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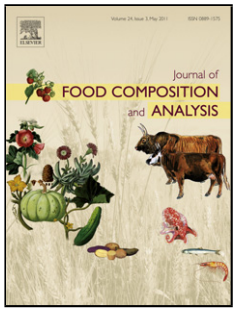

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Influence of rootstock genotype on individual metabolic responses and antioxidant potential of blood orange cv. Tarocco Scirè

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Highlights

- The influence of 10 rootstocks on blood orange metabolic profile was assessed
- 24 phenolic compounds were individually identified and quantified
- Anthocyanins and antioxidant activity are greatly affected by the rootstock
- Role exerted by the rootstock on citrus quality was depicted

Abstract

In fruit crops, tree rootstock plays a relevant role in the tolerance of biotic and abiotic factors, various qualitative traits and biochemical profiles of the juice. In this work, the fruit quality characteristics of sweet orange Tarocco Sciré grafted onto ten rootstocks were evaluated. Analyses were performed on the fruit juice, coupling the quantification of phenolic compounds (anthocyanins, flavanones, flavones and hydroxycinnamic acids) through hyphenated

chromatographic techniques and the assessment of antioxidant potential (measured *in vitro*). The study was conducted for two consecutive years in which the different minimum temperatures recorded during fruit ripening determined a higher accumulation of polyphenols, especially anthocyanins (63.6%), and higher levels of antioxidant activity (48.92) in the colder year. The analyses allowed the identification and quantification of 24 phenolic compounds (6 anthocyanins, 5 flavanones, 1 flavone and 12 hydroxycinnamic acids). The results highlighted a significant effect of both rootstocks and the environment (and their interaction) on the metabolic profile of the juice, with low temperatures being directly involved in juice pigmentation and significant differences among the rootstocks within each year of analysis. In addition to temperature, a significant effect of rootstocks on the metabolic profile of the juice was revealed, with C35 and Bitters being the most effective in enhancing anthocyanin accumulation in the fruit of the grafted variety.

Altogether, the data presented shed light on the significant role exerted by the rootstock on fruit quality, providing useful insights to guide the selection of rootstocks in novel implants.

Keywords: *Citrus sinensis* L.; pigmentation; phenolic compounds; anthocyanins; grafting

Chemical compounds studied in this article

cyanidin 3-*O*-glucoside (PubChem CID: 441667); cyanidin 3-*O*-(6’’-malonyl) glucoside (PubChem CID: 443915); eriocitrin, neoeriocitrin, narirutin (PubChem CID: 442431); hesperidin (PubChem CID: 10621); didymin (PubChem CID: 16760075); vitexin, chlorogenic acid (PubChem CID: 1794427); ferulic acid (PubChem CID: 445858); p-coumaric acid (PubChem CID: 637542); sinapic acid (PubChem CID: 637775).

1. Introduction

Citrus fruits are highly appreciated for their unique flavour (both in terms of taste and aroma), as well as for their high nutraceutical value, by consumers worldwide (Pannitteri et al., 2017). Citrus production takes advantage of the agronomical practice of grafting, often used to confer higher tolerance to biotic and/or abiotic stress to the scion. The increasing wealth of data highlights the strong influence of rootstocks on traits of economic relevance, such as fruit yield, fruit size, juice quality, fruit maturation and postharvest performance (Bowman et al., 2016; Forner et al., 2020; Rodriguez-Gamir et al., 2010). Citrus production has historically relied on the use of sour orange (*Citrus aurantium* L.) as rootstock due to its high resistance to several biotic stresses (especially root rot), abiotic stresses (including drought, salinity, calcareous soils, and mineral deficiency), and good productivity in a wide range of pedoclimatic conditions (Moreno et al., 2008). Mediterranean citriculture is seriously threatened by the spread of citrus tristeza virus (CTV) (Davino et al., 2003) because sour orange shows high susceptibility to the virus. Considering this, new plantings have been established, using mostly citranges [*Citrus sinensis* (L.) Osb × *Poncirus trifoliata* (L.) Raf.] and other *Poncirus*-derived intergeneric hybrids (i.e., 'Swingle' citrumelo) that are tolerant to CTV. However, these rootstocks are susceptible to various environmental and abiotic stress conditions and/or pathogens, and the search for new rootstocks is important in many citrus-producing countries.

Citrus fruits are rich in phenolic compounds in significant quantities. Polyphenols are plant secondary metabolites derived from the shikimate and acetate pathways. The term polyphenol includes several subclasses of molecules that are grouped according to their structure as flavones, flavanones, anthocyanins, stilbenes, chalcones, coumarins, tannins, lignans, and many others (Dewick, 2002). These compounds play pivotal roles in plants, as they may act as phytoalexins, antioxidants, and antifeedants and are involved in plant pigmentation, reproductive processes and protection from UV light. As a component of the food matrix, polyphenols affect the colour, sensory, and nutritional properties of fruits and vegetables and exert a primary role in protection

against several degenerative pathologies in humans (Manach et al., 2004; Heimler et al., 2017). Moreover, polyphenol content in food is remarkably influenced by genetic and environmental factors such as climate, soil, and agronomic practices (Siracusa and Ruberto, 2014), including rootstock selection (Continella et al., 2018; Caruso et al., 2020). In this context, the accumulation of anthocyanin in several blood oranges has been investigated (Rapisarda and Giuffrida, 1994; Butelli et al., 2012; Incesu et al., 2013; Lo Piero, 2015; Caruso et al., 2016); very recently, Morales et al. (2021a) pointed out the strong influence of 8 different rootstocks on phenolics (four anthocyanins, three flavanones and five hydroxycinnamic acids) in the juices of Moro and Tarocco Rosso oranges.

Although the antioxidant potential of *Citrus* species has been extensively investigated (Zhuo et al., 2016; Cömert et Gökmen, 2018), few examples have been reported regarding the effect of rootstocks on the antioxidant activity of blood oranges. Recently, Ordonez et al. (2020) analysed the impact of four different citrus rootstocks on the antioxidant activity of Salustiana and Sanguinelli cultivars. The aim of the present work was to investigate the role of rootstock in influencing polyphenol biosynthesis and accumulation in citrus fruit. We performed evaluations over two harvest years to consider the effects of autumn and winter temperature patterns, the metabolic profile and the antioxidant potential (DPPH and ABTS) of a pigmented sweet orange variety, Tarocco Scirè, grafted on ten different rootstocks, including some recently released rootstocks (Federici et al., 2009) and others that are currently widely used in the Mediterranean basin.

2. Material and methods

2.1 Plant material

The experimental field was established in 2010 in Catania, south Italy (37°17'04.3"N; 14°53'17.3"E; 57 a.s.l.) as a complete randomized block with ten replications per rootstock. Tree

spacing was 5 x 3 m, and plants were subjected to standard cultural practices: pruning every two years, drip irrigation system, fertilizer formula NPK 2–1–1.5, and integrated weed management. The analyses were performed on Tarocco Scirè sweet orange. This variety was grafted onto ten different rootstocks: Bitters (C22), Carpenter (C54) and Furr (C57) citrandarins (hybrids of Sunki mandarin × Swingle trifoliate orange released by the University of California Riverside in 2009), F6P12® and F6P13 (hybrids of *Citrus latipes* and *Poncirus trifoliata* released by CREA-ACM in 2014), Troyer, Carrizo and C35 citranges, Swingle citrumelo, and Severinia [*Severinia buxifolia* (Poir.) Ten.].

