Influence of high pressure homogenization and pasteurization on the *in vitro* bioaccessibility of carotenoids and flavonoids in orange juice

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Abstract

Production of high-quality healthy foods through sustainable methodologies is an urgent necessity. High pressure homogenization (HPH) is an interesting alternative to obtain premium citrus juices, but its effects on bioactive compounds are unclear. There was studied the influence of HPH (150 MPa) and pasteurization (92ºC for 30 s and 85ºC for 15 s) processing on physicochemical properties and *in vitro* bioaccessibility of carotenoids and flavonoids in orange juices. Regarding fresh juice, physicochemical properties of samples remained unchanged although cloudiness was improved by homogenization. Pasteurization did not affect total carotenoids content and retinol activity equivalents (RAE) of juices whereas homogenization yielded a significant reduction (1.37 and 1.35-fold, respectively). Interestingly, particle size reduction from homogenization drastically enhanced (about 5-fold) bioaccessibility of carotenoids including hardly bioaccessible epoxycarotenoids, finding unaltered rates in pasteurized samples. Bioaccessibility of flavonoids was constant in all cases.

Results can promote HPH as an efficient option to obtain health-enhanced foods.

Keywords: Citrus juices; carotenoids; health-promoting foods; high pressure homogenization (HPH); sustainable processing; phytonutrients bioaccessibility.

Chemical compounds

-Z-Violaxanthin (PubChem CID: 6442428)

-Luteolin (PubChem CID: 5280445)

-Zeaxanthin (PubChem CID: 5280899)

-Zeinoxanthin (PubChem CID: 5281234)

42 -β-Cryptoxanthin (PubChem CID: 5281235)

43 -α-Carotene (PubChem CID: 4369188)

44 -β-Carotene (PubChem CID: 5280489)

45 -Phytoene (PubChem CID: 5280784)

46 -Phytofluene (PubChem CID: 6436722)

47 -Narirutin (PubChem CID: 442431)

48 # 1. Introduction

49 Orange juice is the most consumed fruit juice worldwide greatly appreciated by
50 consumers as a delicious natural source of vitamin C, fibre and nutritionally relevant
51 phytochemicals (mainly carotenoids and flavonoids). Epidemiological studies reported
52 that a diet rich in fruit phytochemicals prevents apparition of degenerative diseases,
53 finding that in the particular case of citrus carotenoids and flavonoids such benefits
54 include the protection against oxidizing agents, inflammation and modulation of gene
55 expression (Meléndez-Martínez, 2019; Tripoli, Guardia, Giammanco, Majo, &
56 Giammanco, 2007). Health effects of such phytochemicals depend on the amount
57 consumed and their bioavailability. Intrinsic and extrinsic factors such as the
58 metabolite profile of citrus varieties, human metabolism and food/human genetics,
59 interactions within and between food matrices and industrial processing greatly affect
60 bioaccessibility of these bioactive compounds (Rodríguez-Concepcion et al., 2018). In
61 this line, bioaccessibility is considered a measure of potential bioavailability and can
62 be defined as the amount of a compound released from the food matrix during
63 digestion and potentially available for absorption (Rodríguez-Concepcion et al., 2018).
64 Interest of *in vitro* digestion methods to assess the bioaccessibility of food
65 phytochemicals is undeniable since they can facilitate the understanding of complex
66 interactions naturally occurring under *in vivo* conditions, facilitating the design of
67 commercial products with improved nutritional properties (Etcheverry, Grusak, &
68 Fleige, 2012).

69 Nowadays, consumers demand minimally altered and more natural products hence the
70 interest of the industry to implement innovative non-thermal stabilization technologies
71 such as dynamic high pressure homogenization (HPH) (Martínez-Monteagudo, Yan,
72 & Balasubramaniam, 2017). Similarly, there is an increasing interest to implement

73 innovative and environmentally friendly technologies for the sustainable production of
74 foods alternatively to traditional stabilization processes mainly ruled by thermal
75 treatments (Pereira & Vicente, 2010). Compared to heat processing, a significant
76 reduction of energy (about 15%) was stated for HPH preservation of milk (Valsasina
77 et al., 2017). Moreover, homogenization was also proposed to valorise biomass from
78 different sources such as milk whey and pistachio shell, among others (Marciniak,
79 Suwal, Britten, Pouliot, & Doyen, 2018; Özbek, Fockink, Yanık, Göğüş, & Lukasik,
80 2018).
81

82 The effect of processing on bioaccessibility of carotenoids and flavonoids from
83 foodstuffs seems to be intimately associated to the severity of treatments applied and
84 the food matrix composition (Mapelli-Brahm, Stinco, Rodrigo, Zacarías, & Meléndez-
85 Martínez, 2018; Stinco, Fernández-Vázquez, Escudero-Gilete, Heredia, Meléndez-
86 Martínez, & Vicario, 2012). Regarding the influence exerted by minimally processing
87 HPH treatment on *in vitro* bioaccessibility of carotenoids from foodstuffs, studies were
88 mainly addressed to tomato and tomato-based products with dissenting results
89 (Knockaert et al., 2012; Svelander et al., 2011). Disparate results can be understood by
90 factors outside those induced by the matrix disruption caused by HPH such as the
91 carotenoid type (Panozzo et al., 2013), rheological properties modified by processing
92 (Leite, Augusto, & Cristianini, 2014), the chromoplast substructures and cell wall
93 barriers (Palmero, Panozzo, Colle, Chigwedere, Hendrickx, & Van Loey, 2016). This
94 issue was assessed in some beverages made from the combination of fruit juices with
95 water, milk and soymilk suggesting how *in vitro* bioaccessibility of carotenoids was
96 generally increased by HPH and High Intensity Pulsed Electric fields (HIPEF)
97 compared to traditional thermal treatment (Rodríguez-Roque et al., 2016). To the best

98 of our knowledge, no studies were carried out to evaluate the effect of HPH processing
99 on *in vitro* bioaccessibility of carotenoids from orange juice.
100 Considering phenolics from orange juice, HPH processing does not seem to affect
101 their *in vitro* bioaccessibility (He et al., 2016). Moreover, human studies suggested
102 that *in vivo* bioavailability of orange flavanones was mainly ruled by individual factors
103 rather than technological treatments (Tomás-Navarro et al., 2014).
104 Main objective of this study was to assess the effect of HPH and a traditional
105 pasteurization on the *in vitro* bioaccessibility of carotenoids and flavonoids of Lane
106 Late orange juices. Furthermore, influence of treatments on physicochemical
107 properties of juices was also studied. Results achieved can promote the
108 implementation of more sustainable methodologies by food industry to produce high-
109 quality health-promoting products to cater for the new trends in the demands of
110 consumers.
111

112 ## 2. Experimental

113 ### 2.1. Materials

114 Analytical-grade dichloromethane, acetone and methanol were from Carlo Erba
115 (CARLO ERBA Reagents S.r.l. Milan, Italy). HPLC-grade acetonitrile (ACN) and
116 formic acid (FA) were from Scharlab (Scharlab S.L., Barcelona, Spain). Methanol
117 (MeOH) and methyl *tert*-butyl ether (MTBE) of HPLC-grade were from Merck
118 (Merck KGaA, Darmstadt, Germany). Ultra-pure water was produced by a
119 NANOpureDiamond system (Barnsted Inc.). Mineral salts (KCl, NaCl), sodium
120 bicarbonate, monopotassium-phosphate, magnesium chloride hexahydrate, chlorhydric
121 acid, ascorbic acid (L-AAH, 99% purity grade), ammonium formate, pepsin (porcine
122 gastric mucosa), pancreatin (porcine pancreas), bile salt, α-carotene, β-carotene, β-

123 cryptoxanthin, lutein and zeaxanthin were from Sigma-Aldrich (Merck KGaA,
124 Darmstadt, Germany). Phytoene and Phytofluene standards were extracted from
125 tomato fruits and purified following the procedure of Mapelli-Brahm, Corte-Real,
126 Meléndez-Martínez and Bohn (2017).

127

128 **2.2. Preparation of orange juice assayed**

129 Treatments assayed were decided on the basis of results from previous studies
130 addressing the obtaining of high quality orange juices with extended shelf life through
131 HPH (Carbonell, Navarro, Izquierdo, & Sentandreu, 2013) and pasteurization
132 (Sentandreu, Carbonell, Carbonell, & Izquierdo, 2005) processing.

