**EFFECT OF HIGH-PRESSURE PROCESSING ON CAROTENOIDS, FLAVONOIDS AND
VITAMIN C IN JUICE OF THE SWEET ORANGES 'NAVEL' AND THE RED-FLESHED
'CARA CARA'**

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Abstract

This work examines the effect of high-pressure processing (HPP), applied to either juices or to whole-peeled fruits from ordinary Navel and red-fleshed 'Cara Cara' (CC) sweet oranges, on the extractability and stability of carotenoids, flavonoids, vitamin C and hydrophilic antioxidant activity. HPP at 200 MPa/25°C/1 min (HPP-200) were assayed as pre-treatment on whole-peeled orange fruit before to juicing. Orange juices obtained from untreated and high-pressure-treated peeled oranges (HPP-200) were subsequently processed at 400 MPa/40°C/1 min (HPP-400 and HPP-200-400). HPP-400 applied on freshly-prepared orange juice reduced bioactive compounds, mainly in N-juices, an effect that could be related with the mechanical juicing procedure (jar-blender) used or/and with the pressure-induced activation of detrimental food enzymes. HPP-200 and HPP-200-400 could be considered optimal to produce functionalized N-juices since the concentration of hesperidin (25% and 16%), narirutin (27% and 9%), phytoene (40% and 97%) and phytofluene (9- and 12-fold) were increased, and the vitamin C and the antioxidant activity were unchanged compared to untreated freshly-prepared juice. These two HPP treatments preserved flavonoids and vitamin C in CC-juice, but decreased ~16% total carotenoids, mainly lycopene due to its more exposed deposition in the food matrix. However, all the HP-treated CC-juices presented higher carotenoid content than the corresponding N-juices, and can be then considered an excellent dietary source of these compounds. Therefore, HPP did not modified the profiling of bioactive compounds of 'Navel' (N) and 'Cara Cara' (CC) juices but the concentration of total and individual carotenoids, flavonoids, vitamin C and antioxidant activity was differentially affected depending on the type of bioactive compounds and its position in the food matrix, the orange cultivar and the HPP conditions.

Keywords: HPP; bioactive compounds; antioxidant activity; Navel orange juice; Cara Cara orange juice.

1. Introduction

Citrus is the main fruit-tree crop in the world with a production close to 125 million tons (Mt) in 2017, being Spain the sixth producer in the world with an estimated production of 6.32 Mt. Among *Citrus* species and varieties, sweet orange (*Citrus sinensis*, Osbeck) is the main crop with a total world production of 73.3 Mt (FAOSTAT, 2019). The high consumption of oranges as both juice and fresh-fruit, made of this crop an important dietary source of bioactive compounds such as vitamin C, carotenoids (namely β -cryptoxanthin, lutein, zeaxanthin and β -carotene) and polyphenols (mainly flavanones such as hesperidin, naringenin and narirutin) which may act in concert (additively or synergistically) to provide their antioxidant, anti-inflammatory and other health-related properties (Gironés-Vilaplana, Moreno, & García-Viguera, 2014; Zou, Xi, Hu, Nie, & Zhou, 2016) as the prevention of cardiovascular diseases, atherosclerosis, certain types of cancers, diabetes and lowering blood lipid levels (Liu, Liou, Hong, & Liu, 2017; Lv et al., 2015).

However, the type and concentration of bioactive compounds responsible of the health promoting properties of orange fruits and juices are diverse and vary among the cultivar, environmental and agronomical practices, pre- and postharvest conditions, and processing characteristics (Brasili et al., 2017; Vieira et al., 2018).

Among sweet oranges, there is an increasing interest in the red-fleshed 'Cara Cara' (CC), a spontaneous mutant from the blonde-flesh 'Washington Navel' (N), by the attractive red color of its pulp due to the accumulation of lycopene (Alquézar, Rodrigo, & Zacarías, 2008; Brasili et al., 2017; Lee, 2001; Tao, Wang, Xu, & Cheng, 2012). 'Cara Cara' oranges presented higher carotenoid and flavonoid content and better hydrophilic antioxidant characteristics than the ordinary 'Navel' orange (De Ancos, Cilla, Barberá, Sánchez-Moreno & Cano, 2017; Rodrigo, Cilla, Barberá & Zacarías, 2015). Specifically, the pulp of CC orange presented higher bioactive content than the corresponding fresh juice (De Ancos et al., 2017; Rodrigo et al., 2015). Moreover, the bioaccessible fraction of pulps from CC oranges provided a better protection of the cells from oxidative stress and

77 diminished lipid peroxidation and ROS than their corresponding juices (Cilla et al., 2018). These
78 previous results indicated the convenience of the intake of CC orange pulp (segments or slices) than
79 the corresponding fresh juice considering the content of bioactive compounds, bioaccessibility and
80 protection against cellular oxidative stress. However, it is well known that orange juices are more
81 easily consumed than the whole fruit, especially for children, elderly, athletes and patients, among
82 others. Moreover, it has been reported that the reduction of dietary fiber in fresh squeezed ordinary
83 sweet orange juice increased content in micellar carotenoids and their bioaccessibility compared to
84 fruit pulp (Aschoff et al., 2015).

85 The phytochemical composition and antioxidant characteristics of juices obtained from CC red-
86 fleshed orange rich in carotenoids (Brasili et al., 2017; De Ancos et al., 2017; Rodrigo et al., 2015)
87 has been less studied than that of blood orange juices rich in anthocyanins (Fallico, Ballistreri,
88 Arena, Brighina, & Rapisarda, 2017) or ordinary sweet orange juices (Vieira et al., 2018).
89 Specifically, 'Cara Cara' fresh squeezed juice contained higher concentration of the major
90 flavonoids, hesperidin and narirutin than N-juice (De Ancos et al., 2017). More significantly, total
91 carotenoids were between 5 and 10-times higher in CC- than in N-juice, mainly due to phytoene
92 and phytofluene (5 and 130-fold) and lycopene (190 to 540 µg/100 mL) which was not detected in
93 N-juice (Rodrigo et al., 2015). Despite of the CC-juice is not yet commercially available, different
94 thermal treatments have been assayed showing important losses of bioactive compounds, mainly
95 carotenoids, depending on the type of compounds and orange varieties (N and CC) (Brasili et al.,
96 2017; Lu et al., 2017).

