



Article

Comparative Analysis of Primary and Secondary Metabolites in the Peel of Eight Blood Orange Varieties

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Abstract: The global cultivation of blood oranges is experiencing an increase due to their remarkable nutritional properties. Blood orange by-products, especially the peel, have a high concentration of bioactive compounds with exceptional antioxidant potential, making them an ideal choice for incorporation into various food products. This study aimed to determine the morphological parameters and primary and secondary metabolite content of peel of eight blood orange varieties using ¹H NMR and HPLC-ESI-DAD-MSⁿ. “Tarocco Meli” had the highest weight (367.83 g), caliber (94.13 mm and 88.87 mm), peel thickness (6.73 mm), and peel weight (155.0 g). “Tarocco Rosso”, “Sanguinelli”, and “Tarocco Gallo” had the highest levels of total amino acids (25.57 g kg⁻¹ DW), total organic acids (29.99 g kg⁻¹ DW), and total sugars (68.56 g 100 g⁻¹ DW), respectively. The peel of “Moro” had significantly higher concentrations of total anthocyanins, hydroxycinnamic acids, and flavones (650.67, 263.33, and 449.85 mg kg⁻¹, respectively) compared to the other varieties. In conclusion, “Tarocco Meli” had the most interesting values for morphological parameters, “Tarocco Rosso”, “Sanguinelli”, and “Tarocco Gallo” for primary metabolites, and “Moro” for secondary metabolites. With the increasing interest in utilizing co-products, these findings could be useful in developing functional food products that meet consumer demands for healthier and more sustainable food choices.

Keywords: anthocyanins; blood orange; orange peel; co-products; flavones; flavanones; hydroxycinnamic acids; morphological parameters; primary metabolites; secondary metabolites



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1. Introduction

Citrus fruits, such as the orange species *Citrus sinensis* (L.) Osbeck, are widely acknowledged as highly productive and economically significant crops [1,2]. According to FAOSTAT, the global production of *Citrus* fruits reached 158 million tons in 2020, representing a notable increase of 7.5% compared to 2017 [3]. Oranges, among the various citrus species, hold a prominent position with a worldwide production of 79 million tons [3]. Oranges can be classified into two primary categories: common oranges, extensively cultivated in numerous citrus-producing countries worldwide, and blood oranges, selectively grown in specific regions. Italy, favored by the Mediterranean climate, is renowned for its cultivation of blood oranges, distinguished by their distinctive reddish coloration [2]. The three main blood orange cultivars, namely “Tarocco”, “Moro”, and “Sanguinelli”, enjoy significant recognition as the most important citrus varieties in southern Italy [2]. Italy dedicates approximately 170,000 hectares of land to blood orange production, yielding around

3.4 million tons annually [4]. Each year, around 30 million tons of *Citrus* fruits are processed worldwide for juice extraction [5]. However, this juice production generates significant quantities of co-products, primarily consisting of the peel [6], which are predominantly discarded or utilized for livestock feed or bioethanol production [1]. Interestingly, these co-products contain substantial amounts of untapped bioactive compounds, potentially impacting the cost of the final product [7,8]. In fact, it is estimated that an astonishing 15 million tons of *Citrus* co-products are generated globally each year [9].

In this context, the agri-food industry is facing increasing demands from consumers who seek natural, healthy products that are environmentally friendly [10]. Oranges, including blood oranges specifically, are not only valuable sources of essential nutrients desired by modern consumers, but also reservoirs of bioactive compounds with strong antioxidant properties [11]. Blood orange juice, in particular, exhibits high concentrations of bioactive components such as flavones, flavonols, and hydroxycinnamic acids, with a remarkable richness in anthocyanins [2], which contribute to its potent antioxidant capacity and numerous health benefits [12]. Additionally, anthocyanins play a significant role in the exceptional sensory characteristics of blood oranges and have the potential to act as agents promoting human health by offering protection against cancer and cardiovascular diseases [13]. Therefore, maintaining optimal levels of anthocyanins is essential not only to ensure superior quality but also to enhance consumer acceptance and facilitate effective marketing strategies [14].

However, the richness of bioactive compounds in blood oranges is not limited to their juice; their peel also contains a significant amount of these beneficial compounds [15]. The peel of blood oranges is well-known for its potent natural antioxidants, making it a promising ingredient for various food products as a preservative, similar to the peels of other *Citrus* varieties [16,17]. *Citrus* peel extracts have been utilized to enhance the stability and extend the shelf life of food products [16,17]. For example, a study conducted by [18] demonstrated the remarkable ability of lemon and orange peel extracts to inhibit bacterial growth and prolong the shelf life of cooked meatballs, while also providing notable antioxidant activity to the food. Similarly, ref. [19] investigated the antioxidant properties of kinnow peel and successfully used it as a natural antioxidant in goat meat, effectively reducing fat oxidation during refrigerated storage. Furthermore, ref. [20] incorporated ground *Citrus* peel extract into ground goat meat and evaluated its lipid oxidation after three and six months of storage. The results showed that the meat with *Citrus* peel extract exhibited significantly lower levels of MDA (malondialdehyde) and hydrogen peroxide compared to the control group. In another study, ref. [21] improved the nutritional quality of fish burgers by incorporating microencapsulated orange peel extract. Additionally, ref. [22] conducted a feeding experiment with Angus steers, supplementing their diet with dried *Citrus* peel. This study found that the α -tocopherol content (a form of vitamin E) in the Angus feed supplemented with *Citrus* peel was three times higher than that of the control group. Moreover, the MDA content in the meat of the experimental group was significantly lower than that of the control group. This study further emphasized the significance of bioactive compounds present in *Citrus* peel and highlighted the potential utilization of *Citrus* co-products in the agricultural and food industries, supporting the concept of a circular economy and effective waste management within the fruit industry.

In this way we can deduce that the different *Citrus* co-products, consisting mainly of peel, substantially improve the organoleptic properties of various food bases thanks to the bioactive compounds they contain, which give these foods a high antioxidant capacity, which reduces the unwanted effects that occur during commercial shelf life [1]. However, at present, there is very little information in relation to composition of nutrients and bioactive compounds of blood orange peel and, therefore, their possible applications in different food bases.

The primary aim of this study is to examine for the first time the nutritional and bioactive composition of the peel from eight different blood orange varieties cultivated under identical environmental conditions. The aim is to determine the potential suitability

of these varieties for use in food products. Specifically, the study analyzes the morphological parameters of the fruit and the content of metabolites (primary and secondary) in peel of the following varieties: “Sanguinelli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Tarocco Gallo”, “Tarocco Scirè”, “Tarocco Meli”, and “Moro”, as shown in Figure 1. These varieties were grafted onto *Citrus macrophylla* and were cultivated in the south-eastern region of Spain.

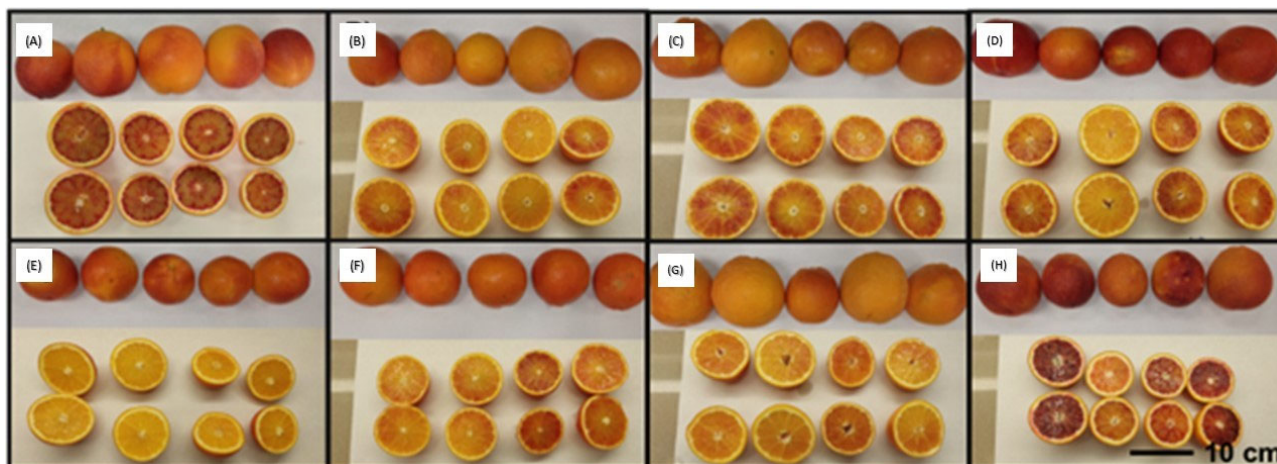


Figure 1. Blood orange fruits of Spanish variety (A) Sanguinelli and Italian varieties (B) Tarocco Sant’Alfio, (C) Tarocco Dalmuso, (D) Tarocco Rosso, (E) Tarocco Gallo, (F) Tarocco Scirè, (G) Tarocco Meli, and (H) Moro.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

In this work, we assessed the morphological characteristics and the content of metabolites (primary and secondary) in the peel of various blood orange varieties, namely “Sanguinelli” (*Citrus sinensis* (L.) cv. Sanguinelli), “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Tarocco Gallo”, “Tarocco Scirè”, “Tarocco Meli” (*Citrus sinensis* (L.) cv. Tarocco), and “Moro” (*Citrus sinensis* (L.) cv. Moro). A total of 200 fruits (25 fruits × 8 varieties) were analyzed, obtained from 24 three-year-old trees (3 trees per variety) grafted onto *Citrus macrophylla* and cultivated in an experimental farm located in Orihuela, Spain (38.06733781, −0.98229272). Throughout the trial, the farm maintained consistent growing conditions, including a pH level of 7.25, an electrical conductivity (EC) of 0.44 dS m^{−1} (20 °C), a temperature of 12 °C, and a relative humidity (RH) of 58%. This was done to minimize external factors that could affect the evaluated parameters. The samples were collected in January 2023, aligning with the commercial consumption period of blood oranges in Spain, which typically spans from January to March. The blood oranges were harvested manually once they reached the stage of commercial maturity [23] and were immediately transported to the laboratory for further processing.

