	40.44	4.33	133.2
Frozen spinach	<i>Spinacia oleracea</i>	nd	10.54	nd	100.1	64.80	16.26	nd	nd	191.7
Field Salad	<i>Valerianella locusta</i>	nd	14.79	nd	69.80	30.92	15.06	nd	nd	130.6
Coffee creamer (10% fat)		nd	21.55	nd	nd	nd	nd	nd	nd	21.55

α -Car: α -Carotene; β -Car: β -Carotene; Lyc: Lycopene; Lut: Lutein; Vio: Violaxanthin; Neo: Neoxanthin; PTE: Phytoene; PTF: Phytofluene, nd: not detectable. All forms represent the more abundant carotenoids isomers (i.e. all-trans). Values are means of duplicate analysis.

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638 **FIGURE HEADINGS**

639 **Figure 1.** Average total carotenoid bioaccessibility (%) between matrices. Each bar represents
640 the relative contribution of the individual carotenoids, detected in each matrix, to the
641 percentage of total carotenoids recovered in the bioaccessible fraction of the digesta, relative
642 to the total carotenoid amount present in the undigested test meal. Different letters represent
643 statistically significant differences ($P < 0.001$). Total number of replicates was 16.

644 **Figure 2.** Effect of divalent cations and sodium on the bioaccessibility of β -carotene across
645 different food matrices. Food matrices were digested in the presence of varying concentrations
646 (mg/L) of 3 different divalent cations (calcium, zinc and magnesium) and of the monovalent
647 cation (sodium). Bioaccessibility values are represented as the percentage of β -carotene
648 recovered from the aqueous micellar fraction at the end of the *in vitro* GI digestion, compared
649 to the amount of β -carotene present in the original matrix. Values represent mean \pm SD of $n =$
650 4. Bars signalled with either * ($P < 0.05$) or ** ($P < 0.01$) were statistically different from the
651 control condition, i.e. [added mineral] = 0 mg/L.

652 **Figure 3.** Graphical representation of trends in bioaccessibility variation of carotenoids from
653 spinach and field salad, as effected by cationic minerals at different concentration ranges. Bars
654 in dark and light grey represent the highest and lowest points, respectively, of the measured
655 bioaccessibility. Concentration range is depicted as C0 to C5, corresponding to the following:
656 Calcium C0 = 0mg/L; C1 = 250mg/L; C2 = 500 mg/L; C3 = 1000 mg/L. Sodium C0 = 0
657 mg/L; C1 = 375 mg/L; C2 = 750 mg/L; C3 = 1500 mg/L. Magnesium C0 = 0 mg/L; C1 = 100
658 mg/L; C2 = 200 mg/L; C3 = 300 mg/L. Zinc C0 = 0 mg/L; C1 = 12.5 mg/L; C2 = 25 mg/L; C3
659 = 50 mg/L; C4 = 100 mg/L; C5 = 200 mg/L. Symbol * above bar indicates statistical
660 significance ($P < 0.05$), compared to controls (no mineral added).

661 **Figure 4.** Graphical representation of the trends in bioaccessibility variation of lycopene and
662 the colourless carotenoids, phytoene and phytofluene, from tomato juice, as an effect of cations
663 at different concentration ranges. Bars in dark and light grey represent the highest and lowest
664 points, respectively, of the measured bioaccessibility. Concentration range is depicted as C0 to
665 C5, which correspond to the following values: Calcium C0 = 0mg/L; C1 = 250mg/L; C2 =
666 500 mg/L; C3 = 1000 mg/L. Sodium C0 = 0 mg/L; C1 = 375 mg/L; C2 = 750 mg/L; C3 =
667 1500 mg/L. Magnesium C0 = 0 mg/L; C1 = 100 mg/L; C2 = 200 mg/L; C3 = 300 mg/L. Zinc
668 C0 = 0 mg/L; C1 = 12.5 mg/L; C2 = 25 mg/L; C3 = 50 mg/L; C4 = 100 mg/L; C5 = 200 mg/L.
669 Symbol * above bar indicates statistical significance ($P < 0.05$), compared to control (no mineral
670 added).

