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

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Abstract

Polyphenols, ascorbic acid content and antioxidant activity of two sweet oranges (Navel-N and Cara Cara-CC) and mandarin (Clementine-M) as well as their bioaccessibilities were evaluated in pulps and compared to those in fresh juice. Thus, pulps of oranges and mandarins displayed higher hesperidin (HES), narirutin (NAR), total flavonoids (TF), total phenols (TP) and antioxidant activity (AAC) than their corresponding juices. Also, CC products presented higher bioactive compounds content than N ones. Bioaccessibility of bioactive compounds and AAC were higher in pulps of both oranges and mandarin than in their corresponding juices. Oranges (N and CC) pulps and juices presented higher bioaccessibilities than mandarin ones.

The postharvest storage of mandarin at 12 ºC during 5 weeks not only produced a significant increase of the bioactive compounds but also an increase of their bioaccessibility. The bioaccessibility of *Citrus* bioactive compounds is necessary for calculating more accurately their daily intake amount.

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

1. Introduction

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

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

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

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

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

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

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

To the author´s knowledge, there are no previously reports evaluating the effect of food matrix (citrus pulp *vs*. citrus fresh juice), orange variety (Navel *vs*. Cara Cara) and postharvest storage (mandarin Clementine control *vs*. five weeks at 12 ºC) on the 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 postharverst 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 (≥ 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), eriodyctiol-*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) choride hexahydrate, phosphate buffered saline, hexadecyltrimethyl-ammonium bromide, 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), and potassium persulfate ($K_2S_2O_8$) 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 ºC 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 µg/ 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, Gainsville, VA).The sample was centrifuged at 9000×g during 15 min at 4 ºC 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, 37x55 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 0.45µm syringe filter and stored at
169 -80 ºC 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 µg/ 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

2.6 Total Phenol and antioxidant activity determinations

2.6.1 Sample extraction

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

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

2.6.2. Total phenol assay

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

Briefly, 0.2 mL of sample extract (or dilutions) was placed into 3 mL test tubes with 1 mL of distilled water and 1 mL of Folin-Ciocalteu reagent and then vortex 5 s. After 5 min, 0.8 mL of sodium carbonate (3.5%, w/v) was added. The mixture was incubated in the dark for 60 min and then 300 µL of the reaction mixture were placed in the microplate well in triplicate. Absorbance was measured at 750 nm in a 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 µg/ 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 ($ABTS^{\bullet+}$) 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 ($ABTS^{\bullet+}$) was produced by reacting ABTS with potassium persulfate ($K_2S_2O_8$) and allowing to stand in darkness at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ 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 $ABTS^{\bullet+}$, and after 20 min in darkness at 30 °C, absorbance was measured at 734 nm in a microplate spectrophotometer (PowerWame 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 ($DPPH^{\bullet}$) 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 ± 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 $[M-H]^-$ 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 $[M-H]^-$ 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 MS^1 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 reveled 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-disustitution (Barreca, Belloco, 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 Gatusso et al. (2007) and Abad-García et al. (2012).

The MS^1 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*-

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

3.2. Flavonoid and total phenolic content

3.2.1. Before GI digestion

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

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

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

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

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

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

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

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

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

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

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

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

3.2.2.Bioaccessibility

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

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

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

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

410 Total flavonoid (TF) bioaccessibility followed the same trend than the major
411 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 ºC 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

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

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

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

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

462 are similar to those reported in a fruit beverage blend including 40% orange juice (26%)
463 (Rodriguez-Roque et al. 2015) and in an exotic fruit juice mixture containing 7.5%
464 orange juice (30%) (Carbonell-Capella, Buniowska, Esteve, & Frígola, 2015). On the
465 other hand, these results are higher than those of other blended fruit juice beverage
466 containing 50% orange juice (11-18%) (Rodríguez-Roque et al., 2013, 2014), but lower
467 than the 90% TP bioaccessibility showed by Cilla et al. (2011) in other fruit juice
468 beverage containing 4.2% w/w of orange concentrate. Different food matrix and *in vitro*
469 GI digestion conditions could explain the differences observed.
470