In the laboratory, the outer surface of the blood oranges underwent thorough cleansing using distilled water to eliminate any potential presence of dirt particles. Subsequently, the color of the peel was measured ($n = 50$) and the weight and size of the fruit ($n = 25$). Destructive analysis was then conducted, where each fruit was cut in half to determine the number of carpels, the peel thickness, and the peel weight ($n = 25$). The juice from each variety of blood orange ($n = 25$) was extracted using a manual commercial juicer (Citromatric Deluxe, MPZ-22, Braum, Madrid, Spain). The remaining peel (albedo + flavedo) was cut into small pieces, divided into six replicates ($n = 6$), with each replicate consisting of 150 g of peel, and freeze-dried using a lyophilizer (LyoMicron, Coolvacuum, Barcelona, Spain) for subsequent metabolomic analysis.

2.2. Fruit Morphological Characterization

The fruit's morphological characteristics were assessed using a modified version of the method outlined by [2], as detailed below. To determine the average weight of the fruit and peel of the blood orange varieties, a digital scale (model BL-600; Sartorius, Göttingen, Germany) was employed. The number of carpels was visually counted. Peel thickness, equatorial diameter, and fruit length were measured using an electronic digital sliding gauge (model CD-15 DC; Mitutoyo, Kawasaki shi, Japan).

2.3. Peel Color Determination

To measure the color parameters, a Minolta C-300 chroma meter (Minolta Corp., Osaka, Japan) was used that was attached to a DP-301 data processor (Minolta Corp.). The color measurements were conducted at two equidistant points from the peel of each fruit, following the guidelines of the Commission Internationale de l'Éclairage (CIE), and recorded as L^* , a^* , b^* . These values were utilized to calculate the hue angle using the following equation:

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

Chroma was calculated using the following equation:

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

Based on findings from a prior investigation [24], hue angle (H°) and chroma (C^*) have been recognized as the most perceptually intuitive color parameters. The color index (CI) was determined using the following equation [25]:

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

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

The primary metabolites, consisting of amino acid groups, organic acids, and sugars, were analyzed with the method described by [26] with slight modifications specified by [27]. The results are presented as $g\ Kg^{-1}$ DW (amino acids and organic acids) or $g\ 100\ g^{-1}$ DW (sugars) of blood orange peel.

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

The individual phenolic compounds in peel of blood orange varieties were made following the method described above by [28]. The results are expressed as $mg\ Kg^{-1}$ DW of blood orange peel.

2.6. Statistical Analysis

The experimental data for primary and secondary metabolites were obtained from 6 replicates ($n = 6$), while 25 replicates ($n = 25$) were used for morphological parameters, and 50 replicates ($n = 50$) for color parameters. Statistical analysis was performed using analysis of variance (ANOVA), and significant differences between mean values were determined by the HSD Tukey test at a significance level of $p < 0.05$, using the SPSS 28.0 software package. Principal component analysis (PCA) was also performed using Stat Graphics Centurion v. 18.1.12, with Pearson's correlation used for analysis.

3. Results

3.1. Morphological Parameters

In this study, we evaluated six of the key morphological parameters that are commonly used to characterize citrus fruits and their relationship with the fruit peel. These parameters include fruit weight, equatorial diameter, fruit length, peel thickness, number of carpels, and peel weight. Our results revealed statistically significant differences ($p < 0.05$) among

the different blood orange varieties studied (Figure 2), indicating significant heterogeneity and variability in some cases.

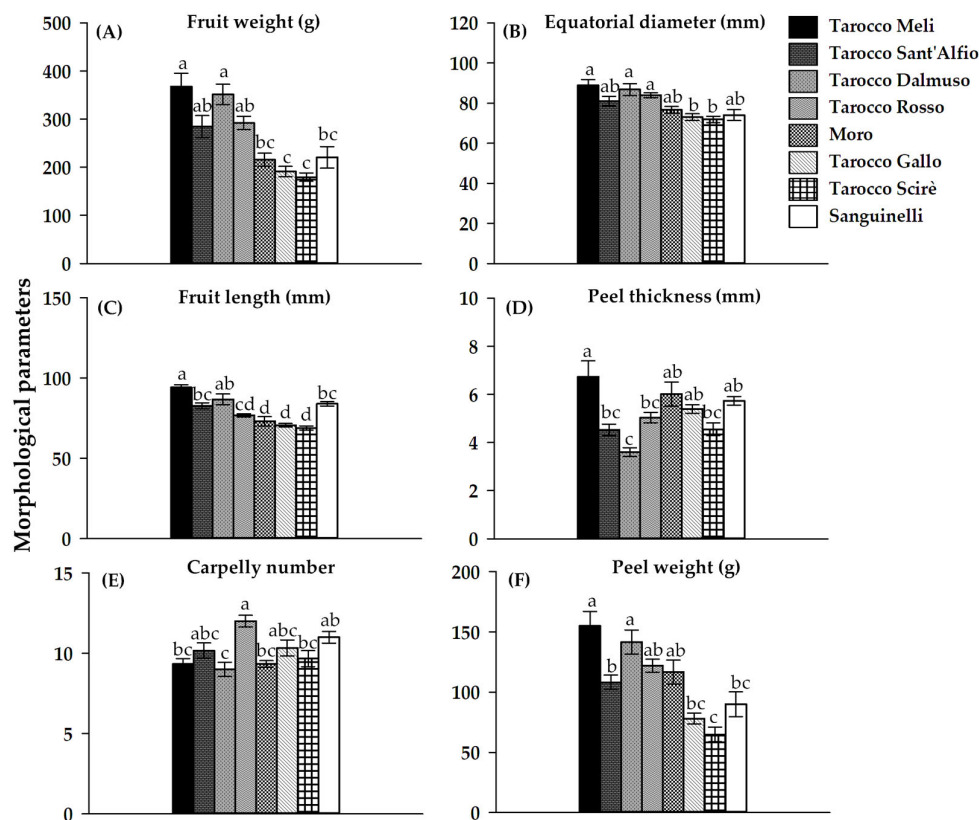


Figure 2. The fruit weight (A), equatorial diameter (B), fruit length (C), peel thickness (D), carpel number (E), and peel weight (F) of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla* were measured. The data are reported as the mean ± standard error (SE) with a sample size of 25 ($n = 25$). Different letters are used to indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

This study investigated various morphological parameters of eight blood orange varieties. Among them, “Tarocco Meli” had the highest fruit weight (367.86 g), while “Tarocco Scirè” had the lowest (179.33 g) (Figure 2A). Similarly, the equatorial diameter and fruit length were highest in “Tarocco Meli” (88.87/94.13 mm) and lowest in “Tarocco Scirè” (71.86/68.95 mm) among all the varieties (Figure 2B,C). Peel thickness also showed significant differences ($p < 0.05$) with “Tarocco Meli” having the highest value (6.73 mm) and “Tarocco Dalmuso” the lowest (3.60 mm) (Figure 2D). Moreover, the number of carpels showed differences between some varieties with “Tarocco Rosso” having the highest value of 12 and “Tarocco Dalmuso” the lowest with 9 (Figure 2E). Lastly, “Tarocco Meli” had the highest weight of peel (155.0 g), while “Tarocco Scirè” had the lowest (64.83 g) (Figure 2F). Overall, the morphological parameters indicate significant differences between the blood orange varieties, with “Tarocco Meli” exhibiting the highest values.

3.2. External Peel Color

Color is a crucial factor in determining fruit quality and is an important consideration for consumers. The main color parameters of the peel, including L^* , a^* , b^* , C^* , H° , and CI (Figure 3), were analyzed for eight varieties of blood oranges. Significant differences ($p < 0.05$) were observed between varieties for each color parameter, with the most variability observed in the CI parameter, which is considered a critical indicator in *Citrus* fruits.