133 Lane Late (Citrus sinensis L. Osb.) orange fruits were harvested in Lliria (Valencia,
134 Spain), collected in May 2017, washed with tap water, drained, sized and finally
135 squeezed in an Exzel industrial extractor from Luzzysa (Luzzysa, El Puig, Valencia,
136 Spain). A paddle finisher (model EPF 06, Luzzysa, El Puig, Valencia, Spain) with
137 holes of 0.4 mm Ø sieved raw juice to obtain 600 L of freshly prepared juice (FJ) that
138 was divided into four different batches (**Fig. S1)**. One batch was homogenized at 150
139 MPa in a continuous high pressure system (NS3015H model, GEA Niro Soavi S.p.A.,
140 Parma, Italy) with a digital thermometer (probe PT-100, range -10/110 ºC) installed in
141 the outlet section to monitor the temperatures reached by the samples. Tap water was
142 used to warm up the HPH system for 30 min operating at 150 MPa at a flow rate of
143 100 L/h. Tempered juice substituted water for 5 min to reach the stationary conditions
144 of 15 s of residence time and 68 ºC at the outlet of the HPH device. Then, the juice
145 was quickly cooled at 7–9 ºC in a plate heat exchanger (Junior model, APV Ibérica
146 S.A., Madrid, Spain) to obtain the H150 sample. A second batch was centrifuged in a
147 Westfalia system (model SAOH 205, GEA Westfalia, Nuremberg, Germany) to

148 separate low pulp (serum) and pulpy (Pu) fractions. The pulpy fraction was
149 homogenized at 150 MPa under the same conditions described above and blended with
150 serum in the appropriate 85/15 proportion to reconstitute the juice that was finally
151 pasteurized at 85 ºC for 15 s to obtain sample CHPuRP8515. Relevance of the
152 CHPuRP8515 sample concerns the capacity of the production line of a juice plant in
153 where the flow rate (volume/time) of reconstituted HPH juice is much higher than the
154 achieved by homogenization of the whole juice (H150).
155 Finally, FJ was pasteurized under strong (92 ºC for 30 s) and mild (85 ºC for 15 s)
156 thermal conditions to yield P9230 and P8515 samples, respectively.
157

158 **2.3. Physicochemical parameters**

159 *2.3.1. Total soluble solids and acidity analyses*

160 Total soluble solids were determined as ºBrix with a digital refractometer (Pal-1,
161 Atago Co. Ltd, Tokyo, Japan) and total acidity was assessed by titration with 0.1 N
162 NaOH (using phenolphthalein 0.1% in 20% EtOH) and expressed as % citric acid
163 (w/v). The Brix to acid ratio and pH of fresh juice were 13.8 and 14.9, respectively
164

165 *2.3.2. Suspended pulp and cloudiness assessment*

166 Determinations followed the methodology proposed by Cheng (2002) and samples
167 were analysed in triplicate expressing the results as average (mean ± Std. dev.).
168

169 *2.3.3. HPLC-DAD analysis of ascorbic acid*

170 It was determined using a liquid chromatograph-diode array (HPLC-DAD) detector
171 Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary
172 pump, vacuum degasser and temperature-controlled autosampler. Separation was

173 achieved on a 250 mm x 4.6 mm i.d., 5 µm Phenomenex Luna (2) C18 column from
174 Phenomenex Inc. (Torrance, CA, USA) with the following conditions: solvent A,
175 water/ACN (95:5) containing 0.1% FA and ammonium formate (25 mM); solvent B,
176 ACN; initially 0% B hold for 10 min, 90% B at 10.5 min and purging up to 20 min,
177 column equilibration with 0% B from 20.5 min up to 35 min; injection volume, 3 µL;
178 flow rate, 1 mL/min; autosampler and column temperatures were 10 ºC and 23 ºC,
179 respectively; DAD detection wavelength, λ = 260 nm. Quantification of L-AAH in
180 samples was through external calibration using a preliminary calibration curve of a
181 commercial standard solution with a correlation coefficient (R^2) of 0.999.
182 Samples were analysed in triplicate and results expressed as average (mean ± Std.
183 dev.).
184
185 *2.3.4. Pectin methyl esterase (PME) activity assay*
186 It was determined following the methodology proposed by Carbonell, Navarro,
187 Izquierdo and Sentandreu (2013) and samples were analysed in triplicate expressing
188 results as average (mean ± Std. dev.).
189
190 *2.3.5 Particle size distribution analysis*
191 It was determined using a Malvern Mastersizer 2000 system (Malvern Instruments
192 Limited, Worcestershire, UK) with a short wavelength blue light source with forward
193 and backscatter detection for enhanced performance in the 0.02–2000 lm range.
194 Values at 1.73 and 1.33 were used as refractive indexes of the juice and the dispersant
195 (water), respectively, using 0.1 for absorption index of cloud particles (Corredig, Kerr,
196 & Wicker, 2001). The equivalent volume mean diameter $D_{[4,3]}$ was calculated as
197 follows:

198 $D_{[4,3]} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3$ (1)

199 where n_i is the number of particles of diameter d_i.

200 Samples were analysed in triplicate and results expressed as average (mean ± Std.
201 dev.).

202

203 *2.3.6. Colour measurement*

204 It was measured with a PC controlled Labscan II Hunter colourimeter (Hunter
205 Associates Lab., Reston, Vi, USA) following the indications from Cerdán-Calero,
206 Izquierdo and Sentandreu (2013). An optical glass cell (3.8 cm high and 6 cm of
207 diameter) containing a 3.5 cm thick layer of sample was covered with the white
208 standard plate (X 78.50; Y 83.32; Z 87.94) to determine diffused reflected light from
209 the cell bottom using a 13 mm diaphragm aperture.

210 The colour parameters of the CIELAB space L*, a* and b* were obtained. L* is an
211 estimation of the lightness. a* takes positive values for reddish colours and negative
212 values for the greenish ones. b* takes positive values for yellowish colours and
213 negative values for the bluish ones. Chroma (C^*_{ab}) and hue-angle (h_{ab}) are calculated
214 from a* and b* and are considered the quantitative and qualitative attribute of
215 colourfulness, respectively. The illuminant D_{65} and a 10° angle of vision were
216 considered The total colour difference among samples assayed was calculated as
217 follows:

218 $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (2)

219 Samples were analyzed in triplicate and results expressed as average (mean ± Std.
220 dev.).

221

222 **2.4. Simulated *in vitro* digestion of juices assayed**

223 It was followed the methodology originally proposed by Minekus et al. (2014) with
224 minor modifications implemented by Stinco et al.(2019) skipping the mastication step.
225
226 *2.4.1. Gastric phase*
227 Five mL of samples, 3.7 mL of simulated gastric fluid (SGF) and 2.5 μL of
228 $CaCl_2(H_2O)_2$ (588 g/L, w/v) were mixed into centrifuge tubes. The pH was adjusted to
229 3 ± 0.1 by adding 0.1 M HCl. Then, 500 μL of porcine pepsin solution (40 mg/mL in
230 SGF) was incorporated into the mixture. The final volume was adjusted to 10 mL with
231 water and it was incubated in a shaker Max Q5000 (Thermofisher scientific Inc.,
232 Waltham, MA) at 37 ºC at 150 rpm for 2 h.
233
234 *2.4.2. Duodenal phase*
235 To stop the gastric digestion, the mixtures were placed in an ice bath. Subsequently, 7
236 mL of simulated duodenal fluid (SDF) and 20 μL of $CaCl_2(H_2O)_2$ (0.3 M) were added.
237 After adjusting pH to 7, additional volumes of pancreatin (1 mL) and porcine bile
238 extract in SDF (1 mL) were added. The final volume was made up to 20 mL with
239 water to reach final concentrations of 390 mg/L and 2.1 g/mL of pancreatin and bile
240 extract respectively in the reaction mixture. Samples were incubated for 2 h at 37 °C in
241 the shaker (Max Q5000 shaker, Thermofisher scientific Inc., Waltham, MA) at 150
242 rpm to mimicking the intestinal digestion. Once completed the digestion, digests were
243 centrifuged for 20 min at 3900 x g at 4 °C in an Allegra X-12R centrifuge (Beckman
244 Coulter, USA). The supernatant was filtered through a 0.22 μm nylon membrane filter
245 (Agilent Technologies, USA) and the aqueous micellar phase were stored at -20 °C in
246 a nitrogen atmosphere until extraction.
247

248 **2.5. Calculation of bioaccessibility**

249 It was calculated as percentage (%) of carotenoids or flavonoids as follows:

250 $$\%Bioaccessibility_{BC} = \frac{M_d}{M_s}\ x\ 100 \qquad (3)$$

251 in where BC was the considered bioactive compound, M_d was the mg of BC in the

252 digestate and M_s was the mg of BC in the sample. In the case of carotenoids, the

253 micellar fraction of the digestate was considered.