671 **Figure 5.** Average spinach-borne β -carotene bioaccessibility (%), and absolute zeta-potential
672 (mV) of spinach aqueous micellar fraction, after digestion with different cations at varying
673 concentrations. Bars represent bioaccessibility, while markers \blacklozenge represent the zeta potential.
674 A: Calcium; B: Sodium; C: Magnesium; D: Zinc. Error bars represent standard deviation of n
675 = 4 replicates.

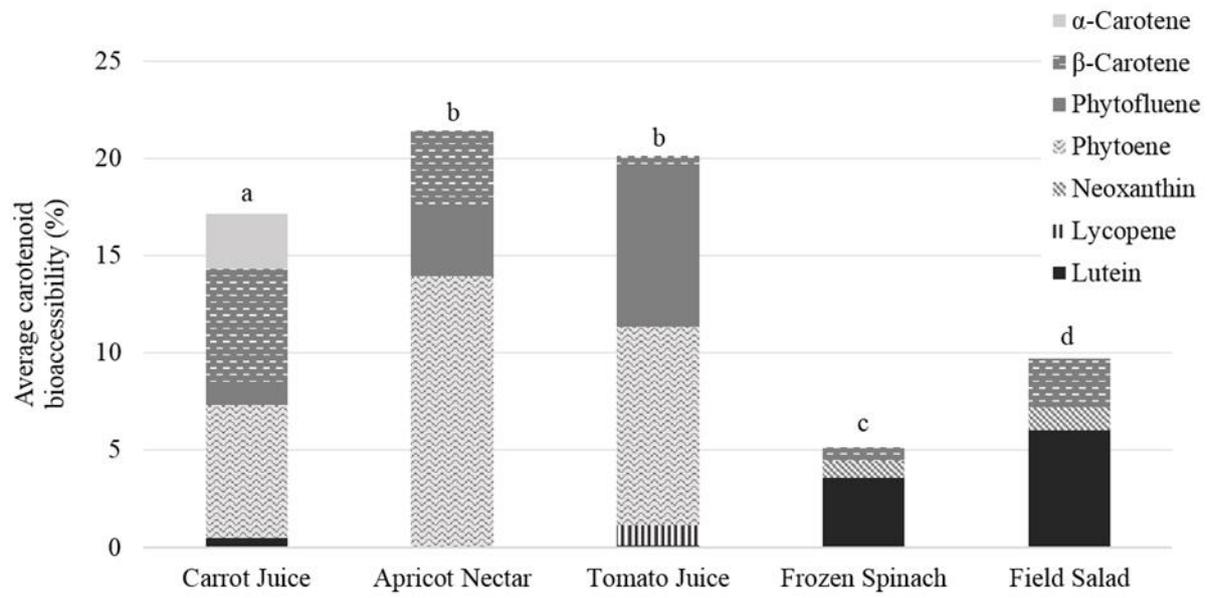
676 **Figure 6.** Average carrot juice-borne β -carotene bioaccessibility (%), and absolute zeta-
677 potential (mV) of carrot juice aqueous micellar fraction, after digestion with different cations
678 at varying concentrations. Bars represent bioaccessibility, while markers \blacklozenge represent the zeta-
679 potential. A: Calcium; B: Sodium; C: Magnesium; D: Zinc. Error bars represent standard
680 deviation of n = 4 replicates.

681 **Figure 7.** Normalised macroviscosity and surface tension values of the aqueous micellar
682 fractions of different food matrices after digestion with varying cationic mineral
683 concentrations.