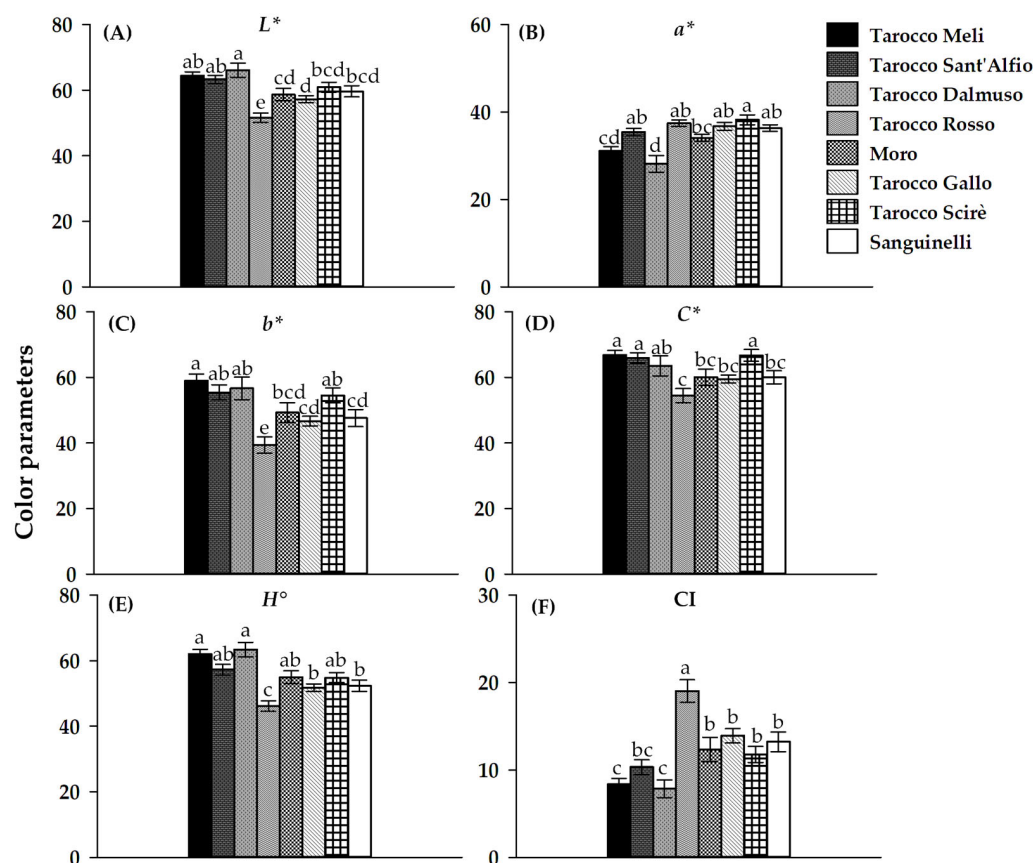


Figure 3. The color parameters L^* (lightness) (A), a^* (redness) (B), b^* (yellowness) (C), C^* (chroma) (D), H° (hue angle) (E), and CI (chroma intensity) (F) were determined in the peel of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla*. The data are reported as the mean \pm standard error (SE) with a sample size of 50 ($n = 50$). Different letters are used to indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

Regarding the L^* parameter, statistically significant differences ($p < 0.05$) were observed between some varieties, with “Tarocco Dalmuso” having the highest value (66.0), and “Tarocco Rosso” obtaining the lowest value (51.57) (Figure 3A). In relation to the a^* and b^* parameters, statistically significant differences ($p < 0.05$) were also observed among the different studied varieties (Figure 3B,C). The highest value of a^* was observed in “Tarocco Scirè” (38.21), while the lowest was in “Tarocco Dalmuso” (28.12) (Figure 3B). The highest value of b^* was observed in “Tarocco Meli” (58.99), and the lowest was in “Tarocco Rosso” (39.33) (Figure 3C). Statistically significant differences ($p < 0.05$) were not observed between “Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, and “Tarocco Scirè”, although there were differences with the rest of the varieties. The highest values of the color parameter C^* were observed in “Tarocco Meli” (66.82), “Tarocco Scirè” (66.69), “Tarocco Sant’Alfio” (65.89), and “Tarocco Dalmuso” (63.48), with no statistically significant differences ($p > 0.05$) among them (Figure 3D). On the other hand, the lowest C^* value was observed in “Tarocco Rosso” (54.40). The highest value of H° was observed in “Tarocco Dalmuso” (63.35) (Figure 3E). Regarding this parameter, no statistically significant differences ($p > 0.05$) were observed between “Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Moro”, and “Tarocco Scirè”. The lowest H° value was observed in “Tarocco Rosso” (46.13). Finally, statistically significant differences ($p < 0.05$) were observed in CI values between “Tarocco Rosso” and the other varieties, with “Tarocco Rosso” having the highest value (19.02) (Figure 3F). In this parameter, no statistically significant

differences ($p > 0.05$) were observed between “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”.

3.3. Primary Metabolites Content in Peel

In the present study, the main primary metabolites in the peel of varieties studied using $^1\text{H-NMR}$ were characterized. The values of seven individual amino acids (4-aminobutyrate, alanine, arginine, asparagine, aspartate, proline, and tyrosine) five organic acids (ascorbate, citrate, formate, malate, and succinate) and three sugars (fructose, glucose, and sucrose) were obtained (Figures 4–6, respectively).

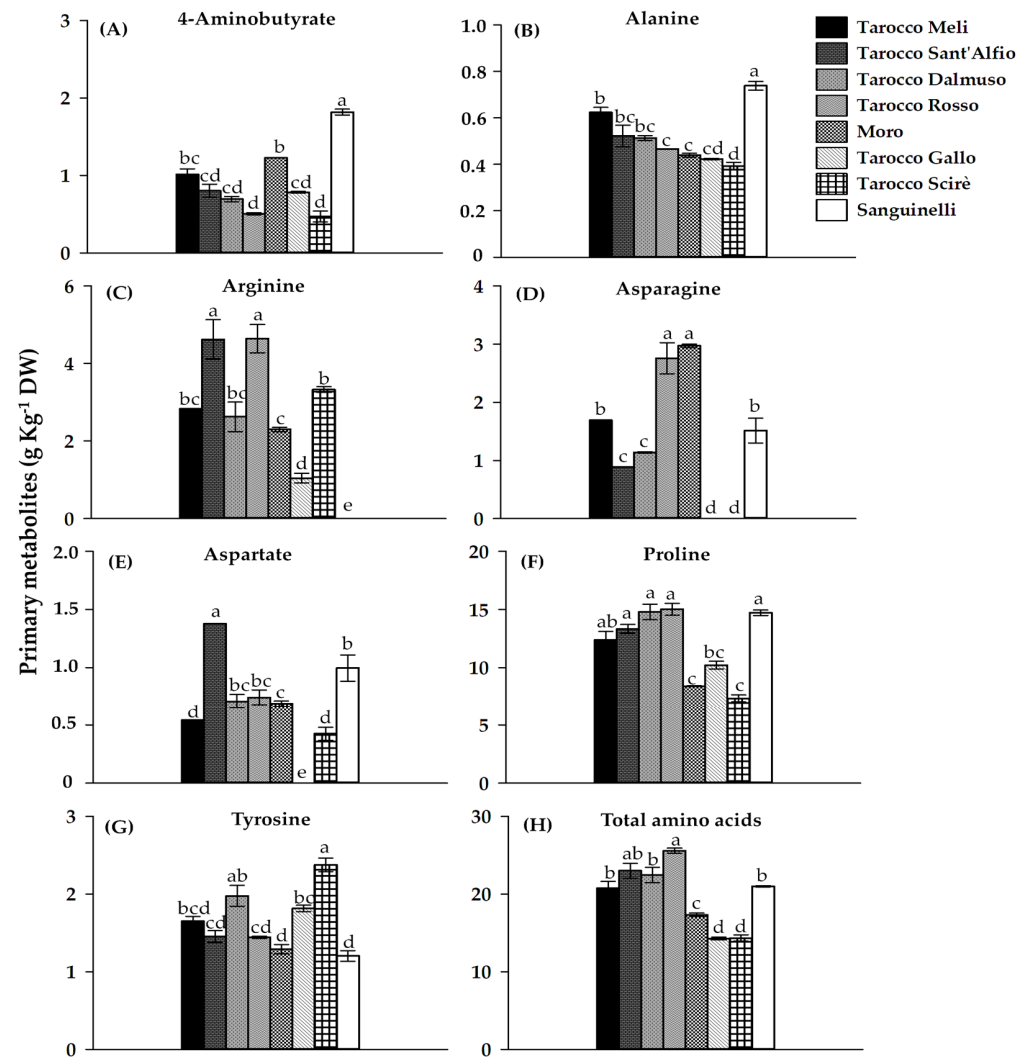


Figure 4. Content of 4-aminobutyrate (A), alanine (B), arginine (C), asparagine (D), aspartate (E), proline (F), tyrosine (G), and total amino acids (H) in peel of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla*. The data are reported as the mean \pm standard error (SE) with a sample size of 6 ($n = 6$). Different letters are used to indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