2.2 Sampling

Fifteen fruits per plant were collected at commercial maturity (first half of March) in 2017 (year I) and 2018 (year II) from three trees per rootstock (Fig. 1). The experimental plot consisted of ten trees per rootstock, which were divided into three biological replicates, each including 3 or 4 trees. We collected 30 fruits from the 4 cardinal points for each biological replicate. Then, three juice samples obtained from the pooled juice of 30 fruits per replicate were used for chemical analyses. The juice was extracted using a domestic squeezer (Citrus Juicer JE290, Kenwood, UK), filtered before analysis, and then used for chromatographic analyses. The juice was stored at -80 °C in 50 mL disposable plastic centrifuge tubes until use.

2.3 Chemicals

All solvents and reagents used in this study were high purity laboratory solvents from VWR (Milan, Italy); HPLC grade water and acetonitrile were also obtained from VWR. Cyanidin 3-O-glucoside, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid and sinapic acid were purchased from Sigma (Sigma–Aldrich., Milan, Italy), while eriocitrin, neoeriocitrin, narirutin, hesperidin, didymin and vitexin were purchased from Extrasynthese (Lyon, France). ABTS+ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) standards were purchased from Sigma (Sigma–Aldrich, Milan, Italy).

2.4 Identification and quantification of Tarocco Scirè biochemical markers via HPLC/DAD and HPLC/ESI/MS analyses

Flavonoids (anthocyanins, flavanones, flavones) and hydroxycinnamic acid derivatives were determined in Tarocco Scirè juice obtained from the different grafting combinations.

Small juice samples (2 mL) were placed in 15 mL plastic sample tubes, and 100 µL of formic acid (98%) was added. Samples were sonicated (DU-32, Argolab) for 5 min and then centrifuged (5417R Eppendorf, Osterode, Germany) at 4000 rpm for 15 min to separate the solid portion of the juices. One of the clear supernatants was transferred into 2 mL HPLC amber vials and immediately analysed. Chromatographic analyses were carried out on an Ultimate3000 UHPLC-focused instrument equipped with a binary high-pressure pump, a photodiode array detector, a thermostatted column compartment and an automated sample injector (Thermo Fisher Scientific, Inc., Milan, Italy). Collected data were processed through a Chromeleon Chromatography Information Management System v. 6.80. Chromatographic runs were all carried out using a reverse-phase column (Gemini C_{18}, 250 x 4.6 mm, 5 µm particle size, Phenomenex Italia s.r.l., Bologna, Italy) equipped with a guard column (Gemini C_{18} 4 x 3.0 mm, 5 µm particle size, Phenomenex Italia s.r.l., Bologna, Italy). Tarocco juice polyphenols were eluted with the following gradient of B (2.5% formic acid in acetonitrile) in A (2.5% formic acid in water): 0 min: 10% B; 20 min: 35% B; 25 min: 10% B. The solvent flow rate was 1 mL/min, the temperature was kept at 25 °C, and the injector volume selected was 40 µL. DAD acquisitions were all performed according to Pannitteri et al. (2017).

To unambiguously identify the chromatographic signals and/or to confirm peak assignments, a series of HPLC/ESI/MS analyses were performed on a selected number of representative samples. In this case, aliquots (5 mL) of the centrifuged juices were freeze-dried (Lyoquest-85, Telstar Italy, Legnano, Milan, Italy), redissolved in 2 mL of HPLC grade water and transferred into 2 mL HPLC amber vials ready for ESI/MS analyses. Chromatographic analyses were performed using the same conditions described above, while ESI mass spectra were acquired by a Thermo Scientific Exactive

Plus Orbitrap MS (Thermo Fisher Scientific, Inc., Milan, Italy) using a heated electrospray ionization (HESI II) interface. LC/ESI/MS settings and mass spectra acquisition were conducted according to our previous work (Pannitteri et al., 2017). All analyses were carried out in triplicate; the results are reported in milligrams (mg) of compound per litre (L) of juice.

2.5 Antioxidant activity ($ABTS^+$ and DPPH• methods) determination

The antioxidant activity was determined by two different methods: ABTS+ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] according to Re et al., 1999 and the DPPH radical method (2,2-diphenyl-1-picrylhydrazyl) according to Brand-Williams et al., 1995.

The $ABTS^+$ and DPPH• methods were used only for 9 rootstocks since trees grafted on *Severinia buxifolia* produced small amounts of fruits. A methanol extract was prepared using 1 mL of each sample juice sample mixed with 10 mL of MeOH/water (80:20, v/v) containing 1% HCl, and the mixture was sonicated at 20 °C for 15 min and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min and centrifuged at 15000 rpm for 10 min. The radical scavenging activity was evaluated using the DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) method and the ABTS+ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation method. The decrease in absorbance of all samples was measured in a UV-visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK) at 515 nm and 730 nm for DPPH• and $ABTS^+$, respectively. A calibration curve was generated with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0 to 20 nmol) from Sigma (Madrid, Spain), and the results were expressed as mmol of Trolox equivalent per kg of fresh weight (mmol TE kg^{-1} FW).

2.6 Statistical analysis

Analysis of variance (ANOVA) was carried out using the 'stat' package of R software. A basic descriptive statistical analysis was followed by an analysis of variance test for mean comparisons. The method used to discriminate among the means (multiple range test) was Fisher's least

significant difference (LSD) procedure at a 95.0% confidence level. Principal component analysis (PCA) was calculated using the 'prcomp' function embedded in the 'stat' R package. PCA results were displayed using the R package 'factoextra'.

3. Results and discussion

3.1 Tarocco Scirè metabolic profile characterization

Peak assignments were performed by evaluating the UV-vis and mass spectral data collected; metabolite identification was confirmed with coinjections of pure analytical standards when available and corroborated with existing literature reports (Fabroni et al., 2016). Fig. S1 and Table 1 report the results obtained: of a total of 24 compounds identified, five metabolites, marked F1-F5, belong to the subclass of flavanones; compounds F6 belong to flavones, compounds C1-C12 belong to the subclass of hydroxycinnamic acids, and six compounds belong to the subclass of anthocyanins (A1-A6). Flavanones are considered *Citrus* genus markers, and in sweet orange, they almost entirely occur as eriodictyol and naringenin glycosides, with glycosylation taking place at position 7 either by rutinose or neohesperidose moiety (Siracusa and Ruberto, 2014). Hesperidin (hesperetin 7-*O*- rutinoside) and narirutin (naringenin 7-*O*- rutinoside) are broadly reported as the main flavanones present in orange juices (Barreca et al., 2016; Rapisarda et al., 2009); our results agreed with the literature, as we found hesperidin (F4) as the main compound in all the samples analysed (see Fig. S2), followed by narirutin (F3) and dydimin (F5). Eriocitrin and its isomer neoeriocitrin (F1 and F2, respectively) were the other flavanones detected, in even smaller amounts. Regarding hydroxycinnamic acids and their derivatives, similar to what was reported in Pannitteri et al. (2017), we tentatively identified and quantified two caffeic acid derivatives (caffeoyl-hexose C1 and chlorogenic acid C5), four p-coumaric acid-based metabolites (C2, C4, C7 including the free acid C11) and free sinapic acid C10. Ferulic acid is undoubtedly the most represented hydroxycinnamic acid with four different derivatives (hexoside C3, quinic acid

derivatives C6, C8 and C9, and finally the free form C12). The *C*-glycosylated flavone vitexin (F6) has been identified as the sole representative of its subclass.

