High-pressure homogenization as compared to pasteurization as a sustainable approach to obtain mandarin juices with improved bioaccessibility of carotenoids and flavonoids

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Abstract

High-pressure technologies are among those with increased interest in the sustainable production of quality-enhanced food products. In this work, Ortanique mandarin juices have been submitted to traditional pasteurization conditions (time/temperature of 65 ºC/15 s, 85 ºC/15 s and 92 ºC/30 s) and energy-saving high-pressure homogenization (HPH, 150 MPa) treatments to compare the effects on the physicochemical composition and *in vitro* bioaccessibility of carotenoids and flavonoids. In general, physicochemical attributes of the homogenized sample were similar to those found in fresh juice, with similar ascorbic acid content and cloudiness but with significant colour differences in all cases. The bioaccessibility of total carotenoids was similar in fresh and pasteurized juices in contrast to the HPH sample that exhibited a five-fold increase, which suggests a positive effect of particle size reduction to favour the action of digestive enzymes. A clear increase in the levels of epoxycarotenoids was detected in the micellar fractions of digested HPH juices, although such carotenoids are not detected in human fluids or tissues . Regarding the bioaccessibility of flavonoids, no significant differences were found in the samples studied. Results obtained can help the implementation of HPH processing to obtain natural beverages with enhanced nutritional properties.

Keywords: Bioaccessibility; citrus juice; carotenoids; flavonoids; high-pressure homogenization, antioxidants.

1. Introduction

Colour is an attribute of food quality with especial relevance in citrus juices It is mainly imparted by carotenoids, precursors of vitamin A and health-promoting compounds (Meléndez-Martínez et al., 2019; Stinco et al., 2012). Citrus products are also good sources of flavonoids (Tripoli et al., 2007). The biological actions of health-promoting compounds are mostly due to the bioavailable fractions. Bioaccessibility, the amount of compound released from the matrix as a result of digestion and available for absorption is one of the main factors governing bioavailability. This parameter can be obtained using *in vitro* simulated digestions. These are simple, inexpensive and reproducible (Fernández-García et al., 2009, Rodríguez-Concepción et al., 2018). The simplicity and high-throughput of these methods are useful to accelerate the optimization of processing techniques aimed at developing products with enhanced bioavailability of their functional components. Stinco et al. (2012) demonstrated how carotenoids from industrially processed orange juices showed an enhanced bioaccessibility compared to their hand-squeezed counterparts as a consequence of the particle-size reduction induced by processing. Positive effects of other industrial practices (i. e. pasteurization, ultra-freezing) on bioaccessibility of carotenoids from orange juice with changes in the microstructure and size of suspended particles was also demonstrated using simulated *in vitro* digestions (Mapelli-Brahm et al, 2018a). These findings were similar to previous results reported in diverse carotenoid-containing matrices. As another example, positive effects of industrially scalable disruptive techniques on carotenoids extractability were evidenced by Xie et al. (2016) studying microalgal cell cultivars treated by high-pressure homogenization (HPH). Such treatment was proposed as a plausible alternative to conventional thermal stabilization for the citrus industry to produce minimally processed juices with extended shelf-life (Carbonell et al., 2013).

HPH processing can be considered a promising option for the commercialization of healthy, safe and attractive high-quality citrus juices. These, continue to be increasingly demanded by the consumers and sought by the agro-food industry. In HPH, a pressurized fluid is forced to flow

63 through a minute gap, resulting in both homogenization and fluid heating effects. Among the
64 advantages of this methodology are that the temperature and pressure can be optimized to achieve
65 pasteurization or sterilization effects and that both homogenization and preservation can take place
66 in the same unit operation (Martínez-Monteagudo et al., 2017).
67 There is a global demand for sustainable foodstuffs and eco-innovative stabilization, mostly non-
68 thermal, technologies such as HPH and pulsed electric fields. These have been implemented at
69 industrial plants (Pereira and Vicente, 2010). Compared to traditional thermal stabilization for the
70 obtaining of bovine milk, the energy consumed by HPH processing has been shown to be
71 significantly lower (about 15%) and environmental benefits increases up to 57-58% have been
72 reported (Smetana et al., 2015). Reduction of the environmental burden achieved is mainly due to
73 the combination of sterilization and homogenization treatments in a single step. Homogenization
74 was proposed as an interesting alternative to valorize biomasses, for instance pistachio shell (Özbek
75 et al., 2018).
76 Despite the beneficial effects and high acceptance of mandarin juice in key population segments
77 such as children (Codoñer et al., 2010) the impact of HPH processing on the potential
78 bioavailability of health-promoting carotenoids and flavonoids from this foodstuff remains unclear.
79 This research aims to assess the effects of HPH processing and traditional thermal treatments on the
80 bioaccessibility of carotenoids and flavonoids from Ortanique mandarin juices. The effect of the
81 treatments in other parameters (ascorbic acid, colour, particle size, cloudiness, pectin
82 methylesterase activity) was also evaluated.
83

84 ## 2. Materials and methods

85 *2.1. Chemicals*

86 HPLC-grade solvents methanol (MeOH), methyl *tert*-butyl ether (MTBE) and acetonitrile (ACN)
87 were acquired from Merck (Merck KGaA, Darmstadt, Germany). The other extraction solvents

were all of the analytical grades from Scharlab (Scharlab S.L., Barcelona, Spain). Water was purified by a NANOpure Dlamond™ system (Barnsted Inc., Dubuque, IO). Pepsin (porcine gastric mucosa), pancreatin (porcine pancreas), bile salt and other reagents used in the in vitro digestion procedures were acquired from Sigma-Aldrich (St. Louis, MO, USA). Flavonoids' standards (purity >98%) were purchased from Extrasynthese (Extrasynthese S. A., Lyon, France). β-carotene (≥95.0% purity), β-cryptoxanthin (≥97% purity), lutein (≥96.0% purity), violaxanthin (≥95.0% purity), zeaxanthin (≥95.0% purity), ascorbic acid (L-AAH, 99% purity), vicenin 2 (98% purity) formic acid (FA) and LC-MS ammonium formate were from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Other carotenoid standards were either isolated from appropriate sources following standard procedures as explained elsewhere (Mapelli-Brahm et al., 2017)

2.2. Juice processing

Mandarin fruits of the Ortanique variety (*Citrus reticulata* x *Citrus sinensis*) were harvested in April 2017 in Lliria (Valencia, Spain). Fruits were washed in tap water, drained, sized and squeezed in an industrial extractor (Exzel model, Luzzysa, El Puig, Valencia, Spain). Raw juice was sieved in a paddle finisher (0.4 mm mesh diameter, model EPF 06, Luzzysa, El Puig, Valencia, Spain) obtaining 200 L of the freshly prepared reference (FJ). Four batches (40 L each) from FJ sample were prepared according to treatments assayed collecting aliquots of 15 L/batch. Severe processing conditions were not considered in this research. To obtain a minimally processed orange juice one batch pre-heated at 31 ºC was processed by HPH at 150 MPa reaching a temperature of 68 ºC for 15 s (Cerdán-Calero et al., 2014). Homogenization was featured by a continuous system (NS3015H model, GEA Niro Soavi S.p.A., Parma, Italy) with a Stellite GR. 20 cobalt alloy valve/seat system and a digital thermometer (probe PT-100, range -10/110 ºC) in the outlet section. The other three batches were conventionally pasteurized at 65 ºC for 15 s, 85 ºC for 15 s and 92 ºC for 30 s using a plate heat exchanger cooled at 7 ºC in the outlet section (Sentandreu et al., 2005).

114 *2.3 Physicochemical parameters*

115

116 *2.3.1 Total soluble solids and acidity*

117 Total soluble solids (ºBrix) were determined with a Pal-1 digital refractometer (Atago Co. Ltd,
118 Tokyo, Japan) and total acidity by titration with 0.1 N NaOH (using phenolphthalein 0.1% in 20%
119 EtOH as an indicator) and the results were expressed as % citric acid (w/v).

