agronomy

MDPI

Article

Fruit Quality and Primary and Secondary Metabolites Content in Eight Varieties of Blood Oranges

María Ángeles Forner-Giner [1], Manuel Ballesta-de los Santos [2], Pablo Melgarejo [2], Juan José Martínez-Nicolás [2], Amparo Melián-Navarro [3], Antonio Ruíz-Canales [3], Alberto Continella [4] and Pilar Legua [2,*]

[1] Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Spain; forner_margin@gva.es
[2] Plant Sciences and Microbiology Department, Miguel Hernández University, Research Group in Plant Production and Technology, Ctra. de Beniel, km 3.2, 03312 Orihuela, Spain; manuel.ballestas@umh.es (M.B.-d.l.S.); pablo.melgarejo@umh.es (P.M.); juanjose.martinez@umh.es (J.J.M.-N.)
[3] Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Ctra. Beniel, km 3.2, 03312 Orihuela, Spain; amparo.melian@umh.es (A.M.-N.); acanales@umh.es (A.R.-C.)
[4] Department of Agriculture, Food and Environment, University of Catania, 95124 Catania, Italy; acontine@unict.it
* Correspondence: p.legua@umh.es; Tel.: +34-966-749-669

Abstract: In Europe, the cultivation of blood oranges for fresh consumption is increasing due to their excellent organoleptic and nutraceutical properties, which give this fruit the status of functional food. Blood orange has a higher content of bioactive compounds, which confer additional benefits for human health. The main morphological and qualitative parameters were studied together with the content of primary metabolites by ^{1}H NMR and secondary metabolites by HPLC-ESI-DAD-MS^n from eight varieties of blood orange grafted on *Citrus macrophylla*. Tarocco Dalmuso was the variety with the highest values of weight (350.6 g), caliber (86.4 mm and 88.6 mm) and juice content (214.2 g). Tarocco Gallo obtained the most interesting qualitative parameters (13.95 °Brix; 22.75 MI). The most intense red juice was in Sanguinelli (a^* = 9.45) and, in crust, it was in Tarocco Scirè (a^* = 40.13). The most abundant primary metabolites were proline, aspartate and asparagine, citric acid and sucrose. The results showed that the juice of the Moro had the highest levels of total flavones and flavanones (90.07 and 592.88 mg L^{-1}, respectively), and Sanguinelli in total anthocyanins (101.06 mg L^{-1}). To conclude, Tarocco Dalmuso obtained the best values of agronomic parameters, and Moro and Sanguinelli in the content of phenolic compounds.

Keywords: amino acid; anthocyanins; blood orange; morphological parameters; organic acid; phenolic compounds; quality parameters; sugars

check for updates

Citation: Forner-Giner, M.Á.; Ballesta-de los Santos, M.; Melgarejo, P.; Martínez-Nicolás, J.J.; Melián-Navarro, A.; Ruíz-Canales, A.; Continella, A.; Legua, P. Fruit Quality and Primary and Secondary Metabolites Content in Eight Varieties of Blood Oranges. *Agronomy* **2023**, *13*, 1037. https://doi.org/10.3390/agronomy13041037

Academic Editors: Giorgia Liguori and Noemi Tel-Zur

Received: 15 March 2023
Revised: 29 March 2023
Accepted: 30 March 2023
Published: 31 March 2023

1. Introduction

Sweet orange (*Citrus sinensis* [L.], Osbeck) is the most important *Citrus* species worldwide. Its world production is 79 million tons [1]. Sweet orange varieties can be divided into two main groups which are white orange, grown in almost all *Citrus*-producing countries of the world, and blood oranges, grown in a few countries (mainly Italy) where favorable climatic conditions induce the synthesis of their characteristic red pigment. The anthocyanins present in the fruit of the blood orange are water-soluble pigments found within the flavonoid family and provide the blood orange with its characteristic red coloration in crust, pulp and juice, as is the case with other fruit trees such as pomegranate [2] or sweet cherry [3]. The origin of *Citrus* fruits and, therefore, blood oranges is unclear. The first known citation of *Citrus* was related in the "Book of History" of ancient China (500 BC). This book refers to the imperial mandate (Emperor Ta Yui, 300 BC) on the acquisition of taxes on two varieties of *Citrus* fruits of different sizes. After arriving in Europe, specifically in Italy, the expansion of *Citrus* fruits to countries such as Spain or France (600 BC) continued according to the book of Etymologies XVII chapter VII, 8, of San Isidoro de Sevilla, referring to *Citrus* fruits as "Arbor Medica" [4]. The most common and widespread varieties of blood oranges in the Mediterranean area are "Sanguinelli" in Spain, and "Tarocco" and "Moro" in Italy. These varieties are consumed worldwide in both fresh produce and juices [5]. The fruits are medium or small in caliber, elongated or rounded, with a thin crust and orange dyed red due to the high concentration of anthocyanins [6].

The red pigments conferred by anthocyanins depend on a range of factors, including variety, rootstocks, maturity, growing region and environment [7,8]. In particular, the contrast between day and night temperature is considered a crucial factor in its synthesis [9,10]. In this sense, the dependence of blood oranges on the cold for the biosynthesis of anthocyanins has been demonstrated, in which a greater difference between day and night temperature leads to a higher concentration of total anthocyanins [11]; therefore, Mediterranean climates are the regions of the planet most suitable for the cultivation of blood oranges. For this reason, Italy is the world's leading producer of blood orange. Specifically, in Sicily, there are almost 40,000 ha dedicated to the cultivation of this fruit and this represents approximately 70% of the total production of sweet orange in Italy.

In Italy, these fruits are mainly consumed fresh thanks to their easy peelability and their optimal balance between organic acids and sugars. The variety "Moro" is the one that presents the most intense red color and its juice offers high levels of bioactive compounds [12], being very important for the human diet as it has an important anti-inflammatory and anticancer effect [13].

In Spain, the cultivation of blood oranges represents approximately 0.7% of the total production of oranges, with a total of 952 ha destined for their cultivation [14]. "Sanguinelli", a spontaneous mutation of "Double Fine" [15], is the most common red orange variety. In addition, with regard to Spain, it is important to note that, although the production of blood orange varieties is still limited, their interest on the part of current consumers is increasing rapidly in recent years [16] mainly due to their high content of bioactive compounds beneficial to human health [17,18].

In fact, health problems are the main concern of today's consumers and they demand a food supply that can improve their well-being. Fruits, in general, and particularly *Citrus* fruits, are important reservoirs of nutrients (amino acids, organic acids or sugars) and other bioactive compounds with antioxidant properties [19]. Among *Citrus* fruits, blood oranges have high concentrations of phytochemicals such as terpenoids (limonoids and carotenoids) or phenolic compounds (flavones, flavanones or phenolic acids), being especially rich in anthocyanins that exhibit a powerful antioxidant activity with great benefits for human health [20]. Anthocyanins give blood oranges high sensory quality and potential health promoters, such as protection against cancer and cardiovascular disease [21]. Therefore, anthocyanin levels have become one of the important quality indices affecting consumer acceptance and marketability of blood oranges [22].

Bearing in mind the above, the main objective of this study is the characterization of eight varieties of blood oranges, which were grown under the same environmental conditions. Specifically, it was intended to analyze the main morphological parameters of the fruit and qualitative of the fruit and its juice together with the content of primary metabolites and secondary metabolites of the juice of the varieties: "Sanguinelli", "Tarocco Sant'Alfio", "Tarocco Dalmuso", "Tarocco Rosso", "Tarocco Gallo", "Tarocco Scirè", "Tarocco Meli" and "Moro", grafted on *Citrus macrophylla* and cultivated under the environmental conditions of southeastern Spain.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

Blood oranges varieties "Sanguinelli" (*Citrus sinensis* (L.) cv. Sanguinelli), which come from a spontaneous mutation of the Double Fine blood orange (*Citrus sinensis* (L.) cv. Double Fine), "Tarocco Sant'Alfio", "Tarocco Dalmuso", "Tarocco Rosso", "Tarocco Gallo", "Tarocco Scirè", "Tarocco Meli" and "Moro" (*Citrus sinensis* (L.) cv. Tarocco and cv. Moro, respectively) were collected from plants grafted on *Citrus macrophylla* and grown in an experimental farm located in Orihuela, Alicante (Spain) (38.06733781, −0.98229272). This farm had an EC of 0.44 dS m^{-1} (20 °C), pH of 7.25, temperature of 12 °C and relative humidity (RH) of 58% at harvest time. In Spain, due to environmental conditions, the stage of commercial consumption of blood oranges runs from January to March. Therefore, the samples considered in this study were harvested in January 2023. In this way, the fruit was harvested manually at the physiological maturity stage, with the aim of ensuring similarity to commercial standards, it was immediately transported to the laboratory starting its analysis that same day.

Initially, for all samples, a manual cleaning of the surface of the fruit was carried out with distilled water, in order to eliminate possible residues of dust and dirt. Subsequently, we proceeded to the external photographic report, crust color (n = 50) and weight and caliber of fruit (n = 25). Next, we proceeded to the destructive analysis of the fruit, in which each fruit was cut in half and determined number of carpels and seeds, crust thickness (n = 25) and internal photographic report. Then, for each variety of blood orange, the juice of 25 pieces of fruit was obtained by a commercial juicer. Previously, the 25 fruit pieces were divided into six replicas (n = 6) for the determination of the qualitative parameters (pH, TSS, TA, MI and juice color) and for metabolomics analysis (15 mL per replica), which were stored at −80 °C until their analysis.

2.2. Fruit Morphological Characterization

The morphological parameters were determined according to [23]. A digital balance (model BL-600; Sartorius, Germany) was used for the determination of fruit and crust weights. Juice weight was determined by the difference between fruit and crust weight. Number of carpels and seeds was visually counted. An electronic digital slide gauge (model CD-15 DC; Mitutoyo, Japan) was used to measure the caliber fruit (equatorial diameter and length of the fruit) and thickness of crust.

2.3. Juice Quality Parameters Dermination

A Crison pH meter model GLP21 (Crison, Barcelona, Spain) was used to measure the pH of blood orange juice; which was previously calibrated with buffer solution code 9463 (pH 4.01) and code 9464 (pH 7.00). An Atago N1 (0.2 °Brix) digital refractometer (model N-1; Ltd., Tokyo, Japan) was used to measure TSS content. An automatic titrator (877 Titrino plus, Metrohm, Herisau, Switzerland) was used to measure the TA of the orange juice; 5 mL of the homogenized blood orange juice was dissolved in 45 mL of distilled H_2O, followed by a pH titration with 0.1 M NaOH to pH 8.1, and the results were expressed as g of citric acid L^{-1} because this is the dominant organic acid in blood oranges [23]. Once the TSS and TA contents had been assessed, the maturity index (MI) was calculated as the TSS/TA ratio and expressed in g of citric acid per 100 mL.