The presence of anthocyanins is a particular feature of blood orange cultivars that has been extensively reported (Hillebrand et al., 2004); in Tarocco Scirè, six different pigments A1-A6 were detected (Table 1 and Fig. S1), and more specifically, two delphinidin derivatives (A1, A3) and a peonidin derivative A6 were detected. The cyanidin nucleus dominated the anthocyanin profiles with three derivatives A2, A4 and A5; A4 (cyanidin 3-*O*-(6"malonyl) glucoside) was the main compound detected within its subclass in all the samples analysed. Anthocyanin profiles correspond to those described by other authors in Tarocco orange (Liang et al., 2011; Barreca et al., 2016). Cebadera-Miranda et al. (2019) identified seven cyanidin derivatives and three delphinidin derivatives. The most frequent anthocyanin compounds recognized in blood orange were the same as those observed in this work (A1-A6) plus cyanidin 3-*O*-sophoroside (Dugo, et al., 2003; Fabroni et al., 2016; Hillebrand et al., 2004; Kelebek et al., 2008).

3.2 Total and individual metabolic changes in Tarocco Scirè juice depending on the rootstock genotype

The total anthocyanin content (mg/L) of the different Tarocco Scirè/rootstock combinations in year I is reported in Table 2. The anthocyanin content largely varied among rootstock genotypes, with C35 having the highest concentration (15.7 mg anthocyanins/L juice), followed by Furr and Bitters (14.6 mg/L and 14.3 mg/L, respectively); F6P13, Troyer and Carpenter, had even lower concentrations (12.4, 12.0 and 11.8, respectively), and were not significantly dissimilar from the previous genotypes. Overall, anthocyanins showed higher variability than the other compound classes investigated. Morales et al. (2021a) observed high anthocyanin contents in Moro in combination with C35 during the first and second harvests occurring in early and late February, detecting a marked decline at the third harvest in late March.

In our study, based only on the anthocyanin profile, the rootstock/scion combination with the lowest pigment content was indeed that with *Severinia buxifolia* (2.2 mg anthocyanins per litre). In contrast, Morales et al. (2021a) observed the lowest anthocyanin contents in Tarocco Rosso blood orange grafted onto C35, *Citrus macrophylla* and Swingle citrumelo.

The rootstock effect on secondary metabolic profiles has been investigated in different fruit tree crops. Tavarini et al. (2011) demonstrated that preharvest factors such as rootstock genotype may influence peach fruit quality, especially in terms of anthocyanin amount. Regarding grapes, Rezazad Bari et al. (2021) and Rumbaugh et al. (2021) detected a significant influence of rootstocks on the content of grape polyphenols, particularly anthocyanins.

As shown in Fig. S3, the content of individual anthocyanins displayed almost the same variations towards all of the rootstocks used, with the sole difference being values corresponding to rootstock Carpenter. Interestingly, this rootstock differentiates anthocyanins A1 and A3 (delphinidin derivatives) from compounds A2, A4 and A5 (cyanidin derivatives). A behaviour similar to that registered for delphinidin derivatives was observed for metabolite A6, the sole peonidin-based pigment detected (see Fig. S3). Previous works present in the literature on different matrices reported dissimilarities in cyanidin- and delphinidin-based pigments depending on geographical variations (Lätti et al., 2008); this phenomenon is biochemically supported, as delphinidin and cyanidin, albeit originating from the same biosynthetic route, seem to differentiate in the B-ring, the precursor coming from the shikimate pathway (see Fig. S4).

Unlike anthocyanins, flavonoids F1-F6 and hydroxycinnamic acids C1-C12 did not show substantial changes among the Tarocco Scirè/rootstock combinations (Table 2). The total flavonoid content peaked at 175 mg/L juice with the genotype Bitters, followed by Carpenter (167 mg/L) and C35 (159 mg/L), while the lowest value was registered for rootstock genotype citrumelo (126 mg/L). When considering the individual metabolite responses within this subclass, a difference emerged between eriodictyol-based flavanones (F1, F2 and F4) and narigenin-based metabolites F3 and F5, and a similar rootstock-dependent trend was observed (Fig. S2). The study

of the biosynthetic pathway of these compounds again offered a possible explanation for the differences registered; in fact, as observed for anthocyanins, naringenin and eriodictyol present slight differences in their biosynthesis, evidenced again in their B-ring derived from p-coumaroyl-CoA and caffeoyl-CoA, respectively (Fig. S4). Accordingly, the influence of the rootstock on some flavonoids, such as naringin, hesperidin, and neohesperidin, was assessed in some blood orange cultivars (Hammati et al., 2018). The flavone vitexin (F6) was the least variant compound among flavonoids (Fig. S2). Hydroxycinnamic acid derivative contents ranged from 86.4 mg/L (lowest value, genotype Carrizo) to 123 mg/L (rootstock genotype F6P13, Table 2); this rootstock generated a peak in the contents of all hydroxycinnamic acids involved excluding sinapic acid C10 (Fig. S5). As already mentioned, no significant rootstock-driven differences were observed within this subclass, with the sole exception of rootstock F6P13 (see Fig. S5).

Recent studies have identified and characterized several structural genes that are involved in the process of biosynthesis of metabolites. Anthocyanin biosynthetic genes are regulated by a complex system of transcription that was thoroughly studied by Butelli et al. (2012) and Lo Piero (2015). Little is known about the effects of rootstock at the molecular level; however, recent advancements have shown that the use of a rootstock can affect scion gene regulation (Liu et al., 2017). Moreover, it seems that scion gene expression might be induced by the movement of proteins and small RNA through the grafting point (Tzarfati et al., 2013; Wu et al., 2019).

3.3 Metabolic changes in the Tarocco Scirè/rootstock combinations over two years of cultivation

The variations in anthocyanins, flavanones and flavones, and hydroxycinnamic acids over two years are reported in Table 2. For all three classes of metabolites, significant differences between years and among the rootstocks tested were detected. The significance of the rootstock effect ranged from 1.3^{-8} (total flavanones/flavones) to 9.33^{-7} (total anthocyanins), while the year effect showed a p-value ranging from 2.4^{-6} (total hydroxycinnamic acids) to values lower than 2^{-16} (total flavanones/flavones). When the interaction of rootstock and year was investigated, the p-value

ranged from 6.1^{-11} (total flavanones/flavones) to 0.007 (total hydroxycinnamic acids). As observed for rootstock genotype, total anthocyanin content was greatly affected by minimum temperature, as explained below in the text; their relative values showed significant differences between the two years of observation, being significantly reduced in the second year: a 63.6% mean decrease with a maximum value for the rootstock genotype C35 (85.7%) and a minimum for F6P12, for which a 39.8% decrease was observed.