120 *2.3.2 Suspended pulp and cloudiness*

121 Both parameters were determined following the methodology proposed by Cheng (2002). Briefly,
122 samples (10 mL) were centrifuged (370 x *g* at 22 ºC for 10 min) in conical graduated tubes.
123 Suspended pulp was expressed as the percentage of pulp (v/v) respect to the total volume of the
124 sample. Cloudiness was determined according to the transmittance (in percentage) of the collected
125 supernatant measured at 650 nm with a UV/visible spectrophotometer (Ultrospec 3300pro,
126 Amersham Bioscience, Pistacaway, NJ, USA) previously adjusted to 100% light transmission with
127 distilled water. Samples were analyzed in triplicate.

128

129 *2.3.3. HPLC-DAD analysis of ascorbic acid*

130 It was performed in an Agilent 1100 chromatographic system (Agilent Technologies, Palo Alto,
131 CA, USA) equipped with a vacuum degasser, binary pump, an UV–vis diode-array detector,
132 automatic injector, temperature-controlled column oven, and autosampler. A stationary phase a
133 C18 column 250 mm x 4.6 mm i.d., 5 µm (Luna Phenomenex Inc., Torrance, CA, USA) and
134 mobile phase solvents (solvent A, water/ACN (95:5) containing 0.1% FA and ammonium formate
135 (25 mM); solvent B, ACN) were used for the method development. Chromatographic conditions
136 were: injection volume, 3 µL; flow rate, 1 mL/min; column oven and autosampler temperatures, 23
137 ºC, and 8 ºC, respectively; DAD detection wavelength, λ = 260 nm.

138 The analysis was performed under isocratic conditions: 0% B for 10 min, washing with 90% B for
139 9.5 min and column equilibration with 0% B for 15 min. External calibration using a commercially

140 available L-AAH standard was considered for quantitation in samples that were analyzed in
141 triplicate expressing averaged results (mean ± Std. dev).
142
143 *2.3.4. Determination of pectin methylesterase (PME) activity*
144 It was assayed to determine stabilization of juices studied following the methodology proposed by
145 Carbonell et al. (2006). Briefly, 5 mL of juice previously adjusted to pH 7.8 with NaOH was added
146 to 20 mL of a 0.5% pectin (high methoxyl pectin, Cargill MRS 150, Cargill Texturizing Solutions,
147 Barcelona, Spain) solution at pH 7.8 containing 0.2 M NaCl. The pH reduction was monitored at 22
148 ºC for 30 min in 30 s intervals. Experimental data (pH vs. time) was fitted to the equation [pH =
149 $(7.8\text{-}a)e^{(-kt)+a}$] where the slope of the curve at zero time corresponded to the enzyme activity.
150 Samples were analyzed in triplicate and values expressed as average (mean ± Std. dev.).
151
152 *2.3.5. Colour measurement*
153 It was measured with a Hunter colourimeter Labscan II model (Hunter Associates Lab., Reston, Vi,
154 USA) according to the methodology proposed by Carbonell et al. (2011). Chroma (C^*_{ab}) and hue-
155 angle (h_{ab}) are calculated from a* and b* and are considered the quantitative and qualitative
156 attribute of colourfulness. The illuminant D_{65} and a 10° angle of vision were considered for
157 measures. The total colour difference among samples assayed was calculated according to CIE
158 (1978):

159 $$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \qquad (1)$$

160
161 The samples were analyzed in triplicate and values expressed as average (mean ± Std. dev.).
162
163 *2.3.6. Particle size distribution analysis*

It was determined using a Malvern Mastersizer 2000 system (Malvern Instruments Limited, Worcestershire, UK) with a short wavelength blue light source in conjunction with forward and backscatter detection to enhance performance in the 0.02–2000 μm range. Values of 1.73 and 1.33 were considered as refractive indexes of the juice and the dispersant (water), setting 0.1 as the absorption index of cloud particles (Corredig et al., 2001). The equivalent volume means diameter $D_{[4,3]}$ μm was calculated as follows:

$$D_{[4,3]} = \sum_i n_i d_i^4 \,/\, \sum_i n_i d_i^3 \qquad (2)$$

where n_i is the number of particles of diameter d_i. Further parameters determined were the specific surface area (SSA, total area of particles divided by the total weight, mg^2/g), the surface weighted mean ($D_{[3,2]}$, μm) and standard percentile readings from the analysis that is the size (μm) of particle below which 10%, 50% and 90% of the sample lies (d(0.1), d(0.5) and d(0.9), respectively). Samples were analyzed in triplicate and values expressed as average (mean ± Std. dev.).

2.4. Simulated static in vitro digestion methodology

The simulated static *in vitro* gastrointestinal digestion assay originally proposed by Minekus et al. (2014) was taken as reference but adapted by Stinco et al. (2019) to liquid samples by the obviation of the oral phase. Briefly, the method simulates gastric phase using pepsin at pH=3 and intestinal phase with bile salts and pancreatin at pH=7 using two electrolytic fluids: Simulated Gastric Fluid (SGF) and Simulated Duodenal Fluid (SDF), both were detailed in Minekus et al (2014).

2.4.1. Gastric phase

Five mL of juices were poured into centrifuge tubes and mixed with 3.7 mL of simulated gastric fluid (SGF) and 2.5 μL of $CaCl_2(H_2O)_2$ (588 g/L, w/v). After adjusting pH to 3 ± 0.1 by addition of HCl 1M, 500 μL of a porcine pepsin solution (40 mg/mL) in simulated gastric fluid (Garrett et al.,

1999) were added. Samples were incubated at 37ºC in a rotating incubator (Max Q5000 shaker, Thermo Fisher Scientific Inc., Waltham, MA) at 150 rpm for 2 h. The volume of samples was adjusted to 10 mL with water after finishing the incubation time.

2.4.2. Duodenal phase

The gastric digestion was stopped by placing the samples in an ice bath. Seven mL of simulated duodenal fluid (SDF) and 20 µL of 0.3M $CaCl_2$ were added and the pH was adjusted to 7.0 with NaOH 1 M. One mL each of pancreatin and porcine bile extract in SDF was added and final volume was made up to 20 mL with water to reach a concentration of 390 mg/L and 2.1 g/mL of pancreatin and bile extract (Garret et al. 1999). Samples were incubated under the same conditions previously described for the gastric phase.

2.4.3. Recovery of the micellar fractions

Micellar fractions were homogenized and centrifuged at 3900 x *g* for 20 min at 4 ˚C according to Granado-Lorencio et al. (2007) in an Allegra X-12R centrifuge (Beckman Coulter, USA). Supernatants were filtered through a 0.22 µm nylon membrane filter (Agilent Technologies, USA) and samples were stored at -20 °C in nitrogen atmosphere until analysis.

2.5. Calculation of bioaccessibility of carotenoids and flavonoids

It was calculated in percentage as follows:

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

where:

BC = bioactive compound

M_d = mg of BC in the digestate

M_s = mg BC in the sample

215 Bioaccessibility has been calculated for both kinds of compounds likewise, although the
216 information obtained needs to be interpreted carefully as they have different chemical structures and
217 digestive behavior. Carotenoids are very lipophilic and need to be emulsified and incorporated into
218 micelles for their uptake in enterocytes. Flavonoids are polyphenols, hydrophilic compounds that
219 can be easily solubilized during digestion. Besides, there is compelling evidence that polyphenols
220 are largely metabolized by the colon microbiota (Bohn et al., 2015).
221
222 *2.6. Carotenoids extraction and analysis*
223 Extraction and saponification of carotenoids from undigested juices were carried out according to
224 Stinco et al. (2012). In the case of digested samples, about 17 mL of the micellar fractions were
225 exhaustively extracted with 10 mL of the mixture of dichloromethane, acetone and MeOH (2:1:1
226 v/v/v), vortexed for 5 min and centrifuged at 3900 x *g* for 10 min at 4 ºC. The procedure was
227 repeated until the colour exhaustion of samples. Supernatants were collected and the resultant
228 solution was evaporated to dryness using a rotary evaporator (Eppendorf ´Concentrator Plus,
229 Hamburg, Germany) at a temperature below 30 °C. Extracts were dissolved in 500µL of
230 dichloromethane and saponified with 500µL of KOH (30% w/v in MeOH) for 60 min. Any trace of
231 the base was removed by NaCl and water washing. Coloured solutions obtained were concentrated
232 to dryness as previously indicated. Extracts were re-dissolved in 50 µL of ethyl acetate and poured
233 into glass vials for HPLC-DAD analysis.
234 Determination of saponified carotenoids from undigested and digested samples was performed by
235 HPLC on a Agilent System 1100 (Agilent, Palo Alto), equipped with UV/VIS diode array detector,
236 which was set at 285 nm for phytoene, 350 nm for phytofluene and 450 nm for the rest of the
237 carotenoids. Separation was carried out in a C30 YMC column (5 µm, 250 × 4.6 mm) (YMC,
238 Wilmington, NC) kept at 20 °C using as mobile phase MetOH (solvent A), TBME (solvent B) and
239 MiliQ water (solvent C). The chromatographic parameters (linear gradient, flow rate, etc) were set
240 in the same conditions as those previously used for carotenoid analyses by according Stinco et al.