97 High-pressure processing (HPP) has been proposed as an alternative system to the traditional
98 thermal processing technologies to obtain safe, nutritive fresh-tasting plant-derived products such as
99 orange juice, avoiding the degradation of nutrients and bioactive compounds (Barba et al., 2015;
100 Plaza et al., 2006; Sánchez-Moreno et al., 2005; Sánchez-Moreno, De Ancos, Plaza, L., Elez-
101 Martínez, & Cano, 2009; Vervoort et al., 2011; Zulueta, Esteve, & Frígola, 2016) as well as
102 inhibiting microbial growth (Georget et al., 2015) and enzyme activity (Terefe, Buckow, &

Versteeg, 2014). Also, HPP affected the natural matrix or the microstructure of food which may results on improved extractability of bioactive compounds and also their bioaccessibility in foods like orange juice alone or immerse in a food complex as beverages mix of fruit juice and milk. Thus, HPP at 400 MPa/40 °C/1 min was enough to increase the concentration of total carotenoids (54%) and total flavanones (35%) extracted from N-juice with pulp (Sánchez-Moreno et al., 2005). Cilla et al. (2012) reported that lutein, zeaxanthin, and neoxanthin+9-Z-violaxanthin improved their concentration (between 53 and 99%) in beverages made with fruit juices (mainly orange juice) and milk or soymilk treated by HPP (400 MPa/40°C/5 min). Similar results were also found by Rodríguez-Roque et al. (2016) that observed an increase of 9-Z-violaxanthin, antheraxanthin, lutein and zeaxanthin (between 12 and 37%) in a high-pressure (HP) treated milk-fruit beverage. Regarding flavonoids, hesperidin and naringenin from a milk-fruit beverages increased (~36%) after HPP at 400 MPa/40°C/5 min (Rodríguez-Roque et al., 2015). Moreover, HPP increased the extraction of carotenoids and polyphenol compounds from vegetable tissues, such as pieces of persimmon, papaya and onion. Thus, HPP at 200 MPa/25°C/1 min increased the total carotenoid content extracted from slices of papaya 'Sunrise' by 53% (De Ancos, Crespo, Sánchez-Moreno, Elez-Martínez, Cano, 2007). Among the different treatments (200-400 MPa/25 °C/1-6 min) assayed with astringent persimmon cubes, HPP at 200 MPa/25 °C/6 min produced the highest increase (86%) in the extraction of total carotenoids (Hernández-Carrion, Vázquez-Gutiérrez, Hernando, Quiles, 2014; Plaza, Colina, De Ancos, Sánchez-Moreno, & Cano, 2012). With respect to flavonoids, HPP at 200, 400 and 600 MPa at 25 °C for 5 min applied to onion pieces increased the extractability of quercetin glycosides by 34%, 21% and 38%, respectively. Therefore, HPP could be a useful technology to obtain functional food or food ingredients of plant origin (González-Peña et al., 2013).

Based on the above mentioned considerations, the aim of this study was to evaluate the application of high-pressure processing (HPP) as a pretreatment on whole peeled orange fruit before juicing, in comparison with the application of HPP directly to freshly prepared orange juice, on the

129 extractability and stability of carotenoid, flavonoid, vitamin C and the hydrophilic antioxidant
130 activity in the red-fleshed 'Cara Cara' (CC) orange juice and the ordinary sweet 'Navel' (N) orange
131 juice.

132

133 **2. Materials and methods**

134 **2.1. Reagents**

135 *Vitamin C analysis:* Glacial acetic acid, metaphosphoric acid and formic acid and L(+) ascorbic
136 acid ($\geq 99\%$ purity) (LAA) were purchased from Panreac Química (Barcelona, Spain).

137 *Flavonoid analysis:* Methanol and acetonitrile (HPLC-grade) were provided by Lab-Scan (Dublin,
138 Ireland). Formic acid and hydrochloric acid were purchased from Panreac Química (Barcelona,
139 Spain). Narirutin (Naringenin-7-*O*-rutinoside) was acquired from Extrasynthèse (France).
140 Hesperidin (hesperitin-7-*O*-rutinoside), eriodictiol-*O*-rutinoside (eriocitrin), naringenin-7-*O*-
141 rutinoside (narirutin), hesperetin-7-*O*-rutinoside (hesperidin), isosakunetin-7-*O*-rutinoside
142 (dydimin), quercetin-3-rutinoside (rutin), apigenin were purchased from Sigma (St. Louis, MO,
143 USA). Stock solutions of 1 mg/mL in methanol of authentic flavonoid standards were prepared.

144 *Carotenoid analysis:* Methanol, chloroform, dichloromethane and acetone (HPLC-grade) were
145 supplied by Scharlau (Barcelona, Spain) and methyl tert-butyl ether (MTBE) by Merck (Darmstadt,
146 Germany). Petroleum ether and diethyl ether were of analytical grade and were supplied by
147 Scharlau (Barcelona, Spain). Commercial standards of β -carotene, and lycopene were purchased
148 from Sigma-Aldrich (Darmstadt, Germany); β -cryptoxanthin and zeaxanthin from Extrasynthese
149 (Lyon, France); all-*E*-violaxanthin and all-*E*-antheraxanthin from CaroteNature (Lupsingen,
150 Switzerland). Standards of phytoene and phytofluene were obtained from flavedo extracts of
151 Pinalate fruits (Rodrigo, Marcos, Alférez, Mallent & Zacarías, 2003), and afterwards purified by
152 thin layer chromatography and the corresponding calibration curves performed (Lado et al., 2015)

153 *Hydrophilic antioxidant activity analysis:* 2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid]
154 (ABTS^{•+}), potassium persulfate (K₂S₂O₈), iron (III) choride hexahydrate, phosphate buffered saline,

155 hexadecyltrimethyl-ammonium bromide, 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]), and Folin-
156 Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-(1-
157 naphthyl)ethylenediaminedihydrochloride and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) were obtained
158 from Fluka Chemie AG (Buchs, Switzerland). L(+)-Ascorbic acid ($\geq 99\%$ purity) and ethanol were
159 obtained from Panreac Química (Barcelona, Spain).

160 **2.2. Preparation of Samples**

161 Ordinary sweet orange 'Washington Navel' (N) (*Citrus sinensis* L. Osbeck) and its spontaneous
162 red-fleshed mutant 'Cara Cara' (CC) (rich in carotenes) at full mature stage (soluble solid, acidity
163 and ripening index were, 12.6 °Brix, 0.957 % and 13.2, respectively, for 'Navel' orange; and soluble
164 solid, acidity and ripening index were, 11 °Brix, 0.934 % and 11.7, respectively, for CC orange)
165 were harvested from adult trees from the Citrus Germplasm Bank at the Instituto Valenciano de
166 Investigaciones Agrarias (IVIA, Moncada, Valencia, Spain). Then, freshly harvested N and CC
167 oranges were transported to ICTAN and stored at 2 °C for two days until they were processed.

