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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 °C 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.

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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-inflammatory, anticancer and cardiovascular protection activities (Aptekmann & Cesar, 2013; Benavente-García & Castillo, 2008; Gironés-Vilaplana, Moreno, & García-Viguera, 2014; Khan, Zill-E-Huma, & Dangles, 2014; Lee, 2013; Liu, Heying, & Tanumihardjo, 2012; Lv et al., 2015; Stinco 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 (Gironés-Vilaplana et al., 2014; González-Molina, Domínguez-Perles, Moreno, & García-Viguera, 2010).

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) (Cano et al., 2008; Dhuique-Meyer, Caris-Veyrat, Ollirtrault, Curk, & Amiot, 2005; Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi, 2007; 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 & Castillo, 2008).

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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 (De Pascual-Teresa et al., 2007; Sánchez-Moreno et al., 2003). 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 (Cilla, González-Sarrías, Tomás-Barberán, Espín, & Barberá, 2009; Cilla et al., 2011, 2012; Gil-Izquierdo, Gil, Ferreres, & Tomás-Barberán, 2001; 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 et al., 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, Barberá, & 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 °C) 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 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 (⩾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-*O*-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 ($K_2S_2O_8$) 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.

2.1.2. Simulated GI digestion

Enzymes and bile salts were purchased from Sigma-Aldrich (St. Louis, MO, USA): pepsin (porcine, 975 units per mg protein), pancreatin (porcine, activity equivalent to 4× USP specifications) and bile extracts (porcine).

2.2. Samples

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

2.3. In vitro GI digestion

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

2.4. Ascorbic acid analysis

Ascorbic acid was extracted and quantified by HPLC according to the procedure described Cilla et al. (2012) using 10 g of sample (fruit pulp, fruit juice and acidified BF). Prior to the extraction of ascorbic acid, the BF (pH 7.6) were acidified with hydrochloric acid to pH 4. Quantification was achieved using an ascorbic acid external standard calibration curve in the range from 5 to 500 μg/mL. Results were expressed as mg of ascorbic acid per 100 g of fresh weight of sample (fruit pulp, fruit juice and BF).

2.5. Flavonoid analysis

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

Polyphenols identification was carried out by HPLC-ESI-MS-QTOF according to the procedure described by Dorta et al.