# 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.

Julia MORALES, Almudena BERMEJO, Pilar NAVARRO, María Ángeles FORNER-GINER, Alejandra SALVADOR*.

InstitutoValenciano de Investigaciones Agrarias, Postharvest Department,

Carretera Moncada-Náquera, Km. 4.5, 46113, Moncada, Valencia, Spain

*Corresponding author. A. Salvador

*E-mail address*: salvador_ale@gva.es

Instituto Valenciano de Investigaciones Agrarias, Postharvest Department, Moncada, 46113,

Valencia, Spain. Tel.: +34-963-424-000, Fax: +34 963-424-001

**Abstract**

The physico-chemical quality parameters (external and internal color, firmness, acidity, total soluble solids, anthocyanins, sugars, hydroxycinnamic acids and flavanones) of 'Moro' and 'Tarocco Rosso' blood oranges grafted onto eight different rootstocks at three harvest time were studied. The rootstocks were 'Carrizo', 'C-35', 'Cleopatra' mandarin, '*Citrus volkameriana'*, '*Citrus macrophylla'*, 'Swingle' citrumelo, 'Forner-Alcaide 5' and 'Forner-Alcaide 13'. All studied parameters were highly rootstock/scion-dependent and showed changes throughout harvest. The content of the main anthocyanins revealed their relation with internal fruit color in both cultivars. The rootstocks that led to fruit with the lowest anthocyanins displayed the least sucrose content. The differences detected in the amount of hydroxycinnamic acids (chlorogenic, ferulic and sinapic) and flavanones (hesperidin, narirutin and didymin) related to anthocyanins content, explained phenylpropanoid pathway.

**Keywords**

Quality, citrus fruit, pigmented oranges, firmness, color, phenolic compounds

## 1. Introduction

In the Mediterranean citrus production area, the importance of blood oranges (*Citrus sinensis* L. Osbeck) is growing given their healthy qualities. Blood oranges are characterized by their high content of anthocyanins, water-soluble polyphenolic compounds which, apart from conferring fruit its characteristic red color, are related to human health properties due to their antioxidant activity (Habibi, Ramezanian, Guillén, Serrano & Valero, 2020). These anthocyanins exert potential action against certain diseases and reduce the risk of several cancer types, heart disease, and low-density lipoprotein (LDL) cholesterol accumulation (Hou, 2003). Indeed, the main aspect that consumers seek in blood oranges is their internal purple color as they associate natural red pigments with health benefits. However, color is related to the content and composition of anthocyanins present in blood oranges, which vastly vary depending on variety, maturity, cultivation region and many environmental conditions, overall ambient temperature and irradiation (Rapisarda, Bellomo & Intelisano, 2001; Crifò, Puglisi, Petrone, Reforgiato Recupero & Lo Piero, 2011). Much research has focused on studying the composition of anthocyanins of a wide variety of blood orange cultivars (Kelebek, Canbas & Selli, 2008), and on understanding the biosynthesis pathway and the factors regulating it as these pigments play an important physiological role in plants, such as protecting them against abiotic stress conditions and pathogen infections (Zhang, Butelli & Martin, 2014). Regarding the anthocyanins pathway, other important compounds markedly influence fruit quality, such as hydroxycinnamic acids, flavanones and sugars (Rapisarda, Carollo, Fallico, Tomaselli & Maccarone, 1998; Li, Van den Ende & Rolland, 2014; Cebadera-Miranda et al., 2019). Although consuming blood oranges has traditionally been in the form of juice, the citrus market is increasingly demanding pigmented oranges for fresh fruit. This means that blood orange production focuses on obtaining fruit that meets the external and internal quality requirements demanded by consumers. Besides internal fruit color, attention must be paid to other quality attributes required by consumers. Firmness is a parameter that has gained much importance in citrus fruit commercialization in recent years. Indeed, blood oranges usually present low firmness values compared to blond oranges, which may be a quality limitation, especially when post-harvest fruit is submitted to long-term shipping or prolonged storage (Pallottino, Menesatti, Lanza, Strano, Antonucci & Moresi, 2012). As in most citrus fruit, sugars and acids content influences the sensory quality of blood oranges and changes during harvest. Most blood oranges maintain a higher acidity content than blond oranges during harvest (Fabroni, Amenta, Timpanaro, Todaro & Rapisarda, 2020).