As already mentioned, there are many induction factors in anthocyanin biosynthesis and accumulation, including environmental factors such as photoinduction, osmotic induction, deficiencies in nitrogen and phosphorous, low pH, wounding, and pathogen infections (Chalker-Scott, 1999). In particular, for blood orange varieties, it is known that a wide day-night thermal range is required to maximize colour formation (Maccarone et al., 1983; Butelli et al., 2012; Lo Piero, 2015). Furthermore, several authors shed light on the role of temperature in the activation of anthocyanin biosynthesis and their accumulation in both the peel and pulp postharvest, which could be relevant to the effects of temperature observed before harvest (Pannitteri et al., 2017; Fabroni et al., 2020). It has been reported that storage at either 4 or 8 °C is a viable option to increase anthocyanin content in blood orange fruit (Rapisarda et al., 2001; Crifò et al., 2012). Recently, Carmona et al. (2017) showed that storage at 9 °C is more effective than storage at 4 °C in enhancing anthocyanin production and thus fruit colour in blood oranges.

In our study, there was a different temperature regime in the field. In particular, according to the analysis of the air temperatures (T) recorded during the ripening period (1 December−15 March), the hours below 9 °C did not differ significantly among years (941 in 2016-17 vs. 865 in 2017-18). Nevertheless, year 1 registered a higher frequency of hours with temperatures below 6 °C compared to the second year (454 vs. 259, respectively). The second year was characterized by high temperatures during the monitored period, which likely determined a severe fall in anthocyanin accumulation for all rootstocks studied compared to the first year in which cooler

climatic conditions occurred, determining higher pigmentation levels, in agreement with what was observed by Continella et al. (2018).

Regarding individual pigment contributions, in year II, anthocyanins A1-A6 decreased in all rootstocks, and no difference emerged between their profiles (Fig. S6). Based on what was observed, it appears that differences in accumulation for different anthocyanins are increased when a wide day-night thermal range occurs during autumn and winter, with more effective minimum temperatures below 6 °C. These findings further support the generally accepted assumption that pigment biosynthesis and accumulation, performed by extremely complex enzymatic networks, is a multiple-step process (Lo Piero, 2015; Carmona et al., 2019).

The hydroxycinnamic acid derivative content was rather stable over the two years (see Table 2 and individual contributions in supplementary material, Fig. S7). In contrast to what was observed for anthocyanins, a general increase in flavonoids was observed; a mean value of 31.4% over 10 rootstock genotypes with a maximum obtained for Severinia (107% increase) and a minimum registered for genotype C35 (8.7%), the sole exception being genotype Bitters which maintained almost the same value (Table 2). Regarding individual flavonoids, also in year II, a similar trend with respect to rootstock genotype was observed for naringenin-based metabolites F3 and F5, with flavone vitexin (F6) showing the lowest variation in the two years (Fig. S8). The different trends observed for anthocyanin and colourless flavonoid content are in accordance with the findings of Crifó et al. (2012) and Lo Piero (2015).

3.4 Determination of antioxidant potential of different Tarocco Scirè/rootstock combinations

Bioactive compounds and antioxidant activity in citrus have been previously investigated during preharvest (Di Matteo et al., 2021), processing (Lo Scalzo et al., 2004) and storage (Habibi et al., 2020). It is well known that the use of rootstocks markedly influences the antioxidant activity of citrus fruits (Aguilar-Hernández et al., 2020; Morales et al., 2021b), but few studies have been reported on blood orange in combination with rootstocks (Ordonez-Diaz et al., 2020).

Furthermore, different components in plant extracts contribute unequally to their total antioxidant ability; in fact, the antioxidant capacity depends on plant extracts and their chemical composition (Zou et al., 2016).

DPPH measurements showed wide variability in the antioxidant activity between rootstocks with Furr/Tarocco Scirè and Swingle citrumelo/Tarocco Scirè characterized by the highest and lowest values, respectively, in the first year (3.59 and 1.42 mmol TE kg^{-1} FW) (Table 3). In the second year, the antioxidant activity of the orange juice sample decreased, except for Carpenter and Swingle citrumelo, both of which showed similar values between the years. $ABTS^{+}$ analyses were remarkably affected by the rootstock in the first year. Troyer/Tarocco Sciré showed the highest value (4.27 mmol TE kg^{-1} FW), followed by Furr (3.83 mmol TE kg^{-1} FW) and F6P13 (3.46 mmol TE kg^{-1} FW) (Table 3). On the other hand, Swingle citrumelo/Tarocco Sciré showed the lowest value, even if it was not significantly different from that of Carrizo, Bitters and C35. During the second year, antioxidant activity decreased in all rootstocks, with values ranging from 1.12 (Furr) to 1.40 (F6P12) mmol TE kg^{-1} FW, and no significant differences among combinations were detected (Table 3).

When the interaction of rootstock and year was investigated, the p-value was significant for both ABTS+ ($<2^{-16}$) and DPPH• (4.29^{-16}). Antioxidant activity was related to the fact that higher concentrations of both anthocyanins and hydroxycinnamic acids were observed in the first harvest year. Indeed, the decrease in antioxidant activity was dependent on many factors, including environmental conditions. Differences in the scion/rootstock interaction were observed in antioxidant activity in different species (Forcada et al., 2019; Ordóñez-Díaz et al., 2020; Aguilar-Hernández et al., 2020). The different methods for determining the antioxidant properties of the juice were incomparable, as already highlighted in previous studies (Zou et al., 2016); however, both methodologies are considered reliable for the determination of antioxidant potential, and under our conditions, the influence of the different rootstocks on the biosynthesis and accumulation of antioxidant-related compounds was clearly evident.

3.5 Principal component analysis (PCA)

To further dissect the differences among the rootstocks tested in terms of the production of metabolites, a multifactorial analysis was conducted on the two years of the data. For the phenotyping carried out in 2017 (harvest year I) (Fig. 2A), the first two principal components explained 64.5% of the cumulative phenotypic variability (Dim1 = 34.9%, Dim2 = 29.6%). While Dim1 was mainly associated with the quantity of metabolites produced (with Dim1 > 0 associated with lower production as observed for F6P12, Citrumelo and Carrizo, Table 2), Dim2 allowed a more precise differentiation according to the different classes of metabolites. Rootstocks characterized by high production of anthocyanins, such as C35, Bitters, Furr and Carpenter, clustered in the upper-left quadrant (Dim1<0 and Dim2 > 0), while samples showing higher synthesis of hydroxycinnamic acids were plotted in the lower-left quadrant (Dim1<0 and Dim2 < 0). A more complex pattern was observed for the loading projections of the flavanones and flavones, with vitexin and eriocitrin pointing towards the left and down, respectively, and the remaining four located towards the upper part of the graph (Fig. 2 A, Table 2). For the analysis performed in 2018 (harvest year II), the first two principal components explained 64% of the total phenotypic variability. Similar to what was observed in 2017, accessions showing high accumulation of metabolites were characterized by negative Dim1 values (Fig. 2 B and Table 2). Moreover, in the second year, the loadings related to the anthocyanins showed high consistency, with all projected towards the same PCA quadrant (lower-left), while flavanones, flavones, and hydroxycinnamic acids pointed mainly towards the upper-left quadrant (Fig. 2B). An exception is represented by eriocitrin, which is characterized by an orthogonal projection compared to the other five flavanones and flavones. The high positive correlation among the six anthocyanin components was further confirmed by the heatmap depicted in Fig. 3, with correlations ranging from 0.91 (delphinidin 3-O-glucoside and peonidin 3-O-(6”- malonyl)glucoside) to 0.99 (delphinidin 3-O-(6”- malonyl)glucoside and cyanidin 3-O-(6”- malonyl)glucoside). Fig. 3 shows the occurrence of

a negative correlation between anthocyanins and most flavanones, flavones, and hydroxycinnamic acids.