168 Four lots of 20 fruits from each type of orange fruits (N and CC) were separated, peeled and
169 processed according to the design showed in Figure 1. Orange citrus juices with low proportion of
170 pulp (8%, w/v) were obtained from unprocessed and high-pressure-processed whole peeled fruits
171 (N and CC) (Figure 1) using a domestic jar blender (Oster 4655, USA) during 6 s, filtered
172 employing a 2 mm steel sieves and packed in portions of 250 mL in high barrier flexible Doypack
173 plastic bags (Polyskin XL, Amcor Flexiblehispania, Granollers, Spain) removing the air from the
174 headspace. Whole peeled fruits (N and CC) were also packed in the same type of plastic bags before
175 HPP.

176 **2.3. High-pressure processing (HPP)**

177 **2.3.1. Processing design**

178 The untreated and High-pressure (HP)-treated samples studied in the present work were obtained
179 according to the experimental design showed in Figure 1:

180 **Untreated Juice:** Juices obtained from whole peeled orange fruits (N and CC) by
181 homogenization in a jar-blender (6 s) and subsequent filtering (2 mm).

182 **HPP-200 Juice:** Whole peeled orange fruits (N and CC) were treated at 200 MPa/25 °C/1 min,
183 and then these HP-treated fruits were homogenization in a jar-blender (6 s) and subsequent filtering
184 (2 mm) to obtain a juice.

185 **HPP-400 Juice:** Juices were obtained from whole peeled orange fruits (N and CC) homogenized
186 in jar-blender (6 s), filtered (2 mm), and then treated at 400 MPa/40 °C/1 min.

187 **HPP-200-400 Juice:** HPP-200 juice were treated at 400 MPa/40 °C/1 min.

188 **2.3.2. High-pressure processing conditions**

189 *Citrus* products (juice and whole peeled fruit) were treated in a hydrostatic pressure unit with a
190 vessel of 2925 mL capacity, a maximum pressure of 900 MPa, and a maximum temperature of 100
191 °C (High Pressure Iso-Lab System, Model FPG7100:9/2C, Stansted Fluid Power LTD., Essex, UK).
192 Citrus products were introduced in the pressure unit vessel filled with pressure medium (water) and
193 processed in the conditions shown above. The compression and decompression rates were both 3
194 MPa/s. Taking into account the adiabatic compression, the maximum temperature in the vessel was
195 25 °C at 200 MPa and 40 °C at 400 MPa. Pressure, time and temperature were controlled by a
196 computer program, being constantly monitored and recorded during the process. These conditions
197 were selected based on previous studies where the nutritional and microbiological stability of high-
198 pressure-processed fruit juices were achieved (Muñoz, De Ancos, Sánchez-Moreno, & Cano, 2007;
199 Rodríguez-Roque et al., 2015).

200 **2.4. Carotenoid determination**

201 Carotenoids from orange juices were extracted, identified and quantified by HPLC-DAD as
202 previously described by Rodrigo et al. (2015). Briefly, juice (2 mL) was weighted in screw-capped
203 polypropylene tubes (15 mL), 4 mL of MeOH plus 5 mL Tris-HCl (50 mM, pH 7.5) (containing 1

204 M NaCl) were added and sample was sonicated 5 min in XUBA3 ultrasonic water bath at room
205 temperature (35 ET AL; Grant Instruments, Cambridge, England). Dichloromethane (DCM) (10 ml)
206 was added to the mixture, stirred for 5 min at 4°C and centrifuged at 3000 x g for 10 min at 4°C.
207 The hypophase was recovered and the aqueous phase re-extracted with DCM until it was colorless.
208 The pooled DCM extracts were dried on a rotary evaporator at 40°C. Samples were saponified in
209 methanolic KOH (6%, w/v) overnight. Saponified carotenoids were recovered from the upper phase
210 after adding water and petroleum ether:diethyl ether (9:1) to the mixture. Extracts were dried and
211 kept at -20°C until further analysis. The carotenoid composition of each sample was analyzed by
212 HPLC with a Waters liquid chromatography system equipped with a 600E pump and a model 2998
213 DAD, and Empower software (Waters, Barcelona, Spain). A C30 carotenoid column (250 x 4.6
214 mm, 5 µm) coupled to a C30 guard column (20 x 4.0 mm, 5 µm) (YMC, Teknokroma, Spain) was
215 used.

216 Chromatographic conditions used are described in Lado et al. (2015) and Rodrigo et al. (2015).
217 The carotenoid peaks were integrated at their individual maximal wavelength, and their contents
218 were calculated using the appropriate calibration curves, as described elsewhere (Lado et al., 2015;
219 Rodrigo et al., 2015).

220 ***2.5. Flavonoids determination***

221 Flavonoids were extracted, separated, identified, and quantified by HPLC-DAD and HPLC-ESI-
222 QTOF-MS from orange juice according to the procedure described by De Ancos et al. (2017) with
223 some modifications. Samples of 20 mL of orange juice with pulp was centrifuged at 9000×g for 15
224 min at 4 °C in a refrigerated centrifuge (Thermo Scientific Sorvall, mod. Evolution RC, Thermo
225 Fisher Scientific Inc., USA) and the supernatant was separated. Then, 4 mL of this solution were
226 loaded on a reversed phase C18 Sep-pack cartridge (200 mg of silica based bonded phase, 37x55
227 µm particle size) (Waters, USA), previously activated with 4 mL of methanol and 4 mL of water.
228 Phenolic compounds were recovered from the cartridge by eluting with 2 mL of methanol and

229 filtered through a 0.45µm syringe filter and stored at -80 °C until HPLC-DAD and HPLC-ESI-
230 QTOF-MS analysis were carried out.

231 Results were expressed as mg of flavonoid per 100 mL of orange juice. Total flavonoids content
232 was determined as the sum of individual compounds quantified by HPLC-DAD (TF-HPLC).

233 ***2.6. Vitamin C determination***

234 Vitamin C (ascorbic acid + dehydroascorbic acid) were extracted and quantified by HPLC
235 according to the procedure described by Plaza et al. (2006) using 10 mL of orange juice with pulp.
236 Total vitamin C was determined by the reduction of dehydroascorbic acid to ascorbic acid using
237 DL-dithiothreitol as reductant agent. Quantification was achieved using an ascorbic acid external
238 standard calibration curve in the range from 5 to 500 µg/ mL. Results were expressed as mg of
239 ascorbic acid per 100 mL of orange juice.