254

255 **2.6. Carotenoid analysis by HPLC-DAD assay**

256 The sampling protocol (extraction from juices and micelles followed by

257 saponification) and carotenoid determination by HPLC-DAD analysis followed the

258 methodology described by Stinco, Fernández-Vázquez, Escudero-Gilete, Heredia,

259 Meléndez-Martínez and Vicario,(2012). Standards were used for identification and

260 external calibration addressing quantification achieving a correlation coefficient (R^2)

261 of 0.999 in all cases. Total carotenoid contents were determined as the sum of

262 individual pigments.

263 The vitamin A activity of juices was expressed in terms of retinol activity equivalents

264 (RAE) to 1 L of sample (Institute of Medicine, 2001) and it was calculated as follows:

265 $$RAE = \frac{\mu g\ \beta\ carotene}{12} + \frac{\mu g\ \ \beta\ cryptoxanthin + \mu g\ \ \alpha\ carotene}{24} \qquad (4)$$

266

267 **2.7. Flavonoid analysis by HPLC-DAD assay**

268 It was followed the methodology proposed by Stinco *et al.* (2015). Before HPLC

269 analysis, undigested juice and digestate were centrifuged at 18000 g for 15 min at 4 ºC

270 and subsequently filtered through a 0.22-µm pore size membrane filter.

271 Standards were used for identification and external calibration addressing
272 quantification achieving a correlation coefficient (R^2) of 0.999 in all cases. Total
273 flavonoid contents were calculated as the sum of individual phenolics.
274
275 **2.8. Statistical Analysis.**
276 Results from flavonoid and carotenoid analyses were given as mean and standard
277 deviation of six independent determinations. One-way analysis of variance (ANOVA)
278 was used to compare the means. Statistically significant differences ($p<0.05$) were
279 determined using the Tukey´s multiple comparison procedure. Correlations between
280 particle size parameters and bioaccessibility of phytochemicals assayed were assessed
281 by simple regressions. Statistical analyses were performed with Statistica v.8.0
282 software.
283

284 3. Results and discussion

285 **3.1. Physicochemical parameters**
286 **Table S1** summarizes physicochemical attributes of the juices assayed. Interestingly,
287 there were not significant differences in the ascorbic acid levels across samples.
288 Residual PME activity of H150 sample was about 40% (0.614 nkat/mL) than initially
289 showed by FJ juice (1.501 nkat/mL) whereas the rest of samples studied achieved
290 approximately a 25-fold reduction (0.045-0.067 nkat/mL). The decrease in the activity
291 of PME in orange juice after treatment by HPH and pasteurization was previously
292 observed in other studies (Carbonell, Navarro, Izquierdo, & Sentandreu, 2013) (**Table**
293 **S1**). In this line, the transmittance of the samples decreased as a result of the
294 treatments indicating how turbidity can be preserved by the particle size reduction
295 (**Fig. S2**) caused by the stabilization treatments as previously suggested by Carbonell,

296 Navarro, Izquierdo and Sentandreu (2013). Thus, while the transmittance of the fresh
297 sample was approximately 13%, those of P9230, P8515 and H150 ranged from 9.5 to
298 10% and the one exhibited by the CHPuRP8515 sample was by far the lowest (~
299 3.3%). This can be understood considering the average diameter of particles ($D_{[4,3]}$,
300 **Table S1**) that was significantly reduced in all treated samples compared to FJ but
301 with special relevance in homogenized juices that showed a 10-fold reduction.
302 Moreover, homogenization yielded samples with the lower mean surface ($D_{[3,2]}$) and
303 higher specific surface areas (SSA) as consequence of having a less coarse and more
304 compactable pulpy material that can affect the distribution of their carotenoid and fiber
305 contents. That is, fresh and pasteurized juices exhibited higher mean particle diameters
306 and so they may tend to settle down more rapidly during storage than in the H150
307 sample as stated by previous results (Leite, Augusto, & Cristianini, 2014**).**
308 **Fig. S3** illustrates the representation of a* and b* colour parameters (**Table S1**) of
309 juices assayed evidencing their clear differentiation (p<0.05) according to the
310 technological treatments applied. The FJ was darker (lower L*) and more reddish
311 (higher a*) than the treated samples. Moreover, it was found how HPH at 150 MPa
312 significantly increased L* and b* but decreased a* values in the same line than
313 previous results found in orange juices (Velázquez-Estrada et al., 2019). Thus,
314 appearance of H150 sample was lighter, less reddish and more yellowish. In general,
315 colour parameters of CHPuRP8515 sample were similar to those of the H150 juice. On
316 the other hand, although the colour parameters of the P9230 and P8515 samples were
317 in general significantly different compared to the FJ, the colour of such samples were
318 closer to the control juice than to the CHPuRP8515 and H150 samples (**Table S1**).
319 Overall, the highest colour differences (>7 CIELAB units) were observed between
320 CHPuRP8515 and H150 samples and the FJ, P9230 and P8515 samples. Since 2.8

321 CIELAB unit has been proposed as a threshold over which untrained people with
322 normal vision are expected to differentiate colour of orange juices visually
323 (Fernández-Vázquez, Stinco, Hernanz, Heredia, & Vicario, 2013), it can be concluded
324 that consumers with normal vision could differentiate some of the juices on the basis
325 of their colours.
326 Variation of colour attributes of samples can be explained to a large extent by the
327 alteration of their carotenoids (pulp-related phytochemicals) profile by processing and
328 changes in the size and structure of pulp particles. In this context, the apparition of
329 aggregative effects from the increased compressibility of smaller particles in HPH
330 juices has been suggested elsewhere (Arena, Fallico, & Maccarone, 2000), which
331 could help explain their lower observable pulp content (**Table S1**). According to
332 Cerdán-Calero, Izquierdo and Sentandreu (2013) , the difference between the color of
333 a fresh juice and that of a juice treated by HPH at 150 MPa could be reduced by
334 including a pre-homogenization at 20 MPa in the process.
335

336 **3.2. Carotenoid content of juices**

337 **Table 1** shows levels of carotenoids before and after the digestion process. Theoretical
338 vitamin A activity was expressed as Retinol Activity Equivalents (RAE). An ANOVA
339 analysis was carried out to evaluate whether the treatments led to significant changes.
340 As expected, the highest level of total carotenoids was detected in FJ sample finding
341 lower results, but not significantly different, in pasteurized juices according to the
342 temperature assayed. These results are in agreement with those obtained by other
343 authors (Aschoff et al., 2015), that reported a slight albeit not significant decrease in
344 total carotenoid levels during the processing of fresh oranges into pasteurized orange
345 juices.

346 Homogenization decreased the total carotenoid content and RAE significantly (1.37
347 and 1.35-fold, that is, 27% and 26%, respectively) compared to FJ. Similarly, Suárez-
348 Jacobo, Rüfer, Gervilla, Guamis, Roig-Sagués and Saldo (2011) described a decrease
349 in the vitamin A content of 18%, 20% and 33% when increasing the homogenization
350 pressure at 100, 200 and 300 MPa. These results suggested that degradation of
351 carotenoids could be mainly due to the cellular disruption promoted by HPH
352 treatments that enhanced their exposure to gastric enzymes and acidic pH, decreasing
353 their stability and favouring their oxidation (Meléndez-Martínez, Vicario, & Heredia,
354 2007). As expected, such negative effects were more pronounced in CHPuRP8515
355 sample since both HPH and pasteurization treatments were applied and led to a
356 reduction of 2.63- and 2.12-fold of its total carotenoid content and RAE, respectively.
357

358 **3.3. Bioaccessibility of carotenoids from juices**

359 **Fig. 1** and **Table 2** summarize results achieved regarding bioaccessibility of juice
360 carotenoids in percentage and the theoretical amount of carotenoids that could be
361 incorporated into micelles after the ingestion of 1L of juice (referred to as carotenoid
362 bioaccessible content, CBC), respectively.
363 Comparable results were found for FJ and pasteurized juices with the exception of β-
364 carotene, (Z)-antheraxanthin isomers and (Z)-luteoxanthin isomers that had a higher
365 bioaccessibility by thermal treatment (**Fig. 1**). These findings followed, in general,
366 previous results about bioaccessibility of carotenoids from orange juices and
367 discrepancies can be explained through the different experimental conditions
368 considered (Mapelli-Brahm et al., 2018; Stinco, Fernández-Vázquez, Escudero-Gilete,
369 Heredia, Meléndez-Martínez, & Vicario,2012).

