



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

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ARTICLE INFO

Keywords:

Sanguinello
Tarocco
Blood oranges
Anthocyanins
Flavonoids
Organoleptic quality

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.) Osbeck) cultivated in the region of Valencia (Comunidad Valenciana, Spain) were

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<https://doi.org/10.1016/j.foodchem.2018.07.094>

Received 11 May 2018; Received in revised form 15 July 2018; Accepted 16 July 2018

Available online 17 July 2018

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considered for study: Sanguinelli (*Citrus sinensis* (L.) cv. Sanguinelli), which comes from a 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 $45^{\circ}/0^{\circ}$ 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 Spherclone ODS (2) 250×4.60 , $5 \mu\text{m}$ Phenomenex column, and a UV-visible detector (Thermo Separation Spectra Series UV100, Madrid, Spain). The mobile phase was $1.8 \text{ mM H}_2\text{SO}_4$ (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 performed 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 $\text{mg}/100 \text{ g}$ 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 $190\text{--}650 \text{ nm}$, with a ternary pump (Jasco PU-1580 HPLC pump), a gradient generator (LG-1580-02 Ternary Gradient Unit) and Ultrabase-C18 column ($5 \mu\text{m}$, $4.6 \times 250 \text{ mm}$). 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 $\text{mg}/100 \text{ g}$ 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 $\text{mg}/100 \text{ g}$ 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

Table 1
Physicochemical parameters (moisture, pH, TA, °Brix and Ripeness index, RI) evaluated in *Citrus sinensis* [L.] Osbeck varieties.

Variety	Batch	Moisture (g/100 g)	pH	TA	°Brix	RI
Sanguinelli	A	82.21 ± 0.75a	3.05 ± 0.01a	14.69 ± 0.23b	13.33 ± 0.15a	14.53 ± 0.65a
	B	87.49 ± 0.08c	3.52 ± 0.04c	13.65 ± 0.47a	14.33 ± 0.06b	16.42 ± 0.52b
	C	85.70 ± 0.06b	3.27 ± 0.01b	13.59 ± 0.52a	14.63 ± 0.06c	16.84 ± 0.62b
Range of Variation:		81.46–87.57	3.04–3.56	13.07–14.92	13.18–14.69	13.88–17.46
T. Rosso	A	79.96 ± 0.22a	2.95 ± 0.01a	16.36 ± 0.06b	13.53 ± 0.21a	13.10 ± 0.48a
	B	85.76 ± 0.01b	3.52 ± 0.01c	13.73 ± 0.20a	13.77 ± 0.12a	15.48 ± 0.49b
	C	85.59 ± 0.17b	3.35 ± 0.01b	13.42 ± 0.34a	15.20 ± 0.10b	17.70 ± 0.34c
Range of Variation:		79.74–85.77	2.94–3.53	13.08–16.42	13.32–15.30	12.62–18.04
T. Ippolito	A	81.12 ± 0.27a	3.07 ± 0.02a	12.78 ± 0.23b	14.13 ± 0.06b	17.28 ± 0.27a
	B	87.46 ± 0.04c	3.75 ± 0.01c	11.27 ± 0.09a	12.63 ± 0.06a	18.91 ± 0.16b
	C	85.45 ± 0.11b	3.67 ± 0.02b	11.65 ± 0.29a	14.73 ± 0.12c	19.76 ± 0.68c
Range of Variation:		80.85–87.50	3.05–3.76	11.18–13.01	12.57–14.85	17.01–20.44

TA: titratable acidity (0.1 N NaOH/100 g); RI (ripeness index (RI): °Brix/Titratable acidity expressed in g/L of citric acid). Results expressed as Mean ± standard deviation n – 1 (n = 9) over 100 g of edible portion. In each column and the same variety. Different letters mean statistical differences between samples (p < 0.05).

during the 2015 season range from 79.74 to 87.57 g/100 g of edible portion. The range of pH values in the samples of blood oranges studied was between 2.95 and 3.67 (Tarocco Rosso and Tarocco Ippolito, respectively, Table 1), highlighting the variety Tarocco Ippolito. These results are similar to other blood orange varieties such as Moro and T. Messina varieties both from Italy (Rapisarda, Lo Bianco, Pannuzzo, & Timpanaro, 2008) and Moro and Sanguinello varieties from Turkey (Kelebek et al., 2008).

All varieties of blood oranges studied showed similar and homogeneous values for soluble solids content. These data are similar with those indicated by other authors such as Kelebek (2008) for Moro (12.0 °Brix) and Sanguinello (12.6 °Brix); and similar values (range: 11.3–13.53) to those described by other authors (Abdelaali et al., 2018; Continellaa et al., 2018).

Ripening index (RI) values, calculated as °Brix/TA, and expressed in grams of citric acid per 100 mL, are similar in all samples analyzed. These values are slightly higher than those described by other authors for other blood oranges varieties, such as Tarocco Scirè (10.5–13.9) according to Continellaa et al. (2018).