241 (2012).

242 Positive assignments were achieved by the comparison of their retention time and UV/vis
243 spectroscopic characteristics with those corresponding to the standards, which were also used for
244 quantitative purposes through external calibration. The total carotenoids content of samples was
245 calculated as the sum of the individual concentration of compounds.

246 The vitamin A activity of samples was expressed in terms of retinol activity equivalents (RAE) to 1
247 L of juice and it was calculated as indicated by the Institute o Medicine (IM, 2001), considering that
248 1 retinol activity equivalent (μg RAE) is equivalent to 12 μg of dietary all-trans-β-carotene and 24
249 μg of other dietary provitamin A carotenoids . Samples were analyzed in triplicate and values
250 expressed as average (mean ± std. dev.).

251

252 *2.7. Flavonoids extraction and analysis*

253 Undigested and micellar fraction samples were centrifuged at 18000 x *g* for 15 min at 4 ºC, the
254 supernatants filtered through a 0.22 μm nylon filter and filtrates poured into vials. Samples were
255 kept at -80 ºC until analysis.

256 Flavonoids were determined by rapid resolution liquid chromatography (RRLC) using an Agilent
257 Technologies 1260 system with a quaternary pump, column and autosampler temperature controller
258 set at 25 ºC and 8 ºC, and coupled to a DAD. The separation was performed according to Stinco et
259 al. (2015) with minor modifications. Briefly, a 50 mm × 4.6 mm i.d., 2.6 μm particle size
260 Phenomenex Kinetex Biphenyl C18 column with SecurityGuard ULTRA UHPLC Biphenyl filter
261 (Phenomenex; Torrance, CA, USA) was used. Mobile phase A was water-FA (99.9/0.1 v/v) and
262 mobile phase B was ACN using the following separation gradient: initially, 0% B; 5% B in 5 min;
263 50% B in 15 min; washing with 100% B for 2 min and column equilibration with 0% B for 3 min.
264 Total running time, was 25 min. Other settings were flow rate, 1.5 mL/min; UV/vis range, 230-400
265 nm; DAD registration wavelengths, 280 and 320 nm for flavanones and flavones. Flavonoids were
266 identified according to their retention time and UV/vis spectra compared to commercial standards

267 also used for quantification purposes by external calibration. Total flavonoids were calculated as the
268 sum of individual compounds. The samples were analyzed in triplicate and values expressed as
269 average (mean ± Std. dev.).
270
271 *2.8. Statistical Analysis*
272 Results were given as mean and standard deviation of independent determinations. All data were
273 subjected to one-way ANOVA and differences between means were identified by Tukey's test
274 (p<0.05) using a statistical software package (Statistica v.8.0.).
275

276 ## 3. Results and discussion

277 *3.1. Physicochemical parameters*
278 **Table 1** summarizes the physicochemical properties of juices assayed. In general, the parameters
279 considered were significantly affected by processing relative to the fresh juice (ºBrix to acid ratio of
280 10.80). The ascorbic acid levels were significantly different across samples. The treatment leading
281 to lower and higher reductions were HPH and pasteurization at 92 °C, respectively. Residual PME
282 activity achieved by the softest thermal and HPH treatments was around 40 % that initially shown
283 by FJ sample. The decrease in the activity of PME in orange juice after treatment by HPH has also
284 been observed by other authors (Carbonell et al., 2013). The turbidity (as evaluated from
285 transmittance) from HPH juice (18.53%) was comparable to that from fresh juice (16.07%),
286 confirming the promotion of cloudiness by particle size reduction at 150 MPa (**Fig. S1**) even with a
287 reduced pulp content in the same line as stated by Carbonell et al (2013) in Lane late orange juice.
288 Mean volume diameter ($D_{[4,3]}$, eq. 2) of the homogenized sample was drastically decreased whereas
289 it was slightly increased in pasteurized samples, evidencing no disaggregation effect on pulp on
290 contrarily to what it was observed by Stinco et al. (2012) in commercial orange juices. This can be
291 understood by the fact that even produced in a pilot plant the Ortanique HPH juice studied did not

292 suffer common shearing effects induced by pumping at industrial plants. In this line, significant
293 (p<0.05) differences in mean surface area diameter ($D_{[3,2]}$) and specific surface area (SSA) were
294 found for the HPH sample in comparison to the other types of juices.
295 As seen in **Fig. S1**, FJ and the pasteurized juices showed a bimodal particle size distribution while
296 the juice treated by HPH presented a monomodal distribution. Indeed, the particle size distribution
297 in the case of the FJ and the pasteurized juices was very similar. The marked decrease in the particle
298 sizes in the juice treated by HPH can be attributed to a more pronounced rupture of the cells so that
299 the cell material would be more uniformly distributed in this sample, whereas in the FJ and
300 pasteurized juices there may be cell clusters consisting of several cells (Velázquez-Estrada et al.,
301 2019).
302 Significant (*p<0.05*) differences were found among juices assayed regarding their colour
303 parameters. Results indicated that fresh juice was the darkest (lowest L* values) and the H150 the
304 lightest (highest L* values), which were the least and most colourful samples (lowest and highest
305 C^*_{ab} values). Juices assayed were clearly grouped and differentiated according to the technological
306 treatment applied by the representation of their a* and b* values (**Fig. 1)**. The samples with the
307 lowest and highest a* values were FJ and P8515, and those with the lowest and highest b* values
308 were FJ and H150. Overall, h_{ab} values found were significantly different among samples assayed
309 and colour. Colour differences (ΔE^*_{ab}, eq. 1) were well over the 2.8 CIELAB unit threshold, where
310 untrained assessors can easily differentiate colour discrepancies in orange juice (Fernández-
311 Vázquez et al., 2013).
312 The changes in colour parameters of HPH-treated juices could be explained to a certain extent by
313 the higher compressibility of their smaller particle size that could favour aggregative effects (Arena
314 et al., 2000) that decreased observable pulp content. Cerdán-Calero et al. (2013) indicated that the
315 colour difference between a fresh juice and a juice treated by HPH at 150 MPa could be reduced by
316 including a pre-homogenization at 20 MPa in the process. In any case and despite colour
317 modifications induced, previous studies demonstrated usefulness of HPH processing to enhance and

318 preserve sensory attributes of citrus juices (Cerdán-Calero et al., 2013).

319

320 *3.2. Carotenoid analysis*

321 *3.2.1 Carotenoid contents in the juices*

322 **Table 2** summarizes the carotenoids contents (mg/L) and Retinol Activity Equivalents (RAE) of
323 juices studied. The carotenoid profile of the mandarin Ortanique included coloured (α-carotene and
324 β-carotene) and colourless carotenes (phytoene and phytofluene), monohydroxycarotenoids
325 (zeinoxanthin and β-cryptoxanthin), dihydroxycarotenoids (lutein and zeaxanthin) and
326 dihydroxycarotenoids with one (antheraxanthin) or two (violaxanthin) 5,6-epoxide groups.
327 Carotenoids with one 5,8-furanoid group resulting from the acid-promoted isomerization of
328 carotenoids with 5,6-epoxide groups into their 5,6-furanoid counterparts, namely mutatoxanthin
329 (formed from antheraxanthin) and luteoxanthin (formed from violaxanthin), were detected. The
330 complexity of the carotenoid profile of Ortanique juice was comparable to those from typical
331 orange varieties (Giuffrida et al., 2019;).

