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Sanguinello and *Tarocco* (*Citrus sinensis* [L.] Osbeck): Bioactive compounds and colour appearance of blood oranges

Laura Cebadera-Miranda[a], Laura Domínguez[a], Maria Inês Dias[b], Lillian Barros[b], Isabel C.F.R. Ferreira[b], Marta Igual[c], Nuria Martínez-Navarrete[c], Virginia Fernández-Ruiz[a], Patricia Morales[a], Montaña Cámara[a,*]

[a] Department Nutrition and Food Science, Faculty of Pharmacy, Complutense University of Madrid (UCM), Plaza Ramón y Cajal, s/n, E-28040 Madrid, Spain
[b] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[c] Universitat Politècnica de València, Food Technology Department, Food Investigation and Innovation Group, Camino de Vera s/n, 46022 Valencia, Spain

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ABSTRACT

Sanguinello and Tarocco (*Citrus sinensis* [L.] Osbeck) are the most common and widespread blood oranges varieties in the Mediterranean climate area. Its interest is increasing mainly due to nutritional and organoleptic properties. In this work, three blood orange varieties cultivated in Spain (Sanguinelli, Tarocco Rosso and Tarocco Ippolito) were characterized in relation to physicochemical parameters and relevant bioactive compounds (vitamin C, organic acids, flavonoids and anthocyanins) as well as colour characterization. All samples showed important vitamin C values (higher than 54.9 mg/100 g of edible portion). Flavonoids represent the largest family of phenolic compounds, being hesperidin, the major flavonoid. Ten different anthocyanins were identified in blood oranges, seven cyanidin derivatives and three delphinidin derivatives, being the most abundant cyanidin 3-(6″-malonylglucoside) and cyanidin 3-glucoside. Blood oranges can show an intense reddish colour in peel whereas the pulp has a yellow-orange colour. Overall, these varieties are good sources of bioactive compounds.

1. Introduction

Blood oranges are the result of a spontaneous genetic mutation that occurred many centuries ago in plants native from China, due to the migratory movements throughout the Mediterranean. It has been cultivated in Sicily since the fifteenth century. Although the original plantation of lemons and bitter oranges in Sicily is attributed to the Arabs, it was the Genovese and Portuguese crusaders who introduced the sweet variety, becoming a basic element of the Sicilian kitchen and receiving even the quality denomination of Protected Geographical Indication (PGI) (Barreca, Gattuso, Laganà, Leuzzi, & Bellocco, 2016).

Sanguinello and Tarocco (*Citrus sinensis* [L.] Osbeck) are the most common and widespread blood oranges varieties in the Mediterranean climate area, mainly southern Italy and Spain (in Europe), and most recently in California (United States of America). These varieties are consumed worldwide in both, fresh and processed products such as fruit juices (Kelebek, Canbas, & Selli, 2008).

Spain has a long tradition in citriculture that began probably in the 7th century (Agustí, 2003). Nowadays Spanish oranges quality is recognized globally due to its good organoleptic properties. Spain is the main citrus producing country in Europe; with orange production in 2017 reaching 3.4 million tons (FAOSTAT, 2017), most of which are exported in the European markets (MAPAMA, 2017). Although the production of blood orange varieties in Spain is limited, its interest is increasing in the recent years mainly due to its nutritional and organoleptic properties (bright colour and pleasant taste).

Considering the importance of citrus production in Spain, the main objective of the present study is to identify and quantify the bioactive compounds present in Sanguinello and Tarocco blood orange varieties, *Citrus sinensis* [L.] Osbeck as well as to evaluate their colour as a relevant parameter for consumer acceptance.

2. Materials and methods

2.1. Samples

Three varieties of blood oranges (*Citrus sinensis* (L.) Obsbeck) cultivated in the region of Valencia (Comunidad Valenciana, Spain) were

* Corresponding author.
E-mail address: mcamara@ucm.es (M. Cámara).

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considered for study: Sanguinelli (*Citrus sinensis* (L.) cv. Sanguinelli), which comes from an spontaneous mutation of blood orange Doble Fina (C. sinensis L. cv. Double Fine) that was firstly detected in 1929 in Almenara (Castellón, Spain); Tarocco Rosso and Tarocco Ippolito (*Citrus sinensis* (L.) cv. Tarocco).

In Spain, due to environmental conditions, blood oranges commercial consumption stage goes from middle of January to the end of March. Samples considered in this study were harvested during 2015 season. Three different batches were collected at very beginning, middle and later period, providing a representative sample of fruits offered to consumers at commercial consumption stage.

Prior analysis, the peel was separated from the pulp. Fresh pulp was homogenized (Ultraturrax®). Aliquots of the homogenized pulp were used for physico-chemical determinations (moisture, pH and titratable acidity), as well as for the determination of vitamin C.

Another portion of homogenized fresh pulp was subjected to freeze drying process. Lyophilized fresh pulp was ground and homogenized, stored in airtight containers, in the dark, at −20 °C, in order to avoid product alteration.

2.2. Chemical characterization

2.2.1. Physico-chemical parameters

Moisture, pH, titratable acidity and grades Brix were determined according to official methods (Horwitz & Latimer, 2005) in homogenized oranges fresh pulp.

Ripeness index was calculated by the °Brix/TA ratio, with TA being titratable acidity expressed as mg of citric acid/100 g of fruit edible portion.