240 ***2.7. Antioxidant activity determinations***

241 ***2.7.1 Sample extraction***

242 A 10 mL of orange juice was homogenized with 10 mL of methanol/water (80:20, v/v) for 2 min
243 at 8000 rpm (Omnimixer, model ES-207, Omni International Inc, Gainesville, VA) and centrifuged
244 at 12000 x g for 15 min at 4° C and the supernatant separated. The pellet was re-extracted with 10
245 mL of methanol/water (80:20, v/v) in the same conditions described before. The two supernatants
246 were pooled together in a volumetric flask and made up to 50 mL with distilled water.

247 This solution was considered as the sample solution for antioxidant activity determinations. If
248 the absorbance of the final sample solution was not in the range of the standard curves, further
249 dilutions were required.

250 ***2.7.2. Total Antioxidant activity (ABTS⁺, FRAP, DPPH[•] and TF-FC)***

251 Total antioxidant activity was determined according to the method of Re et al. (1999) for
252 ABTS⁺), the method of Benzie & Strain (1996) (for FRAP), the method of Sánchez-Moreno,
253 Larrauri and Saura-Calixto (1998) (for DPPH[•]), including an adaptation of the methods to 96-well
254 microplate format as previously described by De Ancos et al. (2017). Also, total phenolic

255 compounds performed according to the Folin-Ciocalteu procedure (TP-FC) was used to quantify the
256 sample's reducing capacity according to the procedure previously described (De Ancos et al.,
257 2017).

258 **2.8 Statistical analysis**

259 The results shown represent mean values $\pm$ standard deviation of three replicates obtained in at
260 least two separate experiments. One-way (type of sample) ANOVA was conducted followed by the
261 Tukey *post hoc* test and Student's t test were used to compare pairs of means and determine
262 statistical significance at the $P \leq 0.05$ level. The correlations within variables were examined by
263 Pearson correlation. All analyses were performed by using the IBM SPSS Statistics 22 Core System
264 (SPSS Inc, an IBM Company).

265

266 **3. Results and discussion**

267 The influence of different high-pressure processing (HPP) on the carotenoids, flavonoids,
268 vitamin C and hydrophilic antioxidant activity of the red-fleshed 'Cara Cara' (CC) juice in
269 comparison with the ordinary sweet orange 'Navel' (N) juice have been studied. HPP-200 (200
270 MPa/25 °C/1 min) was assayed on whole peeled orange fruit before juicing with the aim of
271 evaluating its effectiveness increasing the concentration of bioactive compounds (carotenoids,
272 flavonoids and vitamin C) and antioxidant activity in orange juice with respect to HPP-400 (400
273 MPa/25 °C/1min) applied to freshly juice (Sánchez-Moreno et al., 2005). The application of both
274 treatments, 400 MPa/25 °C/1 min followed by HPP-200 juice (HPP-200-400), was also evaluated to
275 study the effect of two consecutive HPP on the extractability and stability of bioactive compounds
276 (for experimental design see Figure 1).

277

278 **3.1. Effect of HPP on carotenoid content and composition**

279 The profile and concentration of individual and total carotenoids of untreated and HP-treated
280 juices of 'Navel' (N) and 'Cara Cara' (CC) oranges are shown in Table 1. The composition of

individual carotenoids in N- and CC-juices found in the current study is in good agreement with previous results (Lado et al., 2015; Alquézar et al., 2008). Moreover, total carotenoid content in untreated CC-juice was significantly higher ($P \leq 0.05$) than in N-juice (2501 ± 85 and 751 ± 15 $\mu\text{g}/100$ mL, respectively) (Table 1) as described in previous studies (Rodrigo et al., 2015; Brasili et al., 2017). However, these values were 43% and 22% higher than the carotenoid content previously reported for freshly squeezed juice from N or CC oranges, respectively, grown under the same environmental and agronomical conditions (Rodrigo et al., 2015). Therefore, the results of the present study indicate a higher extractability of carotenoids from the membrane cells of juice vesicles by the use of a jar-blender with stainless-steel blades in comparison of a household electric juice squeezed utilized in previous works (Lado et al., 2015; Rodrigo et al., 2015).

HP-treated juices presented similar carotenoid profile than the untreated juices but the concentration of individual and total carotenoids was differentially affected depending the HPP treatment and the orange cultivar (Table 1, Figure 2A). The application of 200 MPa/25 °C/1 min to whole peeled 'Navel' fruit had a positive effect on phytoene and phytofluene, since increased their concentrations in the juice by 40% and 10-times, respectively, compared to untreated juice. The other individual carotenoids of N orange were not significantly affected by the HPP-200 treatment (Table 1). The application of HPP at 400 MPa/40 °C/1 min to HPP-200 N-juice produced an orange juice (HPP-200-400) with similar carotenoid concentration than those found in HPP-200 (Table 1). Therefore, both HPP-200 and HPP-200-400 in N-juice have a positive effect on phytoene and phytofluene. It is interesting to note that these two carotenes are highly hydrophobic (Mapelli-Brahm, Meléndez-Martínez, & Bohn, 2017), and HPP-200 treatment may favour their extractability from the cellular structures (Lado et al., 2015; Mapelli-Brahm, Stinco, Rodrigo, Zacarías, & Meléndez-Martínez, 2018) enhancing their release to the juice. In contrast, HPP-400 treatment in freshly prepared N-juice decreased nearly 35% total carotenoid content compared to untreated juice (Table 1, Figure 2A). This effect was due to a general reduction of the concentration of almost all individual carotenoids (ranging from 30 and 50%), whereas only zeaxanthin and phytofluene were

not affected by HPP-400 (Table 1). Interestingly, the reduction (32%) in the concentration of violaxanthin (both isomers) by HPP-400 in N-juice compared to untreated juice, was diminished to a 15% in HPP-200-400 (Figure 1) (Table 1).