370 Very interestingly, homogenization in H150 sample had a very significant (p<0.05)
371 positive impact on bioaccessibility (about 80%) and CBC (from 3- to 5-fold) of
372 carotenoids lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, lycopene,
373 phytoene and phytofluene compared to FJ (**Fig. 1** and **Table 2**). In this line, RAE
374 activity of H150 sample had a 4.4-fold increase and suggested the relevance of HPH
375 processing to improve the vitamin A status of populations with high consumption of
376 orange juice. Epoxycarotenoids were also found in the micellar fractions from digested
377 H150 sample having a 2- (violaxanthin isomers) to 7.4-fold ((9*Z*)-violaxanthin + Z-
378 isomer of antheraxanthin) CBC enhancement with a 3.86-fold increasing of total
379 carotenoid content compared to FJ (**Table 2**). Relevance of this finding deserves to be
380 highlighted since bioavailability of epoxycarotenoids used to be considered negligible
381 in humans (Meléndez-Martínez et al., 2017). Similarly, bioaccessibility and CBC of
382 carotenoids in CHPuRP8515 sample were significantly (p<0.05) higher than in FJ and
383 pasteurized juices, but lower than that of H150 juice as consequence of the
384 complementary pasteurization treatment assayed (**Fig. 1** and **Table 2**). These firstly
385 reported findings in orange juice demonstrated the relevance of disruptive processes to
386 enhance *in vitro* bioaccessibility of pulp-related phytochemicals such as carotenoids
387 initially hypothesized by Sánchez-Moreno, Plaza, De Ancos, and Cano (2003) in the
388 same food matrix.
389 Since homogenization had a negative effect on carotenoids content from samples
390 assayed (**Table 1**), it is plausible to confer betterment of bioaccessibility and CBC of
391 carotenoids to changes induced by processing on size and structure of pulp particles of
392 juices. Results suggested that particle size reduction featured by homogenization
393 (**Table S1**) greatly increased SSA and boosted extractability of carotenoids. To assess
394 correlations between particle size parameters ($D_{[4,3]}$, $D_{[3, 2]}$ and SSA) and the

395 bioaccessibility of carotenoids, simple linear regression analyses were carried out
396 (**Table S2**). Good correlations between the particle size parameters and
397 bioaccessibility of all carotenoids were found when all the samples were considered
398 altogether. For the correlations between $D_{[4,3]}$ and $D_{[3,2]}$ and bioaccessibility, the
399 correlation coefficients were negative and significant for all cases (r ranging from 0.62
400 to 0.98), while the correlations between SSA and bioaccessibility were positive and
401 significant in all cases (r ranging from 0.55 to 0.86) except for bioaccessibility of α-
402 carotene. According to the signs of the correlation coefficients, the lower the particle
403 size, (ie. lower $D_{[4,3]}$ and $D_{[3,2]}$), the higher the bioaccessibility of carotenoids. On the
404 contrary, the bioaccessibility was directly proportional to SSA. These results were to
405 be expected, since $D_{[4,3]}$ and D[$_{3,2]}$ indicates the diameter of the average volume and
406 the diameter of the average area of a particle, respectively , SSA is related to the
407 density of each particle. Altogether, it can be argued that the reduction in the size of
408 the particles and the subsequent increase in SSA facilitates the release of carotenoids
409 during digestion. Thus, treatments having greater impact on particle size
410 (CHPuRP8515 and H150) can confer better bioaccessibility to carotenoids than non-
411 disruptive thermal treatments. As it can be observed in **Fig. S2**, processing of
412 CHPuRP8515 sample yielded particles of lower size than that shown in H150 sample
413 although, in general, the volume in percentage of particles in CHPuRP8515 juice was
414 lower, explaining to a large extent the differences in bioaccessibility observed between
415 HPH samples assayed.
416 Results suggested that particle size of the food matrix plays a determinant role in the
417 bioaccessibility in of carotenoids. Importantly, the net result of treatments on the
418 actual amount of carotenoids that can be absorbed (referred to as CBC in this work)
419 also depends on how such treatments affect carotenoid retention in juices. In other

420 words, technological treatments that lead to an increased release of carotenoids during
421 digestion can result in an actual increase or decrease CBC depending on how the
422 treatments affected the total content of the carotenoids in the matrix.
423

424 **3.4. Flavonoid content of juices**

425 **Table 3** shows the mean levels of flavonoids found in juices assayed. Results from
426 H150 and CHPuMP8515 samples were significantly (p<0.05) lower (about 18%) than
427 those reached by fresh and pasteurized juices. Controversial results were stated by
428 Velázquez-Estrada et al. (2013) that observed an increase in the flavonoid content
429 (hesperidin and kaempferol) of orange juices treated at 200 and 300 MPa. The total
430 flavonoid content determined in P9230 and P8515 samples were not significantly
431 affected, in agreement to previous results from Sentandreu, Navarro and Sendra (2007)
432 reporting that thermal pasteurization at 90ºC-30 s had a negligible effect on the
433 phenolic content of citrus juices.
434

435 **3.5. Bioaccessibility of flavonoids from juices**

436 As shown by **Fig. 2**, bioaccessibility of flavonoids from the juices assayed was in the
437 range of 3.1% to 11.2%, lower than the bioaccessibility of carotenoids. Overall,
438 bioaccessibility of flavones (**Fig. 2A**) and flavanones (**Fig. 2B**) were not significantly
439 affected by the pasteurization treatments. In the same way, HPH treatment at 150 MPa
440 did not induce significant differences in the bioaccessibility of flavonoids. These
441 results were in agreement with those reported by He et al. (2016) who found that the
442 bioaccessibility of phenolic compounds was not affected in orange juices treated with
443 HPH relative to the untreated juice. It was noticeable how sample CHPuMP8515
444 showed an increased bioaccessibility of total flavones (**Fig. 2A**) and flavanones (**Fig.**

445 **2B**) of 2.5 and 3.3%, respectively, compared to FJ. Bioaccessibility of individual
446 flavonoids were significantly higher than in pasteurized and fresh juices. Findings
447 suggested that after pulp homogenization, these compounds were not protected by cell
448 wall components that could improve extractability of flavonoids, and therefore, their
449 bioaccessibility. Several studies have reported that the processing of plant foods can
450 influence the bioaccessibility of phenolic compounds, mainly through changes in the
451 cell wall structure (Dutra et al., 2017). Likewise, Martínez-Huélamo et al. (2015)
452 suggested that mechanical and thermal treatments of tomato sauce increased
453 bioaccessibility of phenolics.
454 As far as flavonoid bioaccessible contents (FBC) (that is, the amount of flavonoid
455 present in the digestates per liter of juice) the value corresponding to total flavonoids
456 was not significantly affected by the pasteurization (**Table 4**). It was observed a slight,
457 but not significant, increase in juices pasteurized at 92 ºC. Regarding H150 sample,
458 only vicenin-2 was significantly increased compared with untreated juices, as a result
459 of which the bioaccessibility of total flavones was significantly higher too. However,
460 CHPuRP8515 sample exhibited a significant increase in the bioaccessibility of
461 flavanones narigin-d and nariturin compared to FJ. Bioaccessibility of total flavonoids
462 in this juice, albeit not significantly different, was 1.32-fold higher compared to
463 reference (**Table 4**).
464

465 4. Conclusions

466 Although HPH processing (150 MPa) altered colour of juices, no negative effects were
467 found regarding their ascorbic acid content finding in addition, how the particle size
468 reduction induced by homogenization improved cloudiness of samples. Moreover,
469 diminution of the particle size drastically increased (from 4 to 5-fold) bioaccessibility

470 and RAE value of carotenoids from homogenized juices. Very interestingly,
471 epoxycarotenoids, which are not perceived in human plasma or tissues, were readily
472 detected in the micellar fraction of digested homogenized samples. Thus, there was
473 evidenced the relevance of HPH processing to improve incorporation into organism of
474 pulp-linked bioactive nutrients such as carotenoids from natural extracts.
475 In contrast to traditional pasteurization treatments, results of this study can help food
476 industry to adopt greener HPH stabilization technologies for trading high-quality
477 products with enhanced health properties that can satisfy current trends demanded by
478 consumers. Reduction of costs from the use of energy saving homogenization
479 alternatives in addition to their high output rate can favour implementation of HPH
480 processing in a wide range of industrial applications.
481