Color variables were measured using a Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japan) coupled to a DP-301 data processor (Minolta Corp.). Color determinations were made of both the crust and juice according to the Commission Internationale de l' Éclairage (CIE) and expressed as L^*, a^*, b^*. These values were then used to calculate Hue angle degree using the following equation:

$$H^\circ = arctang\ (b^*/a^*) \quad (1)$$

Chroma, indicate of the color intensity or saturation y was calculated using the following equation:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

According to [24], hue angle (H°) and chroma (C^*) have been cataloged as the most intuitive color variables. The calculated color index (CI) used the following equation [25]:

$$CI = 1000\ a^*/L^*\ b^* \quad (3)$$

2.4. Analysis of Primary Metabolites by ^{1}H-Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR)

The primary metabolites were analyzed as described above [26] with slight modifications described below. A volume of 10 mL of the centrifuged blood orange juice (15,000× *g* at 4 °C for 10 min) was passed through a 0.45 μm filter (Millipore, Burlington, MA, USA). Then, an aliquot of 130 μL was mixed to 70 μL of D_2O phosphate buffer (100 mM KH_2PO_4, pH = 6) containing 0.1% of TSP (trimethyl silyl propionic acid sodium salt, *w*/*v*) and to 350 μL of CD_3OD (tetradeuteromethanol). The sample was vortexed for 2 min and filtered, and 600 μL was transferred to an ^{1}H NMR tube for further analysis. All ^{1}H NMR spectra were recorded at 298 K on a Bruker AVIII HD 500 ^{1}H NMR spectrometer (500.16 MHz for ^{1}H) equipped with a 5 mm CryoProbe Prodigy Broadband Observe cryogenic probe (Biospin; Bruker, Bremen, Germany). The results are reported as g or mg L^{-1} of blood orange juice.

2.5. Analysis of Secondary Metabolites by HPLC-Diode Array Detection-Electrospray Ionization-Mass Spectrometry (HPLC-ESI-DAD-MS^n)

The individual phenolic compounds in juice of blood orange varieties analyzed were performed according to [27] with few modifications described below. A volume of 5 mL of blood oranges juice was mixed with 5 mL of MeOH, vortexed for 2 min, and then, the extraction was performed in an ultrasonic bath (2.7 L Ultrasonic cleaner, Toctech, Cantabria, Spain) for 3 min at room temperature. The resulting heterogeneous mixture was centrifuged at 1000× *g* for 5 min and the supernatant passed through a 0.45 μm filter (Millipore, Burlington, MA, USA) prior to injection into the chromatograph system. Chromatographic analyses were carried out on a series 1100 HPLC-ESI-DAD-MS^n Ion Trap (Agilent, Waldbronn, Germany); this HPLC system with DAD detector series 1100 was coupled to a mass spectrometer equipped with an ion trap and an ESI interface). The results are reported as mg L^{-1} of blood orange juice.

2.6. Statistical Analysis

Experimental data are presented as the mean of six (pH, TSS, TA, MI, primary and secondary metabolites) (n = 6), twenty-five (morphological parameters) (n = 25), or fifty (color parameters) (n = 50) replicates per variety. Statistical analysis was performed by analysis of variance (ANOVA) and HSD Tukey test was applied to establish significant differences among mean values at $p < 0.05$, using the SPSS 28.0 software package. Principal component analysis (PCA) using Pearson's correlation was also done using the Stat graphics Centurion v. 18.1.12.

3. Results

3.1. Morphological and Qualitative Parameters

In this study, the main morphological and qualitative parameters were studied together with the content of primary and secondary metabolites from 8 varieties of blood orange ("Sanguinelli", "Tarocco Sant'Alfio", "Tarocco Dalmuso", "Tarocco Rosso", "Tarocco Gallo", "Tarocco Scirè", "Tarocco Meli" and "Moro") grafted on *Citrus macrophylla* and grown under the environmental conditions of southeastern Spain (Figure 1).

Results corresponding to the morphological and qualitative characterization of the blood oranges varieties in terms of fruit weight, equatorial diameter, fruit length, crust thickness, number of carpels, number of seeds, crust weight, juice weight, pH, TSS, TA and MI (TSS/TA) is shown in Table 1. The average weight for eight blood oranges analyzed during the January of 2023 season range from 178.3 to 350.6 g. Tarocco Gallo and Tarocco Scirè showed significantly lower fruit weights (183.2 and 178.3 g, respectively), whereas Tarocco Dalmuso (350.6 g), followed by Taroco Meli (326.2 g) and Tarocco Rosso (288.0 g), had a higher mean fruit weight. Fruit equatorial diameters and length oscillated between 70.1 and 69.1 and 86.4 and 88.8 mm, respectively. The crust thickest was in Tarocco Meli and Moro (6.6 and 5.7 mm, respectively), whereas the thinnest was in Tarocco Dalmuso, Tarocco Scirè and Tarocco Sant'Alfio (3.5, 4.1 and 4.9 mm, respectively).

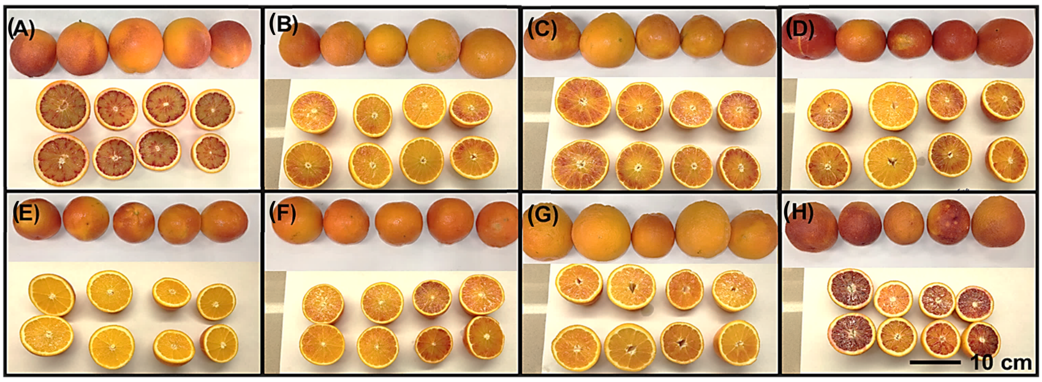

Figure 1. Representative images of the eight blood orange fruits showing the different morphological characteristics of Italian varieties ((**B**) Tarocco Sant'Alfio, (**C**) Tarocco Dalmuso, (**D**) Tarocco Rosso, (**E**) Tarocco Gallo, (**F**) Tarocco Scirè, (**G**) Tarocco Meli and (**H**) Moro) versus Spanish variety ((**A**) Sanguinelli).

Table 1. Morphological and qualitative parameters of eight blood oranges varieties (Sanguinelli, Tarocco Sant'Alfio, Tarocco Dalmuso, Tarocco Rosso, Tarocco Gallo, Tarocco Scirè, Tarocco Meli and Moro) grafted on *Citrus macrophylla*.

Parameters	Sanguinelli	Tarocco Sant'Alfio	Tarocco Dalmuso	Tarocco Rosso	Tarocco Gallo	Tarocco Scirè	Tarocco Meli	Moro
Morphological parameters *								
FW (g)	225.4 bcd	256.5 bc	350.6 a	288 ab	183.2 d	178.3 d	326.2 a	223.5 cd
ED (mm)	74.1 cd	78.4 bc	86.4 a	83.4 bc	72.1 cd	70.1 d	84.5 bc	77.3 bcd
FL (mm)	82 1.6 ab	80.0 b	88.6 a	77.0 b	69.1 c	68.5 c	88.8 a	75.6 bc
CT (mm)	5.3 b	4.9 bc	3.5 d	5.3 bc	5.1 bc	4.1 cd	6.6 a	5.7 ab
NC	11.12 ab	10.41 abc	9.23 c	11.61 a	10.23 abc	9.61 bc	9.34 c	9.35 c
NS	1.6 a	0.1 b	0.0 b	0.3 b	0.0 b	0.0 b	0.0 b	0.0 b
CW (g)	89.8 cde	108.1 bcd	151.2 a	124.3 abc	80.1 de	63.5 e	143.3 ab	109.4 bcd
JW (*w*:*w*)	131.2 bc	176.5 abc	214.2 a	159.5 abc	106.2 c	115.8 c	198.6 ab	110.2 c
Qualitative parameters **								
pH	3.91 b	4.23 ab	4.10 ab	3.91 b	4.42 a	4.27 ab	3.83 b	4.11 ab
TSS (°Brix)	10.11 b	12.32 ab	12.52 ab	13.00 ab	13.95 a	13.00 ab	11.00 ab	11.93 ab
TA (g citric acid L^{-1})	15.43 a	12.81 ab	10.52 bc	9.22 cd	6.13 d	11.13 bc	15.42 a	10.14 bc
MI (TSS/TA)	6.52 e	9.61 cde	11.88 bc	14.09 b	22.75 a	11.68 bcd	7.13 de	11.76 bcd

Note: FW: fruit weight; ED: equatorial diameter; FL: fruit length; CT: crust thickness; NC: number of carpels; NS: number of seeds; CW: crust weight; JW: juice weight; TSS: total soluble solids; TA: titratable acidity and MI: maturity index. Data are the mean (* n = 25 or ** n = 6). Values within the same row that present the same letter are not significantly different according to the HSD Tukey test with a confidence level of 95%.

The range of pH values in the samples of blood oranges varieties studied was between 3.83 and 4.42 (Tarocco Meli and Tarocco Gallo, respectively), highlighting the variety Tarocco Gallo. Concerning the values of TSS and TA, fruits of Tarocco Gallo showed the highest TSS content (13.95 °Brix), whereas Sanguinelli fruits recorded the lowest TSS (10.11 °Brix). At the end of sampling, all the pigmented varieties registered values of total acidity of between 6.13 and 15.43 g citric acid L^{-1} (Tarocco Gallo and Sanguinelli, respectively, Table 1). Maturity index (MI) values, calculated as TSS/TA and expressed in grams of citric acid per 100 mL, are not similar in all samples analyzed. For example, Tarocco Gallo showed the highest MI (22.75), whereas Sanguinelli or Tarocco Meli fruits recorded the lowest MI (6.52 and 7.13, respectively) (Table 1).

3.2. External Crust and Juice Color

Results corresponding to the crust and juice color, all properties are summarized in Table 2. In the crust color parameters, Tarocco Dalmuso variety showed the lowest values of parameter a^* and the highest values of parameter b^* (30.17 and 56.72, respectively). In the following order, Moro, Tarocco Rosso and Sanguinelli showed the lowest L^* value (51.41, 52.38 and 55.69, respectively) and Tarocco Dalmuso showed the highest L^* value. In order to saturation index C^*, Moro variety showed the lowest value (50.31), by the contrary Tarocco Scirè and Tarocco Sant'Alfio showed the highest values (66.37 and 66.29, respectively). Relative to $H°$ parameter, the values were kept in a range between 47.31 and 61.94. The highest color index (CI) values were observed in fruits of Tarocco Rosso (18.85) and Moro (18.84) and the lowest CI value was showed in fruits of Tarocco Dalmuso (8.29).