3.2. Vitamin C and organic acids

Vitamin C was calculated as the sum of ascorbic and

dehydroascorbic acids in the varieties of blood oranges (Table 2). In general, all studied samples shown high concentrations of vitamin C content, highlighting the Sanguinelli and Tarocco Rosso varieties (66.70 and 66.42 mg/100 g, respectively). Regulation (EU) No 1169/2011, related to consumer information on food labelling, indicates a nutrient reference value (NRV) for vitamin C of 80 mg/day. According to this regulation, we can assert that the consumption of 100 g of blood oranges pulp would cover at least 68% of these recommendations, and in all cases, these oranges may be promoted as a natural source of vitamin C, covering more than 15% of such recommendations.

As the amounts of dehydroascorbic acid found in the samples were insignificant (traces), in the following table (Table 2) only the results for ascorbic acid are shown. The AA values obtained are in agreement with those reported for Tarocco and Moro varieties, 53.3 and 66.9 mg/100 mL of juice, respectively (Arena, Fallico, & Maccarone, 2001) and T. Messina and T. Meli (55.37–64.77 mg/100 mL of juice, respectively, Rapisarda et al., 2008). Results obtained in the present study were much higher than those indicated by other authors for other varieties of blood orange, such as Cara Cara and Moro Blood (34.24–31.83 mg/100 g, respectively; Kafkas, Ercisli, Kemal, Baydar, & Yilmaz, 2009).

In relation to the other organic acids, oxalic, tartaric, malic, ascorbic, citric and fumaric acids were characterized in the studied orange fruits (Table 2). It's well know that fruits undergo important

Table 2
Organic acids and vitamin C content in *Citrus sinensis* [L.] Osbeck varieties (mg/100 g per edible portion).

Variety	Batch	Oxalic acid	Tartaric acid	Malic acid	Citric acid	Fumaric acid	Vitamin C
Sanguinelli	A	6.28 ± 0.62a	48.96 ± 4.61a	137.84 ± 1.25a	1020.33 ± 7.03a	0.71 ± 0.03b	54.94 ± 0.08a
	B	17.06 ± 2.22b	72.67 ± 2.43b	132.25 ± 9.62a	1138.41 ± 49.07b	0.23 ± 0.02a	56.05 ± 0.91a
	C	8.50 ± 2.48a	92.44 ± 2.10c	126.28 ± 11.31a	1622.70 ± 83.09c	0.20 ± 0.03a	66.70 ± 0.56b
Range of variation:		5.66–26.17	44.35–94.54	114.97–139.09	1013.30–1705.79	0.17–0.74	54.86–67.26
T. Rosso	A	8.61 ± 0.84a	44.75 ± 3.47a	159.32 ± 2.94b	1236.82 ± 29.14a	0.52 ± 0.07	59.11 ± 0.66a
	B	10.57 ± 2.05a	68.95 ± 5.35b	78.92 ± 2.33a	1169.64 ± 56.29a	tr	65.49 ± 0.35b
	C	7.56 ± 0.34a	86.75 ± 6.81c	163.07 ± 8.28b	1592.82 ± 25.05b	0.17 ± 0.01	66.42 ± 0.54b
Range of variation:		7.22–12.62	41.28–93.56	76.59–171.35	1113.35–1617.87	tr–0.59	58.45–66.96
T. Ippolito	A	17.38 ± 1.77a	35.88 ± 1.23a	122.89 ± 6.55a	979.38 ± 7.14b	0.64 ± 0.13b	57.32 ± 0.29b
	B	18.01 ± 7.81a	64.95 ± 2.45b	116.35 ± 15.83a	864.99 ± 10.17a	0.18 ± 0.11a	55.06 ± 0.34a
	C	15.45 ± 3.37a	89.01 ± 4.93c	241.52 ± 10.12b	1236.31 ± 26.26c	0.29 ± 0.05a	59.17 ± 0.47c
Range of variation:		12.08–25.82	34.65–93.94	100.52–251.64	854.82–1262.57	0.07–0.77	54.72–59.64

tr: traces (< 0.01 mg/100 g ssf). Results expressed as mean ± standard deviation n – 1 (n = 9). In each column, the different letters indicate statistically significant differences (p < 0.05) between batches for each variety. In each column and the same variety, different letters mean statistical differences between samples (p < 0.05).

changes during maturation process strongly affect to organic acid profile as well as other related parameters. Organic acids tend to decrease with maturation of the fruit, mostly because of the use of these compounds as respiratory substrates, as well as, for new substances synthesis. Also it is important to highlight that the difference organic acid profile and amount between samples from different origins may be related to different environmental conditions, since organic acid profile is extremely conditioned by climate conditions (López-Bucio, Nieto-Jacobo, Ramírez-Rodríguez, & Herrera-Estrella, 2000).