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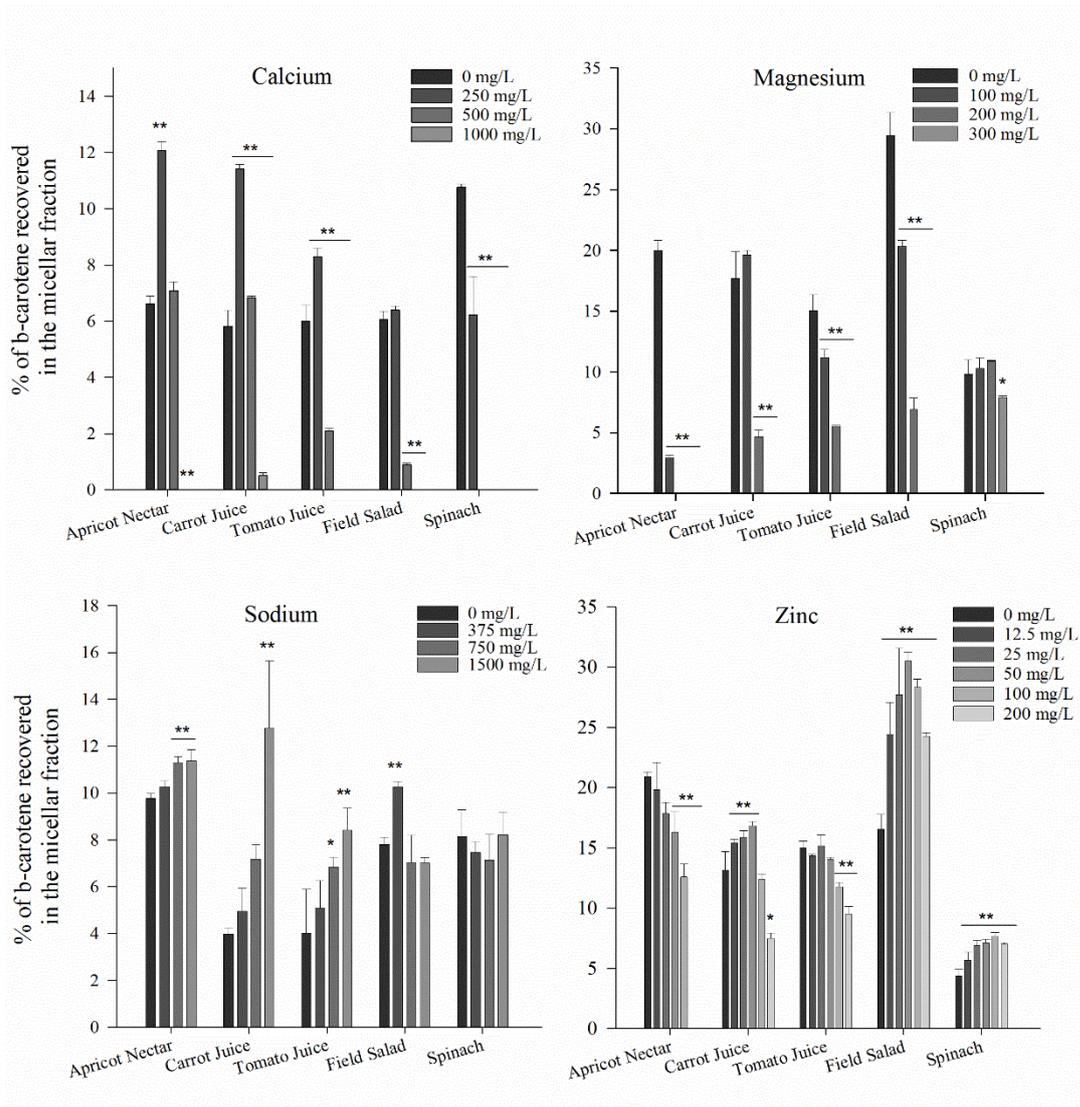


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689 Figure 1

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693 Figure 2

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Calcium C0 C1 C2 C3

Field Salad



Spinach

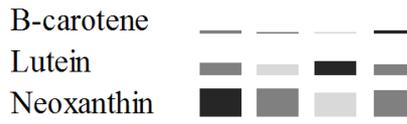


Sodium C0 C1 C2 C3

Field Salad



Spinach



Magnesium C0 C1 C2 C3

Field Salad



Spinach



Zinc C0 C1 C2 C3 C4 C5

Field Salad



Spinach



695

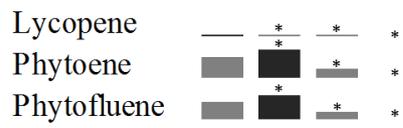
696

697 Figure 3

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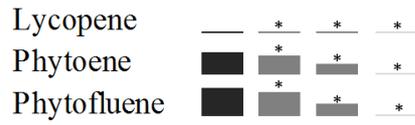
Calcium C0 C1 C2 C3