Table 2. Color parameters in crust and juice of eight blood orange varieties (Sanguinelli, Tarocco Sant'Alfio, Tarocco Dalmuso, Tarocco Rosso, Tarocco Gallo, Tarocco Scirè, Tarocco Meli and Moro) grafted on *Citrus macrophylla*.

Color	Sanguinelli	Tarocco Sant'Alfio	Tarocco Dalmuso	Tarocco Rosso	Tarocco Gallo	Tarocco Scirè	Tarocco Meli	Moro
Crust								
L^*	55.69 b	64.20 a	65.21 a	52.38 b	59.76 ab	59.64 ab	62.61 a	51.41 b
a^*	34.15 bcd	33.74 bcd	30.17 d	36.75 ab	34.78 c	40.13 a	30.69 cd	33.46 bcd
b^*	39.59 b	56.83 a	56.72 a	39.35 b	50.61 a	52.77 a	55.72 a	37.14 b
C^*	52.51 b	66.29 a	64.37 a	54.14 b	61.66 a	66.37 a	63.90 a	50.31 b
$H°$	48.23 d	59.01 abc	61.94 a	46.48 d	55.24 bc	52.66 cd	60.75 ab	47.31 d
CI	16.98 ab	9.57 c	8.29 c	18.85 a	11.95 bc	12.92 bc	9.29 c	18.84 a
Juice								
L^*	39.08 e	46.21 ab	44.91 bc	42.86 d	47.86 a	43.94 cd	44.35 cd	36.30 f
a^*	9.45 a	4.77 c	6.29 b	5.50 bc	1.46 e	5.74 bc	3.34 d	8.51 a
b^*	5.57 d	15.08 ab	13.36 b	10.29 c	17.09 a	13.01 b	13.00 b	3.45 d
C^*	10.99 ef	15.85 ab	14.80 bc	11.67 de	17.16 a	14.33 bc	13.46 cd	9.19 f
$H°$	30.53 d	72.37 b	64.26 c	61.98 d	85.21 a	65.60 c	75.53 b	21.79 e
CI	43.99 b	6.91 cde	10.90 cd	12.43 c	1.74 e	10.59 cd	5.85 de	69.53 a

Note: data are the mean (n = 50). Values within the same row that present the same letter are not significantly different according to the HSD Tukey test with a confidence level of 95%.

In the juice color parameters (Table 2), Tarocco Gallo variety showed the lowest values of parameter a^* (1.46) and the highest b^* values (17.09). The profiles of L^* and $H°$ parameter, show the same behavior with a^* and b^* parameters, being Tarocco Gallo (47.86 and 85.21, respectively), the variety with the highest values analyzed while the Moro (36.30 and 21.79, respectively) showed lower values analyzed. In order to saturation index C^*, except de Moro (9.19) and Tarocco Gallo (17.09), all samples studied showed similar levels. Moro (69.53) and Sanguinelli (49.99) showed the highest color index (CI) values and Tarocco Gallo (1.79) and Tarocco Sant'Alfio (6.91) showed the lowest CI value.

3.3. Primary Metabolites Content in Juice

Figure 2 shows the amino acids, organic acids and sugars total content that were identified and quantified in the varieties. In relation to the amino acids total content (Figure 2A), it was observed that the values were between 3821.64 mg L^{-1} in Tarocco Sant'Alfio and 2256.88 mg L^{-1} in Tarocco Moro. Statistically significant differences (p < 0.05) were observed between some of the total amino acid contents. The highest total contents were observed in Tarocco Sant'Alfio (3821.64 mg L^{-1}) followed by Tarocco Dalmuso (3589.14 mg L^{-1}) and Sanguinelli (3400.44 mg L^{-1}), the lowest total contents were obtained in Tarocco Moro (2256.88 mg L^{-1}) and Tarocco Meli (2300.48 mg L^{-1}). The rest of the varieties showed an amino acids total content that remained between 2456.05 and 2707.04 mg L^{-1}.

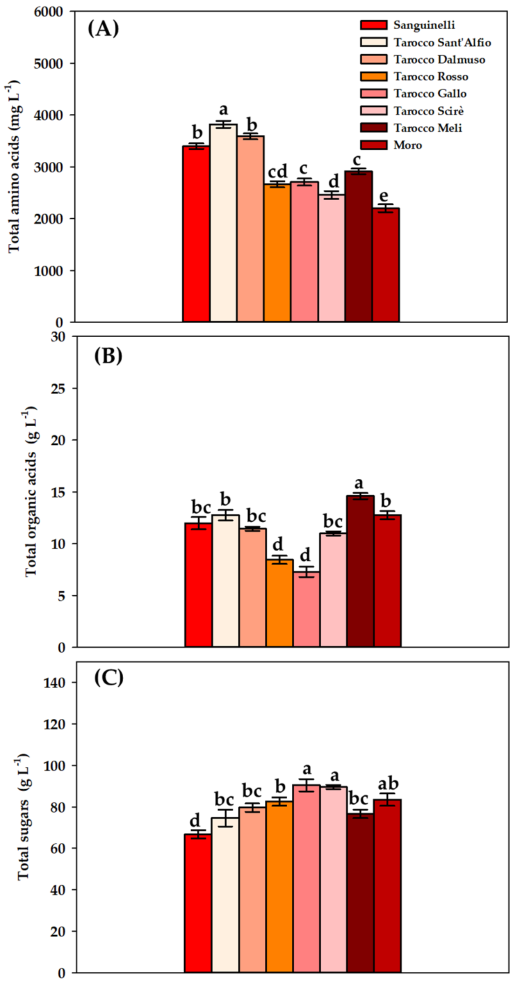

Figure 2. Total content of amino acids (**A**), organic acids (**B**) and sugars (**C**) in juice of eight blood orange varieties (Sanguinelli, Tarocco Sant'Alfio, Tarocco Dalmuso, Tarocco Rosso, Tarocco Gallo, Tarocco Scirè, Tarocco Meli and Moro) grafted on *Citrus macrophylla.* Data are the mean ± SE ($n = 6$). Different letters indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

Regarding the organic acids total contents (Figure 2B), values ranging from 14.60 g L^{-1} in Tarocco Meli to 7.28 g L^{-1} in Tarocco Gallo were obtained. Statistically significant differences ($p < 0.05$) were also observed between the total organic acid content in some of the varieties studied. The highest total contents were observed in Tarocco Meli (14.60 g L^{-1}) followed by Tarocco Sant'Alfio (12.75 g L^{-1}) and Tarocco Moro (12.75 g L^{-1}), and the lowest total contents were obtained in the varieties Tarocco Gallo (7.28 g L^{-1}) and Tarocco Rosso (8.43 g L^{-1}). The rest of the varieties showed an organic acids total content between 11.97 and 10.98 g L^{-1}, without statistically significant differences ($p > 0.05$).

Figure 2C shows the sugars total contents quantified in the varieties of blood oranges. Thus, sugars total contents between 90.44 g L^{-1} in Tarocco Gallo and 66.78 g L^{-1} in Sanguinelli were observed. As in amino acids and organic acids, statistically significant differences ($p < 0.05$) were also observed in sugars between some of the varieties studied. The highest total sugar contents were observed in Tarocco Gallo (90.44 g L^{-1}) followed by Tarocco Scirè (89.56 g L^{-1}) and Tarocco Moro (83.49 g L^{-1}), and the lowest contents were obtained in the Sanguinelli (66.78 g L^{-1}) and Tarocco Sant'Alfio (74.59 g L^{-1}) varieties. The rest of the varieties showed a total content that remained in a range between 82.55 and 79.64 g L^{-1} and were not statistically significant differences ($p > 0.05$).

Table 3 shows the amino acids, organic acids, sugars and others metabolites individual content that were identified and quantified in the eight varieties of blood oranges. In this study, 10 essential amino acids were identified and quantified in terms of the total essential amino acids in blood orange varieties. In Table 3 shows that proline and aspartate were the amino acids that were detected in higher concentrations; their concentrations oscillated from 1093.88 mg L^{-1} in Tarocco Gallo to 651.27 mg L^{-1} in Tarocco Rosso for proline and, 1038.07 mg L^{-1} in Tarocco Dalmuso and 422.94 mg L^{-1} in Tarocco Scirè for aspartate. Arginine and asparagine were the next amino acids that were detected in higher concentrations, with values ranging from 827.15 mg L^{-1} in Tarocco Sant'Alfio to 433.21 mg L^{-1} in Sanguinelli for arginine, and between 1037.01 mg L^{-1} in Tarocco Sant'Alfio and 266.84 mg L^{-1} in Moro for asparagine. Glutamine also presented a high concentration, with values ranging from 249.78 mg L^{-1} in Tarocco Scirè to 334.65 mg L^{-1} in Sanguinelli. Alanine obtained lower concentrations with respect to the concentrations of the amino acids described above, with values between 88.31 mg L^{-1} in Sanguinelli and 29.88 mg L^{-1} in Tarocco Meli. Valine, isoleucine and leucine presented the lowest concentrations of all amino acids identified in blood orange varieties, with values ranging from 5.72 mg L^{-1} in Tarocco Gallo to 12.51 mg L^{-1} in Sanguinelli, 2.42 mg L^{-1} in Tarocco Meli and Tarocco Dalmuso at 4.00 mg L^{-1} in Tarocco Gallo, and from 1.01 mg L^{-1} in Moro to 2.42 mg L^{-1} in Tarocco Sant'Alfio, respectively.

Table 3. Individual content of amino acids, organic acids, sugars and others metabolites in juice of eight blood orange varieties (Sanguinelli, Tarocco Sant'Alfio, Tarocco Dalmuso, Tarocco Rosso, Tarocco Gallo, Tarocco Scirè, Tarocco Meli and Moro) grafted on *Citrus macrophylla*.