332 As expected treatments assayed induced, in general, relevant changes in the carotenoid contents of
333 samples. Total carotenoids decreased as a function of the strength of the pasteurization treatments
334 assayed. Specifically, reductions of 13%, 22% and 30% in total carotenoids relative to the fresh
335 juice were found for samples pasteurized at 68 ºC-15 s, 85 ºC-15 s and 92 ºC-30 s. These
336 observations are in good agreement with the findings of Stinco et al. (2012) and Velazquez Estrada
337 et al. (2013) in industrially produced orange juices. The highest reduction in the total carotenoid
338 levels was observed in the HPH-treated sample (~ 40%). Total carotenoids depletion levels
339 dependent on the pressure applied (100, 200 and 300 MPa) and on the maximal temperature
340 achieved during the treatments have been reported on the higher carotenoid reduction in the HP150
341 sample can be attributed to different factors. On one hand the effect of the thermal treatment
342 undergone by this sample (68 ºC-15 s) and, on the other hand, the higher structural damage of the
343 pulp particles and the cells, exemplified by the marked reduction in particle size already commented

344 (**Table 1**, **Fig. S1**). This effect can facilitate the release of carotenoids from their natural milieu and
345 make them more susceptible to degradation by agents including enzymes, oxidizing agents or acids
346 (Meléndez-Martínez et al., 2007).
347
348 *3.2.2. Bioaccessibility of carotenoids*
349 Although epoxycarotenoids were found in the micellar fractions obtained after the *in vitro*
350 digestions, they are not considered bioavailable as they are not among the carotenoids typically
351 detected in human plasma and tissues (Melendez Martinez et al 2013).
352 **Figures 2A** and **2B** show bioaccessibility (%) of carotenoids and epoxycarotenoids from samples,
353 (eq. 3). It was observed a slight, but not significant, improvement of carotenoid content (**Fig. 2A**) in
354 pasteurized samples over fresh juice, with an average value of ~ 15%. The influence of
355 pasteurization on the bioaccessibility of carotenoids from citrus juices is still a controversial issue
356 finding (Mapelli-Brahm et al. 2018a), and negative (Stinco et al., 2012) effect described in the
357 literature. In our case, pasteurization did not exert any remarkable impact in samples. Contrastingly,
358 HPH treatment drastically improved bioaccessibility of bioavailable carotenoids that varies from
359 92% for α-carotene to 79% for zeinoxanthin. Bioaccessibility of epoxycarotenoids (**Fig. 2B**)
360 showed the same behavior. While pasteurization had no effect, HPH enhanced bioaccessibility with
361 values that ranged from, 96 % for mutatoxanthin epimers to 78% (Z)-violaxanthin isomers. To our
362 knowledge, there is no previous information on the effect of high-pressures on the bioaccessibility
363 of epoxycarotenoids. Since the effects of carotenoids are mostly related to the amounts that can be
364 efficiently absorbed, it is useful to evaluate the potential bioavailability of carotenoids from a
365 certain amount of the product. For this purpose, Mapelli-Brahm et al. (2018b) applied the concept
366 of carotenoid bioaccessible content (CBC) for tomato pulp and powders. In the present study, CBCs
367 were referred considering 1 L of juice. **Table 3** shows carotenoid levels (mg/L) found in the
368 micellar fraction (carotenoid bioaccessible content) after *in vitro* digestion that reflected the
369 potentially absorbable content of carotenoids in the juices assayed. While the pasteurization

370 treatments did not lead to significant changes (p<0.05) in potential bioavailability relative to the
371 fresh juice, the HPH treatment did. Although the pasteurization conditions tested significantly
372 decreased the carotenoid levels of stabilized juices compared to FJ, no significant differences were
373 observed in their CBCs. This observation leads to hypothesize that the loss of carotenoids in the
374 pasteurized juices can be compensated by a higher release of carotenoids and incorporation into
375 micelles during digestion in these juices in comparison to the FJ sample. Previous studies by Stinco
376 et al. (2012) and Mapelli-Brahm et al. (2018a) indicated that the pasteurization of different orange
377 juices can cause changes in pulp particles and even in suborganellar structures that can enhance the
378 bioaccessibility of carotenoids.
379 In this study, homogenization enhanced the "RAE" and carotenoid levels in the micellar fraction
380 considerably compared to fresh juice (about 4-fold and 6-fold higher). The pasteurization conditions
381 did not significantly (p<0.05) affect the potential bioavailability of carotenoids, while HPH improve
382 it markedly. Considering that this latter treatment had a significant negative effect on the levels of
383 carotenoids (p<0.05) as discussed previously, the positive impact on the potential bioavailability of
384 carotenoids can be attributed to the changes caused in the pulp particles by several phenomena,
385 including turbulence, shear, and cavitation (Stang et al., 2001).
386 Comparing these results with those from the particle size distribution, arguably, the disruptive effect
387 of the homogenization treatment is key to explain the enhanced bioaccessibility of carotenoids in
388 the HPH-treated juice. As can be observed in **Table 1** the H150 sample, compared to the FJ sample,
389 had significantly lower $D_{[4,3]}$ and $D_{[3,2]}$ and significantly higher specific surface area (SSA) values.
390 The reduced size of particles and increased SSA can make the carotenoid-containing pulp particles
391 more accessible to the digestive enzymes, facilitating their release during digestion.
392 Significant (p<0.05) positive correlations ranging from 0.89 to 0.96 were obtained between the
393 bioaccessibility of carotenoids and specific surface area (SSA). These results were as expected,
394 since, the greater the surficial area available for the attack by digestive enzymes, the greater the
395 digestion efficiency. On the contrary, a significant negative correlation ranging from 0.82 to 0.99

396 was found between $D_{[4,3]}$ and $D_{[3,2]}$ parameters and bioaccessibility. This suggested that the
397 disruption of the food matrix is determinant in the gastrointestinal absorption of carotenoids.
398 Although there was previous evidence that high pressures can have an important effect on the
399 extractability of carotenoids from orange juices (Sánchez-Moreno et al., 2003), to our knowledge
400 there is no published information about the impact of HPH treatment on the bioaccessibility of
401 carotenoids from mandarin juices.
402 Influence of high-pressure treatments in the bioaccessibility of carotenoids in other alimentary
403 matrices was previously assessed and evidenced how it was mainly modulated by the matrix
404 disruption and other factors such as the carotenoid type (Panozzo et al., 2013), the plastid and cell
405 wall substructures (Palmero et al., 2016), the formation of a fiber network (Colle et al., 2010) and
406 rheological properties (Zhou et al., 2017).
407
408 *3.3. Flavonoid analysis*
409 *3.3.1. Flavonoid contents in the juices*
410 **Table S1** shows the contents of main flavonoids detected in samples reaching similar values than
411 previously reported in juices from the same mandarin variety (Sentandreu et al., 2007). Although
412 some significant differences ($p<0.05$) in the levels of individual and total flavonoids were observed,
413 in general, it was concluded that neither the pasteurization nor the HPH treatments led to marked
414 changes in the flavonoid levels. Similar results have been reported by other authors (Velazquez et
415 al.,2013).
416
417 *3.3.2. Bioaccessibility of flavonoids*
418 Compared to fresh juice, processing had a positive effect on the bioaccessibility of flavonoids (eq.
419 3) in all juices assayed, although pasteurization seemed to provide better results. The improvement
420 achieved was lower relative to carotenoids (**Fig. 3A** for flavones and **Fig. 3B** for flavanones).