482 Acknowledgements

483 Authors thank funding from the Consejería de Economía, Innovación, Ciencia y
484 Empleo, Junta de Andalucía (projects CAROTINCO-P12-AGR-1287 and P11-AGR-
485 7783). Project RTA2014-00034-C04 from the Spanish Instituto Nacional de
486 Investigación y Tecnología Agraria supported contract of E. Sentandreu. Quality
487 assistance from the technical staff of the Service of Biology (SGI, Universidad de
488 Sevilla) is also acknowledged.
489

490 Declaration of competing interest

491 Authors declare no conflict of interest.
492

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668

669 Figure captions

670 **Fig. 1.** Bioaccessibility (%) of carotenoids (A) and epoxycarotenoids (B) from juices
671 assayed: FJ , fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized
672 juice at 85 ºC 15s; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged
673 juice, pulp fraction homogenized at 150 MPa, reconstituted with the serum fraction
674 and pasteurized at 85 ºC 15s. Nomenclature of bioactive compounds: LUT, lutein;
675 ZEA, zeaxanthin; ZEINO, zeinoxanthin; BCR, β-cryptoxanthin; ACAR, α-carotene;
676 BCAR, β-carotene; PT, phytoene; PF, phytofluene; VIO, violaxanthin; ANT,
677 antheraxanthin; LUTEO, luteoxanthin; MUT, mutatoxanthin.
678 Different letters indicate statistically significant differences ($p<0.05$). Number of
679 technical replicates, n=6.
680
681 **Fig. 2.** Bioaccessibility (%) of flavones (A) and flavanones (B) from juices assayed:
682 FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85
683 ºC 15s; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp
684 fraction homogenized at 150 MPa, reconstituted with the serum fraction and
685 pasteurized at 85 ºC 15s.
686 Different letters indicate statistically significant differences ($p<0.05$). Number of
687 technical replicates, n=6.
688
689 **Fig. S1.** Scheme of the operations carried out to obtain orange juices studied: FJ,
690 fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC
691 15s; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp
692 fraction homogenized at 150 MPa, reconstituted with the serum fraction and
693 pasteurized at 85 ºC 15s.
694

695 **Fig. S2.** Particle size distribution of the different samples assayed: FJ, fresh juice;
696 P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; H150,
697 homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp fraction
698 homogenized at 150 MPa, reconstituted with the serum fraction and pasteurized at 85
699 ºC 15s. Number of technical replicates, n=3.

700

701 **Fig. S3.** Representation of the colorimetric a* and b* parameters found in samples
702 assayed: FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized
703 juice at 85 ºC 15s; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged
704 juice, pulp fraction homogenized at 150 MPa, reconstituted with the serum fraction
705 and pasteurized at 85 ºC 15s. Number of technical replicates, n=3.

Table 1. Carotenoid levels (mg/L) and Retinol Activity Equivalents (RAE, in µg/L) found in juices assayed[Ψ].

Carotenoids	Orange juice samples				
	FJ	P9230	P8515	H150	CHPuMP8515
(Z)-ANT isomers	1.65±0.17a	1.32±0.18ab	1.43±0.17ab	1.12±0.07b	0.59±0.08c
all-(E)-VIO + (Z)-VIO isomers	0.35±0.05a	0.24±0.05b	0.28±0.03ab	0.22±0.03b	0.10±0.02c
(Z)-LUTEO isomer	0.76±0.11a	0.76±0.12a	0.78±0.05a	0.64±0.06a	0.33±0.04b
(9Z)-VIO + (Z)-ANT isomer	3.88±0.64a	3.22±0.57a	3.37±0.62a	2.80±0.30a	1.14±0.17b
(Z)-LUTEO isomer	0.59±0.06a	0.45±0.06b	0.50±0.04ab	0.38±0.03bc	0.26±0.03c
MUT- epimer A	0.48±0.06a	0.47±0.05a	0.50±0.01a	0.41±0.04a	0.27±0.01b
LUT	0.51±0.06a	0.50±0.05a	0.53±0.01a	0.43±0.05a	0.28±0.01b
MUT- epimer B	0.86±0.06a	0.78±0.11a	0.89±0.04a	0.76±0.02a	0.41±0.02b
ZEA	1.00±0.06a	0.76±0.10bc	0.82±0.07b	0.62±0.05c	0.41±0.01d
(9Z)- or (9´Z)-ANT	1.80±0.26a	1.43±0.26ab	1.58±0.21ab	1.21±0.15bc	0.68±0.03c
ZEI	0.37±0.04a	0.29±0.05a	0.31±0.04a	0.28±0.02a	0.16±0.01b
BCR	1.01±0.11a	0.86±0.12ab	0.93±0.06ab	0.71±0.06b	0.48±0.03c
ACAR	0.12±0.01a	0.10±0.01ab	0.11±0.01ab	0.10±0.01b	0.09±0.01b
BCAR	0.23±0.02a	0.20±0.03a	0.22±0.01a	0.18±0.01a	0.09±0.02b
PF	0.09±0.01ab	0.08±0.01ac	0.09±0.01ab	0.10±0.01b	0.06±0.01c
PT	0.57±0.06a	0.47±0.06ab	0.51±0.04ab	0.43±0.04b	0.15±0.02c
Total Carotenoids	**14.29±1.68a**	**11.92±1.83ab**	**12.84±1.29ab**	**10.38±0.83b**	**5.50±0.33c**
RAE*	**66.50±6.06a**	**56.82±8.04ab**	**61.42±3.03ab**	**49.02±3.09b**	**31.37±3.07c**

[Ψ]FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; H20, homogenized juice at 20 MPa; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp fraction homogenized at 150 MPa, reconstituted with the serum fraction and pasteurized at 85 ºC 15s.

*Retinol activity equivalent: calculated as RAE (µg/L) = (µg of β-carotene)/12 + (µg β-cryptoxanthin + µ α-carotene)/24.

Different lower case letters in the same row show significant differences (*p<0.05*) among samples.

Nomenclature used for carotenoids: ANT= (antheraxanthin), VIO = (violaxanthin), LUTEO = (luteoxanthin), MUT = (mutatoxanthin), LUT= (lutein), ZEA = (zeaxanthin), ZEINO = (zeinoxanthin), BCR = (β-cryptoxanthin), ACAR= (α-carotene), BCAR= (β-carotene), PT= (phytoene), PF= (phytofluene)

Table 2. Carotenoid Bioaccesible Content (CBC, theoretical amount of carotenoids in mg that could be incorporated into micelles after the ingestion of 1L of juice) of juices assayed[Ψ].

Carotenoids	Carotenoid Bioaccesible Content				
	FJ	P9230	P8515	H150	CHPuMP8515
(Z)-ANT isomers	0.33±0.03a	0.42±0.07a	0.40±0.03a	0.89±0.03b	0.43±0.07a
all-(E)-VIO + (Z)-VIO isomers	0.09±0.01a	0.09±0.02a	0.11±0.01a	0.18±0.02b	0.08±0.01a
(Z)-LUTEO isomer	0.15±0.02a	0.19±0.04a	0.16±0.01a	0.51±0.05b	0.22±0.02a
(9Z)-VIO + (Z)-ANT isomer	0.24±0.06a	0.33±0.07a	0.23±0.06a	1.77±0.09b	0.78±0.10c
(Z)-LUTEO isomer	0.06±0.01a	0.07±0.02ab	0.06±0.01a	0.23±0.03c	0.11±0.01b
MUT- epimer A	0.10±0.01a	0.13±0.03a	0.11±0.01a	0.32±0.03b	0.15±0.01a
LUT	0.11±0.01a	0.14±0.03ab	0.12±0.01a	0.37±0.02c	0.17±0.02b
MUT- epimer B	0.23±0.01a	0.25±0.04a	0.25±0.04a	0.69±0.05b	0.34±0.01c
ZEA	0.16±0.01a	0.19±0.06a	0.16±0.03a	0.49±0.02b	0.24±0.02a
(9Z)- or (9´Z)-ANT	0.15±0.02a	0.17±0.06a	0.15±0.01a	0.77±0.02b	0.36±0.05c
ZEI	0.04±0.01a	0.05±0.01a	0.04±0.01a	0.18±0.01b	0.09±0.01c
BCR	0.13±0.02a	0.17±0.03a	0.15±0.01a	0.56±0.01b	0.25±0.03c
ACAR	0.01±0.01a	0.02±0.01a	0.02±0.01a	0.06±0.01b	0.02±0.01a
BCAR	0.03±0.01a	0.07±0.03ab	0.05±0.01ab	0.140±0.003c	0.07±0.01b
PF	0.02±0.01a	0.02±0.01ab	0.02±0.01ab	0.08±0.01c	0.03±0.01b
PT	0.07±0.01a	0.09±0.02a	0.08±0.01a	0.29±0.01b	0.13±0.02c
Total Carotenoids	**1.95±0.16a**	**2.41±0.52a**	**2.11±0.10a**	**7.52±0.32b**	**3.48±0.36c**
RAE*	**8.48±1.29a**	**13.44±3.68ab**	**11.19±1.20a**	**37.33±0.22c**	**17.20±1.72b**

[Ψ]FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; H20, homogenized juice at 20 MPa; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp fraction homogenized at 150 MPa, reconstituted with the serum fraction and pasteurized at 85 ºC 15s.