Primary Metabolite	Sanguinelli	Tarocco Sant'Alfio	Tarocco Dalmuso	Tarocco Rosso	Tarocco Gallo	Tarocco Scirè	Tarocco Meli	Moro
Amino acids (mg L^{-1})								
Alanine	88.31 a	39.79 c	63.41 b	33.86 c	45.26 bc	47.34 bc	29.88 c	41.43 c
Arginine	433.91 c	827.15 a	681.51 b	739.33 ab	388.42 c	465.06 c	710.58 b	438.98 c
Asparagine	990.21 a	1037.01 a	406.47 c	392.10 c	439.12 bc	342.90 c	613.20 b	266.84 c
Aspartate	811.08 ab	754.97 b	1038.07 a	804.44 ab	426.49 c	422.94 c	616.57 bc	772.48 ab
Glutamine	334.65 a	313.68 a	304.09 a	ND	250.68 ab	249.78 ab	264.89 ab	179.88 b
Isoleucine	3.50 ab	2.62 ab	2.42 b	2.55 ab	4.00 ab	4.76 a	2.42 b	3.23 ab
Leucine	ND	2.42 a	1.71 ab	ND	ND	ND	1.16 b	1.01 b
Proline	691.44 bc	837.48 ab	1041.61 a	651.27 bc	1093.88 a	860.44 ab	667.67 bc	516.77 c
Tyrosine	34.83 c	ND	41.16 b	32.96 c	53.47 a	51.86 a	ND	29.53 c
Valine	12.51 a	6.49 c	8.69 bc	6.53 c	5.72 c	10.97 ab	7.31 c	6.73 c
Organics acids (g L^{-1})								
Citrate	11.96 b	12.75ab	11.43 b	8.43 b	7.28 d	10.98 bc	14.60 a	12.75 ab
Lactate	0.0121 a	0.0098 bc	0.0101 b	0.0079 cd	0.0088 bc	0.0089 bc	0.0065 d	0.0082 bcd
Sugars (g L^{-1})								
Fructose	19.18 c	18.07 c	19.55 c	20.67 bc	26.24 a	25.00 ab	19.66 c	22.54 abc
Glucose	16.64 d	17.16 d	18.24 cd	19.89 bcd	26.20 a	24.08 ab	18.66 cd	21.89 abc
Sucrose	30.02 b	37.85 a	40.14 a	40.32 a	36.49 ab	38.96 a	37.38 a	38.10 a
Myo-inostol	0.94 b	1.51 a	1.71 a	1.67 a	1.51 a	1.52 a	0.89 b	0.96 b
Gluc/Fruc	0.86 b	0.94 ab	0.93 ab	0.99 a	0.99 a	0.96 a	0.94 ab	0.97 a
Gluc/Suc	0.55 bc	0.45 c	0.45 c	0.49 c	0.72 a	0.63 b	0.50 c	0.57 bc
Others (mg L^{-1})								
Choline	9.20 b	7.19 b	13.71 a	13.41 a	12.81 a	12.54 ab	7.21 b	6.38 b
Ethanol	348.45 a	86.49 c	350.84 a	176.07 b	89.43 c	213.96 b	73.87 c	214.10 b
Trigonelline	4.30 b	2.19 c	6.79 a	1.64 c	4.36 b	3.79 b	3.10 bc	4.26 b

Note: data are the mean (n = 6). Values within the same row that present the same letter are not significantly different according to the HSD Tukey test with a confidence level of 95%. ND: not determinate by small quantity (<10 mM).

Two organic acids were separated and identified in the red orange juices of the varieties studied: citric acid and lactic acid (Table 3). Citric acid was the main organic acid found and its concentration oscillated from 14.60 g L^{-1} in Tarocco Meli to 7.28 g L^{-1} in Tarocco Gallo. The rest of the concentrations remained in the range of 8.43 and 12.75 g^{-1}, showing statistically significant differences ($p < 0.05$) in some of these varieties. Lactic acid was the second most abundant identified organic acid in red orange juices of the varieties studied and remained in a range of 0.0065 g L^{-1} in Tarocco Meli to 0.0121 g L^{-1} in Sanguinelli. The rest of the main organic acids present in *Citrus* fruits were not detected by ^{1}H NMR as they presented a concentration lower than 10 mM.

Fructose, glucose, sucrose and myo-inositol were determined as sugar components in red orange juices of the eight varieties studied (Table 3). The main sugar was sucrose and its concentration oscillated from 40.32 g L^{-1} in Tarocco Rosso to 30.02 g L^{-1} in Sanguinelli. The rest of the concentrations remained between 40.14 g L^{-1} in Tarocco Dalmuso and 36.49 g L^{-1} in Tarocco Gallo, showing statistically significant differences ($p < 0.05$) in some of these varieties. The second highest concentration of individual sugars was observed in fructose, whose concentration oscillated from 18.07 g L^{-1} in Tarocco Sant'Alfio to 26.24 g L^{-1} in Tarocco Gallo. The third highest concentration of individual sugars presented by the varieties of blood oranges was glucose, whose concentrations oscillated from 16.64 g L^{-1} in Sanguinelli to 26.20 g L^{-1} in Tarocco Gallo. Myo-inositol was identified and quantified at much lower concentrations than the rest of the sugars detected, showing a range of concentrations that varied from 0.89 in Tarocco Meli to 1.71 in Tarocco Dalmuso. The ratios Glu/Fru and Glu/Suc were maintained, with values ranging from 0.86 (Sanguinelli) to 0.99 (Tarocco Gallo and Tarocco Rosso), and 0.45 (Tarocco Sant'Alfio and Tarocco Dalmuso) to 0.72 (Tarocco Gallo), respectively.

In addition to amino acids, organic acids and sugars, three other individual primary metabolites were also identified: choline, ethanol and trigonelline (Table 3). Of the three metabolites, the one that showed the highest concentration was ethanol, with values ranging from 73.87 mg L^{-1} in Tarocco Meli to 350.84 mg L^{-1} in Tarocco Dalmuso. The second highest concentration of these metabolites was detected in choline, whose concentrations oscillated from 7.19 mg L^{-1} in Tarocco Sant'Alfio to 13.71 mg L^{-1} in Tarocco Dalmuso. Finally, trigonelline was also identified and quantified at concentrations ranging from 1.64 mg L^{-1} in Tarocco Rosso to 4.36 mg L^{-1} in Tarocco Gallo.

3.4. Secondary Metabolites Content in Juice

The total content of anthocyanins, flavones and flavanones is shown in Figure 3. The highest anthocyanin content was observed in Sanguinelli (101.06 mg L^{-1}) and the second highest content in Moro (90.14 mg L^{-1}). The total contents of anthocyanins in both varieties were much higher than those observed in the rest of the varieties analyzed, whose values oscillated between 4.66 mg L^{-1} and 23.8 mg L^{-1} for Tarocco Sant'Alfio and Tarocco Dalmuso, respectively. In Tarocco Gallo, not anthocyanins were detected (Figure 3A).

Flavones oscillated significantly from 62.74 mg L^{-1} to 111.75 mg L^{-1}. The Tarocco Dalmuso variety was the lowest in this chemical marker matrix, followed by Tarocco Sant'Al-fio, Tarocco Meli, Tarocco Gallo, Sanguinelli and Moro whose values were all below 100 mg L^{-1}. The highest values, greater than 100 mg L^{-1}, were found in Tarocco Scirè, followed by Tarocco Rosso (Figure 3B).

Of the three classes of phenolic compounds detected and quantified, flavanones had the highest concentrations (Figure 3C). Total flavanone contents varied significantly from 592.88 mg L^{-1} to 140.66 mg L^{-1}. Considering flavanones, the highest concentration was in Moro (592.88 mg L^{-1}), followed by Sanguinelli (555.02 mg L^{-1}) both varieties without statistically significant differences ($p < 0.05$), while the lowest concentration was in Tarocco Gallo (140.66 mg L^{-1}).

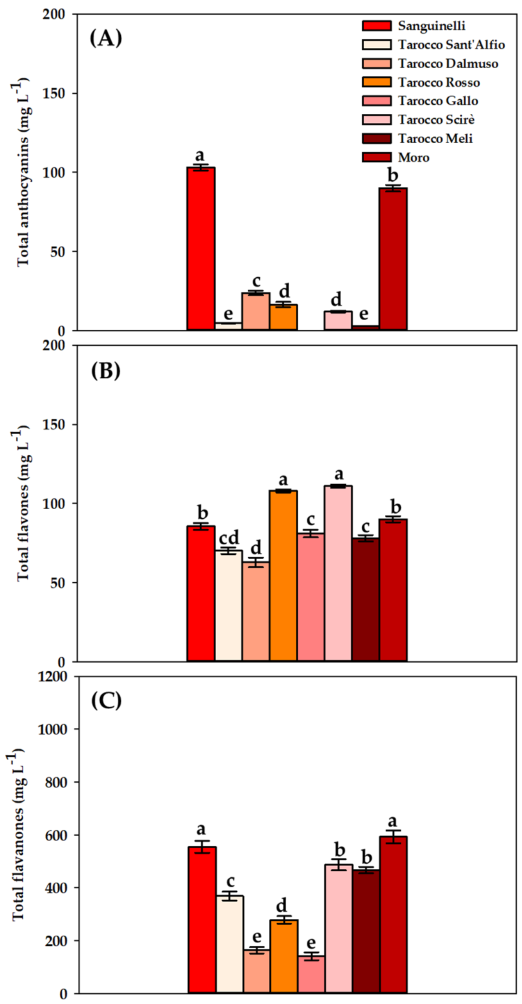

Figure 3. Total content of anthocyanins (**A**), flavones (**B**), and flavanones (**C**) in juice of eight blood orange varieties (Sanguinelli, Tarocco Sant'Alfio, Tarocco Dalmuso, Tarocco Rosso, Tarocco Gallo, Tarocco Scirè, Tarocco Meli and Moro) grafted on *Citrus macrophylla*. Data are the mean ± SE ($n = 6$). Different letters indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

The different subclasses of phenolic compounds found in blood orange varieties are listed in Table 4. In total, 6 individual compounds were detected, quantified and divided into three subclasses: anthocyanins, flavones and flavanones. Cyanidin 3-*O*-sophoroside showed the lowest contents of the 6 flavonoids identified in the varieties studied, with the exception of Sanguinelli and Moro. In this sense, among all the varieties analyzed, the lowest concentrations of anthocyanins were observed in Tarocco Sant'Alfio, with cyanidin 3-*O*-sophoroside values of 1.79 mg L^{-1} and cyanidin 3-*O*-(6″-acetyl-glucoside) values of 2.87 mg L^{-1}, while Sanguinelli and Moro, which had the deepest red juice color, presented the highest content of cyanidin 3-*O*-sophoroside (36.35 and 26.36 mg L^{-1}, respectively) and cyanidin 3-*O*-(6″-acetyl-glucoside) (64.71 and 66.78 mg L^{-1}, respectively) (Table 4).

Among the three flavonoid groups found in the 8 varieties analyzed, the predominant was hesperidin (7-rutinoside), a flavanone glycoside. In our results, the highest values, close to 500 mg L^{-1}, were found in the Sanguinelli (495.05 mg L^{-1}) and Moro (472.79 mg L^{-1}). The rest of the varieties showed values between 371.65 mg L^{-1} (Tarocco Meli) and 92.67 mg L^{-1} (Tarocco Gallo). On the other hand, vicenin 2 (apigenin 6,8-di-*C*-glycoside) was the only flavone identified and quantified, and remained in a range of values between 62.74 (Tarocco Dalmuso) and 111.75 mg L^{-1} (Tarocco Scirè) (Table 4).

Table 4. Individual content of anthocyanins, flavones, and flavanones in juice of eight blood orange varieties (Sanguinelli, Tarocco Sant'Alfio, Tarocco Dalmuso, Tarocco Rosso, Tarocco Gallo, Tarocco Scirè, Tarocco Meli and Moro) grafted on *Citrus macrophylla*.