471 ***3.3. Ascorbic acid***

472 *3.3.1. Before GI digestion*

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

512 acid bioaccessibility (53.4%) These different results could be due to the fact that
513 Aschoff et al. (2015) employed distinct *in vitro* GI digestion conditions including an
514 oral phase and higher centrifugation speed to obtain the BF. In the present study, CC-
515 orange did not show significant differences in the AA bioaccessibility (~85%) between
516 juice and pulps however had significantly higher AA concentration in the BF of juice
517 (50 mg/100 g fw) than in pulp (30.98 /100 g fw). In the case of mandarin, also the
518 ascorbic acid bioaccessibility was significantly higher in pulps (75.8%) than in juice
519 (46%) and this behaviour was observed also after postharvest storage. Thus, the high
520 ascorbic acid bioaccessibility values found in this study for pulps and juices are
521 consistent to those found by other authors (Aschoff et al., 2015) and they are a
522 demonstration of the high vitamin C stability during the digestion process, modulated
523 by the citrus specie (orange and mandarin), food matrix (pulps and juices), and
524 postharvest storage (before and after postharvest storage at 12 ºC). Changes in the
525 ascorbic acid or vitamin C bioaccessibility as consequence of food matrix and
526 processing technology have been well referenced (Cilla el al., 2012; Rodriguez-Roque
527 et al., 2013, 2014, 2015). Thus, ascorbic acid bioaccessibilty values were comprised
528 between 14-70% in blended fruit juices containing orange juice plus whole milk,
529 skimmed milk and soymilk and treated by high pressure processing or pasteurization
530 (Cilla et al., 2012). Other authors determined a vitamin C bioaccessibility value of 15%
531 in a blended fruit juice (Rodriguez-Roque et al., 2013) and between 23% and 11% in
532 fruit based beverage plus soymilk and milk (Rodriguez-Roque et al., 2014, 2015). The
533 higher ascorbic acid bioaccessibility found by Cilla et al. (2012) in comparison with
534 Rodriguez-Roque et al., (2013, 2014, 2015) in similar fruit juices beverages is due to
535 the use by the latter authors of dialysis instead of centrifugation to obtain the

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

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

3.4. Hydrophilic antioxidant activity

3.4.1. Before GI digestion

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

In general, orange N-pulp presented the highest AAC by the three methods (Table 5). Regarding the comparison between orange varieties (N and CC), N-pulp had 27% and 35% higher AAC than CC-pulp measured by $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, respectively, although no significant differences ($p<0.05$) were found between N-pulp and CC-pulp by FRAP method. In juices, the orange CC-juice presented 22% and 17% higher AAC than N-juice measured by $DPPH^{\bullet}$ and FRAP assays, respectively (Table 5). Comparing

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

The postharvest storage of mandarin at 12 ºC for 5 weeks produced a significant increase of AAC in both pulp and juice measured by $ABTS^{\bullet+}$, meanwhile no significant changes were observed with $DPPH^{\bullet}$ and FRAP.

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

The present study showed high antioxidant properties of total phenols, total flavonoids and individual flavanones (HES and NAR) of *Citrus* pulps against FRAP assay that measure total reducing capability of antioxidants based on iron reduction. Also, pulps of oranges and mandarin displayed a high and very similar antioxidant capacity (p<0.05) in the FRAP assays as it was observed in previous studies with unpeeled oranges and mandarins (Girones-Vilaplana et al., 2014), but these authors did not found good correlation between FRAP values and *Citrus* bioactive compounds (TP and vitamin C) perhaps due to the presence of other antioxidant compounds with different chemical 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 $ABTS^{\bullet+}$), 23-54% (by $DPPH^{\bullet}$) and 36-41% (by FRAP) of the antioxidant activity of
593 the non-digested pulps. However, digested juices measured by $ABTS^{\bullet+}$ showed between
594 16-21% higher AAC values than non-digested juices, meanwhile AAC analysed by
595 $DPPH^{\bullet}$ 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 ($DPPH^{\bullet}$, FRAP and $ABTS^{\bullet+}$) 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) ($ABTS^{\bullet+}$, $DPPH^{\bullet}$)
621 than CC-pulp. The postharvest storage of mandarin at 12 ºC for 5 weeks produced a
622 significant increase of NAR, TP, AA and AAC ($ABTS^{\bullet+}$).

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 ºC 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

