**INFLUENCE OF ORANGE CULTIVAR AND MANDARIN POSTHARVEST
STORAGE ON POLYPHENOLS, ASCORBIC ACID AND ANTIOXIDANT
ACTIVITY DURING GASTROINTESTINAL DIGESTION**

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27 Abstract

28 Polyphenols, ascorbic acid content and antioxidant activity of two sweet oranges
29 (Navel-N and Cara Cara-CC) and mandarin (Clementine-M) as well as their
30 bioaccessibilities were evaluated in pulps and compared to those in fresh juice. Thus,
31 pulps of oranges and mandarins displayed higher hesperidin (HES), narirutin (NAR),
32 total flavonoids (TF), total phenols (TP) and antioxidant activity (AAC) than their
33 corresponding juices. Also, CC products presented higher bioactive compounds content
34 than N ones. Bioaccessibility of bioactive compounds and AAC were higher in pulps of
35 both oranges and mandarin than in their corresponding juices. Oranges (N and CC)
36 pulps and juices presented higher bioaccessibilities than mandarin ones.
37 The postharvest storage of mandarin at 12 °C during 5 weeks not only produced a
38 significant increase of the bioactive compounds but also an increase of their
39 bioaccessibility. The bioaccessibility of *Citrus* bioactive compounds is necessary for
40 calculating more accurately their daily intake amount.

41

42

43 **Keywords:** Polyphenols, ascorbic acid, total antioxidant activity, citrus fruits, *in vitro*
44 gastrointestinal digestion

45 1. Introduction

46 *Citrus* fruits and juices play a key role in supplying nutrients and phytochemicals such
47 as vitamin C and polyphenols (mainly flavanones such as hesperidin, narirutin and
48 naringin) that may act in concert (additively or synergistically) to exert their
49 antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities
50 (Benavente-García et al., 2008; Liu, Heying, & Tanumihardjo, 2012; Lee, 2013; Khan,

51 Zill-E-Huma, & Dangles, 2014; Aptekmann & Cesar, 2014; Gironés-Vilaplana, Moreno,
52 & García-Viguera, 2014; Stinco et al., 2015; Lv et al., 2015).

53 In particular, Navel oranges and Clementine mandarins contain a high amount of
54 vitamin C (sum of ascorbic acid and dehydroascorbic acid), with concentrations
55 averaging 46 and 41 mg/100 g fw, respectively (Cano, Medina, & Bermejo, 2008).
56 Ascorbic acid, the most effective and least toxic antioxidant, is involved in vital
57 biological activities including synthesis of collagen, neurotransmitters, steroid
58 hormones, and carnitine, and is responsible for the conversion of cholesterol to bile
59 acids. Also, ascorbic acid intake has been related to reduce risk of cancer and
60 cardiovascular diseases (González-Molina, Domínguez-Perles, Moreno, & García-
61 Viguera, 2010; Gironés-Vilaplana et al., 2014).

62 *Citrus* varieties presented important quantities of flavonoids distributed in different
63 parts of the fruit (flavedo, albedo and juice vesicles) (Tripoli, La Guardia, Giammanco,
64 Di Majo, & Giammanco, 2007). *Citrus* fruits and their juice contain large quantities of
65 flavonoids, mainly flavanones and flavones in their glycosylated form although
66 flavonols have been detected in minor concentration. In general, the most abundant
67 flavanone glycoside identified in oranges and mandarins was hesperetin-7-O-rutinoside
68 (hesperidin) followed by naringenin-7-O-rutinoside (narirutin) (Dhuique-Meyer, Caris-
69 Veyrat, Ollitrault, Curk, & Amiot, 2005; Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi,
70 2007; Cano et al., 2008; Khan et al., 2014). The antioxidant and anti-inflammatory
71 activity, and cardiovascular protection activity of *Citrus* flavonoids and their role in
72 degenerative disease have been widely studied (Benavente-García et al., 2008).

73 The biological activity of *Citrus* phytochemicals depends on several factors such as
74 chemical structure, concentration consumed, food matrix, presence of fat and fiber, type
75 of processing, and their bioavailability mainly determined by human intervention

76 studies (Sánchez-Moreno et al., 2003; De Pascual-Teresa, Sánchez-Moreno, Granado,
 77 Olmedilla, De Ancos, & Cano, 2007). Human studies are the method of choice, but are
 78 expensive, time consuming, difficult to carry out, and the results obtained are not
 79 always generalizable, due to important variability between and even within individuals.
 80 Therefore, simulated *in vitro* gastrointestinal (GI) digestion allows to estimate
 81 bioaccessibility, defined as the amount of a food component released from the food
 82 matrix which constitutes the amount available for absorption. Bioaccessibility can be
 83 used to evaluate the relative bioavailability of bioactive compounds (Cardoso, Afonso,
 84 Lourenço, Costa, & Nunes, 2015).

85 In general, bioaccessibility of *Citrus* hydrophilic constituents such as flavonoids (~19-
 86 43 %) and vitamin C (~21-31%) varied with the food matrix such as orange juice or
 87 fruit-based beverages (containing orange juice) and also with the processing technology
 88 (Gil-Izquierdo, Gil, Ferreres, & Tomás-Barberá, 2001; Cilla, González-Sarriás, Tomás-
 89 Barberán, & Espín, 2009; Cilla, Perales, Lagarda, Barberá, Clemente, & Farré, 2011;
 90 Cilla et al., 2012; Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso,
 91 2013; Rodríguez-Roque et al., 2015). In fact, the bioaccessibility of *Citrus* bioactive
 92 compounds depends on if they are digested as whole fruit or in form of juice (Aschoff,
 93 Kaufmann, Kalkan, Neidhart, Carle, & Schweiggert, 2015). Also, the *Citrus* fruit
 94 species (sweet orange and mandarin) and the postharvest storage could modulate the
 95 carotenoid bioaccessibility during an *in vitro* GI digestion (Rodrigo, Cilla, Barbará, &
 96 Zacarias, 2015).

97 To the author's knowledge, there are no previously reports evaluating the effect of
 98 food matrix (citrus pulp vs. citrus fresh juice), orange variety (Navel vs. Cara Cara) and
 99 postharvest storage (mandarin Clementine control vs. five weeks at 12 °C) on the
 100 bioaccessibility of polyphenols and ascorbic acid in citrus fruits and their antioxidant

activity. In addition, due to the fact that health-effects derived from the intake of sweet oranges and mandarin fruits depends not only on carotenoids but also on phenolic compounds and vitamin C, the aim of the present work was to study the influence of orange cultivar and mandarin postharvest storage on polyphenols (total phenolic content and flavonoids), and hydrophilic antioxidant activity of pulps and juices of two sweet oranges cultivars and one mandarin during an *in vitro* GI digestion.

2. Materials and methods

2.1. Reagents.

2.1.1. Polyphenol, vitamin C and antioxidant activity determinations. Methanol and acetonitrile (HPLC-grade) were provided by Lab-Scan (Dublin, Ireland). Glacial acetic acid, metaphosphoric acid, hydrochloric acid, formic acid, L(+)-ascorbic acid ($\geq 99\%$ purity), sulfuric acid and sodium carbonate were obtained from Panreac Química (Barcelona, Spain). Narirutin (Naringenin-7-*O*-rutinoside) was acquired from Extrasynthèse (France). Hesperidin (hesperitin-7-*O*-rutinoside), eriodictiol-*O*-rutinoside (eriocitrin), naringenin-7-*O*-rutinoside (narirutin), hesperetin-7-*O*-rutinoside (hesperidin), isosakunetin-7-*O*-rutinoside (dydimin), quercetin-3-rutinoside (rutin), apigenin, gallic acid, ascorbic acid, Folin-Ciocalteu's phenol reagent, iron (III) chloride hexahydrate, phosphate buffered saline, hexadecyltrimethyl-ammonium bromide, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]), and potassium persulfate (K₂S₂O₈) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-(1-naphtyl)ethylenediaminedihydrochloride and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) were obtained from Fluka Chemie AG (Buchs, Switzerland). Stock solutions of 1 mg/mL in methanol of authentic flavonoid standards were prepared.

126 **2.1.2. Simulated GI digestion.** Enzymes and bile salts were purchased from Sigma-
 127 Aldrich (St. Louis, MO, USA): pepsin (porcine, 975 units per mg protein), pancreatin
 128 (porcine, activity equivalent to 4 x USP specifications) and bile extracts (porcine).