4. Conclusions

In this work, individual metabolite variations in Tarocco Scirè pigmented orange were investigated as a function of the rootstock genotype used and in relation to the minimum temperature changes over two years; the results showed significant differences in accumulation both between subclasses and within each compound, and such differences can be ascribed to biosynthetic factors. The effect of the environment and, specifically, of the low temperatures, on juice pigmentation has been confirmed: in fact, total anthocyanin content estimation revealed a substantial difference between the two years of study, with a double-fold increase in the colder year. As expected, the different methods used to determine the antioxidant potential of Tarocco Sciré juice gave incomparable results but provided a reliable indication of the behaviour of the different rootstocks/scion combinations. More data seem to be necessary to achieve an unequivocal interpretation of the antioxidant activity of these products.

Overall, the rootstocks C35, Bitters, Carpenter and Furr were the most interesting for pigmented oranges under the tested conditions, as they positively enhanced fruit pulp anthocyanin content. On the other hand, some other rootstocks were considered unsatisfactory for further evaluation because their effect on qualitative fruit parameters was substandard.

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All authors have read and agreed to the published version of the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aguilar-Hernández, M.G., Sánchez-Rodríguez, L., Hernández, F., Forner-Giner, M.Á., Pastor-Pérez, J.J., Legua, P. (2020). Influence of new citrus rootstocks on lemon quality. Agron, 10(7):974.

Barreca D., Gattuso, G., Laganà, G., Leuzzi, U., Bellocco, E., (2016). C- and O-glycosyl flavonoids in Sanguinello and Tarocco blood orange (*Citrus sinensis* (L.) Osbeck) juice: Identification and influence on antioxidant properties and acetylcholinesterase activity, Food Chem, 196, 619-627.

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. J Food Sci Technol, 28, 25–30.

Bowman, K.D., McCollum, G., Albrecht, U., (2016). Performance of 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) on 17 rootstocks in a trial severely affected by huanglongbing. Sci Hortic, 201, 355-361.

Butelli, E., Licciardello, C., Zhang, Y., Liu, J., Mackay, S., Bailey, P., Reforgiato Recupero, G., Martin, C., (2012). Retrotransposons control fruit specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell, 24, 1242–1255

Carmona, L., Alquézar, B., Marques, V. V., Peña, L. (2017). Anthocyanin biosynthesis and accumulation in blood oranges during postharvest storage at different low temperatures. Food Chem, 237, 7–14.

Carmona, L., Alquezar, B., Tarraga, S., Pena L., (2019). Protein analysis of Moro blood orange pulp during storage at low temperatures. Food Chem, 277, 75-83.

Caruso, M., Continella, A., Modica, G., Pannitteri, C., Russo, R., Salonia, F., Arlotta, C., Gentile, A., Russo, G. (2020). Rootstocks influence yield precocity, productivity, and pre-harvest fruit drop of mandared pigmented mandarin, Agron,10(9):1305.

Caruso, M., Ferlito, F., Licciardello, C., Allegra, M., Concetta Strano, M., Di Silvestro, S., Russo, P., Pietro Paolo, D., Caruso, P., Las Casas, G., Stagno, F., Torrisi, B., Roccuzzo, G.,

Reforgiato Recupero, G., Russo, G. (2016). Pomological diversity of the Italian blood orange germplasm, Sci Hortic, 213, 331-339.

Cebadera-Miranda L, Domínguez L, Dias MI, Barros L, Ferreira ICFR, Igual M, Martínez-Navarrete N, Fernández-Ruiz V, Morales P, Cámara M., (2019). Sanguinello and Tarocco (*Citrus sinensis* [L.] Osbeck): Bioactive compounds and colour appearance of blood oranges. Food Chem. 1; 270, 395-402.

Chalker- Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol, 70: 1-9.

Cömert, V., Gökmen, E.D., (2018). Evolution of food antioxidants as a core topic of food science for a century Food Res. Int., 105, 76-93.

Continella, A., Pannitteri, C., La Malfa, S., Legua, P., Distefano, G., Nicolosi, E., Gentile, A. (2018). Influence of different rootstocks on yield precocity and fruit quality of 'Tarocco Scirè' pigmented sweet orange Sci Hortic, 230, 62-67.

Crifò, T., Petrone, G., Lo Cicero, L., & Lo Piero, A. R. (2012). Short cold storage enhances the anthocyanin contents and level of transcripts related to their biosynthesis in blood oranges. Journal of Agriculture and Food Chem, 60(1), 476–481.

Davino, S., Davino, M., Sambade, A., Guardo, M., Caruso, A. The first citrus tristeza virus outbreak found in a relevant citrus producing area of Sicily, Italy. Plant Dis. 2003, 87, 285–289.

Dewick Paul M., (2002). The biosynthesis of C5–C25 terpenoid compounds. Nat Prod Rep, 19, 181-222.

Di Matteo, A., Di Rauso, Simeone G., Cirillo, A., Rao, M. A., Di Vaio, C., (2021). Morphological characteristics, ascorbic acid and antioxidant activity during fruit ripening of four lemon (*Citrus limon* (L.) Burm. F.) cultivars, Sci Hortic, 276,109741.

Dugo, P., Mondello, L., Morabito, D., & Dugo, G. (2003). Characterization of anthocyanin fraction of Sicilian blood orange juice by Micro-HPLC-ESI/MS. J Agr Food Chem, 51, 1373–1376.

Fabroni, S., Amenta, M., Timpanaro, N., Todaro, A., Rapisarda. P., (2020). Change in taste-altering non-volatile components of blood and common orange fruit during cold storage. Food Res Int, 131, 108916.

Fabroni, S., Ballistreri, G., Amenta, M., & Rapisarda, P. (2016). Anthocyanins in different Citrus species: An UHPLC-PDA-ESI/MSn-assisted qualitative and quantitative investigation. J Sci Food Agr, 96, 4797–4808.

Federici, C.T., Kupper, R.S., Roose, M.L., (2009). http://plantbiology.ucr.edu/faculty/new %20citrus%20rootstocks%202009. pdf.

Forcada, C.F., Reig, G., Giménez R., P. Mignard, L. Mestre, M.Á. (2019). Moreno sugars and organic acids profile and antioxidant compounds of nectarine fruits influenced by different rootstocks Sci. Hortic., 248, 145-153.

Forner-Giner M.A., Continella A., Grosser J.W. (2020). Citrus Rootstock Breeding and Selection. In: Gentile A., La Malfa S., Deng Z. (eds) The Citrus Genome. Compendium of Plant Genomes. Springer, Cham., 49-74

Habibi, F., Ramezanian, A., Guillén, F., Castillo, S., Serrano, M., Valero, D., (2020). Changes in bioactive compounds, antioxidant activity, and nutritional quality of blood orange cultivars at different storage temperatures. Antioxidants, 9, 1016.