Secondary Metabolite	Rt (min)	UV/Vis (nm)	$[M+H]^+$ (m/z)	Sanguinelli	Tarocco Sant'Alfio	Tarocco Dalmuso	Tarocco Rosso	Tarocco Gallo	Tarocco Scirè	Tarocco Meli	Moro
Anthocyanins (mg L^{-1})											
Cyanidin 3-*O*-sophoroside	9.7	520	609	36.35 a	1.79 b	8.94 b	8.33 b	ND	5.01 b	0.72 b	23.36 a
Cyanidin 3-*O*-(6″-acetyl-glucoside)	12.9	520	489	64.71 a	2.87 b	14.82 b	8.05 b	ND	7.37 b	2.20 b	66.78 a
Flavones (mg L^{-1})											
Vicenin 2 (apigenin 6,8-di-*C*-glycoside)	10.9	290	593	85.48 d	70.11 dc	62.74 d	108.46 ab	80.93 dc	111.75 a	77.76 dc	90.07 bc
Flavanones (mg L^{-1})											
Narirutin (naringenin-7-rutinoside)	16.2	290	579	49.04 d	67.42 c	44.82 d	69.65 c	43.24 d	88.42 b	68.34 c	92.77 a
Hesperidin (7-rutinoside)	17.6	290	609	495.05 a	285.79 c	112.26 e	193.28 de	92.67 e	160.57 e	371.65 bc	472.79 ab
Didymin (naringenin-40-methyl-ether 7-rutinoside)	22.3	290	593	10.93 bcd	15.55 b	6.39 cd	15.71 b	4.75 d	12.30 bc	27.10 a	27.32 a

Note: data are the mean (n = 6). Values within the same row that present the same letter are not significantly different according to the HSD Tukey test with a confidence level of 95%. ND: not determinate by small quantity.

3.5. Principal Component Analysis (PCA)

The results of the PCA for the classification by varieties showed that the first two main components (PC) explained 73.76% of the total variation in the morphological and qualitative parameters (Figure 4A) and 56.58% for primary and secondary metabolites (Figure 4B). In addition, both figures represented Pearson's correlation among varieties, is a very useful statistical tool to established relationships among attributes that define samples characteristics.

In Figure 4A, PC1 (43.75% of variance explained) was positively correlated mainly with the variables L^*, b^*, $H°$, C^* of crust and juice color. However, it was negatively correlated mainly with the variables CI of crust and juice color and crust thickness. PC2 (30.01% of variance explained) was positively correlated mainly with the variables pH, MI and a^* of crust color. In contrast, PC2 was negatively correlated mainly with the variables fruit weight, fruit length, titratable acidity, crust and weight. Additionally, Figure 4A shows Pearson's correlation between the varieties of blood oranges in the trial in which three groups were established with a correlation >90.0%: (a) Sanguinelli, Tarocco Rosso and Moro, (b) Tarocco Gallo and Tarocco Scirè, and (c) Tarocco Dalmuso, Tarocco Meli and Tarocco Sant'Alfio.

In Figure 4B, PC1 (30.47% of variance explained) was positively correlated mainly with the variables asparagine, aspartate, citric acid, hesperidin, cyanidin 3-*O*-sophoroside and cyanidin 3-*O*-(6″-acetyl-glucoside). In contrast, it was negatively correlated mainly with the variables tyrosine, fructose, glucose, myo-inositol and choline. PC2 (26.11% of variance explained) was positively correlated mainly with the variables alanine, tyrosine, valine, lactic acid and ethanol. However, PC2 was negatively correlated mainly with the variables arginine, sucrose and didymin (naringenin-40-methyl-ether 7-rutinoside). In addition, Figure 4B also shows Pearson's correlation between blood orange varieties in which four groups were established with a correlation >90.0%: (a) Tarocco Gallo and Tarocco Scirè, (b) Tarocco Dalmuso, (c) Tarocco Sant'Alfio, Tarocco Rosso, Tarocco Meli and Moro, and (d) Sanguinelli.

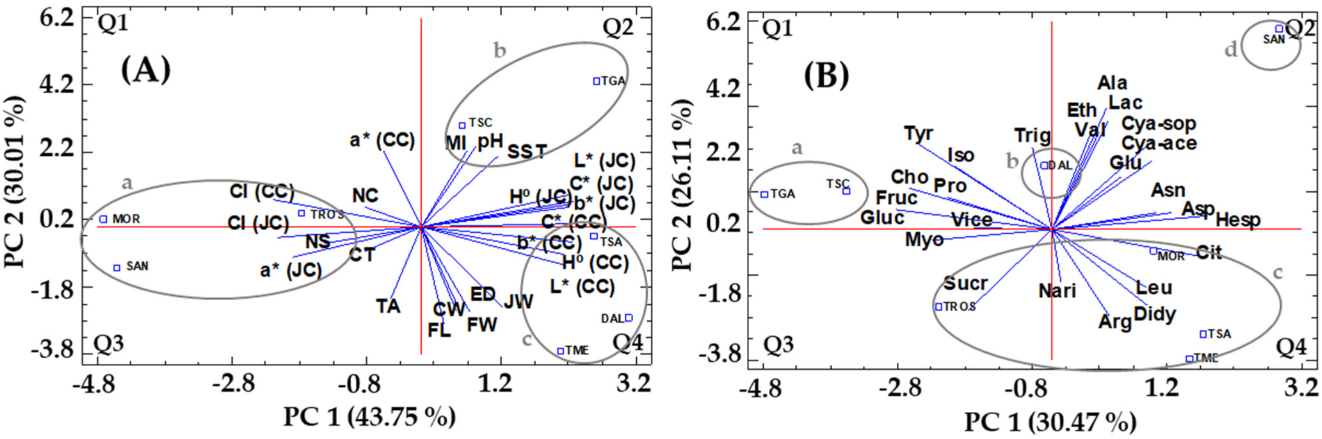

Figure 4. Principal component analysis of morphological and quality parameters (**A**) and individual amino acids, organic acids, sugars and phenolic compounds (**B**) in juice of eight blood orange varieties (SAN: Sanguinelli; SAN: Tarocco Sant'Alfio; DAL: Tarocco Dalmuso; TROS: Tarocco Rosso; TGA: Tarocco Gallo; TSC: Tarocco Scirè; TME: Tarocco Meli; MOR: Moro) grafted on *Citrus macrophylla*. Data set: FW: fruit weight; ED: equatorial diameter; FL: fruit length; CT: crust thickness; NC: number of carpels; NS: number of seeds; CW: crust weight; JW: juice weight; TSS: total soluble solids; TA: titratable acidity and MI: maturity index; L^*, a^*, b^*, $H°$, C^* and CI (CC): crust color; L^*, a^*, b^*, $H°$, C^* and CI (JC): juice color; Ala: alanine; Arg: arginine; Asp: asparagine; Asn: aspartate; Glu: glutamine; Iso: isoleucine; Leu: leucine; Pro: proline; Tyr: tyrosine; Val: valine; Cit: citric acid; Lac: lactic acid; Fruc: fructose; Gluc: glucose; Sucr: sucrose; Myo: myo-inositol; Cho: choline; Eth: ethanol; Tri: trigonelline; Cya-sop: cyanidin 3-*O*-sophoroside; Cya-ace: cyanidin 3-*O*-(6″-acetyl-glucoside); Vice: vicenin 2 (apigenin 6,8-di-*C*-glycoside); Nari: narirutin (naringenin-7-rutinoside); Hesp: hesperidin (7-rutinoside); Didy: didymin (naringenin-40-methyl-ether 7-rutinoside).

4. Discussion

Blood orange fruits are beneficial for human health due to their high content of amino acids, organic acids, sugars or phenolic compounds, among other bioactive compounds. The parameters studied indicated that the genotype substantially influenced the fruit quality and primary and secondary metabolites content. In this sense and in general, statistically significant differences were found ($p < 0.05$) between the different parameters and metabolites analyzed of blood orange varieties.

Morphological parameters of the fruit plays an important role in consumer preferences when buying fruit because different fruit caliber can satisfy diferent types of consumers [28]. In relation to the fruit average weight, Tarocco Dalmuso (350.6 g), Tarosso Meli (326.2 g) and Tarocco Rosso (288 g) (Table 1) were the varieties that presented the highest values, being similar to those described by other authors [23]. Tarocco Scirè and Tarocco Gallo were characterized by the small caliber of the fruit (70.1 mm and 68.5 mm, and 72.1 mm and 69.1 mm, respectively) (Table 1), which limits its use for the fresh market, however, may be suitable for processing and obtaining juice [29]. In this study, some of the varieties were isodiametric such as Moro or Tarocco Scirè, and others were more elongated such as Sanguinelli. Tarocco Meli and Moro showed the thickest crust (6.6 mm and 5.7 mm, respectively) (Table 1). Therefore, these varieties are the most suitable for transport to distant markets due to their greater resistance to mechanical damage [30]; however, they presented the most complicated peelability. In contrast, Tarocco Dalmuso and Tarocco Scirè were the varieties with the thinnest crust (3.5 mm and 4.1 mm, respectively) (Table 1) and easier peelability. These results are in line with those stated by [31], who suggested that Tarocco varieties have a particularly thin crust thickness, which can be interesting from a commercial point of view by providing the piece of fruit with easy peelability, an attribute very attractive to consumers. Another particularly interesting attribute presented by these varieties was the total or almost total absence of seeds along with the high juice content, especially in Tarocco Dalmuso (214.2 g of juice and seedless) and Tarocco Meli (198.6 g of juice and seedless) (Table 1).

pH, TSS, TA and MI are the most relevant in the quality of the juice [23]. In the present study, we observed pH values from 3.83 in Tarocco Meli to 4.42 in Tarocco Gallo (Table 1). These results are similar to other blood orange varieties such as Moro and Tarocco Messina both from Italy [17], Sanguinello from Turkey [5] and Tarocco Rosso and Tarocco Ippolito from Spain [18]. The TSS values in the varieties studied, with the exception of Sanguinelli (10.1 °Brix) and Tarocco Gallo (13.95 °Brix), were shown without statistically significant differences ($p > 0.05$), ranging between 11–13 °Brix (Table 1). These data are similar with those suggested by other authors such as [5] for Moro and Sanguinello; and similar values to those described by other authors [32]. In this sense, [22] stated that the TSS values of blood and white orange varieties generally range between 11–15 °Brix, depending on maturity, environmental conditions or agronomic practices. In relation to the TA values of the blood orange varieties characterized in this study, it was observed that these values were maintained with a total acidity between 6.3 (Tarocco Gallo) and 15.4 (Sanguinelli) g of citric acid L^{-1} (Table 1). The difference in acidity values that occurred between the different varieties studied could probably be due to the difference between the physiological and developmental states of the fruit or by its orientation on the tree [23]. The characteristic taste of red orange juice shows a high acidity, clearly different from the acidity that can be seen in white orange varieties. In fact, at physiological maturity, white orange varieties normally reach values well below 10 g of citric acid L^{-1} thus ensuring sweetness [33]. This is confirmed by the TSS/TA ratio which is defined as maturity index or MI. This ratio is an important qualitative parameter of *Citrus* fruits and is the most widely used method for estimating the *Citrus* maturity level [34]. In this sense, the European Commission (EC) determined the maturity requirements for orange fruits and, among these requirements, the minimum sugar/acid ratio for commercialization is 6.5, regardless of cultivar or fruit type (blood or white orange) [35]. In this study, the highest MI value was observed in Tarocco Gallo (22.75) (Table 1), being very similar to that described by [36] in white orange grafted on rootstock FA41. On the other hand, the lowest values of MI were obtained in Sanguinelli (6.52) and Tarocco Meli (7.13) (Table 1) being similar to those described by [23] in Doble Fina and Entrefina. The rest of the varieties studied were between 9.2 and 14.1 (Table 1); these values being very similar to those described by [32] for other varieties of blood oranges, such as Tarocco Scirè.