129

130 **2.2. Samples.**

131 Fruit pulps and juices from sweet blonde-flesh orange Washington Navel (N) (*C.*
 132 *sinensis* L.) and its spontaneous red-fleshed mutant Cara Cara (CC) (rich in lycopene)
 133 and freshly harvested Clementine mandarin (*C. clementina* L.) (M) (rich in β -
 134 cryptoxanthin) and after a postharvest storage at 12 °C for 5 weeks (M12), were
 135 studied. Origin and treatment of fruit samples has been previously described by Rodrigo
 136 et al. (2015).

137

138 **2.3 In vitro GI digestion**

139 An *in vitro* GI digestion procedure mimicking the physiological situation in the upper
 140 digestive tract including gastric and intestinal steps and obtaining the bioaccessible
 141 fraction (BF) after centrifugation was used to evaluate the bioaccessibility of vitamin C,
 142 polyphenols and hydrophilic antioxidant capacity according to the procedure described
 143 by Rodrigo et al. (2015). Bioaccessibility (BA) is calculated as follows: 100 x (content
 144 in BF /content in non-digested sample).

145

146 **2.4 Ascorbic Acid Analysis**

147 Ascorbic acid was extracted and quantified by HPLC according to the procedure
 148 described Cilla et al. (2012) using 10 g of sample (fruit pulp, fruit juice and acidified
 149 BF). Prior to the extraction of ascorbic acid, the BF (pH 7.6) were acidified with
 150 hydrochloric acid to pH 4. Quantification was achieved using an ascorbic acid external

151 standard calibration curve in the range from 5 to 500 $\mu\text{g}/\text{mL}$. Results were expressed as
152 mg of ascorbic acid per 100 g of sample (fruit pulp, fruit juice and BF).

153

154 *2.5 Flavonoid Analysis*

155 Flavonoids were extracted, identified, and quantified by HPLC-DAD and HPLC-
156 MS-ESI-QTOF from fruit pulp, fruit juice and BF according to the procedure described
157 by Dorta, González, Globo, Sánchez-Moreno, & De Ancos (2014) with some
158 modifications. Previously to the extraction of flavonoids, BF (pH 7.6) was acidified
159 with hydrochloric acid to pH 4. Then, 20 g of sample (fruit pulp, fruit juice and
160 acidified BF) was homogenized with 20 mL methanol/water (80:20, v/v) during 2 min
161 at 8000 rpm with an ultrahomogeniser (Omnimixer, model ES-207, Omni International
162 Inc, Gainesville, VA). The sample was centrifuged at 9000 $\times$ g during 15 min at 4 °C in a
163 refrigerated centrifuge (Thermo Scientific Sorvall, mod. Evolution RC, Thermo Fisher
164 Scientific Inc., USA) and the supernatant was separated. Then, 10 mL of this solution
165 were loaded on a reversed phase C18 Sep-pack cartridge (200 mg of silica based bonded
166 phase, 37 $\times$ 55 μm particle size) (Waters, USA), previously activated with 5 mL of
167 methanol and 5 mL of water. Phenolic compounds were recovered from the cartridge by
168 eluting with 2 mL of methanol and filtered through a 0.45 μm syringe filter and stored at
169 -80 °C until HPLC-DAD and HPL-ESI-MS-QTOF analysis were carried out according
170 to procedure described by Dorta et al. (2014).

171 Polyphenols identification was carried out by HPLC-ESI-MS-QTOF according to
172 the procedure described by Dorta et al. (2014). Phenolic compounds were identified by
173 comparing chromatographic behavior (retention times, UV-Vis spectral properties) and
174 LC-MS spectral data and LC-MS/MS fragmentation patterns with those of authentic
175 commercial standards or related structural compounds (when it was possible). Besides

176 the observed MS and MS/MS spectra and data obtained by QTOF-MS analysis, other
177 main tools for phenolic compounds identification were the interpretation of the
178 observed MS/MS spectra in comparison with those found in the literature (Abad-García,
179 Garmón-Lobato, Berrueta, Gallo, & Vicente, 2012; Gironés-Vilaplana et al., 2014) and
180 several online databases (Phenol-Explorer; ChemSpider, MassBank; MetLin;
181 LipidMaps; MetaboAnalyst; Spectral Database for Organic Compounds).

182 Polyphenols quantification was achieved by HPLC-DAD using external standards
183 calibration curves in the range from 5 to 250 $\mu\text{g}/\text{mL}$ (Dorta et al., 2014). When
184 reference compounds were not available, the calibration of structurally related
185 substances was used including a molecular weight correction factor. Thus, apigenin-6,8-
186 di-*C*-glucoside as equivalents of apigenin and naringenin-7-*O*-rutinoside-4'-*O*-
187 glucoside as equivalents of narirutin. Results were expressed as mg of flavonoid per 100
188 g of sample (fruit pulp, fruit juice and BF). Total flavonoids content (TF) was expressed
189 as the sum of individual compounds.

190

191 **2.6 Total Phenol and antioxidant activity determinations**

192 **2.6.1 Sample extraction**

193 A 10 g of a representative sample (fruit pulp, fruit juice and BF) was homogenized
 194 with 10 mL of methanol/water (80:20, v/v) during 2 min at 8000 rpm (Omnimixer,
 195 model ES-207, Omni International Inc, Gainesville, VA) and centrifuged at 12000 x g
 196 for 15 min at 4° C and the supernatant separated. The pellet was re-extracted with 10
 197 mL of methanol/water (80:20, v/v) in the same conditions described before. The two
 198 supernatants were pooled together in a volumetric flask and made up to 50 mL with
 199 distilled water.

200 This solution was considered as the sample solution for total phenol and antioxidant
 201 activity determinations. If the absorbance of the final sample solution was not in the
 202 range of the standard curves, further dilutions were required.

203 **2.6.2. Total phenol assay**

204 Total phenol determination performed according to the Folin-Ciocalteu procedure
 205 adapted for a 96-microplate determination (Zhang, Zhang, Shen, Silva, & Dennis, 2006)
 206 was used to quantify the sample's reducing capacity due to the high content in the
 207 *Citrus* products of potent antioxidants such as ascorbic acid that can also react with the
 208 Folin-Ciocalteu reagent (Huang, Ou, & Prior, 2005).

209 Briefly, 0.2 mL of sample extract (or dilutions) was placed into 3 mL test tubes with 1
 210 mL of distilled water and 1 mL of Folin-Ciocalteu reagent and then vortex 5 s. After 5
 211 min, 0.8 mL of sodium carbonate (3.5%, w/v) was added. The mixture was incubated in
 212 the dark for 60 min and then 300 µL of the reaction mixture were placed in the
 213 microplate well in triplicate. Absorbance was measured at 750 nm in a
 214 spectrophotometric microplate reader (PowerWame XS, BioTeck, Vicenza, Italy).

Quantification was achieved using a gallic acid external standard calibration curve in the range from 10 to 100 $\mu\text{g}/\text{mL}$. Total phenolic content was expressed as mg of gallic acid equivalents per 100 g fresh weight of sample (fruit pulp, fruit juice and BF).

2.6.3. *2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS^{•+}) scavenging assay*

According to the method described by Re et al. (1999) including an adaptation of the method to 96-well microplate format, ABTS radical cation (ABTS^{•+}) was produced by reacting ABTS with potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) and allowing to stand in darkness at room temperature for 12–16 h before use. The ABTS^{•+} solution (two days stable) was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 10 μL of each phenolic extract were mixed with 290 μL of 7 mM ABTS^{•+}, and after 20 min in darkness at 30 °C, absorbance was measured at 734 nm in a microplate spectrophotometer (PowerWave XS, BioTeck, Vicenza, Italy). Results were compared with a standard curve prepared daily with ascorbic acid (AA), and expressed as μmol of AA equivalents (AAE) per 100 g fresh weight of sample (fruit pulp, fruit juice and BF).

2.6.4. *2,2-Diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging assay*

The method described by Sánchez-Moreno, Larrauri & Saura-Calixto (1998) with modifications, including an adaptation of the method to 96-well microplate format, was followed. Absorbance was measured at 515 nm in a microplate spectrophotometer. All samples were run in triplicate. Results were compared with a standard curve prepared daily with ascorbic acid (AA), and expressed as μmol of AA equivalents (AAE) per 100 g fresh weight of sample (fruit pulp, fruit juice and BF).