In CC-juice, all the HPP assayed produced a moderated reduction, between 12%-20% in total carotenoid content, without significant differences ($P \leq 0.05$) among the treatments (Table 1, Figure 2A). In fact, HPP-400 applied to CC-juice reduced total carotenoid content to lower extent (13%) than in N-juice. Despite this fact, HP-treated CC-juices presented between 3- and 4-times higher total carotenoids than the corresponding HP-treated N-juices (Table 1). Interestingly, while phytoene and phytofluene concentrations in CC-juices were not affected by the three HPP assayed, by 36% of lycopene content was decreased in HPP-200 juice and by 50% in HPP-400 and HPP-200-400 juices (Table 1). Lycopene is a highly apolar carotene responsible for red pigmentation of CC-pulp which accumulates as solid crystalline structures within the chromoplasts of the juice vesicle cells, in contrast to other carotenoids that are stored in small lipid droplets called plastoglobuli (Lado et al., 2015; Lu et al., 2017; Schweiggert & Carle, 2017). The solid crystalline deposition of lycopene may increase the exposure of this carotene to the environment making its structure more unstable and favouring oxidative breakdown. Also, it has been demonstrated that carotenoids bioavailability and bioaccessibility are clearly influenced by their deposition structures in the food matrix (Schweiggert & Carle, 2017; Mapelli-Brahm et al., 2017), and the present results also suggest that this may be a determinant factor for carotenoid stability in the juice upon HPP.

The detrimental effect of HPP-400 treatment on total carotenoid content in both N-juice and CC-juice is not fully consistent with previous works, since an increase or a minor decrease (less than 5%) was reported in juices of ordinary 'Navel' orange processed by similar HPP conditions (Sánchez-Moreno et al., 2005). Different previous results have shown the positive effect of HPP increasing the extraction of carotenoids in orange juice (Barba et al., 2017; Rodrigo et al., 2015; Zuleta et al., 2016). The discrepant results obtained with HPP-400 in the current study may be explained by different factors. Firstly, the different experimental conditions used for fruit juicing.

333 The blender jar used instead of the domestic squeezer increased the efficiency of carotenoids
334 extractability from the pulp and vesicle membrane, and the subsequent HP-treatments assayed were
335 not able to render a further improvement of carotenoid extraction. Secondly, HPP at 400
336 MPa/40°C/1 min in N-juice could favor the degradation of carotenoids caused by the pressure-
337 induced enzyme activation (peroxidase) and thus reducing the concentration of carotenoids
338 extracted by HPP.

339 Considering total carotenoid content and the concentration of the major carotenoids in N- and CC-
340 juices, namely phytoene, phytofluene, lycopene, β -carotene, zeaxanthin and β -cryptoxanthin, HPP-
341 200 and HPP-200-400 treatments may be considered as the best options for both cultivars. It is of
342 particular interest to remark that although the total carotenoid content was reduced in all HP-treated
343 CC-juices compared to untreated juice, the concentration of bioactive carotenoids was not
344 significantly affected, with the exception of lycopene that was reduced between 35-50%. In
345 conclusion, HP-treated CC-juices had higher bioactive carotenoids content than N-juices and were a
346 good source of these compounds.

347

348 **3.2 Effect of HPP on flavonoid content and composition**

349 The profile and concentration of individual and total flavonoids of untreated of HP-treated juices
350 of Navel (N) and Cara Cara (CC) oranges are shown in Table 2. Untreated juice of both N ant CC
351 had statistically ($P \leq 0.05$) similar total flavonoid content (44.31 ± 0.64 and 45.60 ± 5.83 mg/100 mL,
352 respectively) (Table 2). Previous studies reported significant higher total flavonoids content in
353 Navel juice than in CC-juice (De Ancos et al., 2017; Brasili et al., 2017), suggesting that the content
354 of these bioactive compounds could be affected by the environmental and agronomical growing
355 conditions. In the current study, similar flavonoid profile was found in untreated and HP-treated
356 juices of N and CC oranges, being the glycosylated flavanone hesperidin (HES) (~34.5%) and
357 narirutin (NAR) (~26.5%) the majorities. However, the concentration of individual and total

358 flavonoids was differentially affected by the HPP conditions and orange variety (N and CC) (Figure
359 2B, Table 2).

360 The retention of total flavonoid content (TFC) in HP-treated juices compared to those untreated
361 varied significantly ($P \leq 0.05$) depending of the variety, N (between 95.7%-124.2%) and CC (100%)
362 (Figure 2B). Thus, HPP-200 and HPP-200-400 produced a significant increase of total flavonoids
363 by 34% and 7%, respectively, in N-juice in comparison to untreated juice. Thus, HES and NAR in
364 N-juice increased by 24.5% and 27%, respectively, after HPP-200 in comparison with the untreated
365 juice. However, HPP-200-400 (Figure 1) in N-juice also increased but to a lesser extent, the
366 concentration of HES (9%) and NAR (6.5%). The increasing extraction of a bioactive compound
367 from fruits and vegetables treated at 200 MPa/25 °C/1 min has been also described for papaya slices
368 and onion cubes, where a significant increase by 53% of total carotenoid content (De Ancos et al.,
369 2007) and by 21% of quercetin glycosides (González-Peña et al., 2013) were observed. This
370 positive effect of HPP has been attributed to an increase of the membrane permeability and
371 disruption of cell walls and cell organelles that favored the release of bioactive compounds from the
372 tissues and improved their extractability (González-Peña et al., 2013; Vázquez-Gutiérrez et al.,
373 2013; Rodríguez-Roque et al., 2015). This effect of HPP depends on the processing parameters and
374 the food matrix characteristics (Barba et al, 2012; Cilla et al., 2012; Rodríguez-Roque et al. 2015,
375 2016), including the type of cultivar and geographical origin of the fruit (Fernández-Jalao, Sánchez-
376 Moreno, & De Ancos, 2018).

377 As with carotenoids, HPP at 400 MPa/40 °C/5 min applied directly to a freshly obtained N-juice
378 was less effective increasing the extraction of flavonoids than in previous studies (Sánchez-Moreno
379 et al., 2006; Roque et al. 2015), since only produced a significant increase ($p \leq 0.05$) of 4% of HES
380 concentration while NAR and TFC remained unchanged (Table 2). These results agree with those
381 obtained for carotenoids, as discussed above, and it is likely to be due to the procedure used to
382 juicing (jar-blender) and the pressure-induced enzyme activation (peroxidase) that could reduce also
383 the concentration of flavonoids extracted by HPP.

384 Regarding CC-juice, it is remarkable that all the HPP assayed resulted in juices with statistically
385 similar ($P \leq 0.05$) individual and total flavonoid concentration than untreated juice (Table 2, Figure
386 2B). Therefore, the HPP assayed in the current study preserved or indeed increased the extraction of
387 flavonoids and can be used to produce high quality and functional orange juices with increased
388 flavonoids concentration by the selection of appropriate HPP parameters and orange fruit cultivar.