The colorimetric characterization of the blood oranges studied was determined by tri-stimulus colorimetric method based on the CIELAB parameters (L^*, a^*, b^*, C^* and hue) using Hunter Color Flex Colorimeter, with the following specifications: CIE illuminant C, and 45°/0° geometry. The coordinates that define the colour of the sample are: the photometric index (L^*) which varies between 0 (black) and 100 (white); a and b values which range from −100 to +100, being $+a^*$ depending on the intensity of the red colour, $-a^*$ depending on the intensity of the green colour, $+b^*$ depending on the intensity of the yellow colour and $-b^*$ depending on the intensity of the blue colour. Chroma (C^*) is the quantitative colorfulness attribute as it determines the difference degree in comparison to a grey colour with the same lightness for each hue (CIE, 2001). Hue angle (h) is a parameter that defines the colors traditionally as pinkish, yellowish and greenish. C^*ab and hue angle (h_{ab}) was calculated following the Eqs. (1) and (2), respectively:

$$C^*_{ab} = \text{saturation index } (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$h_{ab} = 1/\tan(b^*/a^*) \quad (2)$$

These colour parameters CIELAB were measured in the homogenized pulp and in the peel. The pulp sample was placed in cylindrical glass cuvettes measuring 5 cm in diameter and 1.3 cm in height. External fruit colour was evaluated by three consecutive measurements of three different parts of the fruit: the darkest part of peel, the clearest part and the base of the piece of fresh fruit.

2.2.2. Vitamin C and organic acid content

The extraction of vitamin C and organic acids was carried out in acid medium (Sánchez-Mata et al., 2012) and analyzed and quantified by high performance liquid chromatography (HPLC) in reverse phase with UV–visible detection (Thermo Separation Spectra Series UV100). The equipment consists in an isocratic pump (model PU-II), an AS-1555 automatic injector (Jasco, Japan), a Sphereclone ODS (2) 250 × 4.60, 5 µm Phenomenex column, and a UV–visible detector (Thermo Separation Spectra Series UV100, Madrid, Spain). The mobile phase was 1.8 mM H_2SO_4 (pH = 2.6). For AA analysis a flow-rate of 0.9 mL/min and UV detection at 245 nm was used, while conditions for organic acids were 0.4 mL/min and at 215 nm. The identification was perform using Biocrom 2000 3.0. Software by comparison of the retention times of each chromatographic peak with those of standards products. Values were expressed as mg/100 g of fruits edible.

2.2.3. Carotenoids content: β-carotene and lycopene

Standards of all-translycopene and β-carotene used in this work were from Sigma-Aldrich-Fluka (St. Louis, MO), with a purity of 90%. For identification and quantification purposes, individual working standard solutions were daily prepared by dilution in hexane (Merk, Darmstadt, Germany).

Carotenoids analysis in blood oranges was carried out after extraction by a hexane:acetone:methanol solvent (50:25:25 *v/v/v*) by spectrophotometry of the hexane layer, according to Olives Barba et al. (2006). A Pharmacia Ultrospec 4000 UV/vis spectrophotometer was employed for absorbance measurements (at 446 nm for β-carotene and 502 nm for lycopene) using quartz cells of path length 1 cm.

2.2.4. Phenolic compounds

2.2.4.1. Non anthocyanin flavonoids. The extraction and chemical characterization of flavonoids was carried out following the procedure proposed by Igual, García-Martínez, Camacho, and Martínez-Navarrete (2011). The flavonoids were characterized by high performance liquid chromatography (HPLC) couple to UV–visible detector (MD-1510, Jascos, Italy) with a range of measurement wavelength of 190–650 nm, with a ternary pump (Jasco PU-1580 HPLC pump), a gradient generator (LG-1580-02 Ternary Gradient Unit) and Ultrabase-C18 column (5 µm, 4.6 × 250 mm). The mobile phase was composed of (A) methanol and (B) water and a linear gradient elution was performed starting at 30:70 to reach 100:0 at 70 min, with a flow rate of 1 mL/min. Chromatograms were recorded at 286, 284 and 254 nm and at 25 °C. The standard curves of the reference flavonoids, narirutin (NAT), naringin (NAR), hesperidin (HES), neohesperidin (NEOH), didymin (DID), poncirin (PON), naringenin (NAG) and quercetin (QUER) (Extrasynthese, France) were used to quantify the flavonoids. Naphthalene was used as internal standard. Values were expressed as mg/100 g per edible portion.

2.2.6. Anthocyanins

Sample extraction and characterization was performed following the procedure described by Gonçalves et al. (2017). Double detection was carried out by DAD, using 520 nm as the preferred wavelength, and in a MS connected to the HPLC system via the DAD cell outlet. Anthocyanins were tentatively identified by comparing their UV–vis and mass spectra with available standards and data in our compound library and the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal: cyanidin-3-*O*-glucoside (y = 630.276x 153.83; R^2 = 0.999) and delphinidine-3-*O*-glucoside (y = 557.274x + 126.24; R^2 = 0.999). The results were expressed in mg/100 g of edible portion.

2.3. Statistical analysis

Results were expressed as means of a minimum of triplicate analyses (n = 3) and corresponding standard deviations. Analysis of variance (ANOVA) using Tukey's test was applied to analyse data at the 95% confidence level.

3. Results and discussion

3.1. Physico-chemical parameters

Results corresponding to the characterization of the blood oranges samples in terms of moisture, °Brix, titratable acidity, pH and ripeness index is shown in Table 1.

The average moisture contents for three blood oranges analyzed