2.6.5. *Ferric reducing antioxidant power (FRAP) assay*

The total antioxidant potential of a sample was also determined using the FRAP assay by Benzie & Strain (1996) with certain modifications, including an adaptation of the

method to 96-well microplate format. All samples were run in triplicate at 593 nm in a microplate spectrophotometer. Results were compared with a standard curve prepared daily with ascorbic acid (AA), and expressed as μmol of AA equivalents (AAE) per 100 g fresh weight of sample (fruit pulp, fruit juice and BF).

Statistical analysis

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

3. Results and discussion

3.1. Hydroxycinnamic acids and Flavonoids Identification

In this study, nine main phenolic compounds were identified which belonged to two different phenolic classes: hydroxycinnamic acids derivatives and flavonoids (flavones and flavanones) (Table 1, Figure 1-suppl.).

Hydroxycinnamic acid derivatives. According to the UV-visible spectra, mass spectrometric data and retention time in HPLC, peak **1** and **2** (Table 1, Figure 1-suppl.) were tentatively identified as *O*-hexoxides of ferulic and sinapic acid. They showed the $[\text{M}-\text{H}]^-$ ions at m/z 355 and 387, respectively. These two compounds (**1** and **2**) were also previously identified in orange and mandarin juices by Abad-García et al. (2012).

Flavonoids. Compound **3** (Table 1, Figure 1-suppl.) showed UV-visible spectra typical of flavones and the MS^1 spectra revealed a high intensity $[\text{M}-\text{H}]^-$ ion at m/z 593.

Also, the comparison of the relative absorbance at 270 and 334 nm allowed the identification flavone nature of peak **3**. The absence of the aglycone ion in the MS¹ spectra of this compound indicates that it is a flavone *C*-glycoside rather than *O*-glycoside (Gattuso et al., 2007). This compound **3** showed the fragment ions [M-H-120]⁻ at *m/z* 473 and [M-H-240]⁻ at *m/z* 353 that revealed the hexose nature of two saccharides which are typically of di-*C*-glucoside flavanone. Moreover, the position of the maximum band II in UV-vis spectra at 270-271 nm suggested a 6,8-disubstitution (Barreca, Bellico, Caristi, Leuzzi, & Gattuso, 2011). The compound **3** was identified as apigenin-6,8-di-*C*-glucoside. This compound has been previously identified in sweet orange and tangerine fruits by Gattuso et al. (2007) and Abad-García et al. (2012).

The MS¹ scan spectra and the UV-visible spectrum compared with those of authentic standards determined that compounds **4**, **5**, **6**, **7**, **8** and **9** are flavanone glycosides (Table 1). Eriodyctiol-*O*-rutinoside (compound **5**), quercetin-3-*O*-rutinoside (rutin) (compound **6**), naringenin-7-*O*-rutinoside (compound **7**), hesperetin-7-*O*-rutinoside (compound **8**) and isosakunetin-7-*O*-rutinoside (compound **9**) standards allowed the identification of these flavanones in the orange and mandarin pulps and juices. These flavanones have been widely characterized in orange and tangerine products (Gattuso et al., 2007; Abad-García et al., 2012).

Compound **4** was tentatively identified as Naringenin-7-*O*-rutinoside-4'-*O*-glucoside. The examination of chromatograms in TOF-MS mode revealed that compound **4** gave a [M-H]⁻ ion at *m/z* 741. The MS/MS mode showed an ion fragment at *m/z* 579 that corresponded to the loss of glucose [M-H-162]⁻. Also it was detected an ion fragment at *m/z* 433 [M-H-308]⁻ that corresponded with a rutinoside moiety and *m/z* 271 [M-H-308-162]⁻ that is the characteristic fragmentation pattern of naringenin-7-*O*-

288 rutinoid. Naringenin-7-*O*-rutinoside-4'-*O*-glucoside (compound 4) has been
289 previously described in orange and mandarin fruits by Abad-García et al. (2012).

290

291 3.2. Flavonoid and total phenolic content

292 3.2.1. Before GI digestion

293 *Flavonoids*. Individual and total flavonoid content (TF) of Navel (N) and Cara Cara
294 (CC) oranges and mandarin Clementine (M) are shown in Tables 2 and 3, respectively.

295 The major flavonoid in the pulps and juices of both oranges (N and CC) and mandarin
296 (M) was the flavanone glycoside hesperidin (HES) followed by narirutin (NAR) (Table
297 2). This result agreed with the literature (Dhuique-Meyer et al., 2005; Peterson et al.,
298 2006; Gattuso et al., 2007; Cano et al., 2008; Stinco et al., 2015). In both oranges, N and
299 CC, also were found in decreasing order of concentration, apigenin-6,8-Di-*C*-glucoside,
300 naringenin-7-*O*-rutinoside-4'-*O*-glucoside, eriocitrin, dydimin and rutin. In the case of
301 mandarin, dydimin was the third mayor flavonoid (Table 2). Dydimin has been found in
302 the majority of sweet oranges and mandarins (Khan et al., 2014) while some authors did
303 not detect it (Gironés-Vilaplana et al., 2014).

304 The highest HES concentration was found in CC-pulp (43430 µg/100 g fw), it was
305 1.4- and 3.6-times higher than in N-pulp and M-pulp, respectively. However, NAR
306 concentration was similar in the pulp of both oranges, CC and N (~ 12328 µg/100 g fw),
307 that was 7.5-times higher than in M-pulp (Table 2). Regarding juices, HES
308 concentration of N-orange, CC-orange and M-mandarin juices were 30%, 49% and
309 130%, respectively, lower than in their corresponding pulps. HES and NAR values
310 found in this study agree with literature data where significant differences for HES
311 (60.9-104.7 mg/100 g fw) and NAR (16.4-28.7 mg/100 g fw) content were found
312 among pulps of Navel oranges group (Cano et al., 2008). Also, HES (13.2-60.6 mg/100

g fw) and NAR (2.6-30.0 mg/100 g fw) content significantly varied among pulps of Clementine group (Cano et al., 2008).

In terms of total flavonoid content (TF), the highest value was found in red-fleshed CC-orange pulp (75.15 mg/100 g fw) that was 1.16-times higher than in the blond-flesh N-orange and 4-times higher than in M-mandarin pulp (Table 3). These values agreed with data in the literature for *C. sinensis* (sweet oranges) (Dhuique et al., 2005; Cano et al., 2008) and *C. reticulata* (mandarin) pulps (Del Caro et al., 2004; Cano et al., 2008). Regarding juices, the highest TF content was found in CC-orange juice (48.49 mg/100 g fw) that was 1.18-times higher than in N-orange juices and 6-times higher than in M-juice (Table 3). These values agreed with the results found in the literature for *C. sinensis* juices (Peterson et al., 2006; Escudero-López et al., 2013) and *C. reticulata* juices (Dhuique-Meyer et al., 2005). These significant differences found for individual and TF content in sweet oranges (N and CC) and mandarin (M) agreed with results found in the literature, and could be due to different factors such as the analytical and extraction procedure, the variety of orange and mandarin studied, the development fruit stage, the harvest season and the orange tissue analyzed (Stinco et al., 2015; Chen, Zhang, Pang, Cheng, Deng, & Xu, 2015).

Comparing oranges and mandarin pulps with their corresponding juices, CC-juice, N-juice and M-juice had 1.5-, 1.6- and 2.28-times lower TF than their corresponding pulps indicating a flavonoid loss during juice extraction (Table 3). The loss of flavonoids during *Citrus* juice processing has been previously described (Aschoff et al., 2015). These authors found that the removal of flavonoid rich albedo and juice vesicles during juice extraction produce a decreased of 8-times the flavonoid concentration of the juice in comparison with the segments. The present study found a lower loss in *Citrus* flavonoids content during the extraction of juices since this was approximately 1.5- (in

338 CC) and 2.28-times (in M) lower TF concentration in juices than in pulps. This lower
 339 flavonoid loss found in the present study could be due to the use of different orange
 340 cultivars, maturity stages and procedures for juice production (Chen et al., 2015).