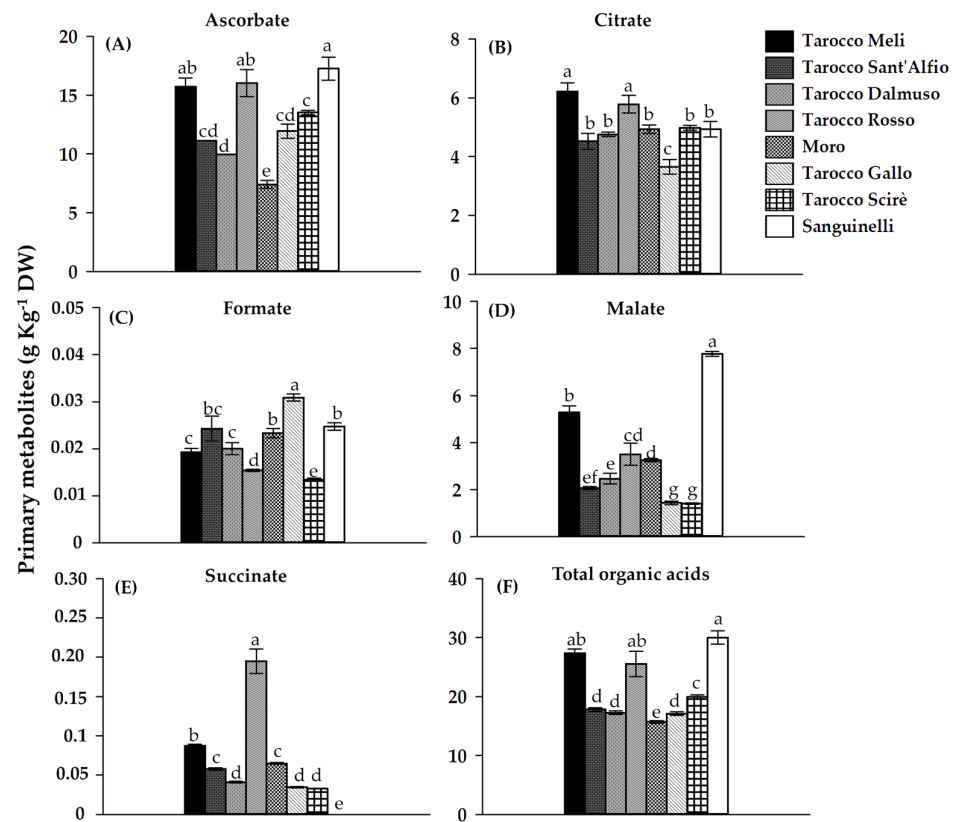


Figure 5. Content of ascorbate (A), citrate (B), formate (C), malate (D), succinate (E), and total organic acids (F) in peel of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla*. The data are reported as the mean ± standard error (SE) with a sample size of 6 ($n = 6$). Different letters are used to indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

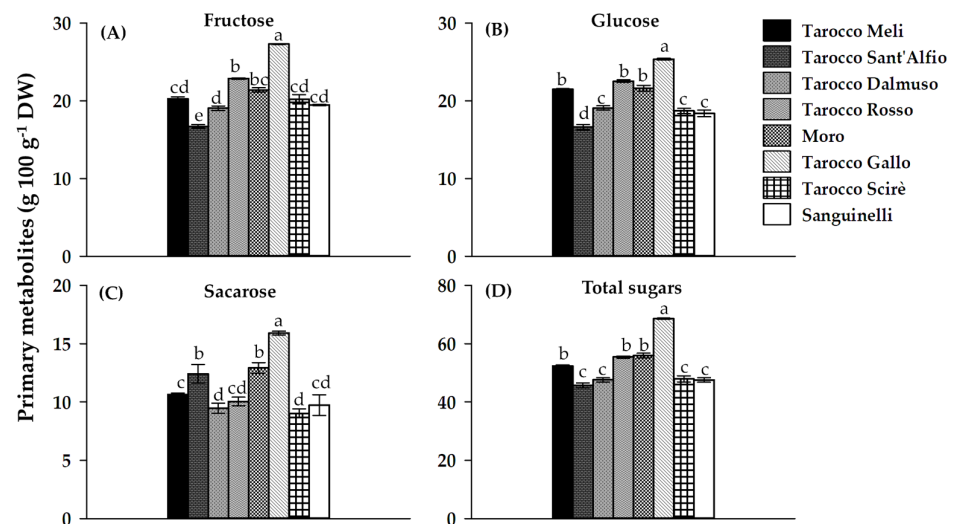


Figure 6. The content of fructose (A), glucose (B), saccharose (C), and total sugars (D) in peel of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla* are presented. The data are reported as the mean ± standard error (SE) with a sample size of 6 ($n = 6$). Different letters are used to indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

Figure 4 displays the identification and quantification of individual amino acids in the peel of the studied orange varieties. Figure 4A presents the values of amino acid 4-aminobutyrate, with “Sanguinelli” exhibiting the highest content (1.81 g Kg⁻¹ DW). Conversely, “Tarocco Scirè” and “Tarocco Rosso” had the lowest values (0.46 and 0.50 g Kg⁻¹ DW, respectively). Statistically significant differences ($p < 0.05$) were observed between some varieties. Regarding the amino acid alanine, once again the “Sanguinelli” variety obtained the highest value (0.73 g Kg⁻¹ DW), while “Tarocco Scirè” had the lowest value (0.39 g Kg⁻¹ DW) (Figure 4B). Statistically significant differences ($p < 0.05$) were observed between some of the varieties for alanine. “Tarocco Sant’Alfio” and “Tarocco Rosso” showed similar values of arginine ($p > 0.05$) (Figure 4C), and had the highest values among the studied varieties (4.62 and 4.63 g Kg⁻¹ DW, respectively). “Tarocco Gallo” had the lowest value (1.04 g Kg⁻¹ DW). Similarly, statistically significant differences ($p < 0.05$) were observed between some of the analyzed varieties for arginine. “Tarocco Rosso” and “Moro” had the highest values of asparagine (2.75 and 2.96 g Kg⁻¹ DW, respectively) (Figure 4D). However, “Tarocco Gallo” and “Tarocco Scirè” had undetectable levels of asparagine, as they were below the detection limit (<10 mM). Statistically significant differences ($p < 0.05$) were observed between some varieties for asparagine. “Tarocco Sant’Alfio” obtained the highest value of aspartate (1.37 g Kg⁻¹ DW) followed by “Sanguinelli” (0.99 g Kg⁻¹ DW). However, aspartate was not detected in “Tarocco Gallo” (below < 10 mM) (Figure 4E). Statistically significant differences ($p < 0.05$) were observed between some varieties in the study for aspartate. The “Sanguinelli”, “Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, and “Tarocco Rosso” varieties had similar proline values, and therefore no statistically significant differences ($p > 0.05$) were observed between them (Figure 4F). “Tarocco Scirè” had the highest value of tyrosine (2.37 g Kg⁻¹ DW), followed by “Tarocco Dalmuso” (1.97 g Kg⁻¹ DW), with no statistically significant differences ($p > 0.05$) between them (Figure 4G). Figure 4H shows the total content of identified and quantified amino acids in the peel of the eight studied varieties. “Tarocco Rosso” had the highest total amino acid content (25.57 g Kg⁻¹ DW), followed by “Tarocco Sant’Alfio” (22.99 g Kg⁻¹ DW) and “Tarocco Dalmuso” (22.44 g Kg⁻¹ DW). On the contrary, the lowest values of total amino acids were observed in “Tarocco Scirè” (14.33 g Kg⁻¹ DW) and “Tarocco Gallo” (14.27 g Kg⁻¹ DW) varieties. Thus, statistically significant differences ($p < 0.05$) were observed between some varieties in the study.