Blood oranges studied were noted for their high content of citric acid, which is the major organic acid, with values ranging from 864.99 to 1622.70 mg/100 g in Tarocco Ippolito and Sanguinelli, respectively (Table 2), being the Tarocco Rosso variety the one with the highest average content. Other authors analyzed the citric acid content in similar varieties of blood oranges, obtained values of 1130 and 1340 mg/100 mL of juice for Moro and Sanguinello varieties, respectively (Kelebek, 2008), data that agree with those obtained in the current study. These values for the content of this organic acid were much higher than other varieties of blood oranges analyzed by other authors, such as the Cara Cara and Moro Blood varieties (421–561 mg/100 mL of juice respectively, Kafkas et al., 2009). The following major organic acids in all analyzed blood oranges were malic and tartaric acids. This content did not show a large difference between varieties, which is in concordance with authors (Braverman, 1933; Vandercook, 1977). Fumaric acid was the minor organic acid in all blood oranges analyzed, other authors also reported that fumaric acid values in this type of citrus fruits were very low and even impossible to quantify (Lee, Carter, Barros, Dezman, & Castle, 1990).

3.3. Carotenoids content

Traditionally it has been considered that the major carotenoids in oranges were violaxanthin, anteraxanthin, lutein, α -cryptoxanthin, β -cryptoxanthin, α -carotene and β -carotene, however, more recent studies have revealed that some of them were mistakenly identified (Meléndez-Martínez et al., 2005, 2008). An exceptional accumulation of lycopene in some citrus fruits has been evidenced in orange and grapefruit mutants, as in Cara Cara, mutant of the Navel orange (*Citrus sinensis* [L]. Osbeck) (Alquézar, Rodrigo, & Zacarias, 2008) and Hong Anliu, mutant of Anliu (*Citrus sinensis* [L]. Osbeck) (Liu, Liu, & Tao, 2006), derived from work done by the National Laboratory for Genetic Improvement of Crops, Huazhong Agricultural University and Citrus Research Institute, RP China.

Results obtained in this study indicate that β -carotene was present in the blood oranges considered in this study. This is in agreement with the work carried out at the Institute of Agrochemistry and Food Technology (IATA) in Valencia and the Spanish Scientific Research Council (CSIC) by Alquézar, Zacarías, and Rodrigo (2009) that indicate the general absence of carotenoid lycopene in citrus fruits.

All varieties analyzed showed similar and homogeneous values of β -carotene content (Table 3). Among the blood oranges samples, Tarocco Ippolito stands out because it presented the highest average value (0.542 mg/100 g), while the Tarocco Rosso variety had the lowest β -carotene content (0.261 mg/100 g), with statistically significant differences ($p < 0.05$) between varieties.

3.4. Phenolic compounds

Tarocco Ippolito was the variety with the highest total flavonoid content, whereas Tarocco Rosso presented the lowest value (statistically significant differences, $p < 0.05$). Comparing our results with those obtained by other authors, the total content of flavonoids was higher than those reported by Lee et al. (1990), which indicated values for a type of blood orange (California) of 68–88 mg/100 mL of juice. In general, the total content of flavonoids is higher than that of other citrus fruits such as grapefruit (141 mg/100 g according to Igual, García-

Martínez, Camacho, and Martínez-Navarrete (2013)). The identified flavonoids in the studied blood oranges were: narirutin, hesperidin, naringin, didimine, quercetin and naringenin according to Kelebek et al. (2008) and Berhow, Tisserat, Kanes, and Vandercook (1998). Table 3 shows the results for the main flavonoids identified and quantified from the different standard curves used. The most abundant flavonoid in the studied blood oranges was hesperidin (values ranging from 78 to 143 mg/100 g in Tarocco Rosso and Sanguinelli, respectively). Tarocco Ippolito and Sanguinelli varieties showed the highest content (range from 99.9 to 119.0 mg/100 g and 92.9 to 143 mg/100 g, respectively). The next major flavonoid was narirutin (37.0–93.0 mg/100 g edible portion, followed by quercetin (28.1–42 mg/100 g edible portion). Tarocco Ippolito variety showed the highest value of narirutin (variation range of 60.9–93.0 mg/100 g) in relation to the other analyzed oranges. Sanguinelli was the variety that presented the highest content of quercetin. On the other hand, naringenin was the minor flavonoid in all varieties studied.

In a study conducted by Barreca et al. (2016), the blood oranges Sanguinello and Tarocco presented a similar profile that obtained in the current study. Hesperidin was the major flavonoid, obtaining values for Sanguinello and Tarocco of 42.6 and 10.6 mg/100 mL of juice, respectively, followed by narirutin values of 6.1 mg/100 mL of juice for Sanguinello and 1.4 mg/100 mL in Tarocco. These results are much lower than those obtained in the blood oranges considered in the present work.