341 Regarding the refrigerated storage of mandarin (M) for five weeks at 12 °C (M12),
 342 necessary to increase the β -cryptoxanthin content (Rodrigo et al., 2015), no significant
 343 differences ($p<0.05$) were found in the majority of the individual and total flavonoid in
 344 M-pulp except for NAR concentration that was approximately 22% higher in M12-pulp
 345 (Table 2 and 3). In juice obtained from M12, an irregular pattern was observed.
 346 Meanwhile HES did not suffer significant changes; NAR was 44% lower in M12-juice
 347 than in M-juice (Table 3).

348 In general, the individual and total flavonoid concentration in pulps and juices of
 349 oranges (N and CC) were significantly ($p<0.05$) higher than in mandarins (M and M12)
 350 (Table 2 and 3). In terms of oranges, pulp and juice of red-fleshed orange-CC had
 351 significantly ($p<0.05$) higher TF content than blonde-fleshed orange-N. Regarding the
 352 comparison between pulp and juice, the individual flavonoid and TF content were
 353 significantly ($p<0.05$) higher in pulps than in their corresponding fresh prepared juices
 354 in all the *Citrus* products studied (N, CC and M). Refrigerated storage at 12 °C for 5
 355 weeks increased 22% the NAR content in M-pulp.

356 *Total phenolic content.* The highest total phenolic content (TP) was found in orange
 357 CC-pulp (102.83 mg/100 g fw) that was 11% and 37% significantly ($p<0.05$) higher
 358 than in N-pulp and M-pulp, respectively (Table 3). CC-juice presented 1.6-times lower
 359 TP concentration than pulp indicating the loss of phenolic compounds or other
 360 antioxidant compounds during juice production. Also, N-juice and M-juice had 1.5- and
 361 1.33-times lower TP concentration than their corresponding pulps (Table 3). The results
 362 of TP obtained in the present study for orange pulps agreed with those reported by

363 Gironés-Vilaplana et al. (2014) but did not agree for mandarin-M pulps perhaps because
 364 of different cultivars of mandarins were studied. The postharvest storage of mandarin-M
 365 for 5 weeks at 12 °C produced a significant increase of 11% and 13% in TP
 366 concentration in juice and pulp, respectively (Table 3).

367 In the present study, HES, NAR, TF and TP content followed the same trend than
 368 carotenoid concentration analyzed in the same *Citrus* products (N, CC and M) (Rodrigo
 369 et al., 2015). Thus, the pulp and juice of red-fleshed mutant orange-CC presented not
 370 only higher total carotenoid content (Rodrigo et al., 2015) but also higher HES, NAR,
 371 TF and TP concentration than the pulp and juice of blonde-fleshed orange-N. These
 372 results provided more evidences about that lycopene accumulation in red-fleshed
 373 orange-CC might be connected with the flavonoid biosynthesis. Although the
 374 mechanism was unclear, it seems that the increasing of flavonoid content depends
 375 greatly on the development fruit stage (Chen et al., 2015).

376 3.2.2. Bioaccessibility

377 The bioaccessibilities (BA) of individual and total flavonoids and total phenolic
 378 compounds are shown in Tables 2 and Table 3. All the individual flavonoids found in
 379 the hydro-methanolic extraction of the non-digested pulps and juices of oranges (N and
 380 CC) and mandarin (M) were detected in their respective BF after the *in vitro* GI
 381 digestion (Table 3). Concerning the bioaccessibility (%) of the major flavonoids, HES
 382 and NAR, significant differences were detected between pulps and juices and between
 383 the two *Citrus* species studied; oranges (N and CC) and mandarin (M). In general, the
 384 individual and total flavonoid bioaccessibilities were higher in pulps and juices of the
 385 two oranges (N and CC) than in the mandarins (Tables 2 and 3). When comparing
 386 between pulp and juice, the result depends on the *Citrus* studied, thus in orange-N, the
 387 bioaccessibility of HES and NAR was higher in juice than in their corresponding pulp

meanwhile the contrary occurred in orange-CC. The same trend was found in mandarin-M than in orange-CC but significantly lower bioaccessibilities for HES and NAR either in pulp or in juice were obtained.

In the comparison between *Citrus*, HES and NAR bioaccessibilities in orange CC-pulp were higher than in orange N-pulp, with the lowest values for mandarin M-pulp (Table 2). Regarding juices, N-juice presented the highest HES and NAR bioaccessibilities. In general, similar trends were also found for the rest of individual flavonoids identified in the pulp and juice of oranges and mandarin (Table 2).

Concerning flavanones, Gil-Izquierdo et al. (2001) found bioaccessibilities of 10.55% and 16.20% for HES and NAR, respectively in the dialyzed fraction of orange juice that was significantly lower than the values obtained in the present study for HES and NAR in orange juice of N and CC (values ranged between 35.40% and 58.15%). The differences could be ascribed to different factors such as the orange cultivar (not specified) and also the different procedure employed to obtain the BF. In the present study, the BF was obtained by centrifugation meanwhile Gil-Izquierdo et al. (2001) employed a dialysis process. Similarly, other studies using dialysis for the measurement of bioaccessibility with fruit beverages containing orange juice (40-50% w/v) reported bioaccessibilities for HES (13.1-18.4%) and NAR (14.4-18.7%) lower than in the present study (Rodríguez-Roque et al. 2013, 2014 and 2015). On the other hand, the HES bioaccessibility in orange pulps and juices (average value 47%) was similar to that found by Cilla et al. (2009) for HES (50%) in a fruit beverage (with 4% of orange concentrate) using solubility for the measurement of bioaccessibility.

Total flavonoid (TF) bioaccessibility followed the same trend than the major flavonoids HES and NAR, and the result depends on the *Citrus* studied (Table 3).

412 Regarding juices, there was higher TF bioaccessibility in oranges than in mandarins
 413 showing N-juice the highest bioaccessibility. Meanwhile in terms of pulps, CC-pulp
 414 showed the highest bioaccessibility. In general, oranges N and CC products (pulp and
 415 juice) have shown higher TF bioaccessibility than mandarin. The postharvest storage of
 416 mandarin-M for 5 weeks at 12 °C produced a significant ($p<0.05$) increase of TF
 417 bioaccessibility in both pulp and juice (M12) (Table 3).

418 The *in vitro* GI digestion decreased the solubility of flavonoids (Table 2 and 3). In line
 419 with this, a limited quantity of soluble flavonoids was bioaccessible (12-19%) in a
 420 fresh-prepared orange juice (Gil-Izquierdo et al., 2001). Also, a decrease in the total
 421 flavanone and total flavone content by at least 38% (62% bioaccessibility) was found in
 422 a digested fruit beverage (4% orange juice concentrate) compared to the non-digested
 423 product (Cilla et al., 2009). Thus, it was found that the *in vitro* GI digestion process,
 424 mainly the transition from the acidic gastric to the mild alkaline intestinal environment,
 425 caused a decrease in the amount of total flavonoids and total phenols in the
 426 bioaccessible fraction, as it is the case for the conversion of flavanones into chalcones
 427 which are less soluble than flavones and therefore less available for absorption under
 428 the *in vitro* digestion (Gil-Izquierdo et al., 2001). In addition to pH, the interaction
 429 between polyphenols and other components of the *in vitro* GI digestion such as enzymes
 430 or other dietary components released during digestion such as iron, other minerals,
 431 dietary fiber or proteins might affect its solubility and bioaccessibility (Rodriguez-
 432 Roque et al., 2014; Cilla et al., 2009).

433 The findings of this study, that have demonstrated significant TF bioaccessibility (BA)
 434 differences between *Citrus* species (oranges vs. mandarin) and oranges varieties (Navel
 435 vs. Cara Cara), are in accordance to those found by Aschoff et al. (2015) for Navel
 436 orange but not for Cara Cara. As the results presented by Aschoff et al. (2015), TF

437 bioaccessibility of Navel-juice (52.51%) was significantly higher than in Navel-pulp
 438 (31.53%). For this reason, the higher TF content of Navel-pulp (64.93 mg/100 g fw) in
 439 comparison to Navel-juice (40.97 mg/100 g fw) (Table 3) offers a relative low health-
 440 promoting benefit because TF concentration in the BF of both pulp and juice was
 441 similar (14.60 and 14.89 mg/100 g fw). However, Cara Cara orange not only had higher
 442 TF concentration in pulp (75.15 mg/100 g fw) than in juice (48.49 mg/100 g fw) but
 443 also CC-pulp showed higher TF bioaccessibility (41.19%) than in juice (30.85%). These
 444 results indicated a relative higher health benefit consuming CC-pulps than CC-juice due
 445 to the higher soluble TF concentration in the BF of pulp (21.45 g/100 g fw) than in juice
 446 (10.59 mg/100 g fw).