Figure 5 illustrates the identified and quantified organic acids in the peel of eight orange varieties. The varieties “Sanguinelli”, “Tarocco Rosso”, and “Tarocco Meli” exhibited the highest values of ascorbate, with no statistically significant differences observed between them ($p > 0.05$) (Figure 5A). Conversely, “Moro” had the lowest ascorbate value (7.40 g Kg⁻¹ DW), which was approximately half of the value observed in “Sanguinelli”, the variety with the highest content. Ascorbate was also the most abundant organic acid detected. The organic acid detected with the next-highest concentration was citrate, with the highest value observed in “Tarocco Meli” (6.21 g Kg⁻¹ DW), followed by “Tarocco Rosso” (5.77 g Kg⁻¹ DW), with no statistically significant differences between them ($p > 0.05$) (Figure 5B). “Tarocco Gallo” had the lowest citrate value, which was statistically different ($p < 0.05$) from the other varieties studied. Figure 5C displays the formate values observed in the study varieties, with “Tarocco Gallo” having the highest value (0.030 g Kg⁻¹ DW), while “Tarocco Scirè” (0.013 g Kg⁻¹ DW) and “Tarocco Rosso” (0.015 g Kg⁻¹ DW) had the lowest values, approximately half of the value observed in “Tarocco Gallo.” Statistically significant differences were observed between the varieties ($p < 0.05$). “Sanguinelli” exhibited the highest malate value (7.77 g Kg⁻¹ DW), which was much higher than the values observed in “Tarocco Scirè” (1.40 g Kg⁻¹ DW) and “Tarocco Gallo” (1.43 g Kg⁻¹ DW), the varieties with the lowest values (Figure 5D). Statistically significant differences ($p < 0.05$) were observed among some blood orange varieties in terms of peel malate values. “Tarocco Rosso” had the highest succinate value (0.19 g Kg⁻¹ DW), followed by “Tarocco Meli” (0.087 g Kg⁻¹ DW), while succinate was not detected in “Sanguinelli” as it was below the detection limit (<10 mM) (Figure 5E).

Statistically significant differences ($p < 0.05$) were observed between some of the varieties studied. Figure 5F presents the values of total organic acids quantified in the peel of the eight studied varieties, with “Sanguinelli”, “Tarocco Meli”, and “Tarocco Rosso” exhibiting the highest values (29.99, 27.35, and 25.52 g Kg⁻¹ DW, respectively), with no statistically significant differences between these three varieties ($p > 0.05$). “Moro” had the lowest value (15.68 g Kg⁻¹ DW), and statistically significant differences ($p < 0.05$) were observed between some of the studied varieties.

Figure 6 shows the values of three individual and total sugars identified and quantified in the peel of the eight varieties studied. In general, fructose and glucose obtained similar values and showed the same trend between varieties. In fact, in both sugars, “Tarocco Gallo” was the variety that obtained the highest values (27.29 and 25.35 g 100 g⁻¹ DW, respectively) and, “Tarocco Sant’Alfio” was the one that obtained the lowest values (16.68 and 16.60 g 100 g⁻¹ DW, respectively) (Figure 6A,B). Statistically significant differences were observed between varieties ($p < 0.05$). Figure 6C shows the sucrose content and, as with fructose and glucose, “Tarocco Gallo” was the variety that obtained the highest value (15.91 g 100 g⁻¹ DW). On the other hand, in general, sucrose contents were slightly lower than those observed in fructose and glucose. Statistically significant differences were observed between sucrose contents of some varieties ($p < 0.05$). Figure 6D shows the values of total sugars identified in peel of the eight varieties studied. As expected, “Tarocco Gallo” had the highest total sugar content (68.56 g 100 g⁻¹ DW) and “Tarocco Sant’Alfio” obtained the lowest content (45.71 g 100 g⁻¹ DW). Statistically significant differences ($p < 0.05$) were also observed between total sugar contents for some of these varieties.

3.4. Secondary Metabolites Content in Peel

The contents of individual secondary metabolites identified and quantified by HPLC-ESI-DAD-MSⁿ in the peel of the studied varieties are presented in Figure 7. A hydroxycinnamic acid (*p*-coumaric acid 4-*O*-glucoside: $R_t = 7.2$ min, $[M - H]^-$ at m/z 325 and UV/vis = 290 nm), three individual anthocyanins (cyanidin 3-*O*-(6''-caffeoyl-glucoside): $R_t = 8.6$ min, $[M - H]^-$ at m/z 609, 399, 355, and UV/vis = 520 nm; cyanidin 3-*O*-sophoroside: $R_t = 9.3$ min, $[M - H]^-$ at m/z 609, 489, 286, and UV/vis = 520 nm; and cyanidin 3-*O*-(6''-acetyl-glucoside): $R_t = 12.5$ min, $[M - H]^-$ at m/z 489, 286 and UV/vis = 520 nm), two flavones (vicenin-2, apigenin 6,8-di-*C*-glycoside: $R_t = 10.8$ min, $[M - H]^-$ at m/z 593, 473, 383, 354, and UV/vis = 290 nm; and apigenin 7-*O*-(6''-malonyl-apiosyl-glucoside): $R_t = 14.2$ min, $[M - H]^-$ at m/z 649, and UV/vis = 290 nm) and four flavanones (naringenin-glucosyl-rutinoside: $R_t = 11.4$ min, $[M - H]^-$ at m/z 741, 579, 433, 271, and UV/vis = 290 nm; narirutin, naringenin-7-rutinoside: $R_t = 15.7$ min, $[M - H]^-$ at m/z 579, 271, and UV/vis = 290 nm; hesperidin 7-rutinoside: $R_t = 17.2$ min, $[M - H]^-$ at m/z 609, 301, and UV/vis = 290 nm; and didymin, naringenin-40-methyl-ether 7-rutinoside: $R_t = 22.0$ min, $[M - H]^-$ at m/z 593, 285, and UV/vis = 290 nm) were identified.

In Figure 7A, it is evident that the “Moro” variety exhibited the highest level of *p*-coumaric acid 4-*O*-glucoside (263.33 mg Kg⁻¹ DW), followed by “Tarocco Meli” (176.25 mg Kg⁻¹ DW), with statistically significant differences ($p < 0.05$). The remaining varieties displayed lower levels, ranging from 107.86 mg Kg⁻¹ DW in “Tarocco Scirè” to 31.30 mg Kg⁻¹ DW in “Tarocco Sant’Alfio”. Regarding the three analyzed anthocyanins, both the “Moro” and “Tarocco Meli” varieties exhibited significantly higher levels, with significant differences ($p < 0.05$) between them, surpassing the levels observed in the other varieties (Figure 7B–D). Additionally, significant differences ($p < 0.05$) were observed between certain other varieties in terms of the levels of the three individual anthocyanins. Figure 7E,F present the levels of two identified flavones: vicenin-2, apigenin 6,8-di-*C*-glycoside, and apigenin 7-*O*-(6''-malonyl-apiosyl-glucoside). In both flavones, the “Moro” variety exhibited significantly higher levels compared to the other varieties ($p < 0.05$), and there were also significant differences ($p < 0.05$) between some of the other varieties for both flavones. Figure 7G–J illustrate the levels of four identified flavanones: naringenin-glucosyl-rutinoside, narirutin, naringenin-7-rutinoside, hesperidin 7-rutinoside, and didymin, naringenin-40-methyl-ether

7-rutinoside. “Tarocco Scirè” exhibited the highest levels of naringenin-glucosyl-rutinoside (39.97 mg Kg⁻¹ DW), hesperidin 7-rutinoside (428.40 mg Kg⁻¹ DW), and didymin, naringenin-40-methyl-ether 7-rutinoside (27.34 mg Kg⁻¹ DW). Moreover, there were no statistically significant differences ($p > 0.05$) between “Tarocco Scirè”, “Moro”, and “Tarocco Meli” for naringenin-glucosyl-rutinoside and narirutin, naringenin-7-rutinoside. Furthermore, in Figure 7, there were no significant differences ($p > 0.05$) between “Tarocco Meli”, “Tarocco Sant’Alfio”, “Moro”, and “Sanguinelli” for didymin, naringenin-40-methyl-ether 7-rutinoside, but significant differences ($p < 0.05$) were observed between these varieties and “Tarocco Scirè”, which exhibited the highest level.