In citrus, one important aspect to consider is the rootstock onto which a cultivar is grafted because it may influence several tree growth and development aspects, including yield, fruit quality, and tolerance to stress caused by biotic and abiotic factors (Filho, Espinoza-Núñez, Stuchi & Ortega, 2007). The search for new better-performing citrus rootstocks than those normally used is one of the main challenges faced by the citrus industry in many countries. In this context, a citrus rootstocks breeding program is being developing at the Instituto Valenciano de Investigaciones Agrarias (IVIA) (Valencia, Spain) and one important objective of this program is to study the effect of rootstock on blood orange. Indeed, the influence of rootstock on blood oranges was recently addressed, with studies focusing on different aspects like yield and fruit quality (Reforgiato Recupero, Russo, Recupero, Zurru, Deidda & Mulas, 2009; Incesu, Çimen, Yesiloglu & Yilmaz, 2013; Continella et al., 2018). Nevertheless, the influence of a specific rootstock on fruit quality is highly dependent on both cultivar and climate conditions. In citrus fruit, and specifically in blood oranges, harvest can be a determining factor of fruit quality. Moreover, as anthocyanins are one of the most valuable parameters in blood oranges, studies about the effect of rootstocks on these pigments are needed, and more studies are necessary to evaluate the way that rootstock influences quality parameters to obtain a better rootstock/scion combination.

The objective of this study was to evaluate the effect of rootstock on the fruit quality of the two blood orange varieties cultivated in Spain, ‘Moro’ and ‘Tarocco Rosso’, throughout harvest. Besides evaluating changes in the main fruit quality attributes, an in-depth study into anthocyanins, hydroxycinnamic acids, sugars and flavanones composition was conducted.

## 2. Material and Methods

### 2.1 Fruit Samples

Fruit samples of ‘Tarocco Rosso’ and ‘Moro’ were collected from an experimental orchard in Museros (39º 57’ 70.95”N; -0º 36’ 06.65” W) on the Spanish Mediterranean coast with sandy soil. This orchard had $EC_{1\text{-}5}$ of 0.407 dS $m^{-1}$ (20ºC) and a pH of 7.05. Relative humidity was above 65%.

Both cultivars were grafted onto ‘Carrizo’ (CC) and ‘C-35’ (C35) citranges, ‘Cleopatra’ mandarin (CL), *Citrus volkameriana* (VK), *Citrus macrophylla* (M), ‘Swingle’ citrumelo (CT) and two hybrid selections, ‘Forner-Alcaide 5’ (FA5), ‘Forner-Alcaide 13’ (FA13), obtained in the rootstock breeding program carried out at the IVIA.

The fruit taken from five trees grafted onto each rootstock, collecting 20 fruits per tree, were harvested at three harvest times: February 7 (H1), February 25 (H2) and March 26 (H3). After each harvest time, fruit was transported to the IVIA where the following physico-chemical analyses were

carried out: external and internal color, firmness, juice, total soluble solids, acidity, maturity index, sugars and phenolic compounds (anthocyanins, hydroxycinnamic acids and flavanones).

### 2.2 Determination of fruit quality attributes

Color determination was made on rind and juice by a Minolta colorimeter (model CR-300, Minolta Co. Ltd, Osaka, Japan). Rind color was measured over 15 fruits per rootstock/scion combination. As blood orange rind coloration is not homogeneous in whole fruit, two measurements were taken in the dark area and two others in the bright area along the equatorial fruit part. Juice color was determined with three juices of five fruit each. The mean values for the 'L', 'a' and 'b' Hunter parameters were calculated with each fruit and expressed as the Citrus Color Index (CCI=1000a/Lb) (Jimenez-Cuesta, Cuquerella & Martínez-Jávega, 1981).

Firmness measurements were taken by a Universal Testing Machine (model 3343, Instron Limited, Buckinghamshire, England) using 15 fruits per rootstock/scion combination. The results were expressed as the percentage of millimeters of the fruit deformation that resulted from a 10 N force on the longitudinal axis at constant speed.

In each fruit lot, three samples of five fruit each were squeezed by an electric juice extractor with a rotating head (Lomi®, Model 4, Lorenzo Miguel, S.L., Madrid, Spain). Titratable acidity (TA) was determined by titration with 0.1 N NaOH solution using phenolphthalein as the indicator and expressed as g citric acid/100 mL of juice. The total soluble solid content (TSS) in juice was measured by a digital refractometer (Atago PR-1, Atago Co., Ltd, Tokyo, Japan) and data were expressed as ºBrix.