447 Although there is no a recommended daily allowance (RDA) for flavonoids it has
 448 been estimated it could be between 250-400 mg/d, respecting the seasonality of food
 449 sources (Peluso & Palmery, 2015). Table 4 showed the percentage of the suggested
 450 daily recommended intake of total flavonoids provided by equivalent portions (120 g)
 451 of pulp and juice of oranges (Navel and Cara Cara) and mandarin (Clementine) and also
 452 taking into account the TF bioaccessibility (Table 3). As shown in Table 4, the values of
 453 the percentage of suggested RDA are significantly lower when the bioaccessibility is
 454 considered (% RDA digested).

455 These results suggested that it is necessary to know the flavonoid bioaccessibility of
 456 *Citrus* fruits for calculating more accurately their daily intake amount. CC-pulp could
 457 better contribute to reach the recommended flavonoid daily intake among the *Citrus*
 458 fruits analyzed in the present study (Table 4).

459 Total phenol (TP) bioaccessibility followed different trend than TF. In general, TP
 460 bioaccessibility in pulps was not significantly higher than in juice (Table 3). The present
 461 results indicated that TP bioaccessibility found in orange juice (average value 25.4%)

are similar to those reported in a fruit beverage blend including 40% orange juice (26%) (Rodríguez-Roque et al. 2015) and in an exotic fruit juice mixture containing 7.5% orange juice (30%) (Carbonell-Capella, Buniowska, Esteve, & Frígola, 2015). On the other hand, these results are higher than those of other blended fruit juice beverage containing 50% orange juice (11-18%) (Rodríguez-Roque et al., 2013, 2014), but lower than the 90% TP bioaccessibility showed by Cilla et al. (2011) in other fruit juice beverage containing 4.2% w/w of orange concentrate. Different food matrix and *in vitro* GI digestion conditions could explain the differences observed.

3.3. Ascorbic acid

3.3.1. Before GI digestion

The concentration of ascorbic acid (AA) in the pulps and juices of both oranges (N and CC) and mandarin (M) were reported in Table 3. AA concentration in juices of oranges and mandarin (53.22 and 61.13 mg/100 g fw, respectively) was significantly higher than in their corresponding pulps (37.55 and 43.79 mg/100 g fw, respectively). These results did not agree with data previously presented by other authors such as Del Caro et al. (2004) that showed higher vitamin C content in segments of sweet orange Salustiana (69.70 mg/100 g fw) than in its juice (59.52 mg/100 g fw). Also, Aschoff et al. (2015) found higher vitamin C content in orange Navel segments (48.6 mg/100 g fw) than in their juices (43.1 mg/100 g fw). Regarding *Citrus* varieties, Navel orange juice showed the lowest ascorbic acid concentration (53.22 mg/100 g fw), meanwhile no significant differences ($p < 0.05$) were found between orange Cara Cara and mandarin Clementine (~57.50 mg/100 g fw) juices. In the present study, AA concentration in orange Navel (53.22 mg/100 g fw) and mandarin Clementine (56.98 mg/100 g fw) juices was significantly higher than those values found by other authors for similar

487 *Citrus* varieties. Thus, Cano et al. (2008) found a vitamin C content in orange Navel and
 488 mandarin Clementine of 47.8 and 38.7 mg/100 g fw, respectively. Also, Aschoff et al
 489 (2015) showed AA content in Navel juice of 43.1 mg/100 g fw. Regarding pulps, Cara
 490 Cara had the lowest AA concentration (37.55 mg/100 g fw), that was significantly lower
 491 ($p < 0.05$) than those found in orange Navel (43.63 mg/100 g fw) and mandarin
 492 Clementine (43.70 mg/100 g fw).

493 Postharvest storage of mandarins for 5 weeks at 12 °C produced a significant increase
 494 of 7% of AA content in the juice obtained from M12 mandarins, meanwhile no
 495 significant changes were recorded in the pulps.

496 The different AA content found by different authors for the same *Citrus* varieties is a
 497 factor well known because AA content in *Citrus* products depends on the specie,
 498 cultivar, climatological conditions harvesting season, besides other pre-harvest and
 499 postharvest conditions (Cardeñosa, Barreira, Barros, Arenas-Arenas, Moreno-Rojas, &
 500 Ferreira, 2015).

501 3.3.2. Bioaccessibility

502 In general, average vitamin C bioaccessible value in pulps was 22% higher than in
 503 juices (Table 3). The alkaline pH and other factors related to *in vitro* GI digestion
 504 (temperature, light, oxygen and enzyme activity) could enhance vitamin C oxidation or
 505 an interaction with metal ions (Rodriguez-Roque et al. 2013). In the case of orange
 506 Navel, with higher vitamin C concentration in juice (53.22 mg/100 g fw) than pulps
 507 (43.63 mg/100 g fw), but with lower ascorbic acid bioaccessibility in juice (74.78%)
 508 than in pulps (88.72%), resulted in a AA concentration released in the BF similar for
 509 both pulps and juice (~ 39 mg/100 g fw). This value was higher to those found by
 510 Aschoff et al. (2015) who presented lower AA concentration in the bioaccessible
 511 fraction of Navel segments and fresh juice (~24.5 mg/100 g fw) due to a lower ascorbic

acid bioaccessibility (53.4%) These different results could be due to the fact that Aschoff et al. (2015) employed distinct *in vitro* GI digestion conditions including an oral phase and higher centrifugation speed to obtain the BF. In the present study, CC-orange did not show significant differences in the AA bioaccessibility (~85%) between juice and pulps however had significantly higher AA concentration in the BF of juice (50 mg/100 g fw) than in pulp (30.98 /100 g fw). In the case of mandarin, also the ascorbic acid bioaccessibility was significantly higher in pulps (75.8%) than in juice (46%) and this behaviour was observed also after postharvest storage. Thus, the high ascorbic acid bioaccessibility values found in this study for pulps and juices are consistent to those found by other authors (Aschoff et al., 2015) and they are a demonstration of the high vitamin C stability during the digestion process, modulated by the citrus specie (orange and mandarin), food matrix (pulps and juices), and postharvest storage (before and after postharvest storage at 12 °C). Changes in the ascorbic acid or vitamin C bioaccessibility as consequence of food matrix and processing technology have been well referenced (Cilla et al., 2012; Rodriguez-Roque et al., 2013, 2014, 2015). Thus, ascorbic acid bioaccessibility values were comprised between 14-70% in blended fruit juices containing orange juice plus whole milk, skimmed milk and soymilk and treated by high pressure processing or pasteurization (Cilla et al., 2012). Other authors determined a vitamin C bioaccessibility value of 15% in a blended fruit juice (Rodriguez-Roque et al., 2013) and between 23% and 11% in fruit based beverage plus soymilk and milk (Rodriguez-Roque et al., 2014, 2015). The higher ascorbic acid bioaccessibility found by Cilla et al. (2012) in comparison with Rodriguez-Roque et al., (2013, 2014, 2015) in similar fruit juices beverages is due to the use by the latter authors of dialysis instead of centrifugation to obtain the

536 bioaccessible fraction and employed different fruits and proportions in the manufacture
537 of beverages.

538 Taking into account that the recommended dietary allowance (RDA) for vitamin C is
539 90 mg/d (IOM 2000), Table 4 showed the percentage of vitamin C (ascorbic acid) RDA
540 provided by equivalent portions (120 g) of pulp and juice of oranges (Navel and Cara
541 Cara) and mandarin (Clementine) before and after digestion taking into account the
542 ascorbic acid content and its bioaccessibility (Table 3). Table 4 showed significantly
543 lowest values of the percentage of RDA when the bioaccessibility is considered. These
544 results suggested that it is necessary to know the bioaccessibility of ascorbic acid of
545 *Citrus* fruits for calculating more accurately its daily intake amount. CC-juice could
546 better contribute to reach the RDA for vitamin C (ascorbic acid) among the *Citrus* fruits
547 analyzed in the present study.