In the agri-food industry, appearance, which includes color, is used decisively as a criterion for selecting fruit pieces [18]. In this sense, the pigmentation of the fruit, an important part of the appearance since it gives it its characteristic color, is highly appreciated for its high nutritional value that is the result of the presence of bioactive compounds [37]. In addition, the color of the rind has been widely used as an indicator of fruit maturity in blood orange varieties [23]. The intensity of the pigmentation of the fruit (crust) and its juice depends on several factors such as: variety, rootstock, soil, stress biotic or abiotic, climate or weather conditions. The instrumental measurement of color in blood orange juice through the analysis of CIELAB color parameters (L^*, a^*, b^*) allows estimating its content of pigments such as anthocyanins [38]. In our study, it was observed that the color of the crust and the juice were not linked (Table 2), so the deeper red color of the crust did not necessarily correspond to the deeper red color of the juice, which confirms the previous results [18,23]. This could be due to the fact that the biosynthesis pathways of anthocyanins, the main pigments that give it the characteristic red color, are not the same in the crust as in juice [39]. In this sense, the heterogeneity of the pigmentation of the crust is a characteristic of most blood orange varieties. According to [40], the most colored area usually corresponds to the north-facing part of the fruit. In general, the color parameters obtained in the present test varied but did not deviate from the values mentioned by other authors for other *Citrus* varieties [41]. This variation may be due to the characteristics of the selected variety or the orientation of the fruit on the tree, as suggested by [23]. When evaluating the values of a^* and CI, the varieties that showed the deepest red color were

Sanguinelli, Tarocco Rosso and Moro for crust and Sanguinelli and Moro for juice (Table 2), according to previous research [18,42]. Thus, looking at all the color parameters evaluated in this assay (L^*, a^*, b^*, C^*, $H°$, CI), CI seems to be more useful to distinguish between these varieties, rather than a^* which is commonly used for color characterization, and CI is considered an objective and reliable parameter for assessing *Citrus* maturity indices [43].

The profile of free amino acids present in the juice has been taken into account as a crucial characteristic that determines the quality and authenticity of the same [44]. In this way, it has been proven that amino acids are responsible for the quality of the taste of food [45]. In addition, amino acids are organic compounds that among other functions have an important role in protein biosynthesis. They are also precursors of volatile compounds characteristic of fruit aroma [46], and secondary metabolism (tyrosine, phenylalanine) [47], or key pieces of primary carbon metabolism due to its participation in pathways such as GABA (glutamate) that greatly influence maturation [48]. In this study, 10 essential amino acids (Ala, Arg, Asp, Asn, Glu, Iso, Leu, Pro, Tyr and Val) were detected and the total amount of essential amino acids varied from 3821.61 to 2256.88 mg L^{-1} in Tarocco Sant'Alfio and Tarocco Moro, respectively (Figure 2). Our results were similar to those observed by [49] in blood orange juice (Table 3). These authors obtained mean values of proline, alanine, valine, isoleucine or arginine, close to 1020.19, 66.52, 17.18, 7.54 and 580.32 mg L^{-1}, respectively.

The overall quality of the juice, organic acids are considered one of the most important and determining factors [23,50]. In our study, statistically significant differences ($p < 0.05$) in total organic acid concentrations were found between the varieties of blood oranges analyzed (Figure 2B). The blood oranges analyzed stood out for their high content of citric acid, which is the main organic acid in *Citrus*, with values between 14.60 and 7.28 g L^{-1} in Tarocco Meli and Tarocco Gallo, respectively. The second organic acid detected and quantified was lactic acid, with values ranging between 0.0121 and 0.0065 g L^{-1} in Sanguinelli and Tarocco Meli, respectively (Table 3). Our results were similar to those obtained by [18] in Sanguinelli, Tarocco Rosso and Tarocco Ippolito varieties, with citric acid values between 10.23–16.22, 11.69–15.92 and 8.64–12.36 g/Kg, respectively. The data obtained in the present study also agree with those obtained by other authors [5] in the Moro and Sanguinello varieties, obtaining values of 11.30 and 13.40 g L^{-1}, respectively. However, [40] they obtained much lower values in citric acid content in the blood orange varieties Cara Cara and Moro, with values of 4.21 and 5.61 g L^{-1}, respectively. The rest of the main organic acids present in *Citrus* fruits were not detected by ^{1}H-Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR) as they presented a concentration lower than 10 mM. In this sense, [23] obtained concentrations of ascorbic acid and malic acid of the order of 80 and 10 fold, respectively, lower than citric acid, the main quantified organic acid. Other authors obtained concentrations approximately 22-fold lower ascorbic acid compared to citric acid [51]. This was due to the fact that the fruits underwent important changes during the ripening process that strongly affected the organic acid profile, as well as other related parameters [18]. Organic acids tend to decrease with fruit ripening, mainly due to the use of these compounds as respiratory substrates, as well as, for the synthesis of new substances. It is also important to note that the difference in organic acid profile and quantity between varieties may be related to the genotype [23] or position of the fruit on the tree [40].

The sugars present in the composition of blood oranges are very important components and determining their quality and organoleptic properties [23]. In addition to the effect on organoleptic properties, it has been observed that sugars are involved in the biosynthesis of anthocyanins [52]. Sugar contents are major factors for fruit flavor and are important breeding traits. The main simple sugars present in *Citrus* fruits are sucrose, glucose and fructose. These three sugars make up approximately 80% of the TSS present in the fruit, and their ratios are approximately 2:1:1 for sucrose:glucose:fructose, respectively [53]. In the present study, sucrose was the main sugar in all varieties (values between 40.32 and 30.02 g L^{-1}, in Tarocco Rosso and Sanguinelli, respectively) followed by fructose (values between 26.24 and 18.07 g L^{-1}, in Tarocco Gallo and Tarocco Sant'Alfio, respectively) and glucose (values between 26.20 and 16.64 g L^{-1}, in Tarocco Gallo and Sanguinelli, respectively) (Table 3). To determine the authenticity of juice samples, the sugar profile and proportions of specific sugars have been suggested as a quality indicator [54], for example, the glucose:fructose ratio should be greater than 0.85. In our study, the glucose:fructose ratios oscillated from 0.99 (Tarocco Rosso and Tarocco Gallo) to 0.86 (Sanguinelli) (Table 3). Generally, in fruits, glucose concentrations are higher than those of fructose; however, oranges contain similar glucose and fructose concentrations or, sometimes, fructose may even be more concentrated than glucose [55] as can be observed in all varieties of our study (Table 3). Therefore, our results were similar and consistent with those reported in other studies [56].

There is now a growing consumer demand for healthy foods that help improve health. Among these healthy foods, red fruits stand out, which are widely recommended by nutritionists and specialists as an important part of healthy diets. Thanks to the widely demonstrated potential to promote the health of the bioactive components of blood orange, juices and vegetable drinks that contain it among its composition obtain greater market added value [4]. The *Citrus* varieties contain multiple classes of phenolic compounds, each possessing a spectrum of chemical behaviors and bioactivities. Such bioactive compounds include anthocyanins, flavones or flavanones, three very important groups of natural antioxidants on which most of their functional properties are based [23]. These secondary metabolites are related to various tasks in the plant; specifically, in fruit, they are related to color, organoleptic and nutritional characteristics, and antioxidant activity [57]. According to [23,42] blood oranges are rich in anthocyanins, water-soluble compounds responsible for their distinctive red/purple coloration. Anthocyanins are the most important bioactive compounds in blood oranges, their importance is related to their powerful antioxidant activity and their effects on nutritional and sensory quality. In this study, two anthocyanins were detected and quantified in the juices of Sanguinelli, Tarocco Sant'Alfio, Tarocco Dalmuso, Tarocco Rosso, Tarocco Scirè, Tarocco Meli and Moro: cyanidin 3-*O*-sophoroside y cyanidin 3-*O*-(6″-acetyl-glucoside) (Tabla 4A). Regarding the total content of anthocyanins, statistically significant differences ($p < 0.05$) were observed between the varieties of Sanguinelli, Tarocco and Moro (Figure 3A). The total anthocyanin content was higher in Sanguinelli (101.06 mg L^{-1}) and Moro (90.14 mg L^{-1}) than in Tarocco Rosso (16.38 mg L^{-1}) (Figure 3A), as previously reported by [23,42], who established that Sanguinelli and Moro are the varieties of blood oranges with the highest content of anthocyanins. As suggested by other authors [23], the different concentrations of the pigments identified in the present study confirm that their accumulation process is influenced by the genotype, rather than by the environment, as they are the same abiotic conditions in all the varieties studied. The relationship between coloration (a^* and CI) and anthocyanin content has been previously reported [23,42]. Consequently, in the present study, the highest color of the juice was observed in Sanguinelli (a^* = 9.45 and CI = 43.99) and Moro (a^* = 8.51 and CI = 69.53) (Table 2) compared to the rest of the varieties analyzed, which was produced by the high content of the individual anthocyanins presented by these two varieties (Table 4). Similarly, the lowest coloration of the juice was in Tarocco Gallo (a^* = 1.46 and CI = 1.74) (Table 2) can be associated with the absence in the content of the determined anthocyanins (Table 4). As previously reported [23], cyanidin 3-*O*-(6″-acetyl-glucoside) was predominant in blood orange, especially in Moro (66.78 mg L^{-1}) (Table 4). On the other hand, we found the highest concentrations of cyanidin 3-*O*-sophoroside in juices from Sanguinelli (36.35 mg L^{-1}). The content of all these phenolic compounds is known to depend strongly on both variety [58], the orientation of the fruit on the tree [23] or day/night temperature [11]. Previous study [59] reported a positive trend for the increase in most bioactive compounds in blood oranges, such as organic acids, flavones and flavanones (didymin, narirutin, and vicenin 2), anthocyanin compounds (cyanidin 3-*O*-glucoside) or antioxidant activity. Flavones and flavanones are the main flavonoids in *Citrus* fruits [18]. In all the varieties studied in the present study, vicenin 2 (apigenin 6,8-di-*C*-glycoside) was the only flavone detected and quantified and, therefore, the one with the highest concentration (Table 4); hesperidin (7-

rutinoside) was the predominant flavanone, followed by narirutin (naringenin-7-rutinoside) and didymin (naringenin-40-methyl-ether 7-rutinoside) (Table 4), which is consistent with what has been previously reported for other blood orange varieties [18,23,42].