Tomato Juice



Magnesium C0 C1 C2 C3

Tomato Juice



Sodium C0 C1 C2 C3

Tomato Juice



Zinc C0 C1 C2 C3 C4 C5

Tomato Juice

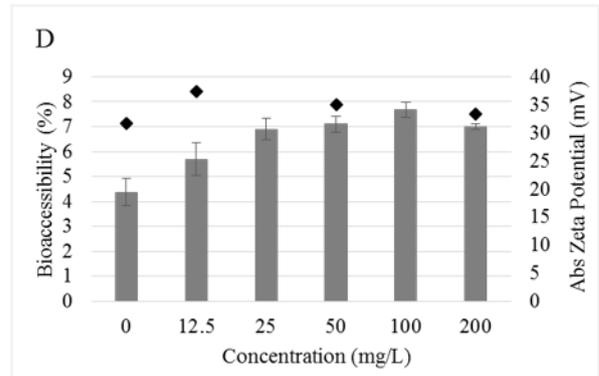
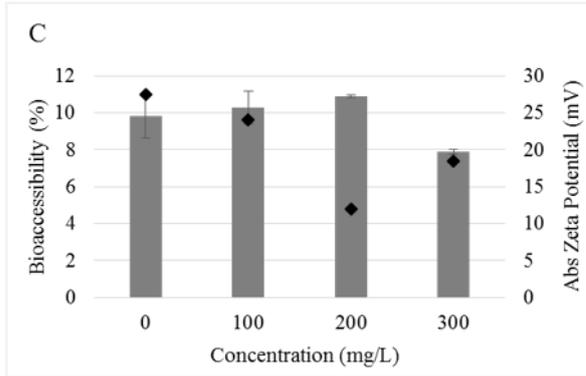
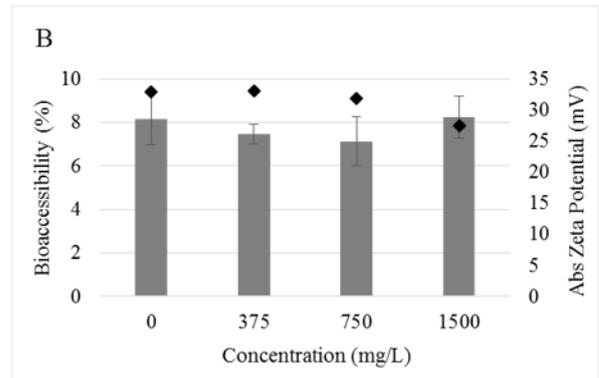
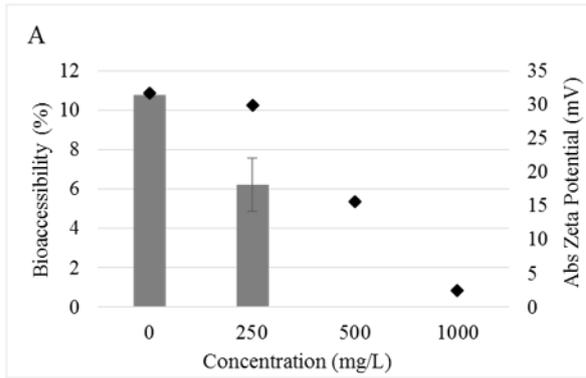