548

549 **3.4. Hydrophilic antioxidant activity**

550 *3.4.1. Before GI digestion*

551 Different methods (DPPH[•], ABTS^{•+} and FRAP) have been employed to determine the
552 antioxidant activity (AAC) of *Citrus* products before and after an *in vitro* GI digestion
553 procedure due to the complex mechanism of action of the antioxidant compounds
554 present in fruits (Table 5) (Stinco et al., 2015).

555 In general, orange N-pulp presented the highest AAC by the three methods (Table 5).

556 Regarding the comparison between orange varieties (N and CC), N-pulp had 27% and
557 35% higher AAC than CC-pulp measured by ABTS^{•+} and DPPH[•], respectively,
558 although no significant differences ($p < 0.05$) were found between N-pulp and CC-pulp
559 by FRAP method. In juices, the orange CC-juice presented 22% and 17% higher AAC
560 than N-juice measured by DPPH[•] and FRAP assays, respectively (Table 5). Comparing

561 oranges and mandarin, N-pulp had 41%, 20% and 38% higher AAC than mandarin M-
 562 pulp according to ABTS^{•+}, DPPH[•] and FRAP methodologies, respectively, meanwhile
 563 juices of N and CC presented similar AAC than mandarin M-juices (Table 5).

564 The postharvest storage of mandarin at 12 °C for 5 weeks produced a significant
 565 increase of AAC in both pulp and juice measured by ABTS^{•+}, meanwhile no significant
 566 changes were observed with DPPH[•] and FRAP.

567 In general, pulps of oranges (N and CC) and mandarin (M) had significantly higher
 568 antioxidant activity than their corresponding juices determined with the three methods
 569 assayed (DPPH[•], ABTS^{•+} and FRAP) (Table 5). For example, N-pulp, CC-pulp and M-
 570 pulp showed 3-, 2.5- and 2-times higher AAC than their corresponding juices,
 571 respectively, by FRAP method (Table 5). Also, pulps, that displayed higher values of
 572 TP, TF and HES and NAR concentration than their corresponding juices (Table 3),
 573 showed a very high and positive correlation between FRAP and these phytochemicals in
 574 pulps (Table 6). In contrast, juices, that had higher ascorbic acid concentration than
 575 pulps, showed good positive correlation between ascorbic acid and ABTS^{•+} ($r^2=0.685$,
 576 $p<0.01$) and DPPH[•] ($r^2=0.784$, $p<0.01$) (Table 6).

577 The present study showed high antioxidant properties of total phenols, total flavonoids
 578 and individual flavanones (HES and NAR) of *Citrus* pulps against FRAP assay that
 579 measure total reducing capability of antioxidants based on iron reduction. Also, pulps of
 580 oranges and mandarin displayed a high and very similar antioxidant capacity ($p<0.05$)
 581 in the FRAP assays as it was observed in previous studies with unpeeled oranges and
 582 mandarins (Girones-Vilaplana et al., 2014), but these authors did not found good
 583 correlation between FRAP values and *Citrus* bioactive compounds (TP and vitamin C)
 584 perhaps due to the presence of other antioxidant compounds with different chemical
 585 structure released from the peel.

586 3.4.2. Bioaccessibility

587 In general, the AAC of the BF of pulps were significantly higher than those of their
 588 corresponding juices (Table 5). After the *in vitro* GI process, a significant loss of
 589 antioxidant bioactive compounds (mainly polyphenols due to the alkaline intestinal
 590 conditions) (Table 3) in pulps and juices of oranges and mandarins were detected. Thus,
 591 the AAC of the BF of oranges and mandarin pulps maintained approximately 70-96%
 592 (by ABTS^{•+}), 23-54% (by DPPH[•]) and 36-41% (by FRAP) of the antioxidant activity of
 593 the non-digested pulps. However, digested juices measured by ABTS^{•+} showed between
 594 16-21% higher AAC values than non-digested juices, meanwhile AAC analysed by
 595 DPPH[•] and FRAP significantly decreased between 64-92% and 16-49%, respectively, in
 596 comparison with the AAC of the non-digested juices (Table 5).

597 Different AAC results were found in the literature for the BF of fruit beverages
 598 containing orange juice. Thus, Cilla et al. (2011) found a significant increase of AAC in
 599 digested fruit beverages in comparison with the non-digested beverages that was not
 600 correlated neither with vitamin C nor polyphenol content in the BF. In contrast,
 601 Rodríguez-Roque et al. (2013, 2014, 2015) found a decrease of the AAC of the fruit
 602 beverage after the digestion process that was modulated by the food matrix and the type
 603 or processing.

604 In the present study, the depletion of AAC in the BF observed could be related with
 605 the changes of the antioxidant compounds analysed (TP, TF, AA, HES and NAR).
 606 Thus, the correlation coefficients between the concentration of these antioxidant
 607 compounds and the antioxidant capacity values (DPPH[•], FRAP and ABTS^{•+}) of the BF
 608 of *Citrus* pulps and juices are shown in Table 6.

609

610 Conclusions

611 The biological activity of polyphenols and ascorbic acid of *Citrus* products depends on
 612 their concentration, bioaccessibility and the citrus matrix in which are engaged. Thus,
 613 the pulps of oranges (N and CC) and mandarin (M) displayed higher hesperidin (HES),
 614 narirutin (NAR), total flavonoids (TF), total phenol (TP) and antioxidant activity (AAC)
 615 than a portion of the same weight of their corresponding juices. Thereby, oranges and
 616 mandarin pulps showed 1.5-2.3-times higher TF content and 2-3-times higher AAC
 617 (FRAP) than their corresponding juices. A comparison between oranges (N and CC)
 618 showed that pulp and juice of red-fleshed orange Cara Cara (CC) orange showed higher
 619 HES, NAR, TF and TP content than the blonde-flesh orange Navel (N) while N-pulp
 620 showed higher ascorbic acid content and antioxidant activity (AAC) (ABTS^{•+}, DPPH[•])
 621 than CC-pulp. The postharvest storage of mandarin at 12 °C for 5 weeks produced a
 622 significant increase of NAR, TP, AA and AAC (ABTS^{•+}).

623 Bioaccessibility of bioactive compounds (HES, NAR, TF, TP, and AA) and AAC of
 624 the bioaccessible fraction were higher in pulps of both oranges and mandarin than in
 625 their corresponding juices. Regarding comparison between *Citrus* products, pulps and
 626 juice of oranges (N and CC) showed higher bioactive compounds bioaccessibilities than
 627 mandarin. In the comparison between the oranges varieties, it was observed higher
 628 bioactive compounds bioaccessibility in CC-pulps than in N-pulps. Also, the
 629 postharvest storage of mandarin at 12 °C for 5 weeks produced a significant increase in
 630 the bioaccessibility of bioactive compounds.

631 In general, the concentration of bioactive compounds in BF was significantly lower
 632 than in the initial *Citrus* products (pulps and juices). All these data point out that it is
 633 necessary to know the bioaccessibility of bioactive compounds of *Citrus* fruits for
 634 calculating more accurately their daily intake amount. The consumption of pulps

635 compared to the same portion of fruit juices would confer a better supply of bioactive
636 compounds and antioxidant activity with potential health benefits.

637

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- 782

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785 **Figure Caption**

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787 **Figure 1.** HPLC-DAD chromatogram of the phenolic compounds at 280 nm from
788 'Navel' orange juice. Peak identification in Table 1.

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Figure 1. HPLC-DAD chromatogram of the phenolic compounds at 280 nm from 'Navel' orange juice. Peak identification in Table 1.