Heimler, D., Romani, A., Ieri, F. (2017). Plant polyphenol content, soil fertilization and agricultural management: a review. Eur Food Res Technol, 243, 1107–1115.

Hemmati, N., Ghasemnezhad, A., Moghaddam, J.F., Ebrahimi, P., (2018). Variation in the content of bioflavonoids of orange as affected by scion, rootstock, and fruit part. Acta Physiol Plant 40, 83.

Hillebrand, S., Schwarz, M., & Winterhalter, P. (2004). Characterization of anthocyanins and pyranoanthocyanins from blood orange [*Citrus sinensis* (L.) Osbeck] juice. J Agr Food Chem, 52, 7331–7338.

Incesu, M., B. Çimen, T. Yeşiloğlu and Bilge Yilmaz, (2013). Rootstock effects on yield, fruit quality, rind and juice color of 'Moro' blood orange. J Food Agric Environ, 11, 867-871.

Kelebek, H., Canbas, A., & Selli, S. (2008). 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, 70, 1710–1716

Lätti, A.K., Riihinen, K.R., Kainulainen, P.S., (2008). Analysis of anthocyanin variation in wild populations of bilberry (*Vaccinium myrtillus* L.) in Finland. J Agr Food Chem, 56,190-6.

Liang, L., Shao-Qian, C., & Si-Yi, P. (2011). Thermal degradation kinetics of three kinds of representative anthocyanins obtained from blood orange. AGRIC SCI China, 10(4), 642–649.

Lo Piero A.R., (2015). The state of the art in biosynthesis of Anthocyanins and its 460 regulation in pigmented sweet orange [(*Citrus sinensis*) L, Osbeck]. J Agr Food Chem, 63, 4031-4041.

Lo Scalzo, R., Iannoccari, T., Summa, C., Morelli, R., Rapisarda, P., (2004). Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice, Food Chem, 85,41-47.

Liu, X.Y., Li, J., Liu, M.M., Yao, Q., Chen, J.Z., (2017). Transcriptome profiling to understand the effect of citrus rootstocks on the growth of “Shatangju” mandarin. PLoS One. 12, 1–22

Maccarone, E., Maccarone, A., Perrini, G., Rapisarda, P. (1983). Anthocyanins of the Moro orange juice. Ann Chim, 73, 533– 539

Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L. (2004). Polyphenols: food sources and bioavailability. Am J Clin Nutr, 79, 727–747.

Morales J., Bermejo A., Navarro P., Forner-Giner M.Á., Salvador A., (2021a). 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, 342,128305.

Morales, J., Salvador, A., Besada, C., Navarro, P., Bermejo, A., (2021b). Physico-chemical, sensorial and nutritional quality during the harvest season of 'Tango' mandarins grafted onto Carrizo Citrange and Forner-Alcaide no. 5. Food Chem., 339, 127781.

Moreno, P., Ambrós, S., Albiach- Martí, M.R., Guerri, J. And Peña, L, (2008), *Citrus tristeza virus*: a pathogen that changed the course of the citrus industry. Mol Plant Pathol, 9, 251-268.

Ordóñez-Díaz, J.L., Hervalejo, A., Pereira-Caro, G., Muñoz-Redondo, J.M., Romero-Rodríguez, E., Arenas-Arenas, F.J., Moreno-Rojas, J.M., (2020). 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 8, 1212.

Pannitteri, C., Continella, A., Lo Cicero, L., Gentile, A., La Malfa, S., Sperlinga, E., Napoli, E.M., Strano, T., Ruberto, G., Siracusa, L., (2017). Influence of postharvest treatments on qualitative and chemical parameters of Tarocco blood orange fruits to be used for fresh chilled juice. Food Chem., 1;230:441-447.

Rapisarda, P., Bellomo, S.E., Intelisano S., (2001). Storage temperature effects on blood orange fruit quality. J Agr Food Chem, 49,3230-3235.

Rapisarda, P., Fabroni, S., Peterek, S., Russo, G., & Mock, H.-P. (2009). Juice of new citrus hybrids (*Citrus clementina* Hort. ex Tan. x *C. sinensis* L. Osbeck) as a souce of natural antioxidants. Food Chem, 117, 212–218.

Rapisarda, P., Giuffrida, A., (1994). Anthocyanins level in Italian blood oranges. Proc. Int. Soc. Citricult 3, 1130–1133.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med, 26, 1231–1237.

Rezazad Bari, L., Ghanbari, A., Darvishzadeh, R., Giglou, M.T., Baneh, H.D., (2021). Discernment of grape rootstocks base on their response to salt stress using selected characteristics in combination with chemometric tools. Food Chem, 365, 130408.

Rodríguez-Gamir, J., Primo-Millo, E., Forner, J.B., Forner-Giner, M.A., (2010). Citrus rootstock responses to water stress. Sci Hortic, 126, 95-102.

Rumbaugh, A.C., Girardello, R.C., Cooper, M.L., Plank, C., Kurtural, S.K., Oberholster, A., (2021). Impact of rootstock and season on red blotch disease expression in Cabernet Sauvignon (*V. vinifera*), Plants, 10, 1583.

Siracusa, L., Ruberto, G., (2014). Chapter II: Plant polyphenol profiles as a tool for traceability and valuable support to biodiversity. In Polyphenols in plants: Isolation, purification and extract preparation, Elsevier Books, 15–33.

Tavarini, S.G.M., Tomas-Barberan, F., Buendia, B., Remorini, D., Massai, R., Degl'Innocenti, E., Guidi, L., (2011). Effects of water stress and rootstocks on fruit phenolic composition and physical/chemical quality in Suncrest peach. Ann Appl Biol, 158, 226-233.

Tzarfati, R., Ben-Dor, S., Sela, I., Goldschmidt, E.E., (2013). Graft-induced changes in microRNA expression patterns in Citrus leaf petioles. Open Plant Sci J., 7, 17–23.

Wu, J., Cao, J., Su, M., Feng, G., Xu, Y., Yi, H., (2019). Genome-wide comprehensive analysis of transcriptomes and small RNAs offers insights into the molecular mechanism of alkaline stress tolerance in a citrus rootstock. Hortic Res., 6, 33.

Zou Z., Wanpeng X., Yan H., Chao N., Zhiqin Z., (2016). Antioxidant activity of Citrus fruits, Food Chem, 196, 885-896.

Captions of figures

Figure 1. Fruits of Tarocco Sciré cultivar harvested at maturity grafted onto 10 rootstocks. From left to right: Carrizo citrange (1), Troyer citrange (2), C35 (3), Bitters (4), Carpenter (5), Furr (6), Swingle citrumelo (7), Severinia buxifolia (8), F6P12 (9), F6P13 (10).

Figure 2. Principal component analysis (PCA) of the 26 parameters analysed in year I (A) and year II (B). Phenolic compounds, antioxidant activity and rootstocks are coloured as categories as specified in the figure legend.