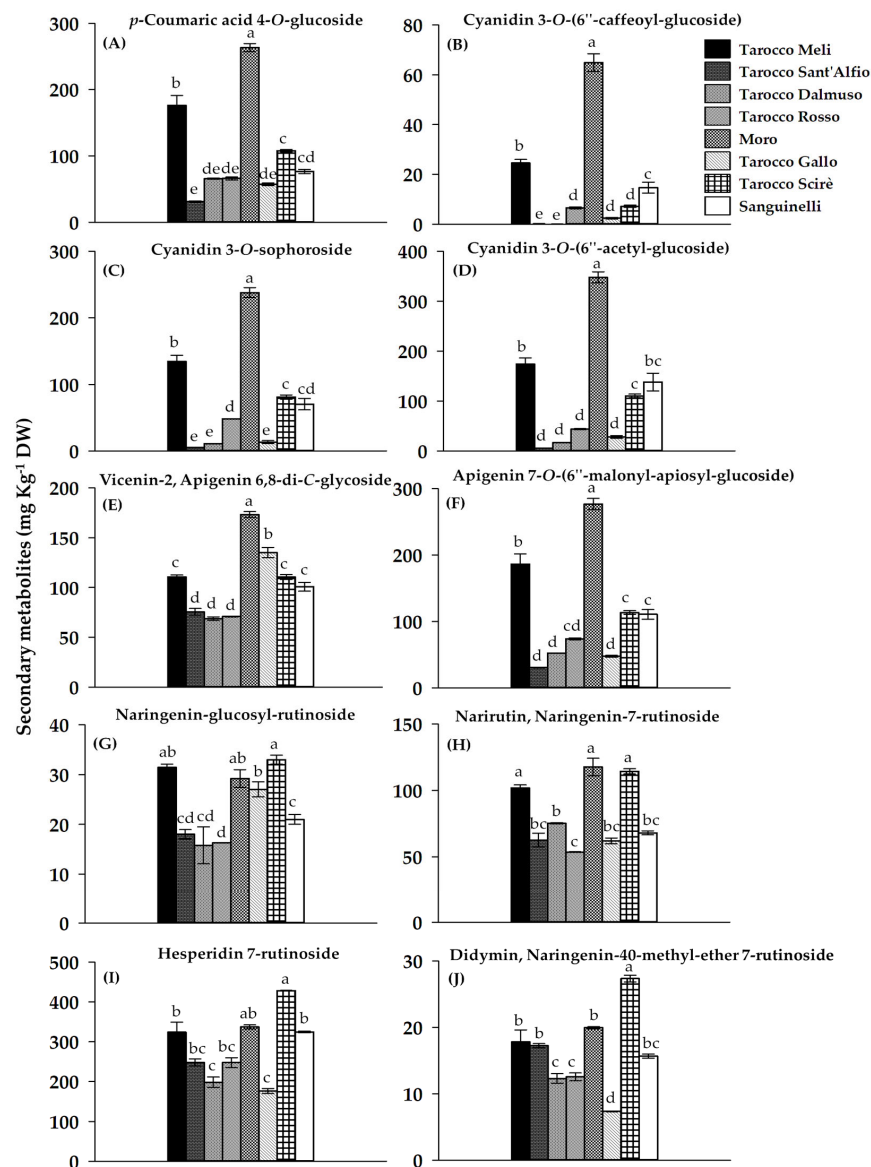


Figure 7. The levels of ten compounds were measured in the peel of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla*. The compounds measured were *p*-coumaric acid 4-*O*-glucoside (A), cyanidin 3-*O*-(6''-caffeoyl)-glucoside (B), cyanidin 3-*O*-sophoroside (C), cyanidin 3-*O*-(6''-acetyl)-glucoside (D), vicenin-2, apigenin 6,8-di-*C*-glycoside (E), apigenin 7-*O*-(6''-malonyl)-apiosyl-glycoside (F), naringenin-glucosyl-rutinoside (G), narirutin, naringenin-7-rutinoside (H), hesperidin 7-rutinoside (I), and didymin, naringenin-40-methyl-ether 7-rutinoside (J). The results, presented as the mean \pm standard error of the mean ($n = 6$), showed significant differences among the varieties (ANOVA, HSD Tukey test; $p < 0.05$), indicated by different letters.

Figure 8 presents the comprehensive analysis of anthocyanins, hydroxycinnamic acids, flavones, and flavanones in the peel of the studied varieties, highlighting their total levels. Among the varieties, the “Moro” variety exhibited the highest contents of anthocyanins (650.67 mg Kg⁻¹ DW), hydroxycinnamic acids (263.33 mg Kg⁻¹ DW), and flavones (449.85 mg Kg⁻¹ DW) (Figure 8A–C). On the other hand, “Tarocco Scirè” displayed the highest total content of flavanones (603.05 mg Kg⁻¹ DW), followed by “Moro” (503.93 mg Kg⁻¹ DW) (Figure 8D). Notably, “Moro” and “Tarocco Meli” stood out with the highest overall levels of secondary metabolites in their peel. Furthermore, statistical analysis revealed significant differences ($p < 0.05$) among the varieties within each group of secondary metabolites.

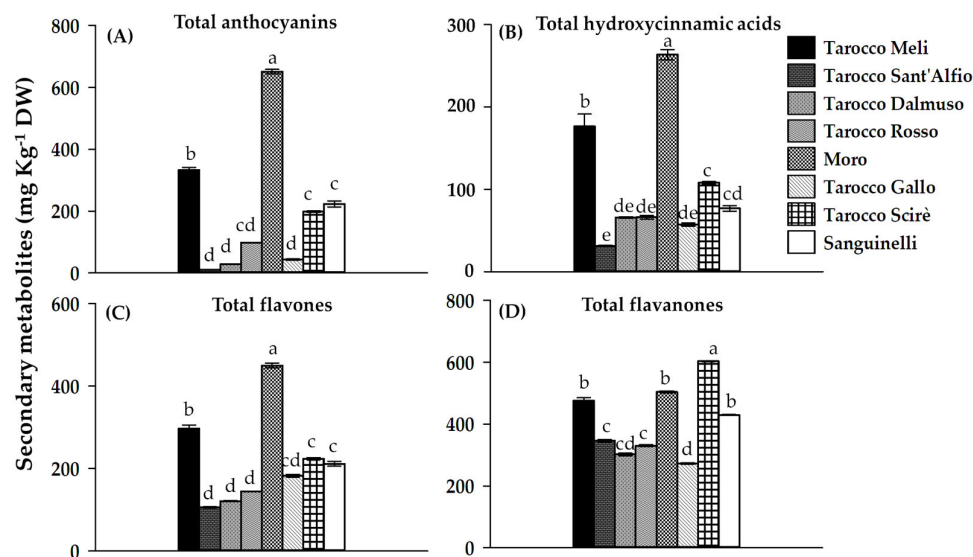


Figure 8. The mean \pm SE ($n = 6$) total content of anthocyanins (A), hydroxycinnamic acids (B), flavones (C), and flavanones (D) in the peel of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla* is presented. The data are reported as the mean \pm standard error (SE) with a sample size of 6 ($n = 6$). Different letters are used to indicate statistically significant differences (ANOVA, HSD Tukey test; $p < 0.05$).

3.5. Principal Component Analysis (PCA)

The results of the principal component analysis (PCA) revealed that the first two principal components (PCs) explained a substantial portion of the total variation in morphological and color parameters (Figure 9A). Specifically, these PCs accounted for 84.22% of the variation in these parameters. Additionally, individual primary metabolites (Figure 9B) contributed to 59.38% of the total variation, individual secondary metabolites (Figure 9C) contributed to 89.69% of the total variation, and total metabolites (Figure 9D) contributed to 74.14% of the total variation. Furthermore, all figures displayed Pearson’s correlation among the different varieties, providing valuable insights into the relationships between the attributes that define the characteristics of the samples.

The results of PCA for variety classification revealed that the first two principal components (PCs) accounted for a significant portion of the total variation in morphological and color parameters (Figure 9A). Specifically, PC1 explained 61.13% of the variance and showed a positive correlation with variables H° , L^* , and b^* , while negatively correlating with variables CI and a^* . PC2 explained 23.09% of the variance and demonstrated a positive correlation with variables C^* and b^* , but a negative correlation with variables peel weight and equatorial diameter. Moreover, Figure 9A depicted Pearson’s correlation among the blood orange varieties, highlighting four distinct groups with a correlation of over 90%.