794 **Table 1.** Major Flavonoids and hydroxycinnamic acid derivatives in pulps and juices of oranges and mandarins characterized by UV-visible
 795 spectrum, HPLC-DAD and HPLC-ESI-QTOF-MS

Compound Number	R _t (min)	λ _{max} (nm)	Precursor Ion m/z [M-H] ⁻	Product Ion (m/z)	MW	Compound Name
Hydroxycinnamic acid derivatives						
1	8.34	304 _{sh} , 330	355		356	Ferulic acid- <i>O</i> -hexoside
2	8.62	332	385		386	Sinapic acid- <i>O</i> -hexoside
Flavonoid compounds						
3	9.13	270 _{small} , 334	593	473 [M-H-120] ⁻ ; 353 [M-H-240] ⁻	594	Apigenin-6,8-di- <i>C</i> -glucoside
4	9.63	284, 330 _{small}	741	579 [M-H-162] ⁻ ; 433 [M-H-308] ⁻ ; 271 [M-H-308-162] ⁻	742	Naringenin-7- <i>O</i> -rutinoside-4'- <i>O</i> -glucoside
5	11.88	284, 325 _{small}	595	449[M-H-146] ⁻	596	Eriodyctiol- <i>O</i> -rutinoside (Eriocitrin)
6	12.89	264 _{small} , 360	609	301 [M-H-308] ⁻	610	Quercetin-3- <i>O</i> -rutinoside (Rutin)
7	14.03	284, 332 _{small}	579	271 [M-H-308] ⁻	580	Naringenin-7- <i>O</i> -rutinoside (Narirutin)
8	15.31	284, 334 _{small}	609	301 [M-H-308] ⁻	610	Hesperetin-7- <i>O</i> -rutinoside (Hesperidin)
9	19.73	284, 334 _{small}	593	285 [M-H-308] ⁻	594	Isosakuranetin-7- <i>O</i> -rutinoside (Dydimin)

799 **Table 2.** Concentration and bioaccessibility of major flavonoids from pulps and juices of sweet oranges and mandarins¹

<i>Citrus</i> Product	<i>Citrus</i> ² Variety	Apigenin-6,8- Di-C-Glucoside (Vicenin 2)	Naringenin-7-O- Rutinoside-4'-O- Glucoside	Eriodyctiol-7- O-Rutinoside (Eriocitrin)	Quercetin-3-O- Rutinoside (Rutin)	Naringenin-7-O- Rutinoside (Narirutin)	Hesperitin-7-O- Rutinoside (Hesperidin)	Isosakunetin-7-O- Rutinoside (Dydimin)
Compound concentration in non-digested product (µg/100 g fw) ³								
Pulp	Navel-N	8041 ± 174dB	6534 ± 510bB	3160 ± 57cB	1076 ± 11cB	12459 ± 327cB	31448 ± 989bB	2210 ± 196dB
	Cara Cara-CC	7487 ± 273cB	6754 ± 335bB	2664 ± 97bB	843 ± 113bB	12198 ± 534cB	43430 ± 2163cB	1777 ± 131bA
	Clementine-M	112 ± 10aA	1391 ± 69aA	1127 ± 72aB	439 ± 67aA	1653 ± 16aB	12439 ± 254aB	1488 ± 14aB
	Clementine-M12	180 ± 47bB	1295 ± 43aA	1030 ± 92aB	495 ± 30aB	2018 ± 82bB	13027 ± 1035aB	1832 ± 92cB
Juice	Navel-N	4234 ± 675dA	2242 ± 67cA	778 ± 74cA	555 ± 47dA	7219 ± 55cA	24179 ± 17bA	1826 ± 68bA
	Cara Cara-CC	3567 ± 200cA	3544 ± 290dA	743 ± 57cA	484 ± 18cA	8221 ± 137dA	29247 ± 1265cA	2678 ± 95cB
	Clementine-M	222 ± 22bB	538 ± 22aA	451 ± 14aA	193 ± 6.79aB	1254 ± 36bA	5354 ± 99aA	106 ± 15aA
	Clementine-M12	29 ± 9aA	1391 ± 69bA	604 ± 37bA	393 ± 20bA	871 ± 61aA	4989 ± 165aA	109 ± 14aA

Bioaccessibility (%)								
Pulp	Navel-N	16.05 ± 2.69aA	38.59 ± 2.47bB	15.68 ± 0.45aA	26.41 ± 0.35bcA	49.04±0.62bA	47.44 ± 2.58cA	53.00 ± 2.15cA
	Cara Cara-CC	26.30 ± 4.71bB	46.86 ± 7.79cB	36.22 ± 1.31bA	50.40 ± 6.73bcA	53.71± 0.76cB	59.04± 0.13dB	55.76 ± 8.2cB
	Clementine-M	Nd	25.91 ± 1.43aB	Nd	22.71 ± 3.69aB	21.76 ± 0.14aB	9.79 ± 0.10aA	2.17±0.11a
	Clementine-M12	Nd	65.22 ± 2.28dB	Nd	56.16 ± 3.44b	56.56± 2.82cB	24.29 ± 2.53bA	18.01±1.34b
Juice	Navel-N	20.36 ± 2.53bB	24.91 ± 3.03dA	55.69 ± 3.76aB	66.16± 5.07bB	58.14 ± 0.49dB	57.16 ± 2.40dB	76.24 ± 1.07bB
	Cara Cara-CC	5.78 ± 0.68aA	6.44 ± 0.64cA	26.20 ± 1.59bB	50.57 ± 4.00bA	38.80 ± 0.58cA	35.40 ± 1.65cA	13.57 ± 0.40aA
	Clementine-M	Nd	4.80 ± 0.13bA	Nd	8.52 ± 1.38aA	2.58 ± 0.37bA	6.38 ± 0.06aB	Nd
	Clementine-M12	Nd	1.93 ± 0.10aA	Nd	Nd	6.07 ± 0.05aA	19.17 0.82bB	Nd

800

801 ¹Data are expressed as the mean ± SD (n = 4). ² Navel-N and Cara Cara-CC are two varieties of sweet orange fruit. Clementine-M and Clementine-M12 are a variety of
802 mandarin before and after 5 weeks storage at 12 °C, respectively. ³ Different lower case letters in the same column and *Citrus* product indicate significant differences (*p*
803 <0.05) within citrus varieties. Different capital letters in the same column and *Citrus* variety indicate significant differences (*p* <0.05) within *Citrus* product (pulp and juice).
804 Nd, not detected

805

Table 3. Concentration and bioaccessibility of ascorbic acid (AA), total phenols (TP) and total flavonoids (TF) from pulps and juices of sweet oranges and mandarins¹

<i>Citrus</i>	<i>Citrus</i> ²	AA ⁴	TP	TF
Product	Variety	Compound concentration in non-digested product (mg/100 g fw) ³		
Pulp	Navel-N	43.63 ± 0.49bA	95.18 ± 3.35cB	64.93 ± 1.61bB
	CaraCara-CC	37.55 ± 1.15aA	102.83 ± 1.67dB	75.15 ± 2.99cB
	Clementine-M	43.79 ± 1.41bcA	75.04 ± 2.95aB	18.57 ± 0.18aB
	Clementine-M12	40.78 ± 0.21abA	85.15 ± 2.29bB	19.87 ± 0.78aB
Juice	Navel-N	53.22 ± 0.48aB	63.92 ± 3.94aA	40.97 ± 0.78bA
	CaraCara-CC	56.47 ± 1.41abB	62.66 ± 1.33aA	48.49 ± 1.48cA
	Clementine-M	56.98 ± 1.00bB	56.18 ± 2.82bA	8.15 ± 0.10aA
	Clementine-M12	61.13 ± 0.35cB	62.20 ± 4.26aA	8.39 ± 0.23aA
Bioaccessibility (%)				
Pulp	Navel-N	88.72 ± 4.48bB	29.13 ± 0.54dA	31.53 ± 0.47bA
	CaraCara-CC	82.41 ± 4.60abA	27.36 ± 0.65cA	41.19 ± 1.02cB
	Clementine-M	75.81 ± 4.42aB	23.18 ± 0.78aB	11.13 ± 0.21aB
	Clementine-M12	75.26 ± 1.00aB	26.46 ± 0.59bA	31.49 ± 3.28bB
Juice	Navel-N	74.78 ± 0.54cA	31.20 ± 4.66bA	52.51 ± 1.82dB
	CaraCara-CC	89.23 ± 3.37dA	28.88 ± 0.74bA	30.85 ± 0.77cA
	Clementine-M	46.34 ± 3.35aA	19.51 ± 2.18aA	10.02 ± 0.02aA
	Clementine-M12	67.30 ± 1.66bA	28.85 ± 2.18bA	15.20 ± 3.31bA

808

809 ¹Data are expressed as the mean ± SD (n = 4). ²Navel-N and Cara Cara-CC are two varieties of sweet
810 orange fruit. Clementine-M and Clementine-M12 are a variety of mandarin before and after 5 weeks
811 storage at 12 °C, respectively. ³Different lower case letters in the same column and citrus product for

812 compound concentration or bioaccessibility, indicate significant differences ($p < 0.05$) within citrus
813 varieties. Different capital letters in the same column and citrus variety for compound concentration or
814 bioaccessibility, indicate significant differences ($p < 0.05$) within *Citrus* product (pulp and juice).⁴ AA=
815 Ascorbic Acid.