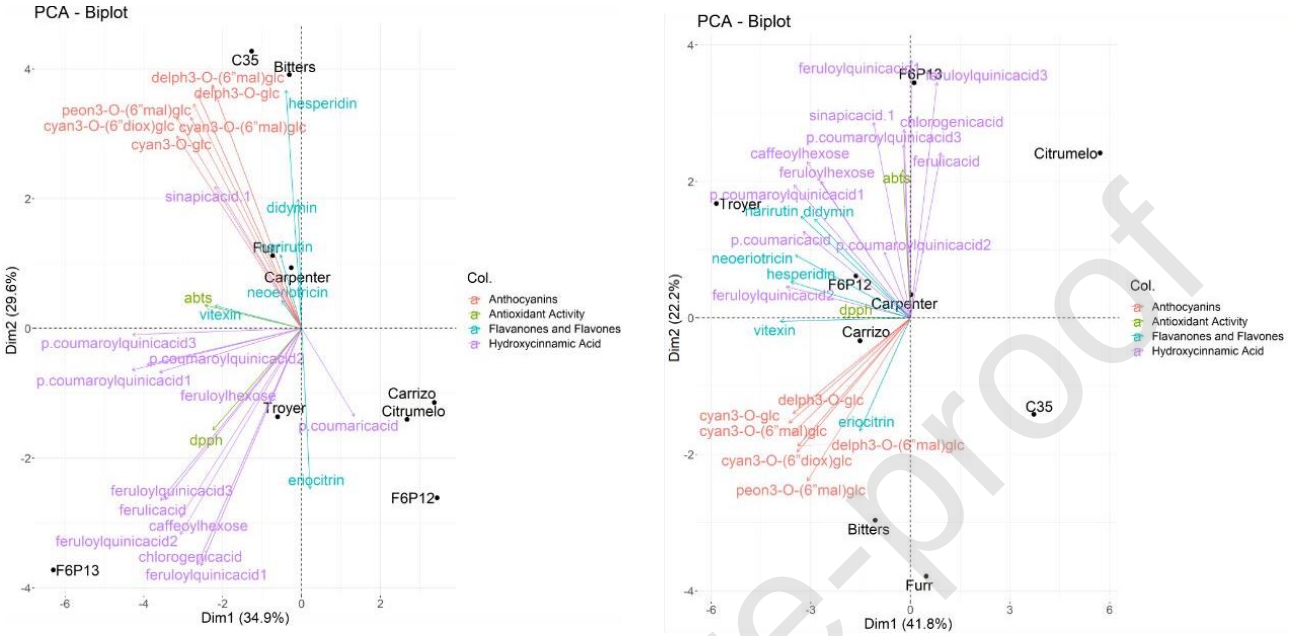

Figure 3. Heatmap of the pairwise correlations between individual phenolic compounds and antioxidant activity (26 traits in total). Colors reflect the correlation level between two traits ranging from dark red (positive correlation) to white (no correlation) and dark blue (negative correlation). Correlations values exceeding the significance threshold level (p value > 0.05) were crossed.

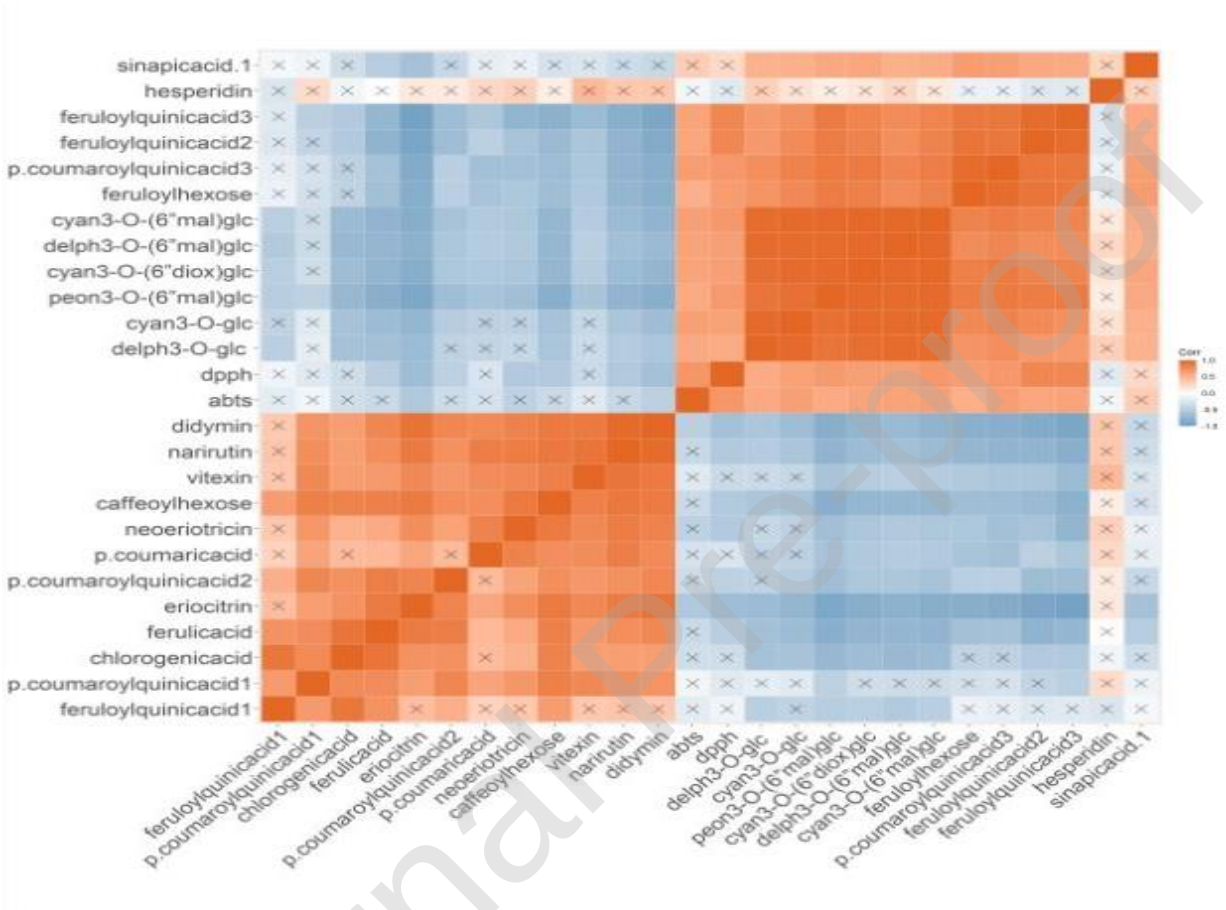

Table 1. Peak list and diagnostics, as obtained through HPLC/DAD and HPLC/ESI-MS analyses, for Tarocco Scirè orange juice biochemical markers. Peak letters and numbers refer to Figure S1.