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

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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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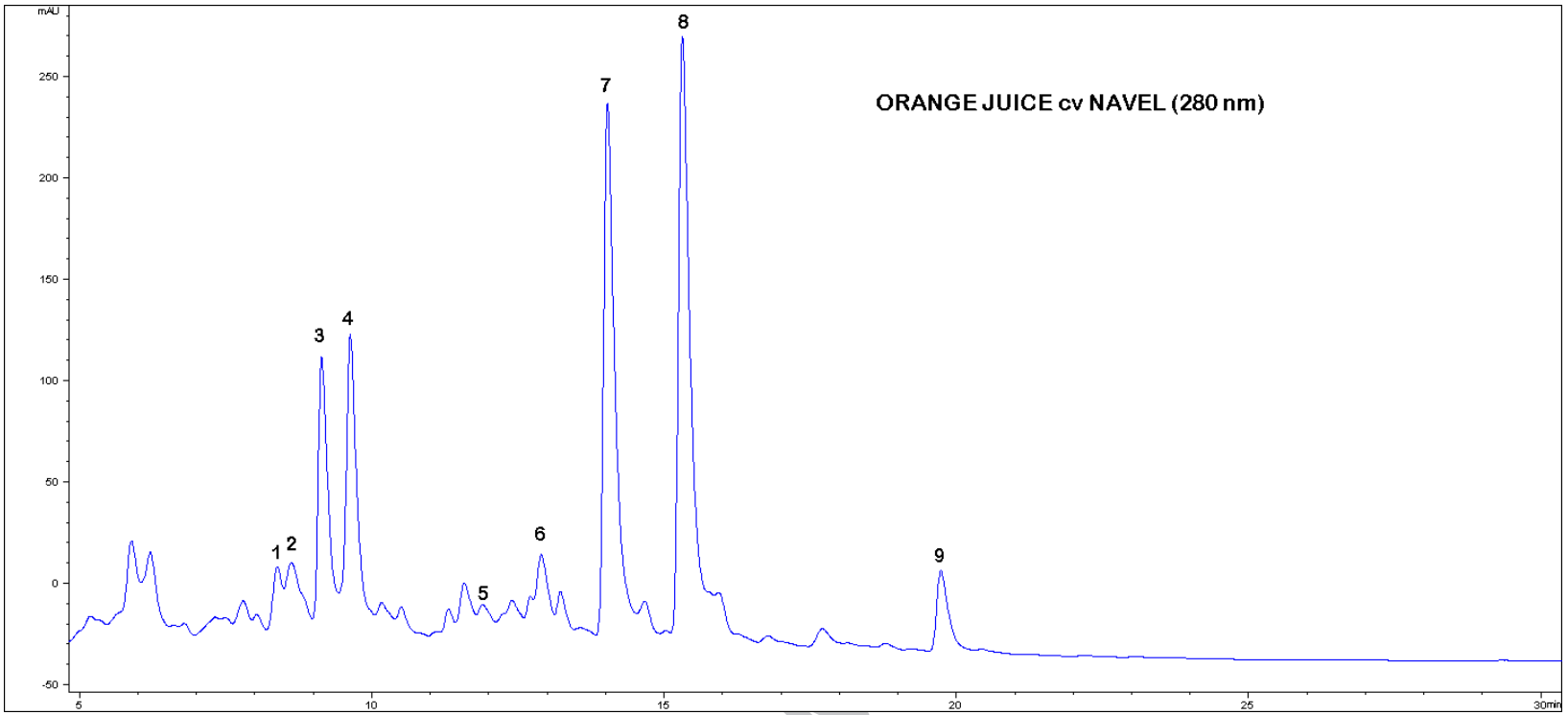

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791 **Figure 1**. HPLC-DAD chromatogram of the phenolic compounds at 280 nm from 'Navel' orange juice. Peak identification in Table 1.

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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-*O*-hexoside
2	8.62	332	385		386	Sinapic acid-O-hexoside
Flavonoid compounds						
3	9.13	270_{small}, 334	593	473 $[M-H-120]^-$; 353 $[M-H-240]^-$	594	Apigenin-6,8-di-*C*-glucoside
4	9.63	284, 330_{small}	741	579 $[M-H-162]^-$; 433 $[M-H-308]^-$; 271 $[M-H-308-162]^-$	742	Naringenin-7-*O*-rutinoside-4´-*O*-glucoside
5	11.88	284, 325_{small}	595	449$[M-H-146]^-$	596	Eriodyctiol-*O*-rutinoside (Eriocitrin)
6	12.89	264_{small}, 360	609	301 $[M-H-308]^-$	610	Quercetin-3-*O*-rutinoside (Rutin)
7	14.03	284, 332_{small}	579	271 $[M-H-308]^-$	580	Naringenin-7-*O*-rutinoside (Narirutin)
8	15.31	284, 334_{small}	609	301 $[M-H-308]^-$	610	Hesperetin-7-*O*-rutinoside (Hesperidin)
9	19.73	284, 334_{small}	593	285 $[M-H-308]^-$	594	Isosakuranetin-7-*O*-rutinoside (Dydimin)

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799 **Table 2.** Concentration and bioaccessibility of major flavonoids from pulps and juices of sweet oranges and mandarins[1]

Citrus Product	*Citrus*[2] Variety	Apigenin-6,8-Di-*C*-Glucoside (Vicenin 2)	Naringerin-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

[1]Data 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 mandarin before and after 5 weeks storage at 12 ºC, respectively. [3]Different lower case letters in the same column and *Citrus* product indicate significant differences (p <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). Nd, not detected