*Retinol activity equivalent: calculated as RAE (μg/L) = (μg of β-carotene)/12 + (μg β-cryptoxanthin + μ α-carotene)/24.

Different lower case letters in the same row show significant differences (*p<0.05*) among samples.

Nomenclature used for carotenoids: ANT= (antheraxanthin), VIO = (violaxanthin), LUTEO = (luteoxanthin), MUT = (mutatoxanthin), LUT= (lutein), ZEA = (zeaxanthin), ZEINO = (zeinoxanthin), BCR = (β-cryptoxanthin), ACAR= (α-carotene), BCAR= (β-carotene), PT= (phytoene), PF= (phytofluene)

Table 3. Flavonoid levels (mg/L) found in juices assayed[Ψ].

Flavonoids	Orange juice samples				
	FJ	**P9230**	**P8515**	**H150**	**CHPuMP8515**
Vicenin-2	10.07±0.19a	10.27±0.13ab	10.256±0.12ab	10.57±0.12bc	10.91±0.26c
Apigenin-d	7.82±0.04a	7.64±0.13a	7.89±0.208a	7.17±0.20b	6.08±0.10c
∑Flavones	**18.65±0.25a**	**18.60±0.27a**	**18.68±0.34a**	**18.33±0.21ab**	**17.54±0.40b**
Naringin-d	13.81±0.39a	14.60±0.25a	14.40±0.37a	14.03±1.04a	13.11±0.53a
Narirutin	16.61±0.05ab	16.68±0.26ab	17.33±0.53b	15.68±0.43ac	15.03±0.66c
Hesperidin	251.28±9.30a	271.02±4.27a	262.64±5.24a	207.66±5.49b	205.03±19.40b
Didymin	6.42±0.42abc	7.14±0.39b	7.01±0.63ab	5.87±0.106bc	5.55±0.45c
∑Flavanones	**288.12±9.51a**	**309.44±4.83a**	**301.37±5.08a**	**243.24±6.32b**	**238.72±20.99b**
∑Flavonoids	**306.77±9.76a**	**328.04±5.10a**	**320.06±5.42a**	**261.57±6.32b**	**256.26±21.35b**

[Ψ]FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; H20, homogenized juice at 20 MPa; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp fraction homogenized at 150 MPa, reconstituted with the serum fraction and pasteurized at 85 ºC 15s.

Different lower case letters in the same row show significant differences ($p<0.05$) among samples.

Table 4. Flavonoid Bioaccesible Content (FBC, theoretical amount of flavonoids in mg present in the digestates after the ingestion of 1L of juice) of juices assayed$^{\Psi}$.

Flavonoids	Flavonoid Bioaccessible Content				
	FJ	**P9230**	**P8515**	**H150**	**CHPuMP8515**
vicenin-2	0.82±0.05a	0.89±0.05ab	0.79±0.07a	1.01±0.06bc	1.15±0.09c
apigenin-d	0.25±0.02a	0.26±0.02a	0.25±0.01a	0.29±0.03a	0.29±0.03a
∑Flavones	**1.07±0.07ab**	**1.15±0.07ab**	**1.04±0.08a**	**1.30±0.09bc**	**1.44±0.12c**
naringin-d	0.78±0.10a	0.91±0.20a	0.90±0.09a	0.91±0.11a	1.46±0.23b
narirutin	1.01±0.06a	1.05±0.13a	1.01±0.05a	1.19±0.09ab	1.38±0.16b
hesperidin	16.33±1.16a	18.04±2.13a	16.17±1.32a	17.09±2.16a	19.69±1.44a
didymin	0.46±0.05a	0.56±0.06a	0.47±0.05a	0.56±0.06a	0.59±0.07a
∑Flavanones	**18.58±1.36a**	**20.55±2.50a**	**18.55±1.51a**	**19.74±2.41a**	**23.11±1.81a**
∑Flavonoids	**19.65±1.44a**	**21.70±2.57a**	**19.595±1.59a**	**21.04±2.49a**	**24.55±1.92a**

$^{\Psi}$FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; H20, homogenized juice at 20 MPa; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp fraction homogenized at 150 MPa, reconstituted with the serum fraction and pasteurized at 85 ºC 15s.

Different lower case letters in the same row show significant differences (p<0.05) among samples.