### 2.3. Sugars analysis

Extraction and characterization was carried out following the procedure as previously described by Bermejo, Pardo, Morales, and Cano (2016). Carbohydrates were analyzed by HPLC equipped with a refractive index detector (Waters, Barcelona, Spain) using a 5-µm Tracer Carbohydrate column (250 mm x 4.5 mm) (Teknokroma, Barcelona, Spain). The mobile phase was acetonitrile:water (75:25) at a flow rate of 1 mL/min. Compounds were identified by comparing their retention time with standards and quantified using an external calibration curve. Fructose, glucose and sucrose sugars were obtained from Sigma (Sigma Co., Barcelona, Spain). The results were expressed as g/L.

## 2.4. Phenolic compounds analysis

### 2.4.1. Anthocyanins

Sample extraction was performed following the procedure as previously described by Laribi et al. (2013). Anthocyanins were analyzed by HPLC equipped with a photodiode array detector (Merck Hitachi, Germany) in a Licospher 100 RP-18 column, preceded by a precolumn (4 mm x 4 mm) and confirmed by HPLC-MS using an HPLC equipped with a ZQ2000 mass detector (Waters, Barcelona, Spain). The mobile phase was 5% formic acid (A) and methanol (B) in a linear gradient starting with 15% B and reaching 35% B in 30 min at a flow rate of 1 mL/min. Chromatograms were recorded at 520 nm absorbance. The HPLC-MS analysis was run and worked under electrospray ion positive conditions. Full data acquisition was performed by scanning 200 to 800 uma in the centroid mode. Cyanidin 3-glucoside ($R_T$=12.5 min $[MH]^+$ 449 m/z), was identified by comparing to an authentic pure standard obtained from Sigma (Sigma Co., Barcelona, Spain). Delphinidin 3-glucoside ($R_T$=10.5 min, $[MH]^+$ 465 m/z), cyanidin 3-(6"-malonyl)-glucoside ($R_T$=18.0 min $[MH]^+$ 535 m/z) and cyanidin 3-(6"-dioxalyl)-glucoside ($R_T$=21.5 min $[MH]^+$ 593 m/z), were tentatively identified based on their retention times, absorption spectrum characteristics and mass spectrum data with available standards and the data described in the literature (Kelebek, et al., 2008). For the quantitative analysis, an external calibration curve with available standard cyanidin 3-glucoside was carried out. The results were expressed as mg/L of juice.

### 2.4.2. Hydroxycinnamic acids and flavanones

The extraction method was performed following the procedure as previously described by Bermejo et al. (2016). Compounds were analyzed by HPLC equipped with a photodiode array detector and a ZQ2000 mass detector (Waters, Barcelona, Spain) in a reverse-phase column $C_{18}$. The gradient mobile phase was acetonitrile (A) and 0.6% acetic acid (B), starting with 10% A for 2 min, reaching 75% in 28 min and then back to the initial condition to be held for 5 min (total run time 35 min). The flow rate was 1 mL/min. Chromatograms were recorded at the absorbance of 200-400 nm. The HPLC-MS analysis was carried out and worked under electrospray ion negative and positive conditions. Full data acquisition was performed by scanning 150-800 uma in the centroid mode.

The major hydroxycinnamic acids in the fruit juice were quantified, two free acids (sinapic and ferulic) and a conjugated acid (chlorogenic). Chlorogenic acid ($R_T$=10.6 min $[M-1]^+$ 353 m/z) was identified by comparing to an authentic pure standard purchased from Sigma-Aldrich (Barcelona, Spain). Ferulic acid ($R_T$=10.8 min $[M-1]^+$ 193 m/z) and sinapic ($R_T$=11.2 min, $[M-1]^+$ 223 m/z) were tentatively identified by comparing their UV-vis and mass spectra based on available standards and

the data described in the literature (Kelebek et al., 2008). For the quantitative analysis, an external calibration curve with available hydroxycinnamic acid standards was carried out. The results were expressed as mg/L of juice.

Flavanone compounds were identified on the basis of comparing their retention times, UV-Vis spectra and mass spectrum data with authentic standards using an external calibration curve with narirutin ($R_T$=14.2 min $[MH]^+$ 581 m/z), hesperidin ($R_T$=14.8 min $[MH]^+$ 611 m/z) and didymin ($R_T$=17.6 min $[MH]^+$ 595 m/z). Narirutin was purchased from Extrasynthesis (Genay, France), hesperidin from Sigma-Aldrich (Barcelona, Spain) and didymin from ChromaDex (Irvine, CA, USA). The results were expressed as mg/L of juice.