Anthocyanins - 520 nm					
Compound code	**Rt, min[a]**	**Compound identification**	**λmax, nm[b]**	**MW**	**MS (ESI^+/ESI^-) data, m/z[c]**
A1	7.00	delphinidin 3-O-glucoside	524, 320sh, 278	465	465 $(M)^+$*
A2	7.92	cyanidin 3-O-glucoside[d]	515, 278	449	449 $(M)^+$, 287*
A3	9.62	delphinidin 3-O-(6”- malonyl)glucoside	520, 328, 284	551	551 $(M)^+$*
A4	10.68	cyanidin 3-O-(6”- malonyl)glucoside	517, 330, 279	535	535 $(M)^+$, 449*, 287
A5	11.16	cyanidin 3-O-(6”- dioxalyl)glucoside	517, 278	593	593 $(M)^+$, 449*, 287
A6	12.69	peonidin 3-O-(6”- malonyl)glucoside	518, 330, 278	549	549 $(M)^+$, 463*, 301
	Flavanones and flavones - 280 nm				
F1	12.08	eriocitrin[d]	328, 283	596	595 $(M-H)^-$
F2	13.97	neoeriocitrin[d]	328, 284	596	595 $(M-H)^-$
F3	15.41	narirutin[d]	329, 284	580	579 $(M-H)^-$,433*, 271
F4	16.57	hesperidin[d]	326, 284	610	609 $(M-H)^-$ *, 463, 301
F5	21.43	didymin[d]	328,283	594	593 $(M-H)^-$
F6	10.19	vitexin[d]	338, 270	432	431 $(M-H)^-$ *,311
	Hydroxycinnamic acids - 330 nm				
C1	5.43	caffeoyl-hexose	328, 300sh	342	341 $(M-H)^-$ *, 179
C2	6.04	p-coumaroylquinic acid 1[e]	312	338	337 $(M-H)^-$*, 191
C3	6.73	feruloyl-hexose	326, 300sh	356	355 $(M-H)^-$
C4	7.17	p-coumaroylquinic acid 2[e]	312	338	337 $(M-H)^-$, 191*
C5	8.05	chlorogenic (3- caffeoylquinic) acid[d]	325, 300sh	354	353 $(M-H)^-$*, 191
C6	8.67	feruloylquinic acid 1[e]	323, 300sh	368	367 $(M-H)^-$, 191*
C7	8.85	p-coumaroylquinic acid 3[e]	312	338	337 $(M-H)^-$, 191*
C8	9.62	feruloylquinic acid 2[e]	322, 300sh	368	367 $(M-H)^-$*, 191
C9	10.02	feruloylquinic acid 3[e]	323, 300sh	368	367 $(M-H)^-$, 191*
C10	12.31	sinapic acid[d]	323	224	223 $(M-H)^-$
C11	14.26	p-coumaric acid[d]	311	164	163 $(M-H)^-$
C12	15.23	ferulic acid[d]	323, 298sh	194	193 $(M-H)^-$

[a] as average of 10 rootstocks x 3 replicates x 2 years = 60 analytical measurements; [b] from HPLC; [c] main peaks marked with an asterisk; [d] co-injection with pure analytical standards; [e] correct isomer not determined

Table 2. Content (mg L^{-1}) of Tarocco Scirè juice anthocyanins, flavanones and flavones and hydroxycinnamic acids measured on fruits on different rootstocks in year I and II. P value resulting from the two-way analysis of variance (ANOVA) considering rootstock, year and their interaction as fixed effect.

	Total anthocyanins (mg L^{-1})			**Total flavanones/flavones (mg L^{-1})**			**Total hydroxycinnamic acids (mg L^{-1})**		
rootstock genotype	year I[a]	year II	variation %	year I	year II	variation %	year I	year II	variation %
Carrizo	9.1 bc	4.8 abcd	- 46.8	132 de	186 bc	40.6	86.4 b	82.6 b	- 4.5
Troyer	12.0 ab	6.7 a	- 43.8	154 abcd	204 b	32.5	97.2 b	94.0 b	- 3.2
Furr	14.6 a	5.1 abc	- 65.1	144 cde	176 cde	22.6	93.9 b	77.8 b	- 17.1
Citrumelo	9.4 bc	1.7 ef	- 82.0	126 e	160 e	27.2	91.8 b	85.6 b	- 6.8
Carpenter	11.8 ab	4.0 abcde	- 66.1	167 ab	185 bc	11.2	96.3 b	86.2 b	- 10.5
F6P13	12.4 ab	2.6 cdef	- 78.6	135 de	186 cd	37.3	123 a	90.8 a	- 26.2
S. buxifolia	2.2 d	0.7 f	- 69.4	133 de	275 a	107.3	94.6 b	83.6 b	- 11.7
F6P12	5.9 cd	3.6 bcde	- 39.8	151 bcd	191 bc	26.7	88.8 b	84.0 b	- 5.4
C35	15.7 a	2.2 def	- 85.7	159 abc	173 de	8.8	92.6 b	78.7 b	- 15.1
Bitters	14.3 a	6.0 ab	- 58.3	175 a	174 cde	-0.4	91.0 b	83.0 b	- 8.8
mean	***10.7±4.2***	***3.7±1.9***	***- 63.6***	***148±16***	***191±32***	***31.4***	***95.6±10.2***	***84.6±4.9***	***- 10.9***
ANOVA-factors	***p value***								
rootstock	***< 0.0001***			***< 0.0001***			***< 0.0001***		
year	***< 0.0001***			***< 0.0001***			***< 0.0001***		
rootstock*year	***0.002***			***< 0.0001***			***0.007***		

[a] Values followed by the same letter, within the same column, are not significant different according to Fisher's Least Significant Difference (LSD) procedure at 95.0 % confidence level.

Table 3. Content (mmol TE kg^{-1} FW) of DPPH• and $ABTS^{+}$ measured in Tarocco Sciré juices from fruits grown on different rootstocks in year I and II. P value resulting from the two-way analysis of variance (ANOVA) considering rootstock, year and their interaction as fixed effect.

	DPPH• (mmol TE kg^{-1} FW)			**$ABTS^{+}$ (mmol TE kg^{-1} FW)**		
Rootstock genotype	year I	year II	variation %	year I	year II	variation %
Bitters	2.68 c	1.70 ab	-36.45	2.16 fg	1.38 a	-36.17
C35	2.37 d	1.81 a	-23.72	2.25 f	1.32 a	-41.43
Carpenter	1.62 f	1.69 ab	4.08	2.59 e	1.36 a	-47.33
Carrizo	2.24 de	1.64 ab	-26.69	2.08 gh	1.39 a	-33.12
Citrumelo	1.46 g	1.47 b	0.84	1.94 h	1.35 a	-30.44
F6P12	2.20 e	1.66 ab	-24.58	2.87 d	1.40 a	-51.34
F6P13	3.48 b	1.76 ab	-49.48	3.46 c	1.37 a	-60.32
Furr	3.59 a	1.60 ab	-55.48	3.83 b	1.12 a	-70.67
Troyer	2.10 e	1.79 ab	-14.93	4.27 a	1.30 a	-69.48
mean	***2.23±1.04***	***1.68±0.10***	***34.24± 20.24***	***2.83±0.84***	***1.33±0.08***	***-48.92±15.18***
ANOVA-factors	***p value***					
rootstock	***< 0.0001***			***< 0.0001***		
year	***< 0.0001***			***< 0.0001***		
rootstock* year	***< 0.0001***			***< 0.0001***		

Values followed by the same letter, within the same column, are not significant different according to Fisher's Least Significant Difference (LSD) procedure at 95.0 % confidence level.