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Table 4. Percentage (%) of the flavonoid recommended daily allowance (RDA) and percentage of the vitamin C (ascorbic acid) recommended dietary allowance (RDA) provided by equivalent portions (120 g) of orange (Navel and Cara Cara) and mandarin (Clementine)

<i>Citrus</i> portion (120 g)	Total Flavonoids (TF)			Ascorbic Acid (AA)		
	Content in portion (mg)	%RDA ¹ Non-digested	%RDA ² Digested	Content in portion (mg)	%RDA ³ Non-digested	%RDA ⁴ Digested
1Whole peeled Navel orange	114.22	31.2-19.5	9.8-6.1	52.36	58.2	51.9
Navel orange juice	76.70	19.6-12.3	9.7-6.1	63.86	70.9	53.1
1Whole peeled Cara Cara orange	123.40	39.7-22.5	14.8-9.3	45.06	50.1	41.2
Cara Cara orange juice	75.19	23.3-14.5	7.2-4.5	67.76	75.3	67.2
2 Whole peeled Clementine mandarin	90.05	8.9-5.6	1-0.6	52.55	58.4	44.3
Clementine mandarin juice	67.42	3.9-2.4	3-2	68.38	75.9	32.6

¹Percentage (%) of flavonoid RDA (250-400 mg/d) provided by the non-digested product.

²Percentage (%) of flavonoid RDA (250-400 mg/d) taking into account the TF bioaccessibility (Table 3). ³Percentage (%) of vitamin C RDA (90 mg/d) provided by the non-digested product.

⁴Percentage (%) of vitamin C RDA (90 mg) taking into account the AA bioaccessibility (%) (Table 3)

829 **Table 5.** Antioxidant activity (AAC) of non-digested and digested (bioaccessible fraction) pulps
 830 and juices of sweet oranges and mandarin¹

<i>Citrus</i>	<i>Citrus</i> ²	Non-Digested <i>Citrus</i> Product		
Product	Variety	(Equ $\mu\text{M AA}/100 \text{ g fw}^4$)		
		ABTS ⁺⁺	DPPH [•]	FRAP
Pulp	Navel-N	504.67 $\pm$ 14.3cB	356.99 $\pm$ 9.18cB	475.38 $\pm$ 9.15bB
	Cara Cara-CC	398.44 $\pm$ 3.80bB	264.30 $\pm$ 1.98aB	467.49 $\pm$ 4.79bB
	Clementine-M	357.36 $\pm$ 7.09aB	298.14 $\pm$ 7.53bB	343.69 $\pm$ 9.38aB
	Clementine-M12	402.45 $\pm$ 11.2bB	304.30 $\pm$ 8.22bB	371.88 $\pm$ 15.65aB
Juice	Navel-N	289.90 $\pm$ 8.67aA	164.00 $\pm$ 5.19aA	159.76 $\pm$ 10.75aA
	Cara Cara-CC	283.76 $\pm$ 4.98aA	200.45 $\pm$ 0.46bA	186.80 $\pm$ 4.78bA
	Clementine-M	291.68 $\pm$ 0.33aA	198.51 $\pm$ 3.63bA	170.96 $\pm$ 11.48abA
	Clementine-M12	355.42 $\pm$ 9.31bA	203.90 $\pm$ 5.29bA	173.28 $\pm$ 16.10abA
Bioaccessible Fraction (corresponding to 100 g fw <i>Citrus</i> product)				
(Equ $\mu\text{M AA}^4$)				
		ABTS ⁺⁺	DPPH [•]	FRAP
Pulp	Navel-N	352.93 $\pm$ 1.18abB	110.67 $\pm$ 5.80cB	186.96 $\pm$ 3.89bB
	Cara Cara-CC	354.36 $\pm$ 1.19abB	142.22 $\pm$ 2.43dB	169.48 $\pm$ 13.50bB
	Clementine-M	344.51 $\pm$ 3.76aA	69.63 $\pm$ 1.87aB	141.13 $\pm$ 5.56aB
	Clementine-M12	360.03 $\pm$ 3.48bB	100.75 $\pm$ 6.88bB	150.79 $\pm$ 10.92aB
Juice	Navel-N	336.55 $\pm$ 7.62acA	58.63 $\pm$ 3.28dA	134.89 $\pm$ 11.0bA

Cara Cara-CC	337.05 ± 0.74aA	23.45 ± 1.25bA	95.26 ± 4.57aA
Clementine-M	353.89 ± 1.66bcB	14.80 ± 1.58aA	98.95 ± 0.80aA
Clementine-M12	332.26 ± 2.74aA	44.90 ± 3.63cA	92.51 ± 4.11aA

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832 ¹Data are expressed as the mean ± SD (n = 4). ² Navel and Cara Cara are two varieties of orange fruit.
833 Clementine-C and Clementine-M12 are a variety of mandarin before and after 5 weeks storage at 12 °C,
834 respectively. ³Different lower case letters in the same column and citrus product indicate significant
835 differences ($p < 0.05$) within citrus varieties. Different capital letters in the same column and citrus
836 variety indicate significant differences ($p < 0.05$) within citrus product (pulp and juice).⁴ AA= Ascorbic
837 Acid.

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841 **Table 6.** Pearson`s correlation coefficient between HES, NAR, TF, TP and AA content and AAC
 842 determined by ABTS⁺⁺, DPPH[•] and FRAP methods in non-digested and digested (bioaccessible
 843 fraction) pulps and juices of sweet oranges and mandarin

<i>Citrus</i> Product	<i>Citrus</i> ¹ data	Non-Digested <i>Citrus</i> Product		ABTS ⁺⁺
		DPPH [•]	FRAP	
Pulp	HES	0.406 ^c	ns	0.857^a
	NAR	0.669^a	ns	0.950^a
	TF	0.398 ^c	ns	0.816^a
	TP	0.494 ^b	ns	0.870^a
	AA	0.414 ^c	0.808^a	ns
	ABTS ⁺⁺	1	0.782^a	0.786^a
	DPPH [•]	0.782^a	1	0.273
	FRAP	0.786^a	0.273	1
Juice	HES	-0.593 ^b	-0.440 ^c	ns
	NAR			
	TF	-0.363 ^c	-0.950^a	-0.363 ^c
	TP	ns	-0.358 ^c	ns
	AA	0.685^a	0.784^a	0.203
	ABTS ⁺⁺	1	0.357 ^c	ns
	DPPH [•]	0.357 ^c	1	0.413 ^c
	FRAP	ns	0.413 ^c	1
Bioaccessible Fraction				
		ABTS ⁺⁺	DPPH [•]	FRAP
Pulp	HES	0.238	0.855^a	0.576 ^b
	NAR	0.223	0.860^a	0.678^a
	TF	0.235	0.862^a	0.626^a
	TP	0.509 ^b	0.948^a	0.815^a
	AA	ns	ns	0.534 ^b

	ABTS ⁺⁺	1	0.563 ^b	ns
	DPPH [•]	0.563 ^b	1	0.682^a
	FRAP	ns	0.682^a	1
Juice	HES	-0.357 ^c	0.511 ^b	0.674^a
	NAR	-0.341 ^c	0.472 ^c	0.674^a
	TF	-0.346 ^c	0.536 ^b	0.713^a
	TP	-0.690^a	0.727^a	0.458 ^b
	AA	-0.742^a	0.274	ns
	ABTS ⁺⁺	1	-0.636^a	ns
	DPPH [•]	-0.636^a	1	0.634^a
	FRAP	ns	0.634^a	1

844

845 ¹HES (Hesperidin), NAR (Narirutin), TF (Total flavonoids), TF (Total phenols), AA (Ascorbic
846 acid);^a significant level $p<0.01$; ^b significant level $p<0.05$; ^c significant level $0.05<p<0.1$; ns, no
847 significant.

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852 **Highlights**

853 Pulps had higher HES, NAR, TF and TP contents and antioxidant activity than juices

854 Postharvest storage of mandarin increased NAR, TP, AA and AAC

855 Higher bioaccessibility of HES, NAR, TF, AA and AAC can be found in pulps vs.

856 juices

857 Postharvest storage of mandarins increased bioaccessibility of bioactive compounds

858 *Citrus* bioactive compounds' bioaccessibility would allow more accurate RDA values

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