### 2.5. Statistical analysis

Data were subjected to an analysis of variance based on two factors (harvest time × rootstock) for each cultivar. The mean values of the evaluated parameters were compared by the least significant difference test (LSD) at a significance level of 5%. ($P < 0.05$). Principal component analysis (PCA) using Pearson's correlation was also carried out. These analyses were performed using the statistical software Statgraphics Centurion XVII.II software (Manugistics Inc., Rockville, MD, USA).

## 3. Results and Discussion

### 3.1 Firmness, Total Soluble Solids and Acidity

Citrus fruit firmness is a quality parameter which the market is increasingly demanding, despite not being included in current quality standards. Hence one important weakness of some blood oranges cultivars is their low firmness, which makes their commercialization as fresh fruit difficult (Pallottino et al., 2012).

In this study, both evaluated cultivars 'Tarocco Rosso' and 'Moro' showed high firmness at all the harvest times (Table 1). Only 'Moro' grafted onto FA13, and 'Tarocco Rosso' grafted onto C35 and onto M, exhibited a significant loss of firmness at the third harvest. Nevertheless, all the fruit had commercial market values. In 'Moro', the maximum deformation values were 2.3%, shown for FA13 while in 'Tarocco' the highest value was 3.3% observed in M. In both cultivars, the fruit grafted onto CT had the highest firmness values with the least percentage deformation values close to 1.6%. Similar values were observed in C-35 for 'Moro' and in FA5 and CC for 'Tarocco Rosso'.

The influence of rootstock on sugars and acid content has been related to the inherent rootstock differences that affect plant water relations. These differences include root distribution, water uptake ability, hydraulic conductivity, and leaf or stem water potentials (Castle, 1995; Barry, Castle & Davies, 2004).

The TSS content in the blood orange juice fell within the of 9.3-10.8 ºBrix range for 'Moro' and within the 9.9-11.8 ºBrix range for 'Tarocco Rosso' (Table 1). These values were slightly lower than those measured in other studies done with blood oranges, such as 'Tarocco Scirè', 'Tarocco Rosso', 'Tarocco Ippolito' and 'Sanguinello', which ranged between 12.57 and 15.30 (Continella et al., 2018; Cebadera-Miranda et al., 2019).

In both cultivars, the TSS content was affected by harvest and rootstock, with significant interaction between these factors. In 'Moro', the TSS content at the first harvest ranged between 9.3 ºBrix in FA13 and 10.4 ºBrix in CC. At the following harvests all the fruit exhibited increased TSS, except for the fruit grafted onto CC and VK, without significant differences among harvests. At third harvest, minor differences in TSS content among rootstocks were shown, and ranged from 10.1 ºBrix in the fruit from M to 10.8 ºBrix in the fruit from FA5, CC and CL. In 'Tarocco Rosso', the rise in TSS content during harvest was significant in the fruit grafted onto FA5, CC and CL. In this cultivar, and similarly to 'Moro', the lowest TSS values were detected in the fruit from M, with average value close to 9.9 ºBrix values, followed by C-35 fruit with average value close to 10.3 ºBrix. The highest values were recorded for the fruit from FA5.

An effect of harvest and rootstocks was also observed in fruit acidity. The acidity of 'Moro' fruit at first harvest, ranged from close to 1.35 g of citric acid/100 mL in VK and FA13 to 1.60 g of citric acid/100 mL in CT (Table 1). In all cases, fruit acidity lowered with harvest advance, overall after second harvest, except in the fruit from M whose levels remained until the third harvest. The most pronounced decline was recorded for the CT-fruit, but had the highest values at the third harvest, similarly to those for the M-fruit, close to 1.40 citric acid/100 mL. The acidity values of 'Tarocco Rosso' were lower than for 'Moro' and ranged at the first harvest from 1.00 g of citric acid/100 mL in the M-fruit to 1.21 g of citric acid/100 mL in the FA13 fruit. All the fruit also exhibited loss of acidity as harvest advanced. Similar to 'Moro', M-fruit acidity remained constant, but C35-fruit acidity did not vary in 'Tarocco Rosso'. At third harvest the highest acidity values were for the fruit grafted onto CT and C35, with the lowest acidity for the fruit from FA13, M, and VK.