Regarding anthocyanin compounds, the peak characteristics (retention time, λ_{\max} in the visible region, mass spectral data) and tentative identification and quantification of anthocyanin compounds in Tarocco samples are present in Table 4. Ten compounds were identified in all samples, seven cyanidin derivatives (peaks 2, 3, 5, 6, 7, 9, and 10) and three delphinidins derivatives (peaks 1, 4 and 8). Peaks 1, 2, 3, 4, 6, 7 and 9 were previously identified by other authors in blood orange samples (Destani, Cassano, Fazio, Vincken, & Gabriele, 2013; Dugo, Mondello, Morabito, & Dugo, 2003; Fabroni, Ballistreri, Amenta, & Rapisarda, 2016; Hillebrand, Schwarz, & Winterhalter, 2004; Kelebek et al., 2008; Lee, 2002). All the compounds were identified according with their MS characteristics (molecular ion and MSⁿ fragmentation pattern), UV spectra and where tentatively identified comparing this data with information reported in the literature. Nonetheless, compounds 5, 8 and 10, to the best of our knowledge, were not previously described in orange blood samples. Peak 5 was tentatively identified as a cyanidin (malonyl) diglucoside, presenting a pseudomolecular ion $[M+H]^+$ at m/z 697, releasing a unique MS² fragment at m/z 287 ($[M+H-410]^+$), which might correspond to the joint successive loss of a malonyl group (–86 mu) and two glucoside units (–324 mu). Peak 8 was tentatively identified as delphinidin-3-rhamnoside, presenting a pseudomolecular ion $[M+H]^+$ at m/z 449 and a unique MS² fragment at m/z 303 (delphinidin aglycone) corresponding to the loss of a rhamnoside unit ($[M+H-146]^+$). Peak 10 showed a pseudomolecular ion $[M+H]^+$ at m/z 679 and a MS² fragment at m/z 287 ($[M+H-392]^+$), which might correspond to the successive loss of a malonyl (–86 mu), dioxalyl (–144 mu) and hexoside (–162 mu) units, being tentatively identified as cyanidin malonyl-(dioxalyl)-hexoside. Dugo et al. (2003) reported the presence of a similar compound in blood orange fruit juice, however it only identifies the aglycone (cyanidin).

In general, all varieties studied showed high values in the individual content of each of the identified compounds, as well as the total content of anthocyanins. All Spanish blood oranges studied showed similar and homogeneous values in the total anthocyanins content, all of which showed values in the range of 6.95–25.26 μ g/100 g of edible portion. Sanguinelli sample presented the highest concentration of anthocyanin compounds (25.26 μ g/100 g edible portion), mainly due to peaks 3 (cyanidin 3-glucoside) and 6 (cyanidin 3-(6"-malonyl) glucoside). The most abundant peak in all samples was peak 6 (cyanidin 3-(6"-malonyl) glucoside), followed by peak 3 (cyanidin 3-glucoside). Of course, the three blood oranges Sanguinelli, Tarocco Rosso and Tarocco Ippolito

Table 3
Content of major flavonoids and β -carotene present in *Citrus sinensis* [L.] Osbeck varieties (mg/100 g per edible portion).

Variety	Batch	Narirutine	Hesperidin	Naringenin	Quercetin	Total flavonoids	β -carotene
Sanguinelli	A	57.4 \pm 1.0b	97.0 \pm 4.1a	1.04 \pm 0.0c	37.9 \pm 1.4b	193.0 \pm 6.0b	0.418 \pm 0.086a
	B	60.4 \pm 1.2c	140.0 \pm 3.0c	0.94 \pm 0.0b	40.0 \pm 2.0b	241.0 \pm 7.0c	0.366 \pm 0.022a
	C	41.1 \pm 4.1a	111.0 \pm 2.0b	0.48 \pm 0.1a	29.4 \pm 1.3a	182.0 \pm 1.0a	0.381 \pm 0.015a
Range of variation:		37.0–65.6	92.9–143.0	0.47–1.04	28.1–42.0	181.0–248.0	0.366–0.418
T. Rosso	A	53.1 \pm 2.0c	96.0 \pm 2.1b	1.00 \pm 0.4a	31.8 \pm 0.4a	181.0 \pm 0.0a	0.295 \pm 0.023a
	B	41.0 \pm 3.0a	100.0 \pm 6.1b	1.42 \pm 0.0b	36.0 \pm 3.0b	178.0 \pm 12.0a	0.261 \pm 0.082a
	C	46.9 \pm 0.7b	84.0 \pm 6.0a	nd	35.9 \pm 1.0b	166.0 \pm 5.1a	0.386 \pm 0.077a
Range of variation:		37.0–55.1	78.0–106.1	nd–1.42	31.4–39.0	160.9–181.0	0.261–0.386
T. Ippolito	A	62.0 \pm 1.1a	104.0 \pm 4.1a	2.4 \pm 0.3b	38.0 \pm 3.0a	209.0 \pm 9.0a	0.588 \pm 0.055a
	B	91.0 \pm 2.0c	116.0 \pm 3.0b	1.08 \pm 0.1a	38.8 \pm 0.7a	258.0 \pm 7.0b	0.487 \pm 0.050a
	C	68.2 \pm 0.4b	106.4 \pm 0.5a	1.07 \pm 0.1a	39.7 \pm 0.5a	216.0 \pm 1.0a	0.550 \pm 0.035a
Range of variation:		60.9–93.0	99.9–119.0	1.06–2.07	35.0–40.2	200.0–265.0	0.487–0.588