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701 Figure 4

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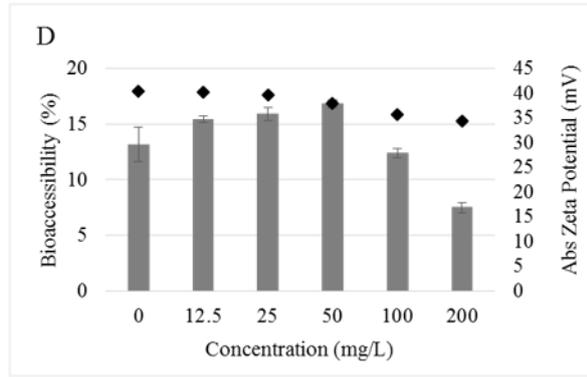
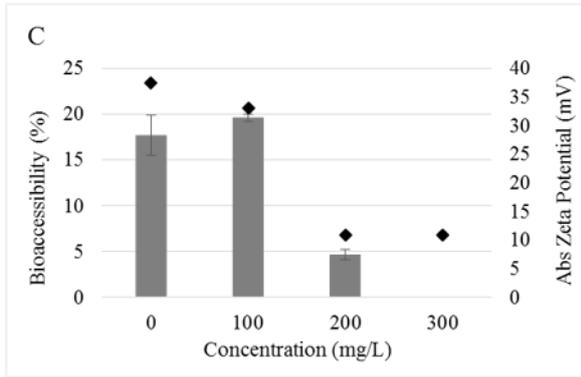
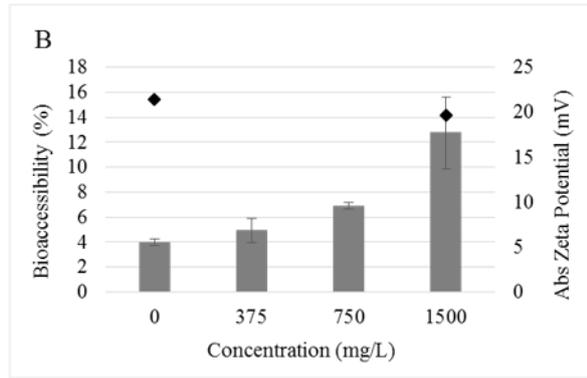
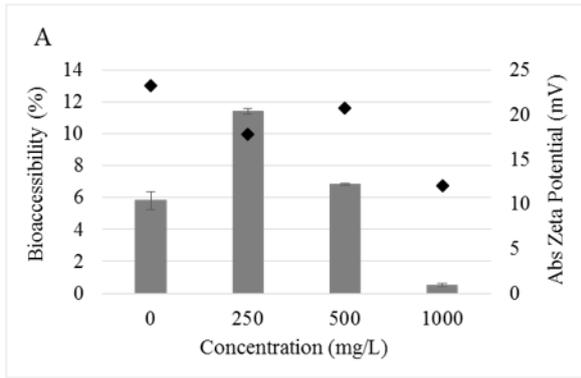