389

390 **3.3. Effect of HPP on vitamin C**

391 Vitamin C content in untreated N-juice (60.18 ± 4.51 mg LAsA /100 mL) was 15% higher than in
392 CC-juice (50.96 ± 1.06 mg LAsA/100 mL) (Table 3). These results agree with those previously
393 found for N- and CC-juices (Plaza et al., 2006; Cano et al., 2008; Aschoff et al., 2015; De Ancos et
394 al., 2017; Brasili et al., 2017; Lu et al., 2018). In general, HPP protect better the thermo-labile
395 molecule of ascorbate than thermal treatments (TT) in vegetable products (Barba, Esteve, &
396 Frígola, 2010). In the current study, less than 5% of vitamin C (ascorbic acid + dehydroascorbic
397 acid) reduction was observed by most of the HP-treatments assayed in both N- and CC-juices
398 (Figure 2C). This low vitamin C depletion is in agreement with the results described in the literature
399 where losses lower than 10% of the initial content were usually observed after HPP on different
400 fruit products, included orange juices (Sánchez-Moreno et al., 2005; Plaza et al., 2006; Esteve &
401 Frígola, 2008). In the present study, only HPP-400 (400 MPa/25 °C/1 min) in N caused a reduction
402 of 30% of vitamin C (Figure 3C). This result may be related to the pressure-induced activation of
403 food enzymes (peroxidase, ascorbate oxidase) as it has been previously described in Muscadine
404 grape juice treated at HPP at 400 MPa/15 min, where the increase of polyphenoloxidase (PPO)
405 activity was related with the loss of 80% of initial ascorbic acid (Del Pozo-Insfran, Del Follo-
406 Martínez, Talcott, & Brenes, 2007). In fact, Cano, Hernández & De Ancos (1997) reported a low
407 peroxidase (POD) inactivation in orange (50%) after HPP at 400 MPa/32 °C/15 min and Vervoort et
408 al. (2011) showed a less efficient POD inactivation (13%) after HPP at 600 MPa/5°C/1 min.
409 Additionally, the action of orange juice enzymes could have been favoured by the increase of the

oxygen dissolved in the juice due to the juicing procedure used in the present study (Escobedo-Avellaneda, Gutiérrez-Urbe, Valdez-Fragoso, Torres & Welte-Chanes, 2015). Nevertheless, the HPP-200 treatment to whole peeled orange did not produce any increase in the vitamin C extraction as it has been previously described when different HPP were applied to comminute orange resulting in a significant increases of vitamin C (Escobedo-Avellaneda et al., 2015). This result may be explained by the increase in the disintegration of the orange tissue produced by the juicing procedure used in the current study that favoured vitamin C extraction, and the HPP may not be able to produce additional increases on the extraction of this compound. Then, the results discussed above showed that the stability of vitamin C in N- and CC-juices HP-treated depend not only on the HPP parameters but also on the food matrix characteristics of the orange variety and the juicing procedure (blender jar).

421

3.4. Effect of HPP on hydrophilic antioxidant activity

The effect of HPP on the hydrophilic antioxidant activity (AA) from N- and CC-juices was evaluated by four different methods such as total phenolic by Folin-Ciocalteu (TP-FC) and FRAP (sample's reducing capacity) and by DPPH[•] and ABTS^{•+} (sample's radical scavenging capacity) (Table 3). Untreated N-juice showed significant higher AA than CC-juice by the four methodologies assayed [DPPH[•] (46%), ABTS^{•+} (9%), FRAP (12%) and TPC-FC (16%)]. These results may be in agreement with the higher vitamin C content (15%) found in N-juice. In general, HPP-200-400 maintained the AA in N-juice except DPPH[•] value that decreased by 12%. Also, HPP-400 (18-26%) and HPP-200 (11-23%) decreased the AA in N-juice by the four methods assayed (Table 3).

In CC-juice, HPP-200 and HPP-200-400 maintained the radical scavenging capacity (DPPH[•] and ABTS^{•+}) but significant reduced (11-14%) with HPP-400. In contrast, the reducing capacity of CC-juice (TC-FC and FRAP) significant increased by 7-13% after HPP-400 and HPP-200-400 (Table

3). These results showed that HPP-200-400 was the best treatment to maintain the antioxidant activity in N- and CC-juices.

In accordance with the results obtained for carotenoids, flavonoids and vitamin C, HPP-400 was the treatment that caused the largest reduction in the antioxidant activity of N- and C- juices. This result disagreed with others obtained previously with a similar HPP applied on orange juice (Plaza et al., 2006; Esteve & Frígola, 2008). Different authors have connected the decrease of AA of plant-derived products with the loss of bioactive compounds as a consequence of different HPP (Del Pozo-Insfran et al., 2007; Rodriguez-Roque et al., 2015). The decrease of the antioxidant activity after processing could be explained by the oxidation of certain bioactive compounds, such as vitamin C, phenolic and carotenoid compounds or by the activation of detrimental food enzymes such as peroxidase or polyphenoloxidase. In general, the effect of HPP on AA values from N- and CC-juices measured by the four methods were different depending on the food matrix (orange cultivar) and HPP parameters. These results agree with the data found in the literature for AA in HPP orange juices (Barba et al., 2012; Plaza et al., 2006).

According to data showed in Table 5, positive correlations were found in N-juices between total vitamin C (VITC) and ABTS^{•+} ($r^2 = 0.595$), TPC-FC ($r^2 = 0.455$), DPPH[•] ($r^2 = 0.455$) and FRAP ($r^2 = 0.444$). Total carotenoids (TCAROT) correlates positively with ABTS^{•+} ($r^2 = 0.755$), TPC-FC ($r^2 = 0.455$), DPPH[•] ($r^2 = 0.375$) and FRAP ($r^2 = 0.372$) (Table 4). Statistically significant positive correlations were found between TP-FC and FRAP ($r^2 = 0.808$) (sample's radical scavenging capacity) and between DPPH[•] and ABTS^{•+} ($r^2 = 0.738$) (sample's reducing capacity). Therefore, vitamin C and carotenoids seem to be the parameters mostly influencing AA in N-juice. Nevertheless, the AA values did not show positive correlations with total flavonoids (TF-HPLC) in N-juices.

Regarding CC-juices, no positive correlations were found between vitamin C, total carotenoids, total flavonoids and the four AA values analyzed. In fact, only positive correlations in CC juices were found between TP-FC and FRAP ($r^2 = 0.655$) and between DPPH[•] and ABTS^{•+} ($r^2 = 0.542$).