nd: Not detected. Results expressed as mean \pm standard deviation n – 1 (n = 9). In each column and the same variety, the different letters indicate statistically significant differences ($p < 0.05$) between batches for each variety.

showed the highest content of both compounds, ranging from 2.75 to 10.85 $\mu\text{g}/100\text{ g}$ of edible portion of cyanidin 3-(6"-malonyl) glucoside (major compound) and 2.32–8.18 $\mu\text{g}/100\text{ g}$ of cyanidin-3-glucoside edible portion. This anthocyanins profile corresponds to that described by other authors for similar varieties (Tarocco and Sanguinello) from Italy (Barreca et al., 2016), California (Lee, 2002). It also corresponds to other varieties of blood oranges from China (Liang, Shao-Qian, & Si-Yi, 2011). On the other hand, Barbagallo, Palmeri, Fabiano, Rapisarda, and Spagna (2007) published a study analyzing the anthocyanins content in Taroccos from Italy (15.6 and 11.5 mg/100 mL juice). Liang et al. (2011) also conducted a study on Taroccos from China, which showed that the majority anthocyanins in this type of fruit are cyanidin 3-(6"-malonyl) glucoside and cyanidin-3-glucoside, as reflected in the present study (Shao-Qian, Si-Yi, Xiao-Lian, & Hong-Fei, 2010).

3.5. Colour characterization. Study of correlations between pigments and colour measurement

Appearance, including fruit colour, is generally used as a selection criterion throughout the supply and consumer chain. The intensity of the pigmentation depends on several factors such as: variety, soil type, climate, weather conditions of the campaign, etc. For example, warm summers followed by cold winters produce fruits of more intense pigmentation. Peel and pulp colour is not uniform, and it does not always correspond to a blood zone of the peel with red pulp below that zone. This more coloured area usually appears in the part of the fruit with north orientation (Kafkas et al., 2009).

As the accumulation of lycopene is quite unusual in this type of oranges, and lycopene has not been identified in samples analyzed, the characteristic red colour of blood oranges should be attributed to a combination of β -carotene and anthocyanic compounds (Barreca et al., 2016; Rapisarda, Bellomo, & Intrigliolo, 2001).

The instrumental measurement of blood orange colour by the analysis of CIELAB colour parameters (L^* , a^* , b^* , C^* and hue) can give an estimation of its pigments content as carotenoids and anthocyanins (Meléndez-Martínez, Gómez-Robledo, Melgosa, Vicario, & Heredia, 2011).

Regarding the pulp colour parameters (Table 5), Tarocco Rosso variety showed the lowest values of parameter a^* (lower intensity of red colour) and the highest b^* values (higher intensity of yellow-orange colour). The profiles of L^* (brightness) and hue parameter, show the same behaviour with a^* and b^* parameters, being Tarocco Rosso, the variety with the highest values while the others two varieties analyzed showed lower and similar levels between them. Regarding saturation index C , all samples studied showed similar levels. In general, the

colour parameters obtained show variations, but not deviate from the values mentioned by other authors for other citrus varieties (Choi, Kima, & Leeb, 2002). Such variation may be due to the characteristics of the product, the selected variety and the time of harvesting.

In order to evaluate if the selected colour parameters are predictive values of the pigment content of the analyzed samples, a study of the correlations between the different CIELAB parameters (L^* , a^* , b^* , C^* and h) of the pulp and the pigment content, carotenoid and anthocyanin compounds were carried out (Table 6). Correlation study shows that all colour parameters are strongly correlated with both, total anthocyanin content and β -carotene content in the pulp. As expected, the parameter a^* showed positive correlation with the content of these compounds (0.613 and 0.709 for β -carotene and total anthocyanins content, respectively), since this parameter is the one that measures the intensity of the orange-reddish colour, characteristic colour of these pigments.

On the other hand, the parameters L^* , b^* , chroma C^* and hue, show a strong negative correlation with the total content of β -carotene and anthocyanins. It means that the colour fruits with a tendency to yellow and away from red, along with a more vivid and clear colour, could indicate a lower content of these pigments. Nevertheless, β -carotene showed no significant correlation ($p > 0.05$) with the parameter C^* .