In general, PCA analysis allowed to highlight the influence of genotype on the biosynthesis of primary and secondary metabolites (Figure 4). The results of the PCA analysis confirmed the strong influence of the variety on the morphological and quality parameters and on the classes of primary and secondary metabolites investigated. The most abundant primary metabolites were in the amino acid group proline, aspartate, and asparagine, respect to organic acids, citric acid and, in sugars, sucrose followed by fructose and glucose. The highest contents of amino acids, organic acids and sugars were in Tarocco Sant'Alfio, Tarocco Meli and Tarocco Scirè and Tarocco Gallo, respectively. The predominant flavonoid in the oranges studied was hesperidin (7-rutinoside) being Sanguinelli the variety with the highest content. As for anthocyanins, two different compounds were identified in blood oranges—the most abundant was cyanidin 3-*O*-(6″-acetyl-glucoside) being much higher in Moro and Sanguinelli than in the rest of the varieties studied.

5. Conclusions

Blood oranges contain high concentrations of antioxidants and bioactive compounds such as anthocyanins. In Spain, its cultivation is scarce and not widespread; however, consumer interest is increasing substantially due to its numerous benefits for human health. For this reason, it is a necessary and timely characterization and comparison between the main varieties of blood orange consumed in Spain (one of the main oranges producing and exporting countries in the world) versus the most widespread and consumed varieties internationally. Tarocco Meli and Moro showed the thickest crust; therefore, they were best suited for transport to distant markets being more resistant to mechanical damage. Tarocco Dalmuso showed the highest juice content, being the most suitable variety for domestic consumption. Tarocco Gallo was the variety that obtained the best balance between organic acids and sugars, presenting the highest MI value; therefore, it presented the most interesting sensory properties. The color of the crust and juice varied significantly depending on the variety. Its reddish coloration was mainly due to the presence of two pigments of anthocyanic nature: cyanidin 3-*O*-samphoroside and cyanidin 3-*O*-(6″-acetyl-glucoside). The color study showed that there is no direct relationship between the coloration of the crust and the coloration of the juice. The highest content of amino acids, organic acids and sugars was found in Tarocco Sant'Alfio and Tarocco Dalmuso, Tarocco Meli and Moro, and Tarocco Meli and Tarocco Scirè, respectively, being the most important varieties in terms of nutritional content. Moro and Sanguinelli juice obtained the highest content of phenolic compounds, being especially interesting for consumers who demand fruits with a high content of bioactive compounds. In general, the knowledge generated in this work can be used for the agri-food industry by identifying some of the most interesting varieties of blood oranges from the agronomic and/or nutritional point of view. Despite this, more research is needed, particularly on the possible effect of biotic and abiotic stresses or rootstock/graft combinations on bioactive compounds that make them attractive to the agri-food industry and consumers.

Author Contributions: Conceptualization, M.B.-d.l.S. and P.L.; methodology, A.R.-C. and A.M.-N.; software, M.B.-d.l.S., A.C. and A.R.-C.; validation, P.M. and P.L.; formal analysis, P.M. and J.J.M.-N.; investigation, M.B.-d.l.S., J.J.M.-N. and P.L.; resources, M.Á.F.-G. and P.L.; data curation, A.M.-N. and A.R.-C.; writing—original draft preparation, M.B.-d.l.S.; writing—review and editing, M.B.-d.l.S. and P.L.; visualization, A.C. and M.B.-d.l.S.; supervision, M.Á.F.-G. and P.L.; project administration, P.L.; funding acquisition, P.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by: Generalitat Valenciana, Spain (reference: CIAICO/2021/310).

Data Availability Statement: All data are available via email request to the corresponding author.

Acknowledgments: The authors are grateful to Metabolomics Platform of CEBAS–CSIC for the excellent technical assistance.

Conflicts of Interest: The authors declare not conflict of interest.

References

1. Food and Agriculture Organization of the United Nations. FAOSTAT Statistical Database. Rome, Italy. 2020. Available online: www.faostat.fao.org (accessed on 30 October 2021).
2. Pareek, S.; Valero, D.; Serrano, M. Postharvest Biology and Technology of Pomegranate. *J. Sci. Food Agric.* **2015**, *95*, 2360–2379. [CrossRef] [PubMed]
3. Serradilla, M.J.; Akšic, M.F.; Manganaris, G.A.; Ercisli, S.; Gonzalez-Gomez, D.; Valero, D. Fruit chemistry, nutritional benefits and social aspects of cherries. In *Cherries: Botany, Production and Uses*; CABI: Boston, MA, USA, 2017; pp. 420–441. Available online: https://www.cabi.org/horticulture/ebook/20173212321 (accessed on 7 August 2017).
4. Habibi, F.; García-Pastor, M.E.; Puente-Moreno, J.; Garrido-Auñón, F.; Serrano, M.; Valero, D. Anthocyanin in blood oranges: A review on postharvest approaches for its enhancement and preservation. *Rev. Food Sci. Nutr.* **2022**, *12*, 1–13. [CrossRef] [PubMed]
5. Kelebek, H.; Canbas, A.; Selli, S. Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from cvs. Moro and Sanguinello (*Citrus sinensis* (L.) Osbeck) grown in Turkey. *Food Chem.* **2008**, *107*, 1710–1716. [CrossRef]
6. Tadeo, F.R.; Terol, J.; Rodrigo, M.J.; Licciardello, C.; Sadka, A. Fruit growth and development. In *The Genus Citrus*; Woohead Publishing: Oxford, UK, 2020; pp. 245–269. [CrossRef]
7. Lo Piero, A.R. The state of the art in biosynthesis of anthocyanins and its regulation in pigmented sweet orange [(*Citrus sinensis*) L. Osbeck]. *J. Agric. Food Chem.* **2015**, *63*, 4031–4041. [CrossRef]
8. Lana, G.; Modica, G.; Las Casas, G.; Siracusa, L.; La Malfa, S.; Gentile, A.; Sicilia, A.; Distefano, D.; Continella, A. Molecular insights into the effects of rootstocks on maturation of blood oranges. *Horticulturae* **2021**, *7*, 468. [CrossRef]
9. Pannitteri, C.; Continella, A.; Lo Cicero, L.; Gentile, A.; La Malfa, S.; Sperlinga, E.; Napoli, E.M.; Strano, T.; Ruberto, G.; Siracusa, L. Influence of postharvest treatments on qualitative and chemical parameters of Tarocco blood orange fruits to be used for fresh chilled juice. *Food Chem.* **2017**, *230*, 441–447. [CrossRef]
10. Habibi, F.; Ramezanian, A.; Guillén, F.; Castillo, S.; Serrano, M.; Valero, D. Changes in bioactive compounds, antioxidant activity, and nutritional quality of blood orange cultivars at different storage temperatures. *Antioxidants* **2020**, *9*, 1016. [CrossRef]
11. Butelli, E.; Licciardello, C.; Zhang, Y.; Liu, J.; Mackay, S.; Bailey, P.; Reforgiato-Recupero, G.; Martin, C. Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. *Plant Cell* **2012**, *24*, 1242–1255. [CrossRef]
12. Grosso, G.; Galvano, F.; Mistretta, A.; Marventano, S.; Nolfo, F.; Calabrese, G.; Buscemi, S.; Drago, F.; Veronesi, U.; Scuderi, A. Red orange: Experimental models and epidemiological evidence of its benefits on human health. *Oxidative Med. Cell Longev.* **2013**, *2013*, 157240. [CrossRef]
13. Proteggente, A.R.; Saija, A.; De Pasquale, A.; Rice Evans, C. The compositional characterisation and antioxidant activity of fresh juices from sicilian sweet orange (*Citrus sinensis* L. Osbeck) varieties. *Free Radic. Res.* **2003**, *37*, 681–687. [CrossRef]
14. Ministerio de Agricultura, Pesca y Alimentación. Anuario de Estadística. Madrid, Spain. 2019. Available online: https://www.mapa.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/2019/ (accessed on 3 August 2019).
15. Porras, I.C.; Conesa-Martínez, A.; Martínez-Nicolás, J.; Jara-Rodríguez, F.J.; Manera-Bassa, F.; Sánchez-Valladares, A.; Brotons, J.M. Estudio Preliminar de Diversas Variedades de Naranjas Sanguinas. Parte i: Parámetros de Calidad y del Colour Externo de los Frutos. *Levane Agric.* **2014**, *420*, 19–22. Available online: https://redivia.gva.es/bitstream/handle/20.500.11939/7217/2014_Porras_Estudio.pdf?sequence=1&isAllowed=y (accessed on 3 September 2014).
16. Simons, T.J.; McNeil, C.J.; Pham, V.D.; Suh, J.H.; Wang, Y.; Slupsky, C.M.; Guinard, J.X. Evaluation of California-grown blood and Cara Cara oranges through consumer testing, descriptive analysis, and targeted chemical profiling. *J. Food Sci.* **2019**, *84*, 3246–3263. [CrossRef] [PubMed]
17. Rapisarda, P.; Bianco, M.L.; Pannuzzo, P.; Timpanaro, N. Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [*Citrus sinensis* (L.) Osbeck]. *Postharvest Biol. Technol.* **2009**, *49*, 348–354. [CrossRef]
18. Cebadera-Miranda, L.; Domínguez, L.; Dias, M.I.; Barros, L.; Ferreira, I.C.F.R.; Igual, M.; Martínez-Navarrete, N.; Fernández-Ruiz, V.; Morales, P.; Cámara, M. Sanguinello and Tarocco (*Citrus sinensis* [L.] Osbeck): Bioactive compounds and colour appearance of blood oranges. *Food Chem.* **2019**, *270*, 395–402. [CrossRef] [PubMed]
19. Lu, X.; Zhao, C.; Shi, H.; Liao, Y.; Xu, F.; Du, G.; Xiao, H.; Zheng, J. Nutrients and bioactives in Citrus fruits: Different Citrus varieties, fruit parts, and growth stages. *Crit. Rev. Food Sci. Nutr.* **2021**, *5*, 1–24. [CrossRef]
20. Zhao, C.; Wang, F.; Lian, Y.; Xiao, H.; Zheng, J. Biosynthesis of Citrus flavonoids and their health effects. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 566–583. [CrossRef]
21. Fallico, B.; Ballistreri, G.; Arena, E.; Brighina, S.; Rapisarda, P. Bioactive compounds in blood oranges (*Citrus sinensis* (L.) Osbeck): Level and intake. *Food Chem.* **2017**, *215*, 67–75. [CrossRef]
22. Lado, J.; Gambetta, G.; Zacarias, L. Key determinants of *Citrus* fruit quality: Metabolites and main changes during maturation. *Sci. Hortic.* **2018**, *233*, 238–248. [CrossRef]
23. Butelli, P.; Modica, G.; Porras, L.; Conesa, A.; Continella, A. Bioactive compounds, antioxidant activity and fruit quality evaluation of eleven blood orange cultivars. *J. Sci. Food Agric.* **2021**, *102*, 2960–2971. [CrossRef]
24. McGuire, R.G. Reporting of objective color measurements. *HortScience* **1992**, *27*, 1254–1255. [CrossRef]