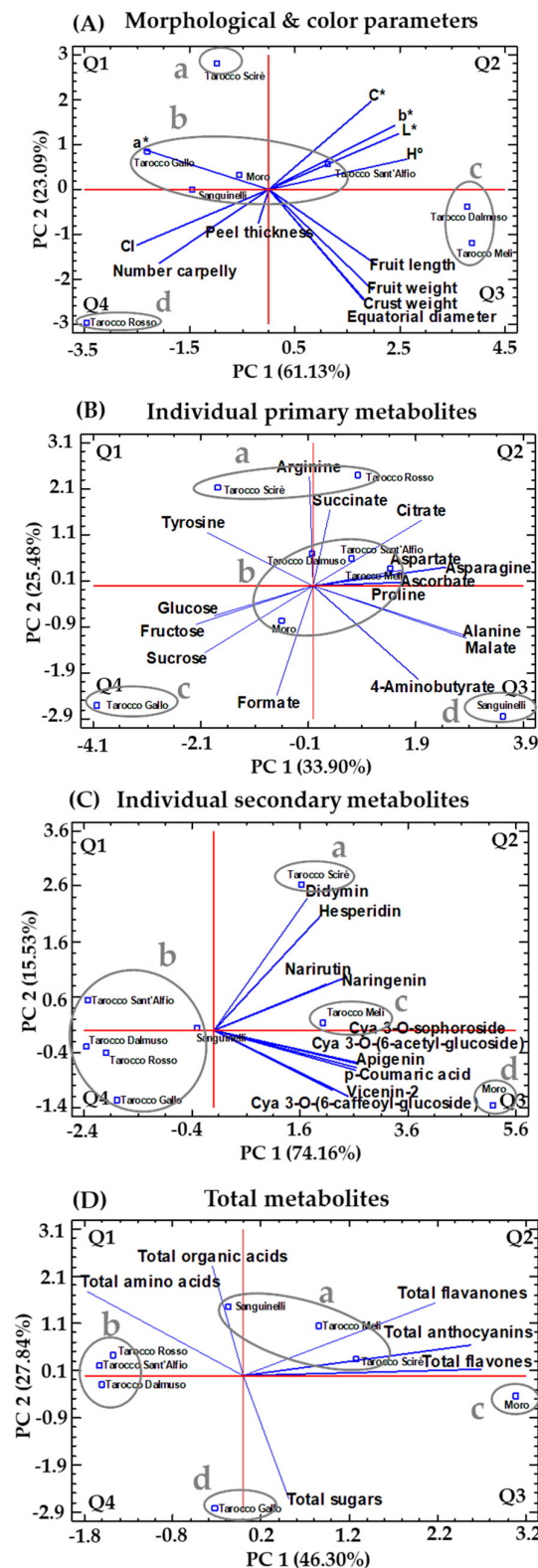


Figure 9. PCA was conducted to analyze the morphological and color parameters (A), individual primary metabolites (B), individual secondary metabolites (C), and total metabolites (D) in the peel of eight blood orange varieties (“Tarocco Meli”, “Tarocco Sant’Alfio”, “Tarocco Dalmuso”, “Tarocco Rosso”, “Moro”, “Tarocco Gallo”, “Tarocco Scirè”, and “Sanguinelli”) grafted onto *Citrus macrophylla*.

For primary metabolites (Figure 9B), PC1 accounted for 33.90% of the variance and exhibited a positive correlation with variables malate and alanine, while negatively correlat-

ing with variables fructose, glucose, and sucrose. PC2 explained 25.48% of the variance and showed a positive correlation with variables arginine and citrate, but a negative correlation with variables formate and 4-aminobutyrate. Figure 9A,B also present Pearson's correlation among the blood orange varieties, delineating four groups with a correlation of over 90%.

In the case of secondary metabolites (Figure 9C), PC1 explained 74.16% of the variance and exhibited a positive correlation with all variables, without any negative correlations observed. PC2 accounted for 15.53% of the variance and displayed a positive correlation with variables hesperidin 7-rutinoside, didymin, and naringenin-40-methyl-ether 7-rutinoside, but a negative correlation with variables vicenin-2 and apigenin 6,8-di-C-glycoside. Similarly, Figure 9C showcased the Pearson's correlation among the blood orange varieties, with four distinct groups exhibiting a correlation of over 90%.

Regarding total metabolites (Figure 9D), the first component explained 46.30% of the variance and displayed a positive correlation with the variables total flavones, anthocyanins, and flavanones, but a negative correlation with the variable total amino acids. The second component accounted for 27.84% of the variance and demonstrated a positive correlation with total organic acids, while negatively correlating with total sugars. Additionally, Figure 9D presents Pearson's correlation among the blood orange varieties, revealing four groups with a correlation of over 90%.

4. Discussion

Blood oranges are a distinct variety of oranges known for their characteristic dark red color in both the pulp and peel, which is attributed to the presence of anthocyanin pigments. Apart from their rich vitamin and mineral content, blood oranges are also a valuable source of bioactive compounds, including phenolic compounds and organic acids, primarily found in the fruit's peel [2,15]. The conducted study demonstrated that the genotype of the blood oranges under investigation had a significant influence on various morphological parameters and the content of primary and secondary metabolites present in the peel, such as peel thickness and weight. Furthermore, notable statistically significant differences ($p < 0.05$) were observed among the analyzed blood orange varieties concerning their morphological parameters and metabolite content in the peel. These findings emphasize the significance of selecting appropriate genotypes to obtain fruits with optimal characteristics suitable for both the food and pharmaceutical industries, as well as for fresh commercialization. Thus, these results hold considerable relevance for blood orange producers and consumers alike.

Morphological parameters are important for the characterization of *Citrus* fruits, as they can provide valuable information about their quality, yield, and potential uses in the food and nutraceutical industry, thus playing an important role in consumer preferences when buying fruit because different fruit calibers can satisfy different types of consumers [29,30]. These parameters include weight, caliber, peel thickness, and peel weight, among others. Additionally, they are important indicators of the nutritional and physiological status of the tree and can be used to determine the optimal harvest time [31]. In particular, these parameters are related to the sensory and nutritional quality of *Citrus* fruits, as well as their ability to resist diseases and pests [32]. The present study examined the morphological parameters of different varieties of blood oranges (Figure 2). Statistically significant differences ($p < 0.05$) were observed in weight, equatorial diameter, fruit length, peel thickness, number of carpels, and peel weight among the different varieties. The "Tarocco Meli" variety obtained the highest values in most of the morphological parameters studied, while the "Tarocco Scirè" variety obtained the lowest values. The results were confirmed by demonstrating a positive correlation between the morphological parameters and the "Tarocco Meli" variety, and vice versa with the "Tarocco Scirè" variety, through the use of PCA (Figure 9A). These findings confirm that genotype significantly influences fruit morphology in blood oranges [2]. Thus, we suggest that morphological parameters are a useful tool for evaluating fruit quality and tree yield in citriculture. It is important

to consider these parameters in genotype selection, nutritional and physiological status evaluation of the tree, post-harvest management, and fruit commercialization.

Color is a key parameter in the agri-food industry, especially in the selection of fruits [33]. The appearance of a fruit can greatly affect consumer preference and purchase decisions [34]. Color parameters, such as C^* , H° , and L^* , are used to evaluate the appearance of fruits [33]. These parameters can also provide information about the maturity, ripeness, and quality of the produce. In addition to affecting consumer preference, color parameters can also be used to assess the nutritional value of fruits [35]. For example, the antioxidant activity of fruits is often correlated with their color intensity [36,37]. Fruits with darker colors, such as blueberries, have higher levels of anthocyanins, which are potent antioxidants [38]. In addition, the color of the peel has been widely used as an indicator of fruit maturity in blood orange varieties [39]. Therefore, the color parameters of fruits and vegetables are an important criterion for selecting produce in the agri-food industry. The appearance of fruits and vegetables can influence consumer choice and can also provide valuable information about the maturity and quality of the produce. The use of color parameters as a selection criterion is decisive, as it ensures that only the highest quality produce is selected for sale. The color of the fruit is due to pigmentation with compounds such as anthocyanins or carotenoids, which are produced by the plant during its growth and development [40]. The synthesis of these pigments can be influenced by various factors such as genetics, environment, or agricultural practices [39,41,42]. Pigmentation can also have implications for human health since fruit pigments contain antioxidant compounds that are believed to have beneficial health effects [43]. Therefore, fruit pigmentation can be an important factor in food selection for people looking to improve their health through nutrition. Therefore, attention should be paid to pigmentation in fruit production and marketing. This study analyzed color parameters (L^* , a^* , b^* , C^* , H° , and CI) in different varieties of blood oranges and found significant differences ($p < 0.05$) between them. "Tarocco Dalmuso", "Tarocco Scirè", and "Tarocco Meli" had the highest values in different color parameters, while "Tarocco Rosso" had the lowest values for several parameters but the highest CI value (Figure 3). This variation in color among the different varieties was supported by the PCA, which revealed a substantial distance between "Tarocco Sant' Alfio" and "Tarocco Rosso" (Figure 9A). We suggest that differences in the peel coloration among different varieties of blood oranges were due to the genetic variability of different genotypes, since they were grown under the same environmental factors, such as temperature, light intensity, and soil moisture. We also observed that the amount and type of anthocyanins produced, the pigments responsible for the red or purple coloration in the pulp of blood oranges, varied according to the genotype of the blood orange, and therefore, the peel coloration. These results can be used by the agri-food industry for the selection of blood orange varieties, as their cultivation relies heavily on the quality and intensity of their peel color, which in turn affects their commercial appeal and consumer acceptance [34].

Blood oranges are known for their characteristic red pigmentation, which is due to the presence of anthocyanins in their fruit flesh [2,39]. However, recent research has highlighted the importance of the nutritional quality of the fruit peel [15], which is typically discarded as waste. There are 20 different amino acids that are used by the body to synthesize proteins, nine of which are considered essential, meaning that they must be obtained from the diet. While most attention has traditionally been given to the amino acid content of the fruit flesh, recent studies have shown that the peel can also provide a significant amount of these important nutrients [15,44]. Additionally, *Citrus lemon*, *Citrus limetta* or *Citrus maxima* peel has been shown to contain other non-essential amino acids such as glutamic acid and aspartic acid [45], which are important for brain function and the regulation of the immune system [46,47]. Tryptophan, methionine, histidine, lysine, cysteine, arginine or tyrosine have also been shown to have antioxidant capacity [48], which can help to protect cells from damage caused by free radicals. This study analyzed amino acid content in the peel of eight blood orange varieties, revealing significant differences ($p < 0.05$) between some varieties (Figure 4). These findings demonstrate the variability in amino acid profiles

among blood orange varieties and their potential impact on nutritional value. These results were corroborated by the PCA in which the distance between the varieties was observed for each amino acid (Figure 9B). In this way, the peel of blood oranges contains a variety of important amino acids, which have been shown to be essential for various biological processes in humans [49]. Given the potential health benefits of these nutrients, further research into the nutritional value of fruit peel is warranted, and efforts should be made to promote the consumption of these often-discarded parts of the fruit.