421 Considering flavonoid bioaccessible contents (FBC) (that is, the amount of flavonoid present in the
422 digestates per litre of juice) significant changes in the bioaccessibility of individual compounds,
423 total flavones, total flavanones, and total flavonoids were found across samples (**Table S2**). In
424 general, the treatments led to increases in the bioaccessibility. Considering total flavonoids the most
425 remarkable increase was observed in the P6515 sample (~ 2-fold increase relative to the fresh
426 juice). The HPP treatment increased the bioaccessibility compared to fresh juice by 1.43 fold
427 although this increase was not statistically significant. Our findings were in good agreement with
428 those reported by He et al. (2016), who observed a clear bioaccessibility increase of total phenolics
429 in pasteurized (80 ºC-30 min and 90 ºC-30 s) orange juices but not a significant increase in HPH
430 treated samples compared to the fresh juice. In humans, it was observed that flavanones from an
431 HPH Ortanique juice exhibited a better absorption than its fresh and pasteurized counterparts in
432 individuals with high excretion capabilities, finding no significant differences in medium- and low-
433 excretion volunteers (Tomás-Navarro et al., 2014). Previous investigations indicated that structural
434 changes caused by tomato sauce processing treatment positively affected bioaccessibility of
435 phenolic compounds (Martínez-Huélamo et al. 2015). Although in the present study a clear
436 association between particle size reduction caused by the treatments tested and enhanced
437 bioaccessibility for carotenoid was observed, this was not the case for flavonoids.
438

439 ## 4. Conclusions

440 High-pressure homogenization and pasteurization have different effects on physicochemical
441 parameters, ascorbic acid content and the contents and *in vitro* bioaccessibility of carotenoids and
442 flavonoids of Ortanique mandarins. While HPH treatments had a better effect on ascorbic acid
443 retention, pasteurization showed a significantly higher positive effect on bioaccessibility of
444 flavonoids. The most remarkable increase was observed in sample pasteurized at 65 °C (~ 2-fold
445 increase relative to the fresh juice). HPH reduced significantly the carotenoid content but showed a

positive effect on their bioaccesibility (around 5-fold considering total carotenoids) probably due to changes in the particles that favored the attack by digestive enzymes, as it can be inferred from correlations between bioaccessibility values and particle size or specific surface area. .Such positive effect was also remarkable in the case of epoxycarotenoids which were readily detected in micellar fractions studied despite they are not considered bioavailable. As a negative aspect color was significantly affected by this treatment, This study points to the usefulness of the energy-saving HPH treatment to produce health-promoting mandarin juices mainly through the enhancement of the bioavailability of their carotenoids.

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Conflict of interest

The authors declare no conflict of interest.

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633
634

Figure captions

Figure 1. Representation of the colorimetric a* and b* values of the different juices assayed. Number of technical replicates, n=3.

Figure 2. Bioaccessibility (%) of carotenoids (A) and epoxycarotenoids (B) from the different types of samples studied. Nomenclature used for juices: FJ, fresh juice; P9230, pasteurized juice at 92 ºC-30s; P8515, pasteurized juice at 85 ºC-15s; P8515, pasteurized juice at 65 ºC-15s; H150, homogenized juice at 150 MPa.
Different letter within the bars indicate statistically significant differences (p<0.05). Number of technical replicates, n=3.

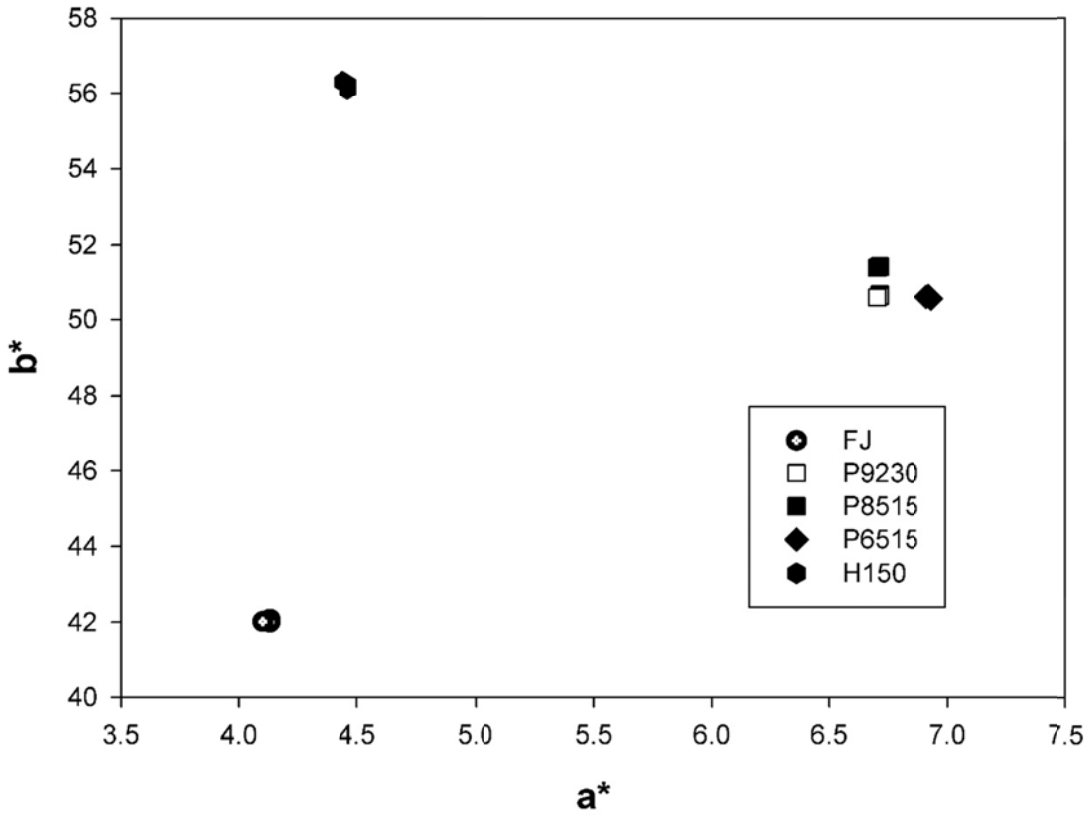
Figure 3. Bioaccessibility (%) of flavonoids (A, flavones; B, flavanones) from the juices studied. Nomenclature used: FJ, fresh juice; P9230, pasteurized juice at 92 ºC-30s; P8515, pasteurized juice at 85 ºC-15s; P8515, pasteurized juice at 65 ºC-15s; H150, homogenized juice at 150 MPa.
Different letter within the bars indicate statistically significant differences (p<0.05). Number of technical replicates, n=3.

Figure S1. Particle size distribution of the different types of samples assayed. Representation made with averaged results from three technical replicates.

Highlights

- Bioaccessibility of carotenoids and phenolics from mandarin juices was studied.
- Pasteurization and energy-saving high-pressure homogenization (HPH) were compared.
- Five-fold increases in total carotenoids bioaccessibility by HPH processing observed.
- Overall, bioaccessibility of flavonoids remained unchanged by both treatments.
- HPH is a sustainable option to obtain juices with improved nutritional properties.