Figure 1. Carla M. Stinco

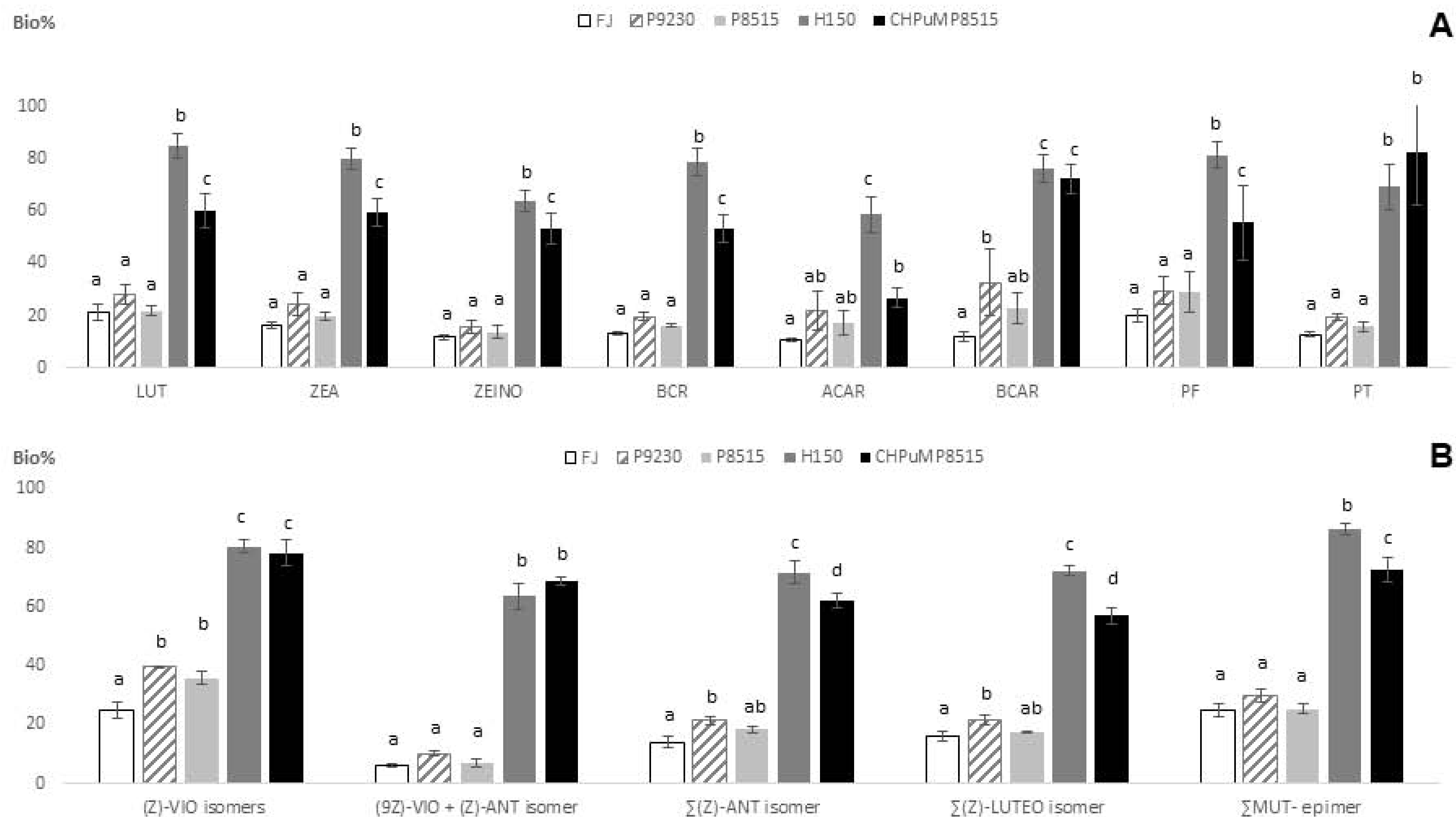

Figure 2. Carla M. Stinco

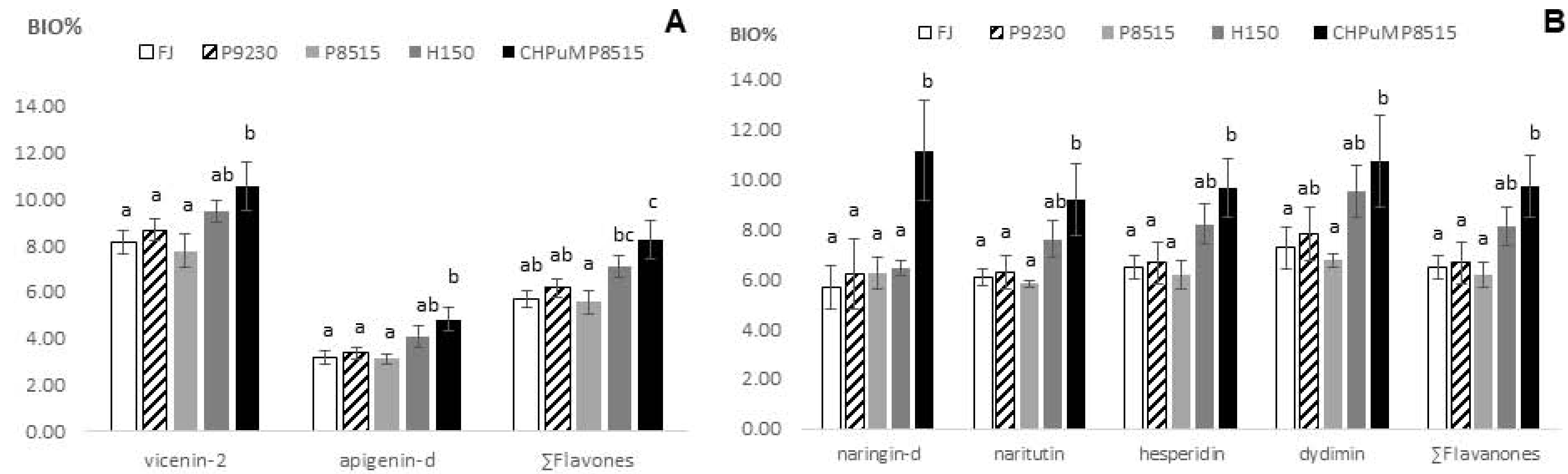

Table S1. Physicochemical parameters[Ψ] of orange juices assayed.

Parameters	FJ	P9230	P8515	H150	CHPuRP8515
Pulp (%)	8.83±0.59[a]	7.56±0.31[a]	8.01±0.01[a]	2.01±0.35[c]	2.33±0.23[c]
Transmitance (%)	13.02±0.91[a]	9.65±0.49[b]	9.50±1.34[b]	9.97±0.39[b]	3.32±0.08[c]
Ascorbic acid (mg/L)	428.70±0.35[a]	413.07±0.73[a]	414.17±0.02[a]	425.91±0.78[a]	388.07±0.66[a]
PME activity (nkat/mL)	1.501±0.023[a]	0.045±0.001[d]	0.067±0.001[d]	0.614±0.001[c]	0.061±0.001[d]
L*	44.69±0.01[f]	45.73±0.07[e]	46.71±0.01[d]	50.27±0.01[a]	49.91±0.04[f]
a*	5.33±0.01[a]	4.95±0.01[d]	5.07±0.01[b]	3.24±0.01[f]	3.52±0.02[d]
b*	54.06±0.01[c]	51.06±0.27[d]	50.91±0.11[d]	58.49±0.01[a]	58.50±0.01[a]
C^*_{ab}	54.32±0.01[c]	51.30±0.027[d]	51.17±0.11[d]	58.58±0.01[a]	58.61±0.01[a]
h_{ab}	84.36±0.01[e]	84.46±0.03[d]	84.31±0.01[e]	86.83±0.01[a]	86.55±0.02[b]
ΔE^*_{ab} FJ	–	3.20[c]	3.75[b]	7.42[a]	7.09[a]
ΔE^*_{ab} P9230	3.20[c]	–	1.02[d]	8.87[a]	8.65[a]
ΔE^*_{ab} P8515	3.75[c]	1.02[d]	–	8.56[a]	8.38[a]
ΔE^*_{ab} H150	7.42[b]	8.87[a]	8.56[a]	–	0.45[d]
ΔE^*_{ab} CHPuRP8515	7.09[a]	8.65[a]	8.38[a]	0.45[d]	–
¥$D_{[4,3]}$ (µm)	540.89±2.97[a]	369.50±4.10[b]	355.13±7.13[b]	70.36±3.52[c]	52.05±3.14[c]
¥$D_{[3,2]}$ (µm)	108.84±1.53[a]	67.82±2.87[b]	64.14±0.85[b]	31.18±2.70[c]	14.54±0.96[d]
¥SSA (m^2/g)	0.06±0.01[d]	0.09±0.01[d]	0.09±0.01[d]	0.19±0.02[c]	0.42±0.03[a]
¥d(0.1) (µm)	70.16±1.70[a]	35.11±1.86[b]	28.86±0.83[b]	18.96±3.29[c]	9.79±1.96[d]
¥d(0.5) (µm)	480.13±2.06[a]	325.16±4.46[b]	320.28±7.28[b]	60.28±3.29[c]	39.81±1.92[c]
¥d(0.9) (µm)	1096.51±4.16[a]	768.54±6.37[b]	732.53±12.74[b]	137.55±6.47[c]	114.68±7.47[c]

[Ψ] FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; H150, homogenized juice at 150 MPa; CHPuRP8515, centrifuged juice, pulp fraction homogenized at 150 MPa, reconstituted with the serum fraction and pasteurized at 85 ºC 15s. Different letters within the same row indicate statistically significant differences ($p < 0.05$).

[¥] $D_{[4,3]}$, volume mean diameter; $D_{[3,2]}$, surface area mean diameter; A_s, specific surface area, d(0.1), d(0.5), and d(0.9), standard percentile reading.

Table S2. Summary of the simple regression analysis* between the particle size parameters and the carotenoids bioaccessibility found in orange juices assayed.

Bioaccessibility(%)	**D[4,3]**	**D [3, 2]**	**As**	**d (0.1)**	**d (0.5)**	**d (0.9)**
(*Z*)-VIO isomers	***-0.975***	***-0.918***	***0.813***	***-0.817***	***-0.977***	***-0.978***
(9Z)-VIO + (Z)-ANT isomer	***-0.942***	***-0.873***	***0.859***	***-0.734***	***-0.946***	***-0.947***
∑(*Z*)-LUTEO isomer	***-0.919***	***-0.821***	***0.705***	***-0.694***	***-0.921***	***-0.926***
∑MUT- epimer	***-0.922***	***-0.827***	***0.736***	***-0.694***	***-0.924***	***-0.929***
∑(*Z*)-ANT isomer	***-0.943***	***-0.856***	***0.759***	***-0.734***	***-0.946***	***-0.949***
LUT	***-0.878***	***-0.765***	***0.620***	***-0.643***	***-0.880***	***-0.886***
ZEA	***-0.908***	***-0.805***	***0.666***	***-0.688***	***-0.908***	***-0.915***
ZEINO	***-0.924***	***-0.829***	***0.743***	***-0.701***	***-0.925***	***-0.930***
BCR	***-0.892***	***-0.783***	***0.628***	***-0.667***	***-0.892***	***-0.900***
ACAR	***-0.739***	***-0.628***	0.336	***-0.565***	***-0.736***	***-0.748***
BCAR	***-0.966***	***-0.915***	***0.796***	***-0.824***	***-0.968***	***-0.968***
PF	***-0.861***	***-0.758***	***0.557***	***-0.668***	***-0.860***	***-0.868***
PT	***-0.909***	***-0.852***	***0.849***	***-0.723***	***-0.913***	***-0.912***
TC	***-0.940***	***-0.852***	***0.765***	***-0.724***	***-0.942***	***-0.946***
RAE	***-0.913***	***-0.816***	***0.656***	***-0.708***	***-0.914***	***-0.920***

*Numbers in italic and bold indicate the existence of significant correlations ($p<0.05$).