Regarding to peel colour of samples analyzed (Table 6), the parameter a^* show similar values for all varieties studied in a reduced range (27.16–39.10). These values for the a^* parameter are close to those found in the pulp. However, the parameter b^* together with the brightness and saturation index C^* profiles showed the lowest value for the Sanguinelli variety, which means that Sanguinelli variety show in their peel a redder colour than the other peel samples analyzed. In this sense, no direct relationship was found between the external and internal coloration of these fruits studied. It was observed that the peel follows a different behavior to the pulp. In view of the results, it can be found varieties with an intense yellow-orange coloration (higher parameter b^*) and reddest pulp (higher parameter a^*), as it happens in the Tarocco Ippolito variety and alternatively, it can be found varieties with a more reddish coloration on their peel, and more yellow-orange pulp, as in the case of Tarocco Rosso variety.

4. Conclusions

We can affirm that the blood oranges Sanguinelli and Tarocco Rosso are good sources of vitamin C and could cover the 100% of nutrient reference value (NRV). Cítric acid is the predominant organic acid. Tarocco Ippolito variety showed the highest β -carotene and total flavonoid content. The predominant flavonoid in the studied oranges was hesperidin being Sanguinelli the variety with the highest content of this

Table 4
Tentative identification and quantification ($\mu\text{g}/100\text{ g}$) of the anthocyanins in *Citrus sinensis* [L.] Osbeck varieties.

Peak	Rt (min)	λ_{max} (nm)	[M+H] ⁺ (m/z)	MS ² (m/z)	Tentative identification	Sanguinelli			T. Rosso			T. Ippolito		
						A	B	C	A	B	C	A	B	C
1	15.7	526	465	303 (100)	Delphinidin 3-glucoside	0.022 ± 0.003f	0.083 ± 0.001d	0.19 ± 0.01c	tr	0.054 ± 0.003e	0.093 ± 0.003d	tr	0.30 ± 0.01b	0.44 ± 0.03a
2	16.0	526	611	287 (100)	Cyanidin 3-sophoroside	0.29 ± 0.01g	0.19 ± 0.02h	0.45 ± 0.02d	0.50 ± 0.01c	0.396 ± 0.001e	0.58 ± 0.02b	0.57 ± 0.02b	0.32 ± 0.02f	1.15 ± 0.04a
3	18.2	520	449	287 (100)	Cyanidin 3-glucoside	4.68 ± 0.01c	2.90 ± 0.06e	8.18 ± 0.04a	2.32 ± 0.10g	2.68 ± 0.07f	3.85 ± 0.12d	4.66 ± 0.11c	3.82 ± 0.14d	6.85 ± 0.13b
4	24.9	518	551	303 (100)	Delphinidin 3-(6-malonyl) glucoside	0.11 ± 0.01f	0.161 ± 0.005d	0.42 ± 0.01b	tr	0.114 ± 0.001ef	0.124 ± 0.004e	0.08 ± 0.02g	0.28 ± 0.01c	0.60 ± 0.05a
5	27.4	520	697	287 (100)	Cyanidin (malonyl) diglucoside	0.343 ± 0.002c	0.189 ± 0.004e	0.45 ± 0.03b	0.26 ± 0.01d	0.27 ± 0.01d	0.33 ± 0.01c	0.43 ± 0.03b	0.18 ± 0.01e	0.58 ± 0.06a
6	29.3	520	535	287 (100)	Cyanidin 3-(6-malonyl) glucoside	8.211 ± 0.002c	4.21 ± 0.20f	10.95 ± 0.24a	2.75 ± 0.20h	3.29 ± 0.17g	4.65 ± 0.28e	6.31 ± 0.37d	4.19 ± 0.22f	9.14 ± 0.37b
7	31.3	522	593	287 (100)	Cyanidin 3-(6-dioxalyl) glucoside	0.778 ± 0.005d	0.54 ± 0.02f	1.20 ± 0.01a	0.34 ± 0.02h	0.44 ± 0.01g	0.56 ± 0.02f	0.93 ± 0.06c	0.66 ± 0.04e	0.99 ± 0.07b
8	33.6	526	449	303 (100)	Delphinidin 3-rhamnoside	0.15 ± 0.01c	0.126 ± 0.004d	0.27 ± 0.03b	0.010 ± 0.003f	0.100 ± 0.001e	0.10 ± 0.01e	0.15 ± 0.03c	0.119 ± 0.004de	0.48 ± 0.04a
9	34.4	522	549	301 (100)	Peonidin 3-(6-malonyl) glucoside	1.242 ± 0.004b	0.67 ± 0.01e	1.88 ± 0.01a	0.40 ± 0.02h	0.540 ± 0.002g	0.69 ± 0.02de	0.98 ± 0.03c	0.61 ± 0.01f	0.70 ± 0.06d
10	35.4	518	679	287 (100)	Cyanidin malonyl-(dioxalyl)-hexoside	0.993 ± 0.003b	0.62 ± 0.01d	1.27 ± 0.04a	0.38 ± 0.02g	0.468 ± 0.005f	0.60 ± 0.04d	0.98 ± 0.04b	0.51 ± 0.01e	0.90 ± 0.06c
Total Anthocyanins						16.81 ± 0.01c	9.70 ± 0.33g	25.26 ± 0.23a	6.95 ± 0.39i	8.35 ± 0.24h	11.58 ± 0.54e	15.09 ± 0.73d	11.01 ± 0.48f	21.80 ± 0.90b
Range of variation						8.67–25.26		6.95–12.12				10.53–22.70		