Other authors did not find any correlations between vitamin C and hydrophilic AA values determined by ORAC method in HPP comminuted oranges (Escobedo-Avellaneda et al., 2015) or between FRAP, ABTS^{•+} and AA values after ultra-high pressure homogenization of orange juice (Velázquez-Estrada et al., 2013). In addition to the participation of vitamin C, total flavonoids and total carotenoids in the AA, it is necessary to take into account the release of other antioxidants and the possible synergistic and antagonistic effects between all of them, that may depend on the food matrix and the HPP conditions (Rodriguez-Roque et al., 2015).

468

469 **Conclusions**

HPP at 200 MPa/25°C/1 min pre-treatment on whole-peeled Navel orange fruits before to juicing resulted efficient to obtain a functionalized orange juice (HPP-200) since it did not modify vitamin C content and significantly ($p<0.05$) increased total flavonoids (34%), hesperidin (25%), narirutin (27%), phytoene (40%) and phytofluene (9-fold) concentration respect to untreated freshly juice. This pre-treatment applied on Cara Cara fruit did not modified either flavonoids or vitamin C content but decreased ~16% total carotenoids, mainly lycopene. The higher instability of lycopene in HP-treated CC-juices could be related with the solid crystalline deposition of this carotenoid in the food matrix in contrast to the storage of other carotenoids in more protected small lipid droplets.

The application of HPP at 400 MPa/40 °C/1 min on HPP-200 juices of the two varieties (N and CC) resulted in orange juices (HPP-200-400) with similar bioactive composition than the corresponding HPP-200 one. In the case of Navel, HPP-200-400 juice presented higher content in colorless bioactive carotenes, phytoene (97%) and phytofluene (12-fold), than untreated freshly prepared juices.

It is remarkable that HPP-400 applied to untreated freshly prepared juice decreased significantly the bioactive compounds, mainly in N-juices. This effect could be related with the mechanical

485 juicing procedure (jar-blender) used in the present study or with the pressure-induced activation of
486 detrimental enzymes (peroxidase, ascorbate oxidase).

487 Then, HPP-200 and HPP-200-400 were considered optimal to produce functionalized N-juices
488 since some bioactive compounds were increased (flavonoids and colorless carotenoids) and the
489 antioxidant activity were unchanged compared to untreated freshly prepared juice. However, these
490 two treatments in CC-juices maintained their hydrophilic bioactive compounds (vitamin C and
491 flavonoids) but decreased ~ 16% the total carotenoids content compared to untreated juice.
492 However, it is remarkable that all the HP-treated CC juice presented higher carotenoid content than
493 the corresponding N-juices, so they are considered an excellent dietary source of these compounds.

494 Therefore, the HPP assayed in this work did not modified the profiling of bioactive compounds of
495 'Navel' (N) and 'Cara Cara' (CC) juices but the concentration of total and individual carotenoids,
496 flavonoids, vitamin C and antioxidant activity was differentially affected depending on the type of
497 bioactive compounds, their position in the food matrix, the orange cultivar and the HPP conditions.
498 All these variables should be considered to obtain a HP-processed juices with a composition in
499 bioactive compounds and antioxidant activity similar or improved compared to a freshly prepared
500 juice.

501

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510

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682

Figure 1. Flow diagram of experimental design

698

699 **Figure 2.** Effect of HPP on total carotenoids (A), total flavonoids (B) and vitamin C (C) of Navel and Cara
700 juices expressed as the % of retention compared to the corresponding untreated juice.

701 **Table 1.** Concentration of individual and total carotenoids (μg / 100 mL) of untreated and HP-treated orange juices

Carotenoid	Navel (N) Juice				Cara Cara (CC) Juice			
	Untreated	HPP-200	HPP-400	HPP-200-400	Untreated	HPP-200	HPP-400	HPP-200-400
Phytoene	35.7 $\pm$ 5.8a	51.2 $\pm$ 7.2b	17.5 $\pm$ 3.4c	70.2 $\pm$ 11.2b	1252.4 $\pm$ 11.2A	1194.1 $\pm$ 22.1A	1324.7 $\pm$ 74.1A	1287.8 $\pm$ 58.3A
Phytofluene	1.5 $\pm$ 0.1a	13.1 $\pm$ 0.7b	1.4 $\pm$ 0.2a	17.4 $\pm$ 3.9b	245.8 $\pm$ 14.2A	238.5 $\pm$ 16.9A	228.4 $\pm$ 8.9A	220.4 $\pm$ 21.6A
Lycopene	N.D.	N.D.	N.D.	N.D.	319.4 $\pm$ 51.2A	204.8 $\pm$ 25.1B	153.4 $\pm$ 8.6C	171.4 $\pm$ 11.8B
β -carotene	N.D.	N.D.	N.D.	N.D.	45.1 $\pm$ 5.3A	35.4 $\pm$ 8.4A	22.4 $\pm$ 4.1B	35.9 $\pm$ 5.3A
β -Cryptoxanthin	65.4 $\pm$ 9.2a	75.2 $\pm$ 9.3a	41.2 $\pm$ 6.2b	79.2 $\pm$ 9.2a	80.1 $\pm$ 11.2A	74.2 $\pm$ 6.7A	61.2 $\pm$ 5.4A	68.4 $\pm$ 6.6A
Zeaxanthin	21.8 $\pm$ 3.4a	20.2 $\pm$ 3.2a	15.2 $\pm$ 2.4a	19.4 $\pm$ 4.1a	36.8 $\pm$ 3.1A	27.6 $\pm$ 2.1A	29.4 $\pm$ 4.1A	31.2 $\pm$ 4.3A
Antheraxanthin	93.8 $\pm$ 11.2a	99.6 $\pm$ 11.4a	75.4 $\pm$ 4.2b	88.5 $\pm$ 3.4a	135.2 $\pm$ 11.4A	111.5 $\pm$ 9.7A	85.2 $\pm$ 9.B2	79.9 $\pm$ 8.8B
All- <i>E</i> - Violaxanthin	85.1 $\pm$ 9.4a	82.1 $\pm$ 10.2a	39.5 $\pm$ 8.1b	65.1 $\pm$ 7.1c	48.6 $\pm$ 3.5A	24.3 $\pm$ 3.3B	52.4 $\pm$ 6.1A	40.0 $\pm$ 8.1A
9- <i>Z</i> -Violaxanthin	411.5 $\pm$ 21.3a	420 $\pm$ 15.4a	303.1 $\pm$ 9.5b	351.2 $\pm$ 15.4c	285.9 $\pm$ 16.2A	219.5 $\pm$ 15.4B	219.7 $\pm$ 14.5B	214.3 $\pm$ 9.8B
Total carotenoids	751.4$\pm$15.4a	775$\pm$23.2a	495$\pm$10.4b	725$\pm$22.5a	2501.9$\pm$85.2A	1998.5$\pm$54.8B	2199.2$\pm$75.4B	2148.8$\pm$69.4B

702 Data are mean $\pm$ SD (n=4). Different lowercase letters (Navel juice) and uppercase letters (Cara Cara juice) on the same line represent significant
703 differences ($p < 0.05$) for a carotenoid among different treatments. N.D., not detected.