Fig. 1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b*
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6.0
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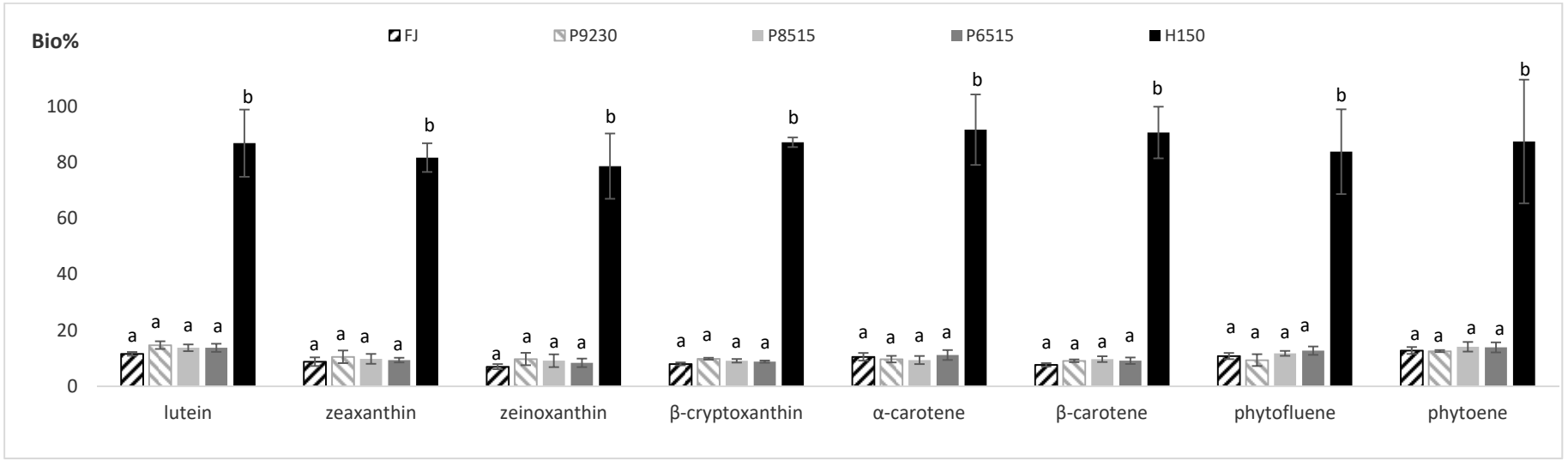
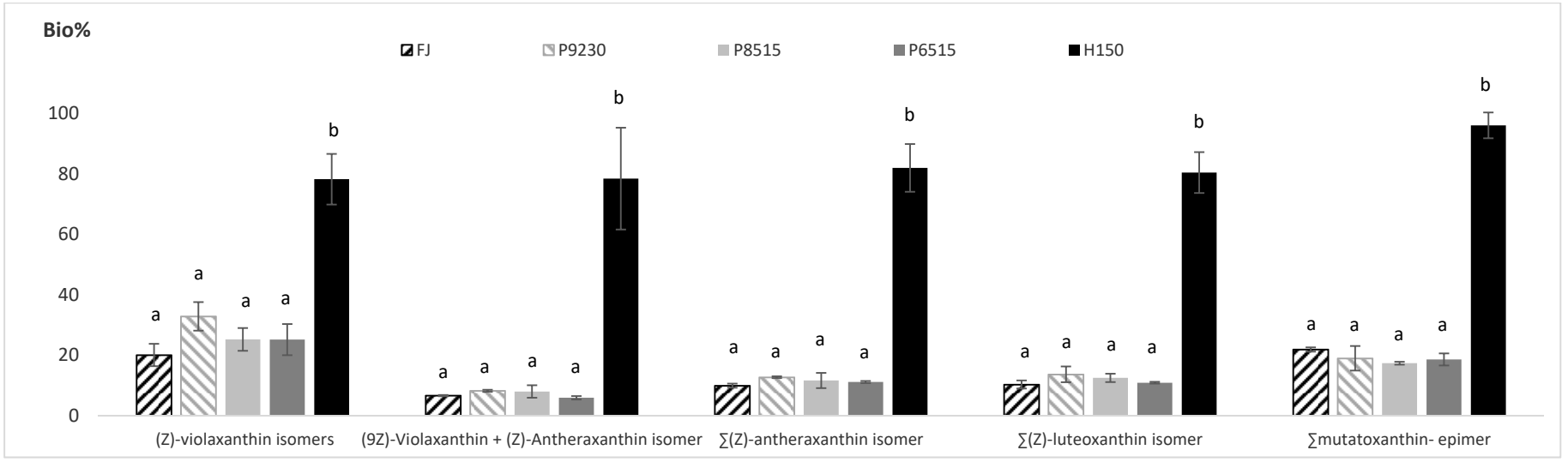
7.0
7.5
a*
FJ
P9230
P8515
P6515
H150

Fig. 2

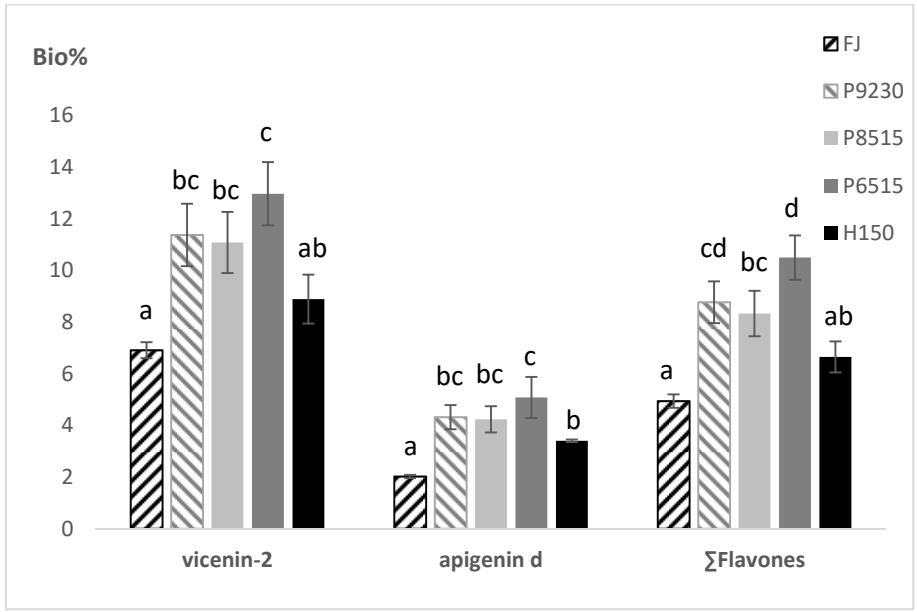

Bio%
FJ
P9230
P8515
P6515
H150
100
80
60
40
20
0
lutein
zeaxanthin
zeinoxanthin
β-cryptoxanthin
α-carotene
β-carotene
phytofluene
phytoene

(A)


Bio%
FJ
P9230
P8515
P6515
H150
100
80
60
40
20
0
(Z)-violaxanthin isomers
(9Z)-Violaxanthin + (Z)-Antheraxanthin isomer
∑(Z)-antheraxanthin isomer
∑(Z)-luteoxanthin isomer
∑mutatoxanthin- epimer

(B)

Fig 3


Bio%
FJ
P9230
P8515
P6515
H150
16
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12
10
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6
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a
bc
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c
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b
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cd
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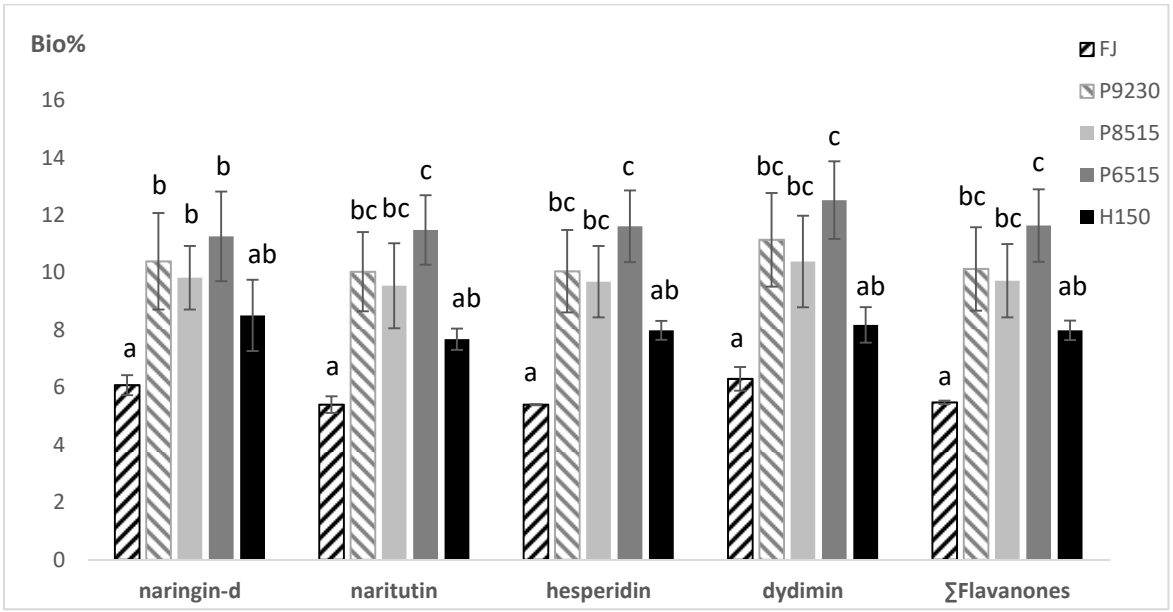
d
ab
vicenin-2
apigenin d
∑Flavones

(A)


Bio%
FJ
P9230
P8515
P6515
H150
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c
ab
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bc
bc
c
ab
a
bc
bc
c
ab
naringin-d
naritutin
hesperidin
dydimin
∑Flavanones

(B)

Table 1. Physicochemical and colorimetric parameters (including colour differences ΔE^*_{ab}) of Ortanique juices assayed [a].