Retention time (Rt), wavelengths of maximum absorption in the UV–vis region (λ_{max}), pseudomolecular and MS² fragment ions (in brackets), (< LOQ = 4.32 $\mu\text{g}/\text{mL}$, LOD = 1.30 $\mu\text{g}/\text{mL}$). Calibration curves: cyanidin 3-glucoside ($y = 243287x - 1E + 06$, $R^2 = 0.995$). In each column, the different letters indicate statistically significant differences ($p < 0.05$) between batches for each variety.

Table 5
Pulp colour parameters in *Citrus sinensis* [L.] Osbeck varieties.

Variety	Batch	L*	a*	b*	C*	h (hue)
Sanguinelli	A	46.96 ± 1.30c	29.93 ± 0.10a	31.39 ± 0.03c	43.38 ± 0.61b	46.35 ± 0.92c
	B	41.29 ± 0.06b	32.85 ± 0.38b	28.42 ± 0.69b	43.44 ± 0.74b	40.86 ± 0.37b
	C	39.68 ± 0.04a	34.54 ± 0.07c	24.34 ± 0.04a	42.25 ± 0.05a	35.18 ± 0.09a
Range of variation:		39.64–48.26	29.83–34.61	24.30–31.42	42.20–44.24	35.09–47.27
Tarocco Rosso	A	56.14 ± 0.02c	20.70 ± 0.93a	44.96 ± 0.02b	49.49 ± 0.02b	65.28 ± 0.02c
	B	49.65 ± 0.01a	26.93 ± 0.01c	39.03 ± 0.07a	47.42 ± 0.06a	55.40 ± 0.05a
	C	53.52 ± 0.03b	23.33 ± 0.02b	45.24 ± 0.12c	50.90 ± 0.12c	62.71 ± 0.04b
Range of variation:		49.64–56.16	21.63–26.94	38.96–45.36	47.36–49.51	55.35–65.30
Tarocco Ippolito	A	49.32 ± 2.62c	30.33 ± 0.18a	38.06 ± 0.02c	48.68 ± 3.87c	51.36 ± 1.61c
	B	41.64 ± 0.13b	32.07 ± 1.51b	25.49 ± 0.13b	40.28 ± 0.18b	39.25 ± 0.02b
	C	36.85 ± 0.02a	33.86 ± 0.02c	20.79 ± 0.04a	39.73 ± 0.02a	31.55 ± 0.06a
Range of variation:		36.83–51.94	30.15–33.88	20.75–38.08	39.71–52.55	31.49–52.97

Parameters CIELAB: L*, a*, b*, C*: Chroma and hue angle. Results expressed as mean ± standard deviation. In each column and the same variety, different letters mean statistical differences per each sample ($p < 0.05$).

flavonoid. Regarding anthocyanins, ten different compounds were identified in the blood oranges, seven cyanidin derivatives and three delphinidin derivatives, the most abundant was cyanidin 3-(6"-malonylglucoside) and cyanidin 3-glucoside. All varieties analyzed (Sanguinelli, Tarocco Rosso and Tarocco Ippolito) presented high total anthocyanins content. Overall, blood orange varieties are good sources of bioactive compounds mainly vitamin C.

The colour of the pulp of these oranges ranges from yellow-orange to reddish or more intense. Its coloration is due mainly to its content in carotenoids (β -carotene), and to the presence of other water-soluble

pigments of anthocyanic nature (mainly cyanidin 3-(6"-malonyl) glucoside and cyanidin 3-glucoside).

Finally, regarding colour determinations Sanguinelli and Tarocco Ippolito were the varieties with redder coloration in their pulp (higher a* and lower b*), with the brightest colours (higher C*); while the Tarocco Rosso is the one variety with more yellow-orange coloration (higher b* and lower a*) and high luminosity (L*). The colour study demonstrated that there is no direct relationship between external and internal coloration; oranges could be found with a higher reddish colour in their peel whereas their pulp presents a yellow-orange colour,

Table 6
Peel colour parameters *Citrus sinensis* [L.] Osbeck varieties.