Organic acids, which are abundant in *Citrus* fruits, including oranges, have been recognized for their beneficial effects on human health [2]. The peel of oranges, in particular, is rich in organic acids and offers various health advantages [50]. Among these organic acids, ascorbic acid (vitamin C) plays a vital role in preventing chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative disorders [51]. Orange peel is known to be a significant source of ascorbic acid, which possesses antioxidant, anti-atherogenic, anti-carcinogenic, and immunomodulatory properties [50]. Moreover, vitamin C has been linked to a reduced risk of stomach, lung, and colorectal cancers [52]. Apart from their health benefits, the organic acids present in orange peel have practical applications in the food industry. They serve as natural preservatives and flavor enhancers, imparting distinct tastes and aromas to food products [53]. This study focused on analyzing the organic acid content in eight different varieties of oranges. Among them, “Sanguinelli”, “Tarocco Rosso”, and “Tarocco Meli” exhibited the highest levels of ascorbic acid, while citrate was the most abundant organic acid overall. “Tarocco Gallo” displayed the highest formate content, and “Sanguinelli” had the highest malate content. Additionally, “Tarocco Rosso” showed the highest succinate value. Regarding the total organic acid content, “Sanguinelli”, “Tarocco Meli”, and “Tarocco Rosso” showcased the highest levels. These findings highlight the variations in organic acid content among different blood orange varieties, as depicted in Figure 5. The PCA analysis further confirmed the differences, illustrating the proximity of ascorbic acid to “Tarocco Meli”, citric and succinic acid to “Tarocco Rosso” (Figure 9B), and total organic acids to “Sanguinelli” (Figure 9D). It is worth noting that the disparity in organic acid profiles and quantities among varieties may be attributed to factors such as genotype [2,39] or fruit position on the tree [54]. These factors contribute to the significant variations observed among the different organic acids in the analyzed varieties.

The results of this study suggest that the organic acids present in blood orange peel offer a range of health benefits to humans, including the prevention of chronic diseases and the alleviation of muscle pain and fatigue. Furthermore, these organic acids can be utilized in the food industry as natural preservatives and flavor enhancers, contributing to the overall appeal of food products. The sugar content in the peel of blood oranges has been the subject of scientific research due to its potential application in the food industry. According to [44], the sugars present in the peel of oranges are mainly fructose, glucose, and sucrose, and their values vary depending on the variety of orange. The authors of [55] also highlighted the importance of sugars in the peel of oranges for the food industry. In particular, it has been found that these sugars can be used as a source of carbon for the production of biopolymers, such as pectin, which is a food additive used as a thickener and stabilizer. Additionally, orange peel has been shown to be a source of fermentable sugars for the production of bioethanol, biogas, or bioenergy [56]. In this sense, the use of blood orange peel as a source of sugars can be a sustainable and profitable alternative for the production of bioproducts in the food industry. This study analyzed the sugar content in the peel of eight orange varieties (Figure 6). Fructose and glucose had similar values among the varieties, with “Tarocco Gallo” having the highest values and “Tarocco Sant’Alfio” having the lowest. Sucrose content was slightly lower, with “Tarocco Gallo” having the highest value. The total sugar content was the highest in “Tarocco Gallo” and lowest in “Tarocco Sant’Alfio”. Statistically significant differences ($p < 0.05$) were observed between varieties for all sugars measured. The PCA has confirmed these results by positively correlating fructose and glucose or the total sugar content with “Tarocco

Gallo" (Figure 9B,D). Therefore, we continue to assert that the sugar content in the peel of blood oranges is an important aspect to consider for its possible application in the food industry. The sugars present in the peel can be used as a source of carbon for the production of biopolymers and other biochemical products. Further research is required to explore the potential of blood orange peel as a source of sugars for the food industry.

The demand for healthy food is growing, and among the options available, red fruits are gaining popularity due to their well-established health benefits [10]. Nutritionists and specialists recommend including red fruits, such as blood oranges, in a healthy diet because they contain bioactive components that have been proven to enhance health [11]. Additionally, *Citrus* varieties, including blood oranges, have a wide range of phenolic compounds such as anthocyanins, flavones, or flavanones [2,39,57]. These natural antioxidants provide functional properties to the fruit, including color, taste, and nutritional benefits [58]. Anthocyanins are water-soluble compounds that are responsible for the distinctive red/purple coloration of the peel of blood oranges [37]. They are the most important bioactive compounds in blood oranges, providing powerful antioxidant activity that positively impacts the nutritional and sensory quality of the fruit. In this sense, it has been observed that orange peel has been shown to contain a large amount of bioactive compounds that have antioxidant and antimicrobial properties [6]. These bioactive compounds also have antimicrobial properties and can be used as natural preservatives in different foods [1,39]. This study examined the phenolic compound content of various blood orange varieties and found that genotype had a greater impact than environmental factors on the accumulation of these compounds. The "Moro" variety had the highest levels of several phenolic compounds, including *p*-coumaric acid 4-*O*-glucoside, anthocyanins, and flavones, followed by "Tarocco Meli", while "Tarocco Scirè" had the highest content of flavanones. Significant differences ($p < 0.05$) were observed in the levels of these compounds between the different varieties. Overall, "Moro" and "Tarocco Meli" had the highest total levels of secondary metabolites in their peel, indicating their potential health benefits (Figures 7 and 8). According to the results of the PCA, the blood orange variety known as "Moro" showed a strong positive correlation with various phenolic compounds, including individual (Figure 9C) or total (Figure 9D) anthocyanins, hydroxycinnamic acids, flavanones, and flavones. This suggests that this blood orange variety contains a higher amount of these compounds compared to other varieties. Overall, these results highlight the importance of genotype in the accumulation of phenolic compounds in blood oranges and their potential to provide health benefits when consumed. In addition, the results of the PCA can provide a useful tool for identifying blood orange varieties with peel with higher levels of phenolic compounds for use in the food industry and in the production of dietary supplements. As previously noted, the concentration of phenolic compounds in fruits such as blood oranges can be strongly influenced by a variety of factors. These include the genotype [2], rootstocks [59], as well as factors such as the orientation of the fruit on the tree [39] or day/night temperature variations [60]. These findings underscore the importance of considering multiple variables when analyzing the nutritional composition of fruits. Given the complex interactions between various factors in the accumulation of phenolic compounds in *Citrus* fruits such as blood oranges, continued research in this area is crucial. Such studies can help identify specific genetic, environmental, and processing factors that affect the nutritional composition of fruits and may inform the development of strategies to optimize the content of bioactive compounds in fruits. Understanding the impact of genetics on the accumulation of pigments such as anthocyanins is crucial for the development of desirable varieties of fruits and vegetables with enhanced nutritional and functional properties. On the other hand, these compounds can be used as natural ingredients in different foods to improve their quality and shelf life, reducing the reliance on chemical preservatives in the food industry, which has benefits for both human health and the environment.

5. Conclusions

The high content of primary and secondary metabolites in the peel of blood oranges is highly beneficial for human health due to its antioxidant properties. Currently, there is increasing interest in the application of blood orange metabolites in the agri-food and nutraceutical industries. For example, extracts from blood orange peel can serve as natural food preservatives and active ingredients in nutraceutical products. The incorporation of blood orange peel extract into food bases can provide several health benefits due to its high content of bioactive compounds. Moreover, blood orange peel extract can enhance the sensory profile of foods by providing a pleasant taste and distinctive mouthfeel. It can also prolong the shelf life of food products due to its natural antioxidant and antimicrobial properties. This study successfully achieved its objectives by providing sufficient knowledge to confirm the suitability of the peel from the eight studied blood orange varieties as a potential high-value co-product that can be incorporated into various food matrices. This study found that the peel from “Tarocco Meli” was the most productive in quantitative terms, while the peels from “Tarocco Rosso”, “Sanguinelli”, and “Tarocco Gallo” varieties were the most suitable in qualitative terms for primary metabolites. Additionally, the peel from the “Moro” variety had the highest concentration of secondary metabolites, making it the peel with the highest antioxidant value, meeting the demands of current consumers. These findings suggest that incorporating blood orange peel into food matrices can have potential benefits for human health due to its high concentration of bioactive compounds. Furthermore, utilizing the peel in food production can help reduce food waste. Therefore, the results of this study could be useful for the food industry to develop novel functional food products that can meet consumer demands for healthier and more sustainable food choices.

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