[b]Parameters	FJ	P9230	P8515	P6515	H150
pulp (%)	4.45±0.03a	4.77±0.15a,b	5.00±0.00b,c	5.30±0.15c	2.00±0.00d
transmitance (%)	16.07±0.59a	8.63±0.04b	9.88±0.04c	9.50±0.06b,c	18.53±0.04d
ascorbic acid (mg/L)	383.0±1.15a	353.0±0.58b	363.7±0.88c	358.0±0.58d	368.0±0.58e
PME activity (nkat/mL)	0.418±0.014a	0.000b	0.090±0.023c	0.154±0.015d	0.164±0.021d
L*	36.08±0.01a	40.74±0.02b	41.18±0.01c	40.71±0.01b	45.30±0.01d
[c]C^*_{ab}	42.23±0.02a	51.07±0.02b	51.87±0.01c	51.05±0.02b	56.4±0.05d
[c]h_{ab}	84.41±0.01a	82.46±0.01b	82.33±0.01c	82.34±0.01c	85.47±0.01d
ΔE^*_{ab} FJ	–	10.12	11.04	10.10	16.93
$D_{[4,3]}$ (µm)	329.46±5.89a	367.60±2.60b	352.36±2.99b	362.07±4.79b	55.58±2.47c
$D_{[3,2]}$ (µm)	48.42±1.11a	61.25±0.35b	57.09±0.35b	57.59±0.78b	31.55±1.62c
SSA (m^2/g)	0.12±0.01a	0.10±0.01b	0.11±0.01a,b	0.10±0.01a,b	0.19±0.01c
d(0.1) (µm)	18.59±0.58a	28.53±0.31b	23.71±0.25c	23.78±0.55c	16.49±0.77a
d(0.5) (µm)	282.45±7.14a	325.59±2.42b	304.84±3.76c	318.42±4.65bc	48.04±2.28d
d(0.9) (µm)	715.95±9.60a	769.45±5.84b	752.46±5.04b	765.91±9.44b	106.58±4.45c

[a] FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; H150, homogenized juice at 150 MPa.
Different letters within the same row indicate statistically significant differences ($p < 0.05$).
[b]Description of parameters detailed in main text.
[c]Calculated from a* and b* values found in samples (see **Fig. 1**).

Table 2. Carotenoid contents (mg/L) and Retinol Activity Equivalents (RAE, in µg/L) of the Ortanique juices studied.

Carotenoids	FJ	P9230	P8515	P6515	H150
antheraxanthin isomers	1.798±0.065 c	1.119±0.141 a	1.349±0.045 ab	1.507±0.033 bc	1.090±0.252 a
all-(*E*)-violaxanthin + (*Z*)-violaxanthin isomers	0.464±0.027 c	0.237±0.053 a	0.299±0.008 ab	0.357±0.039 b	0.289±0.036 ab
luteoxanthin + (*Z*)-antheraxanthin isomer	0.970±0.084 c	0.724±0.053 ab	0.737±0.044 ab	0.861±0.042 bc	0.635±0.123 a
(9*Z*)-violaxanthin +antheraxanthin	5.344±0.183 c	2.947±0.511 a	3.709±0.100 ab	4.071±0.318 b	2.923±0.527 a
(*Z*)-luteoxanthin isomer	0.610±0.073 bc	0.502±0.072 ab	0.589±0.068 abc	0.705±0.064 c	0.423±0.031 a
mutatoxanthin- epimer A	0.695±0.052 c	0.570±0.015 ab	0.571±0.004 ab	0.624±0.018 bc	0.486±0.079 a
lutein	0.731±0.057 c	0.600±0.017 ab	0.602±0.004 ab	0.659±0.020 bc	0.508±0.087 a
mutatoxanthin- epimer B	1.047±0.027 ab	0.994±0.080 ab	0.972±0.104 ab	1.101±0.066 b	0.828±0.151 a
zeaxanthin	1.662±0.053 d	1.185±0.032 ab	1.366±0.035 bc	1.492±0.154 cd	0.981±0.104 a
(9*Z*)- or (9´*Z*)-antheraxanthin	3.333±0.219 d	2.174±0.193 ab	2.594±0.088 bc	2.711±0.267 c	1.855±0.174 a
zeinoxanthin	0.758±0.080 c	0.526±0.032 ab	0.614±0.011 abc	0.663±0.071 bc	0.451±0.089 a
β-cryptoxanthin	4.288±0.044 d	2.888±0.176 ab	3.383±0.046 bc	3.740±0.354 cd	2.337±0.295 a
α-carotene	0.185±0.007 b	0.141±0.024 a	0.151±0.011 ab	0.171±0.011 ab	0.141±0.012 a
β-carotene	1.856±0.054 c	1.340±0.097 b	1.458±0.019 b	1.612±0.188 bc	0.993±0.147 a
phytofluene	2.120±0.078 c	1.643±0.090 b	1.644±0.086 b	1.797±0.042 b	1.300±0.154 a
phytoene	3.495±0.222 d	2.971±0.155 bc	2.869±0.184 b	3.433±0.089 cd	2.295±0.186 a
∑Total Carotenoids	29.347±1.057 d	20.564±1.279 ab	22.907±0.520 bc	25.504±1.650 cd	17.535±2.149 a
RAE	341.063±3.263 d	238.026±16.294 b	268.767±3.002 bc	297.312±30.186 cd	185.996±24.375 a

[Φ] FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; P8515, pasteurized juice at 65 ºC 15s; H150, homogenized juice at 150 Mpa.

Different letters within the same row indicates statistically significant differences ($p < 0.05$).

Table 3. Carotenoid levels (mg/L) and Retinol Activity Equivalents (RAE, in ug/L) in the micellar fraction (carotenoid bioaccesible content) after in vitro digestion of the Ortanique juices studied.

Carotenoids	ZF	P9230	P8515	P6515	H150
antheraxanthin isomers	0.263±0.025 a	0.219±0.019 a	0.230±0.031 a	0.263±0.029 a	0.865±0.066 b
all-(*E*)-violaxanthin + (*Z*)-violaxanthin isomers	0.092±0.012 a	0.076±0.009 a	0.075±0.013 a	0.089±0.014 a	0.224±0.009 b
luteoxanthin + (*Z*)-antheraxanthin isomer	0.106±0.007 a	0.109±0.015 a	0.107±0.012 a	0.112±0.008 a	0.516±0.045 b
(9*Z*)-violaxanthin +antheraxanthin	0.354±0.011 a	0.241±0.038 a	0.294±0.069 a	0.241±0.037 a	2.256±0.375 b
(*Z*)-luteoxanthin isomer	0.055±0.002 a	0.058±0.017 a	0.059±0.011 a	0.058±0.006 a	0.328±0.024 b
mutatoxathin- epimer A	0.080±0.003 a	0.082±0.007 a	0.078±0.007 a	0.085±0.011 a	0.402±0.033 b
lutein	0.084±0.003 a	0.088±0.008 a	0.083±0.008 a	0.091±0.011 a	0.436±0.036 b
mutatoxanthin- epimer B	0.302±0.021 a	0.212±0.042 a	0.190±0.016 a	0.235±0.027 a	0.859±0.194 b
zeaxanthin	0.146±0.026 a	0.125±0.029 a	0.133±0.022 a	0.140±0.028 a	0.804±0.123 b
(9*Z*)- or (9´*Z*)-antheraxanthin	0.244±0.023 a	0.199±0.034 a	0.226±0.056 a	0.206±0.041 a	1.532±0.224 b
zeinoxanthin	0.053±0.012 a	0.051±0.014 a	0.056±0.014 a	0.055±0.012 a	0.350±0.051 b
β-cryptoxanthin	0.341±0.019 a	0.284±0.027 a	0.307±0.018 a	0.329±0.044 a	2.034±0.217 b
α-carotene	0.019±0.002 a	0.014±0.001 a	0.014±0.001 a	0.019±0.004 a	0.130±0.027 b
β-carotene	0.142±0.012 a	0.122±0.008 a	0.141±0.013 a	0.147±0.030 a	0.893±0.077 b
phytofluene	0.229±0.031 a	0.152±0.028 a	0.193±0.021 a	0.228±0.032 a	1.081±0.150 b
phytofluene	0.446±0.060 a	0.372±0.008 a	0.402±0.028 a	0.476±0.058 a	1.981±0.332 b
∑Total Carotenoids	**2.957±0.220 a**	**2.404±0.251 a**	**2.587±0.295 a**	**2.774±0.305 a**	**14.539±1.719 b**
RAE	**26.850±1.347 a**	**22.537±1.548 a**	**25.121±1.788 a**	**26.791±4.493 a**	**164.539±15.377 b**