Figure S1. Carla M. Stinco

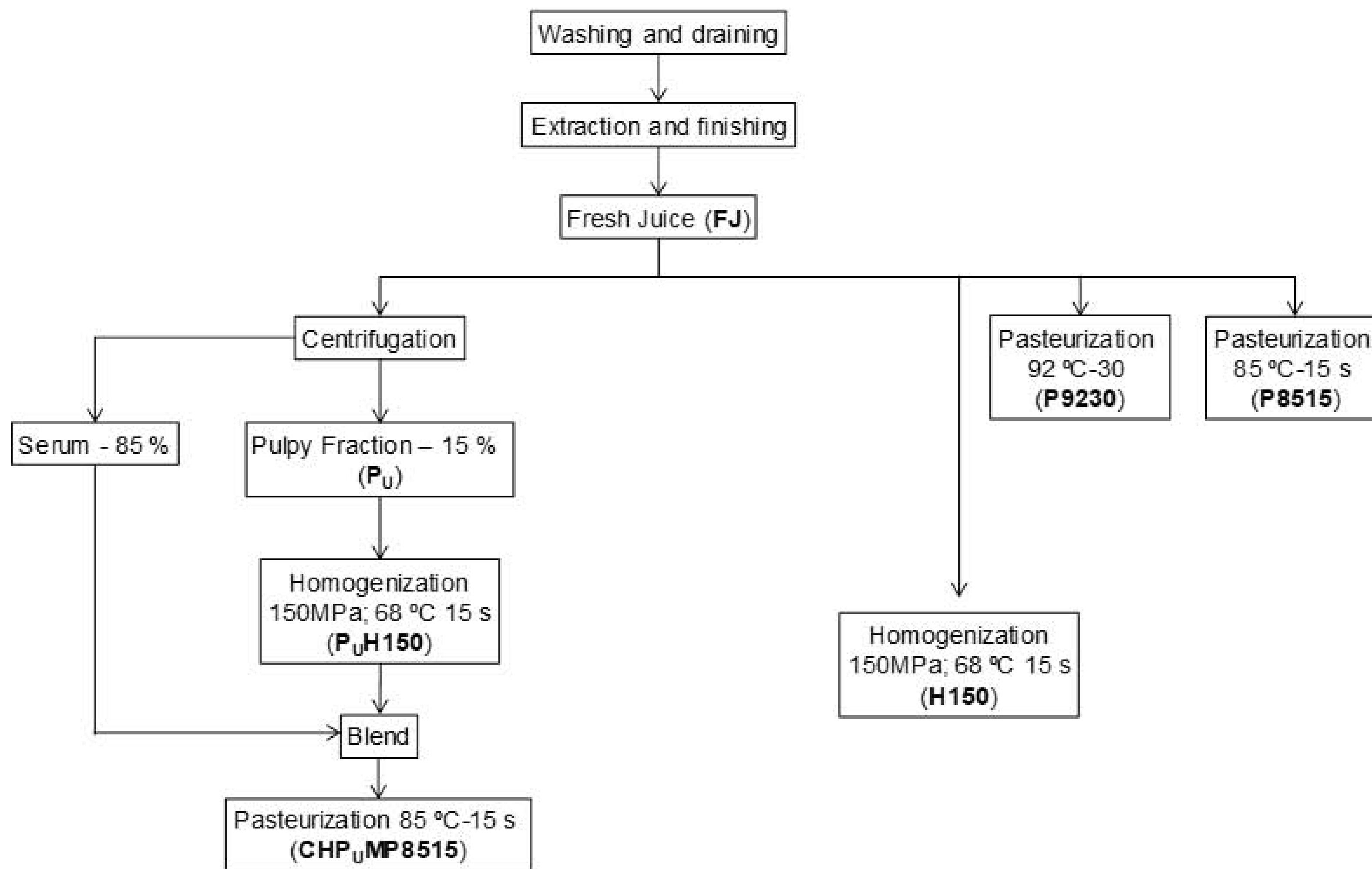

Figure S2. Carla M. Stinco

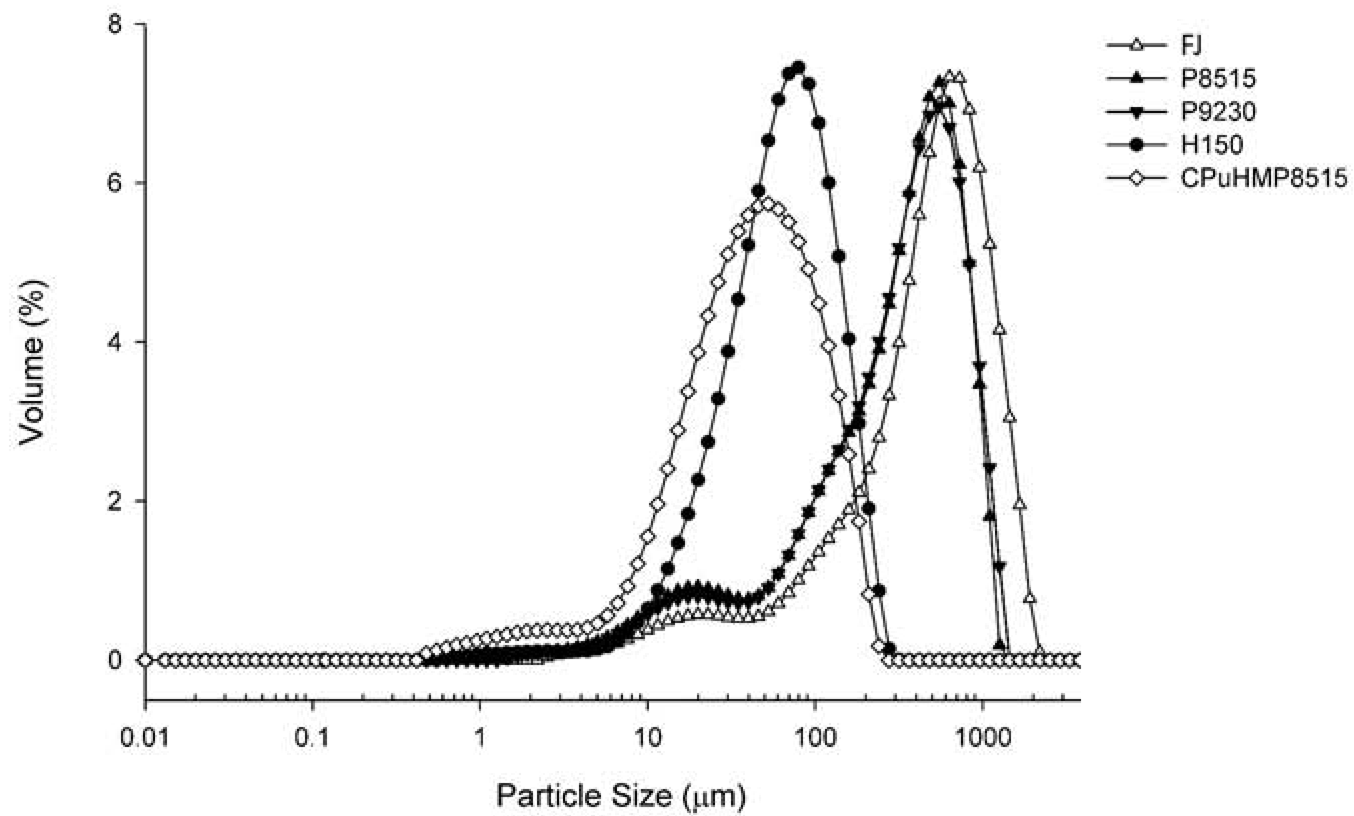

Figure S3. Carla M. Stinco