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705 Figure 5

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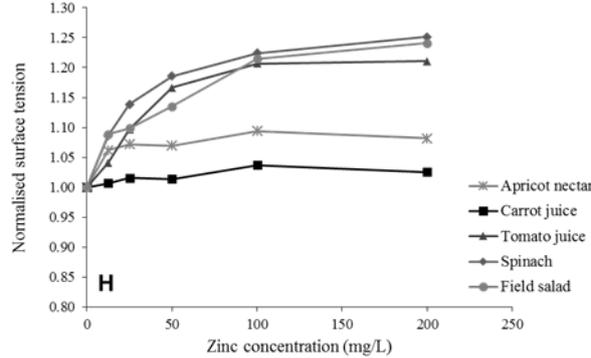
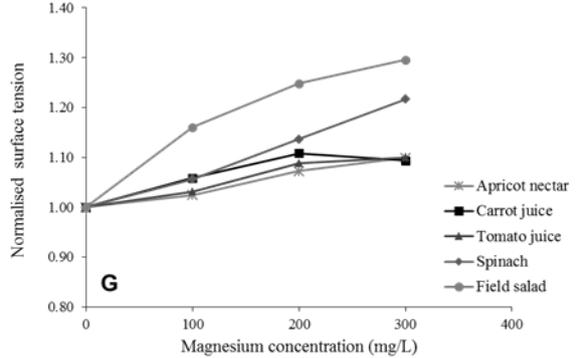
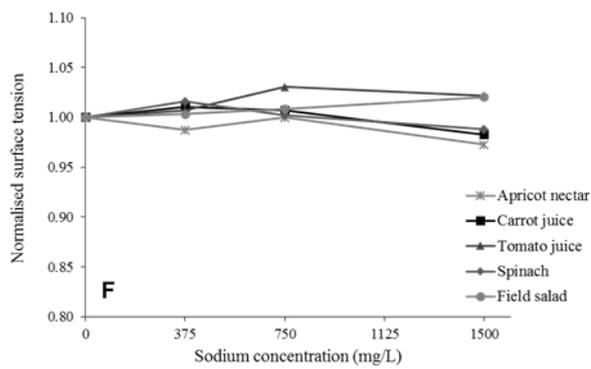
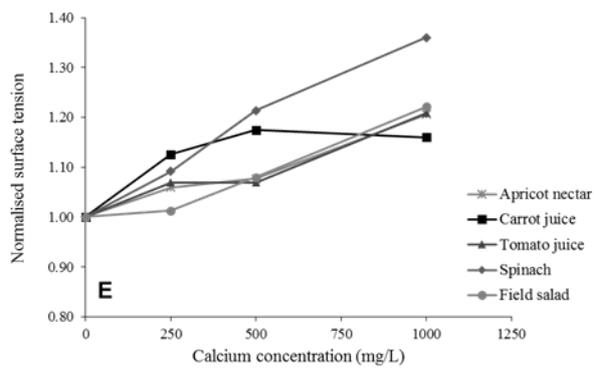
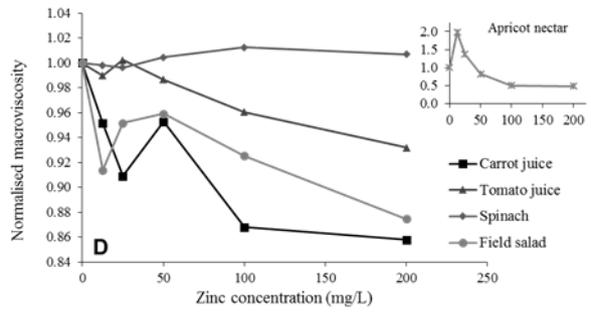
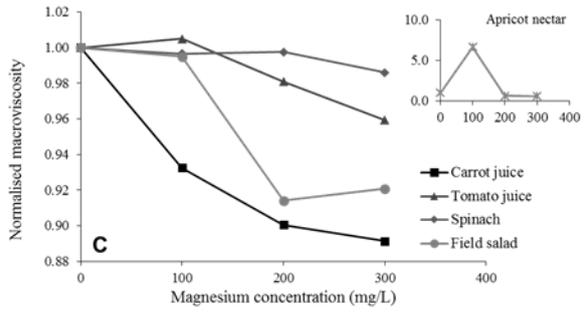
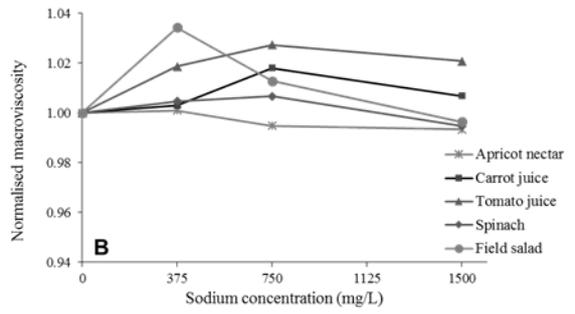
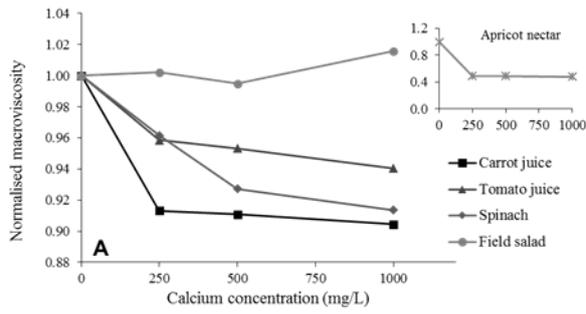


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709 Figure 6

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713 Figure 7