$^{\Phi}$ FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; P8515, pasteurized juice at 65 ºC 15s; H150, homogenized juice at 150 Mpa.

Different letters within the same row indicates statistically significant differences ($p < 0.05$).

Table S1. Flavonoid contents (mg/L) in the juices assayed.

Flavonoids	FJ	P9230	P8515	P6515	H150
vicenin-2	26.79±0.22a	27.96±0.45ab	27.87±0.24ab	28.03±0.80b	27.29±0.25ab
apigenin-d	11.36±0.13a	9.91±0.36b	10.39±0.13bc	9.23±0.33d	10.90±0.19ac
∑Flavones	**42.11±0.36a**	**41.12±1.38ab**	**42.33±0.24a**	**39.09±1.98b**	**42.07±0.15ab**
naringin-d	13.61±0.19a	12.96±0.64a	13.56±0.17a	13.40±0.37a	13.25±0.28a
narirutin	33.55±0.31a	33.39±1.13a	34.08±0.71a	32.50±1.16a	33.68±0.59a
hesperidin	251.28±0.49ab	250.93±6.89ab	252.57±1.01b	238.78±8.32a	248.53±1.00ab
didymin	19.41±0.38a	19.52±0.71a	19.9414±0.67a	19.00±0.80a	19.81±0.28a
∑Flavanones	**317.85±0.93a**	**316.80±8.39a**	**320.16±2.49a**	**303.68±10.46a**	**315.27±1.12a**
∑Flavanoids	**359.96±1.28ab**	**357.92±9.77ab**	**362.49±2.72b**	**342.77±11.58a**	**357.34±1.26ab**

$^{\Phi}$ FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; P8515, pasteurized juice at 65 ºC 15s; H150, homogenized juice at 150 Mpa.
Different letters within the same row indicates statistically significant differences ($p < 0.05$).

Fig. S1

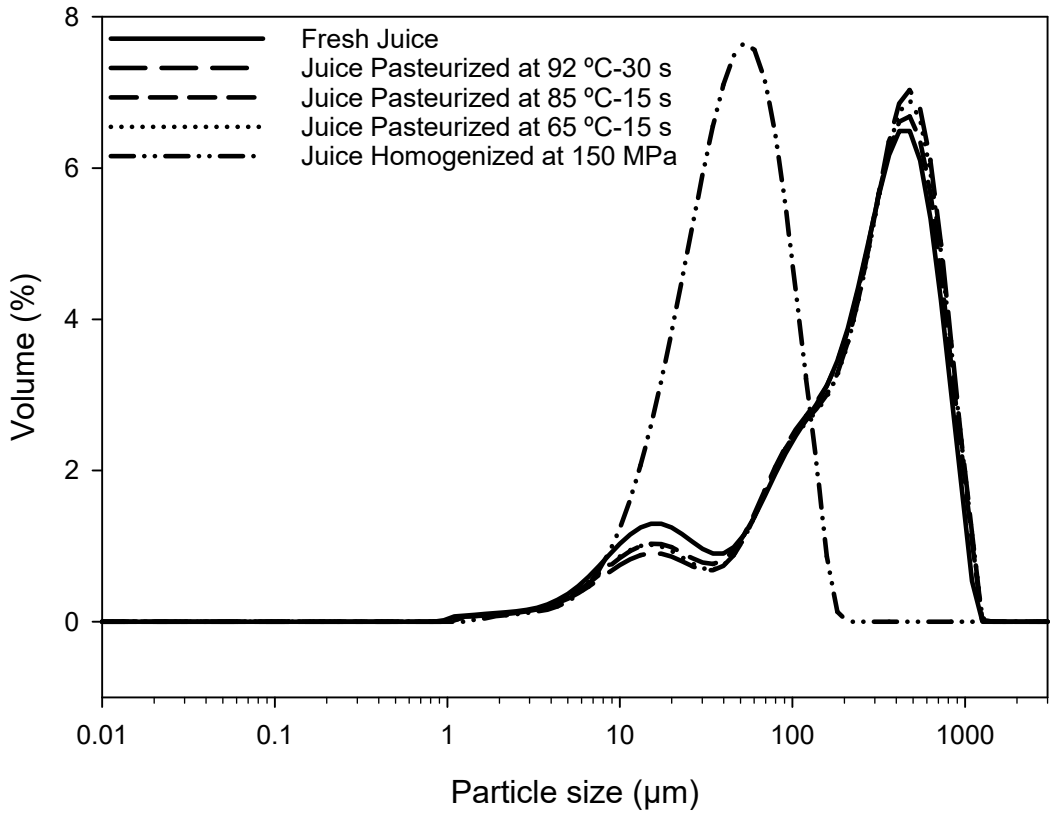
Fresh Juice
Juice Pasteurized at 92 ºC-30 s
Juice Pasteurized at 85 ºC-15 s
Juice Pasteurized at 65 ºC-15 s
Juice Homogenized at 150 MPa
Volume (%)
0
2
4
6
8
0.01
0.1
1
10
100
1000
Particle size (µm)

Table S2. Flavonoid levels in the micellar fraction (flavonoids bioaccesible content) after *in vitro* digestion of the Ortanique juices assayed. The results are expressed as mg of flavonoid in the digestate per L of juice.

Flavonoids	FJ	P9230	P8515	P6515	H150
vicenin-2	1.85±0.10a	3.18±0.37bc	3.09±0.31bc	3.64±0.41c	2.43±0.24ab
apigenin-d	0.23±0.07a	0.43±0.06b	0.44±0.06b	0.47±0.09b	0.37±0.01ab
∑Flavones	**2.08±0.10a**	**3.61±0.43bc**	**3.53±0.36bc**	**4.11±0.c**	**2.80±0.25ab**
naringin-d	0.83±0.04a	1.35±0.23b	1.33±0.14b	1.51±0.25b	1.13±0.16ab
narirutin	1.81±0.09a	3.36±0.56bc	3.25±0.45bc	3.74±0.51c	2.59±0.10ab
hesperidin	13.57±0.07a	25.27±4.12bc	24.46±3.05bc	27.78±3.75c	19.85±0.75ab
didymin	1.22±0.08a	2.18±0.38bc	2.07±0.25bc	2.38±0.31c	1.62±0.11ab
∑Flavanones	**17.43±0.19a**	**32.15±5.29bc**	**31.10±3.88bc**	**35.42±4.81c**	**25.18±0.99ab**
∑Flavanoids	**19.51±0.29a**	**35.76±5.71bc**	**34.62±4.23bc**	**39.53±5.31c**	**27.97±1.08ab**

$^{\Phi}$ FJ, fresh juice; P9230, pasteurized juice at 92 ºC 30s; P8515, pasteurized juice at 85 ºC 15s; P8515, pasteurized juice at 65 ºC 15s; H150, homogenized juice at 150 Mpa.

Different letters within the same row indicates statistically significant differences ($p < 0.05$).