704

705 **Table 2.** Concentration of individual and total flavonoids (mg / 100 mL) of untreated and HP-treated orange juices

Flavonoids	Navel (N) juice				Cara Cara (CC) juice			
	Untreated	HPP-200	HPP-400	HPP-200-400	Untreated	HPP-200	HPP-400	HPP-200-400
Apigenin-6,8-Di-C-Glucoside -Vicenin 2	5.47±0.07b	6.27±0.12a	4.80±0.25c	5.62±0.22b	6.25±0.86A	6.00±0.15A	6.40±0.65A	6.07±0.67A
Narigenin-7-O-Rutinoside-4'-O-Glucoside	6.43±0.44b	7.97±0.54a	5.65±0.23c	6.91±0.24b	6.17±0.79A	6.45±0.17A	6.59±0.80A	6.37±0.76A
Eriodyctiol-7-O-Rutinoside - Eriocitrin	0.99±0.02b	1.30±0.04a	0.89±0.03b	0.99±0.20b	1.03±0.17A	1.01±0.14A	1.08±0.11A	1.05±0.12A
Quercetin-3-O-Rutinoside - Rutin	2.55±0.08b	2.93±0.05a	2.21±0.03c	2.37±0.28bc	1.74±0.21A	1.73±0.06A	1.91±0.14A	1.87±0.18A
Naringenin-7-O-Rutinoside - Narirutin	11.14±0.08c	13.87±0.17a	10.49±0.45c	12.16±0.53b	12.80±1.41A	12.62±0.34A	13.28±1.09A	12.58±6.6A
Hesperetin-7-O-Rutinoside - Hesperidin	15.57±0.12d	19.68±0.41a	16.14±0.08c	18.13±0.44b	15.51±2.18A	14.68±0.46A	16.66±2.52A	16.57±2.11A
Isosakunetin-7-O-Rutinoside - Dydimin	2.17±0.04c	2.77±0.01a	2.23±0.03c	2.49±0.16b	2.10±0.20A	1.97±0.10A	2.15±0.32A	2.16±0.23A
Total flavonoids	44.31±0.64c	59.47±1.28a	42.41±1.10c	47.38±2.06b	45.60±5.83A	44.48±1.41A	48.08±5.62A	46.66±5.31A

706 Data are mean ± SD (n=4). Different lowercase letters (Navel juice) and uppercase letters (Cara Cara juice) on the same line represent significant
707 differences ($p<0.05$) for a flavonoid among different treatments.

708

709 **Table 3.** Vitamin C and antioxidant characteristics of untreated and HP-treated orange juices

		Navel (N) juice				Cara Cara (CC) juice			
		Untreated	HPP-200	HPP-400	HPP-200-400	Untreated	HPP-200	HPP-400	HPP-200-400
Vitamin C	(mg	60.18±4.51a	59.25±8.67a	42.05±5.84b	55.65±1.22a	50.96±1.06A	49.63±0.79A	50.25±0.27A	48.80±6.80A
AsA/100 mL)									
TPC-FC	(mg	25.03±0.56a	20.84±1.25b	20.56±0.80b	26.87±1.38a	21.00±0.77B	19.80±0.53B	23.23±0.59A	23.68±0.36A
GAE/100 mL)									
DPPH	(μmol	352.22±2.1a	236.09±8.1d	260.19±3.4c	309.82±1.5b	190.15±6.1A	194.84±2.4A	169.47±2.8B	209.40±7.7A
AsAE/100 mL)									
ABTS	(μmol	413.00±6.7a	368.06±17b	318.84±13c	423.18±7.2a	378.03±8.8A	380.91±2.9A	325.13±11B	389.59±4.0A
AsAE/100 mL)									
FRAP	(μmol	312.25±10a	225.31±5.6b	234.89±1.6b	292.30±13a	274.13±4.1C	281.37±1.0B	286.31±4.0AB	293.95±7.2A
AsAE/100 mL)									

710 Data are mean ± SD (n=4). AsA= Ascorbic acid. AsAE= Ascorbic acid equivalents. TPC-FC= Total Phenolic Compounds determined by Folin
711 Ciocalteu assay. GAE= Gallic acid equivalents. Different lowercase letters (Navel juice) and uppercase letters (Cara Cara juice) on the same line
712 represent significant differences (p<0.05) for each determination among different treatments.

Table 4. Pearson's correlation coefficients (r^2) among total carotenoids (TCAROT), total flavonoids determined by HPLC (TF-HPLC), vitamin C (VITC), and antioxidant activity (TPC-FC, DPPH[•], ABTS^{•+} and FRAP) for untreated and HP-treated Navel juices

	VITC	TCAROT	TF-HPLC	TPC-FC	DPPH [•]	ABTS ^{•+}	FRAP
VITC	1	0.677 ^{**}	0.368	0.455	0.375	0.595 [*]	0.444
TCAROT	0.677 ^{**}	1	0.650 ^{**}	0.414	0.290	0.750 ^{**}	0.372
TF-HPLC	0.368	0.650 ^{**}	1	-0.121	-0.490	0.112	-0.329
TPC-FC	0.455	0.455	-0.121	1	0.756 ^{**}	0.816 ^{**}	0.808 ^{**}
DPPH [•]	0.375	0.375	-0.490	0.756 ^{**}	1	0.738 ^{**}	0.963 ^{**}
ABTS ^{•+}	0.595 [*]	0.755 ^{**}	0.112	0.816 ^{**}	0.738 ^{**}	1	0.781 ^{**}
FRAP	0.444	0.372	-0.329	0.808 ^{**}	0.963 ^{**}	0.781 ^{**}	1

*Pearson's correlation coefficient at level $p<0.05$; **Pearson's correlation coefficient at level $p<0.01$