806 **Table 3.** Concentration and bioaccessibility of ascorbic acid (AA), total phenols (TP) and total
807 flavonoids (TF) from pulps and juices of sweet oranges and mandarins[1]

Citrus Product	*Citrus*[2] Variety	AA[4]	TP	TF
		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 [1]Data are expressed as the mean ± SD (n = 4). [2]Navel-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 ºC, respectively. [3]Different 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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819 **Table 4.** Percentage (%) of the flavonoid recommended daily allowance (RDA) and percentage of
820 the vitamin C (ascorbic acid) recommended dietary allowance (RDA) provided by equivalent
821 portions (120 g) of orange (Navel and Cara Cara) and mandarin (Clementine)

	Total Flavonoids (TF)			Ascorbic Acid (AA)		
***Citrus* portion (120 g)**	**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

822 [1]Percentage (%) of flavonoid RDA (250-400 mg/d) provided by the non-digested product.
823 [2]Percentage (%) of flavonoid RDA (250-400 mg/d) taking into account the TF bioaccessibility
824 (Table 3). [3]Percentage (%) of vitamin C RDA (90 mg/d) provided by the non-digested product.
825 [4]Percentage (%) of vitamin C RDA (90 mg) taking into account the AA bioaccessibility (%) (Table
826 3)

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829 **Table 5.** Antioxidant activity (AAC) of non-digested and digested (bioaccessible fraction) pulps
830 and juices of sweet oranges and mandarin[1]

Citrus Product	*Citrus*[2] Variety	Non-Digested *Citrus* Product (Equ µM AA/100 g fw[4])		
		ABTS$^{\bullet+}$	DPPH$^{\bullet}$	FRAP
Pulp	Navel-N	504.67 ± 14.3cB	356.99 ± 9.18cB	475.38 ± 9.15bB
	Cara Cara-CC	398.44 ± 3.80bB	264.30 ± 1.98aB	467.49 ± 4.79bB
	Clementine-M	357.36 ± 7.09aB	298.14 ± 7.53bB	343.69 ± 9.38aB
	Clementine-M12	402.45 ± 11.2bB	304.30 ± 8.22bB	371.88 ± 15.65aB
Juice	Navel-N	289.90 ± 8.67aA	164.00 ± 5.19aA	159.76 ± 10.75aA
	Cara Cara-CC	283.76 ± 4.98aA	200.45± 0.46bA	186.80 ± 4.78bA
	Clementine-M	291.68 ± 0.33aA	198.51 ± 3.63bA	170.96 ± 11.48abA
	Clementine-M12	355.42 ± 9.31bA	203.90 ± 5.29bA	173.28 ± 16.10abA
		Bioaccessible Fraction (corresponding to100 g fw *Citrus* product) (Equ µM AA[4])		
		ABTS$^{\bullet+}$	DPPH$^{\bullet}$	FRAP
Pulp	Navel-N	352.93 ± 1.18abB	110.67 ± 5.80cB	186.96 ± 3.89bB
	Cara Cara-CC	354.36 ± 1.19abB	142.22 ± 2.43dB	169.48 ± 13.50bB
	Clementine-M	344.51 ± 3.76aA	69.63 ± 1.87aB	141.13 ± 5.56aB
	Clementine-M12	360.03 ± 3.48bB	100.75 ± 6.88bB	150.79 ± 10.92aB
Juice	Navel-N	336.55 ± 7.62acA	58.63 ± 3.28dA	134.89 ± 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

831

832 [1]Data 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 ºC,
834 respectively. [3]Different 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 $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP methods in non-digested and digested (bioaccessible
843 fraction) pulps and juices of sweet oranges and mandarin

Citrus Product	*Citrus*[1] data	Non-Digested *Citrus* Product DPPH$^{\bullet}$	FRAP	$ABTS^{\bullet+}$
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
	$ABTS^{\bullet+}$	1	**0.782**[a]	**0.786**[a]
	$DPPH^{\bullet}$	**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
	$ABTS^{\bullet+}$	1	0.357[c]	ns
	$DPPH^{\bullet}$	0.357[c]	1	0.413[c]
	FRAP	ns	0.413[c]	1

		Bioaccessible Fraction $ABTS^{\bullet+}$	$DPPH^{\bullet}$	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]

	$ABTS^{\bullet+}$	1	0.563[b]	ns
	$DPPH^{\bullet}$	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
	$ABTS^{\bullet+}$	1	**-0.636[a]**	ns
	$DPPH^{\bullet}$	**-0.636[a]**	1	**0.634[a]**
	FRAP	ns	**0.634[a]**	1

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

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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