Variety	Batch	Part of the fruit	L*	a*	b*	C*	h (hue)
Sanguinelli	A	Light	59.09 ± 7.98	33.01 ± 1.59	53.41 ± 13.67	63.11 ± 11.36	57.39 ± 7.53
		Dark	57.87 ± 4.11	32.93 ± 1.52	51.38 ± 7.44	61.08 ± 7.01	57.15 ± 2.67
		Base	61.81 ± 3.01	32.00 ± 1.62	56.98 ± 4.31	65.40 ± 3.60	60.61 ± 2.51
	B	Light	52.86 ± 1.22	34.67 ± 0.23	44.69 ± 1.61	56.57 ± 1.37	52.18 ± 0.90
		Dark	44.99 ± 6.62	32.79 ± 3.09	30.90 ± 10.13	45.25 ± 9.26	42.44 ± 6.34
		Base	46.01 ± 1.21	32.37 ± 0.53	34.85 ± 1.15	47.57 ± 1.20	47.11 ± 0.48
	C	Light	57.90 ± 10.22	30.91 ± 3.75	51.73 ± 15.20	60.89 ± 11.41	57.86 ± 10.23
		Dark	41.91 ± 4.24	30.07 ± 2.09	26.06 ± 7.04	39.92 ± 6.13	40.30 ± 5.93
		Base	43.21 ± 6.14	32.13 ± 2.41	29.89 ± 10.84	44.16 ± 9.26	41.87 ± 7.63
Range of variation:			37.07–68.12	27.16–34.90	19.02–67.08	33.79–74.47	34.24–64.94
Tarocco Rosso	A	Light	62.06 ± 6.49	34.64 ± 4.26	57.07 ± 7.35	67.02 ± 4.50	58.50 ± 6.06
		Dark	55.06 ± 4.95	34.38 ± 1.83	46.36 ± 7.35	57.90 ± 5.15	53.13 ± 5.38
		Base	62.91 ± 2.35	32.88 ± 1.22	58.64 ± 3.56	67.26 ± 2.61	60.66 ± 2.30
	B	Light	50.19 ± 4.70	35.61 ± 3.02	41.26 ± 7.62	54.60 ± 7.20	48.92 ± 4.02
		Dark	50.39 ± 1.27	38.20 ± 1.25	41.58 ± 2.42	56.50 ± 1.03	47.40 ± 2.56
		Base	58.38 ± 3.22	35.54 ± 2.37	54.87 ± 6.77	65.45 ± 6.01	56.92 ± 3.35
	C	Light	59.35 ± 4.74	33.60 ± 2.84	53.44 ± 5.92	63.27 ± 3.80	57.66 ± 10.23
		Dark	49.10 ± 3.31	35.67 ± 1.91	38.16 ± 4.01	52.34 ± 1.71	46.84 ± 5.93
		Base	60.58 ± 3.42	35.78 ± 2.60	57.25 ± 4.87	67.61 ± 3.32	57.90 ± 7.63
Range of variation:			45.79–68.55	30.76–38.90	34.15–64.42	47.40–71.52	52.94–67.89
Tarocco Ippolito	A	Light	60.37 ± 5.62	33.41 ± 1.46	54.74 ± 10.15	64.34 ± 8.05	58.14 ± 5.64
		Dark	48.37 ± 4.71	36.26 ± 1.08	36.24 ± 6.89	51.45 ± 4.54	44.66 ± 5.81
		Base	59.87 ± 0.76	34.55 ± 1.86	53.56 ± 0.80	63.75 ± 0.67	57.18 ± 1.72
	B	Light	61.95 ± 5.53	34.30 ± 5.04	59.23 ± 8.01	68.76 ± 5.08	59.64 ± 6.65
		Dark	54.69 ± 9.27	34.44 ± 2.44	45.39 ± 16.59	57.74 ± 12.2	51.26 ± 11.07
		Base	57.83 ± 3.63	37.03 ± 1.30	53.19 ± 5.75	64.84 ± 5.36	55.04 ± 2.16
	C	Light	51.51 ± 5.93	37.34 ± 1.76	40.95 ± 9.01	55.74 ± 5.60	47.15 ± 7.43
		Dark	56.05 ± 8.87	36.55 ± 1.91	47.61 ± 11.98	60.46 ± 8.25	51.71 ± 8.71
		Base	60.47 ± 3.28	33.80 ± 3.33	55.75 ± 3.49	65.29 ± 1.89	58.73 ± 3.87
Range of variation:			43.66–67.48	29.26–39.10	29.35–67.24	46.91–73.84	38.85–66.29

CIELAB Parameters: L*, a*, b*, C*: Chroma and hue angle.

as in the case of Tarocco Rosso.

Acknowledgment

Authors are grateful to OTRI UCM-ANEKOOP S. Coop. (322-2015) project for financial support, and Dr. Ángel del Pino researcher for samples collection. The authors are also grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013) and L. Barros contract; also to FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E.

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