25. Jimenez-Cuesta, M.; Cuquerella, J.; Martínez-Javega, J.M. Determination of a colour index for *Citrus* fruit degreening. In Proceedings of the International Society of Citriculture Citriculture, IV Congress, Tokyo, Japan, 9–12 November 1981; Volume 2, pp. 750–753.
26. Choi, Y.H.; Kim, H.K.; Linthorst, H.J.M.; Hollander, J.G.; Lefeber, A.W.M.; Erkelens, C.; Nuzillard, J.M.; Verpoorte, R. NMR metabolomics to revisit the tobacco Mosaic virus infection in *Nicotiana tabacum* leaves. *J. Nat. Prod.* **2006**, *69*, 742–748. [CrossRef] [PubMed]
27. Legua, P.; Forner-Giner, M.Á.; Nuncio-Jáuregui, N.; Hernández, F. Polyphenolic compounds, anthocyanins and antioxidant activity of nineteen pomegranate fruits: A rich source of bioactive compounds. *J. Funct. Foods.* **2016**, *23*, 628–636. [CrossRef]
28. Musacchi, S.; Serra, S. Apple fruit quality: Overview on pre-harvest factors. *Sci. Hortic.* **2018**, *234*, 409–430. [CrossRef]
29. Ordóñez-Díaz, J.L.; Hervalejo, A.; Pereira-Caro, G.; Muñoz-Redondo, J.M.; Romero-Rodríguez, E.; Arenas-Arenas, F.J.; Moreno-Rojas, J.M. Effect of Rootstock and Harvesting Period on the Bioactive Compounds and Antioxidant Activity of Two Orange Cultivars ('Salustiana' and 'Sanguinelli') Widely Used in Juice Industry. *Processes* **2020**, *8*, 1212. [CrossRef]
30. Caruso, M.; Ferlito, F.; Licciardello, C.; Allegra, M.; Strano, M.C.; Di Silvestro, S.; Russo, M.P.; Paolo, D.P.; Caruso, P.; Las Casas, G.; et al. Pomological diversity of the Italian blood orange germplasm. *Sci. Hortic.* **2016**, *213*, 331–339. [CrossRef]
31. Rapisarda, P.; Russo, G. Fruit quality of five Tarocco selections grown in Italy. In Proceedings of the International Society of Citriculture 9th Congress, University of California, Riverside, CA, USA, 3–7 December 2000; Volume 2, pp. 1149–1153.
32. Continellaa, A.; Pannitteria, C.L.; Malfa, S.; Leguab, P.; Distefanoa, G.; Nicolosia, E.; Gentilea, A. Influence of different rootstocks on yield precocity and fruit quality of 'Tarocco Scirè' pigmented sweet orange. *Sci. Hortic.* **2018**, *230*, 62–67. [CrossRef]
33. Legua, P.; Bellver, R.; Forner, J.; Forner-Giner, M.A. Plant growth, yield and fruit quality of 'Lane Late' navel orange on four *Citrus* rootstocks. *Span. J. Agric. Res.* **2011**, *9*, 271–279. [CrossRef]
34. Legua, P.; Bellver, R.; Forner, J.B.; Forner-Giner, M.A. Trifoliata hybrids rootstocks for 'Lane Late' navel orange in Spain. *Sci. Agric.* **2011**, *68*, 548–553. [CrossRef]
35. *EN 32011R0543*; Commission Implementing Regulation (EC) N° 543/2011 of 7 June 2011 Laying Down Detailed Rules for the Application of the Council Regulation (EC) No 1243/2007 in Respect of the Fruit and Vegetables Processed Fruit and Vegetables Sectors. European Union: Luxembourg, 2011.
36. Hervalejo, A.; Cardeñosa, V.; Forner-Giner, M.A.; Salguero, A.; Pradas, I.C.; Moreno, J.M.; Arenas-Arenas, F.J. Preliminary data on influence of six *Citrus* rootstocks on fruit quality of "lane late" navel orange. *Acta Hortic.* **2015**, *1065*, 363–366. [CrossRef]
37. De Pascual, T.S.; Sanchez-Ballesta, M.T. Anthocyanins: From plant to health. *Phytochemistry* **2008**, *7*, 281–299. [CrossRef]
38. Meléndez-Martínez, A.J.; Gómez-Robledo, L.; Melgosa, M.; Vicario, I.M.; Heredia, F.J. Color of orange juices in relation to their carotenoid contents as assessed from different spectroscopic data. *J. Food Compos. Anal.* **2011**, *24*, 837–884. [CrossRef]
39. Lo Cicero, L.; Puglisi, I.; Nicolosi, E.; Gentile, A.; Ferlito, F.; Continella, A.; Lo Piero, A.R. Anthocyanin levels and expression analysis of biosynthesis-related genes during ripening of sicilian and international grape berries subjected to leaf removal and water deficit. *J. Agric. Sci. Technol.* **2016**, *18*, 1333–1344.
40. Kafkas, A.; Ercisli, S.; Kemal, K.N.; Baydar, K.; Yilmaz, H. Chemical composition of blood oranges varieties from Turkey. To comparative study. *Pharmacogn. Mag.* **2009**, *5*, 329–335. [CrossRef]
41. Choi, M.H.; Kima, G.H.; Leeb, H.S. Effects of ascorbic acid retention on juice colour and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. *Food Res. Int.* **2002**, *35*, 753–759. [CrossRef]
42. Morales, J.; Bermejo, A.; Navarro, P.; Forner-Giner, M.Á.; Salvador, A. Rootstock effect on fruit quality, anthocyanins, sugars, hydroxycinnamic acids and flavanones content during the harvest of blood oranges 'Moro' and 'Tarocco Rosso' grown in Spain. *Food Chem.* **2021**, *342*, 128305. [CrossRef]
43. Lado, J.; Rodrigo, M.J.; Zacarías, L. Maturity indicators and *Citrus* fruit quality. *Stewart Postharvest Rev.* **2014**, *10*, 1–6.
44. AIJN. *The AIJN Code of Practice, AIJN—European Fruit Juice Association*; AIJN: Antwerp, Belgium, 2018.
45. Susan, S.S.; Sennewald, K.; Gagnon, J. Comparison of taste qualities and thresholds of D- and L-amino acids. *Physiol. Behav.* **1981**, *27*, 51–59. [CrossRef]
46. Rambla, J.L.; Tikunov, Y.M.; Monforte, A.J.; Bovy, A.G.; Granell, A. The expanded tomato fruit volatile landscape. *J. Exp. Bot.* **2014**, *65*, 4613–4623. [CrossRef]
47. Pellaud, S.; Mène-Saffrané, L. Metabolic origins and transport of vitamin E biosynthetic precursors. *Front. Plant Sci.* **2017**, *8*, 1959. [CrossRef]
48. Aghdam, M.S.; Fard, J.R. Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (*Fragaria anannasa* cv. Selva) by enhancing GABA shunt activity. *Food Chem.* **2017**, *221*, 1650–1657. [CrossRef]
49. Wistaff, E.A.; Beller, S.; Schmid, A.; Neville, J.J.; Nietner, T. Chemometric analysis of amino acid profiles for detection of fruit juice adulterations—Application to verify authenticity of blood orange juice. *Food Chem.* **2021**, *1*, 128452. [CrossRef] [PubMed]
50. Al-Jaleel, A.; Zekri, M.; Hammam, Y. Yield, fruit quality, and tree health of "Allen Eureka" lemon on seven rootstocks in Saudi Arabia. *Sci. Hortic.* **2005**, *105*, 457–465. [CrossRef]
51. Kelebek, H.; Selli, S.; Canbas, A.; Cabaroglu, T. HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. *Microchem. J.* **2009**, *91*, 187–192. [CrossRef]
52. Abdullah, M.; Cheng, X.; Shakoor, A.; Cao, Y.; Lin, Y.; Cai, Y.; Shangao, J. New opinion of sugar and light crosstalk in the induction of anthocyanins biosynthesis in fruits. *Int. J. Agric. Biol.* **2018**, *20*, 2465–2474. [CrossRef]

53. Lee, H.S.; Coates, G.A. Quantitative study of free sugars and myoinositol in *Citrus* juices by HPLC and literature compilation. *J. Liq. Chromatogr. Relat. Technol.* **2014**, *14*, 2123–2141. [CrossRef]
54. Castellari, M.; Versari, A.; Spinabelli, U.; Galassi, S.; Amati, A. An improved HPLC method for the analysis of organic acids, carbohydrates and alcohols in grape musts and wines. *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 2047–2056. [CrossRef]
55. Kelebek, H.; Selli, S. Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (*Citrus sinensis* L. Osbeck) orange juice. *J. Sci. Food Agric.* **2011**, *91*, 1855–1862. [CrossRef] [PubMed]
56. Sicari, V.; Pellicanò, T.M.; Giuffrè, A.M.; Zappia, C.; Capocasale, M. Bioactive compounds and antioxidant activity of *Citrus* juices produced from varieties cultivated in Calabria. *J. Food Meas. Charact.* **2016**, *10*, 773–780. [CrossRef]
57. Simón-Grao, S.; Gimeno, V.; Simón, I.; Lidón, V.; Nieves, M.; Balal, R.M.; Carbonell-Barrachina, R.; Manera, A.; Hernandez, F.J.; García-Sánchez, F. Fruit quality characterization of eleven commercial mandarin cultivars in Spain. *Sci. Hort.* **2014**, *165*, 274–280. [CrossRef]
58. Todaro, A.; Cavallaro, R.; La Malfa, S.; Continella, A.; Gentile, A.; Fischer, U.A.; Carle, R.; Spagna, G. Anthocyanin profile and antioxidant activity of freshly squeezed pomegranate (*Punica granatum* L.) juices of Sicilian and Spanish provenances. *It. J. Food Sci.* **2016**, *28*, 464–479. [CrossRef]
59. Reche, J.; Almansa, M.S.; Hernández, F.; Amorós, A.; Legua, P. Physicochemical and antioxidant capacity of jujube (*Ziziphus jujuba* Mill.) at different maturation stages. *Agronomy* **2011**, *11*, 132. [CrossRef]