### 3.2 External and Internal Color

Blood oranges cultivars are characterized by the presence of phenolic compounds belonging to the anthocyanin class (Rapisarda et al., 2001). Although these compounds accumulate mainly in fruit

flesh in some cultivars purple or red shaded peel may be observed (Rodrigo, Alquézar, Alós, Lado & Zacarías, 2013). This accumulation is sometimes due to the synthesis of anthocyanins when fruit is exposed to adverse environmental conditions, such as visible and UVB radiation, cold temperature and water stress (Muccilli et al., 2009). Therefore, this can lead to significant differences in external color between the peel area exposed to UVB radiation, whose coloration is the darkest, and the shaded peel area. For this reason, two different coloration areas were measured separately in this study: the most pigmented area (dark area) and the area with less color (bright area) (Fig. 1).

In both cultivars, in the two areas measured an important effect of the harvest and rootstock was detected and in any case significant interaction between these factors was found.

In 'Moro', the CCI of the bright area ranged between 12.0 and 14.7 at first harvest and a significant but slight increase took place as harvest advanced. The highest CCI values were observed during the second harvest in the fruit grafted onto FA5 and M. Moreover, at this last harvest, the lowest CCI was exhibited by the fruit form FA13, C35 and CT. The CCI of the dark area at the first harvest ranged from 16.1 in the CT-fruit to close to 28 in fruit from CL and VK. In general, an increase in the CCI values was observed in the second harvest before dropping at the third harvest. Fruit from FA13, C35 and CT, with the lowest values, exhibited slight changes throughout the harvest period.

In 'Tarocco Rosso', the bright area showed CCI values ranged between 13.1 in fruit from both C35 and FA13 fruit to 17.8 in the M-fruit at first harvest. This area exhibited an increase of CCI in the second harvest and dropped at the third harvest. The lowest values during the studied period were found in C35-fruit followed by VK-fruit. The highest values were shown in M-fruit that exhibited the smallest changes with the harvest advance. The CCI of the darkest area, at first harvest, ranged between 22.2 in C35-fruit to close to 33.8 in fruit from CL and VK. In fruit from all rootstocks, important coloration decrease occurred at the third harvest. The smallest reduction was shown in fruit from C35, CT and M. The C35-fruit exhibited the lowest CCI values.

Although the anthocyanin content in peel was not measured, the increased peel color observed in 'Moro' at second harvest suggested that an increase in anthocyanin content took place. Likewise, in both cultivars, the significant reduction in peel color at the third harvest could be associated with anthocyanin degradation. In previous studies, an increase in total anthocyanins during harvest to a final reduction in late ripening stages has been reported in different blood orange cultivars (Barbagallo, Palmeri, Fabiano, Rapisarda & Spagna, 2007).

The external color changes herein observed could be related to the variation in environment temperature. It is known that cool nights and warm days are required to stimulate anthocyanin synthesis (Barry, Caruso & Gmitter, 2020). Figure supplementary 1 shows the maximum

249temperature and cold hours per day during the study period. Between the first and second harvest,
250the maximum temperature came close to 18ºC with an average 10 h of cold hours. These conditions
251could have induced anthocyanin synthesis in the 'Moro' fruit, overall in fruit from FA5, CC, M and
252VK, which would lead to the major color increase. The increased peel coloration observed in
253'Moro' would corroborate that this cultivar is one that most strongly depends on prevailing climate
254conditions for full color development (Butelli et al., 2012).
255After second harvest, the cold hour average fell to 5 h and the mean maximum temperature rose to
25622ºC. This scenario could trigger anthocyanin degradation with the consequent drop in external
257color observed in both cultivars. It has been reported that fruit are apparently able to actively
258degrade anthocyanins under warm conditions (Steyn, 2008). The fact that the fruit from rootstocks
259like FA13, C35 and CT in Moro and C35, CT and M in 'Tarocco Rosso', show minor changes in
260external color during harvests suggests that these rootstocks induce low sensitiveness to
261temperature.
262Regarding internal color, 'Moro' presented higher CCI values than 'Tarocco Rosso' (Fig. 1). Of the
263studied blood oranges, 'Moro' has been reported as a variety with the highest internal color values
264(Mondello, Cotroneo, Errante, Dugo & Dugo, 2000). A significant effect of harvest and rootstock
265was found in 'Moro'. At first harvest, the color juice of the 'Moro' fruit grafted onto CL had the
266highest CCI values, close to 93.0, while the CT-fruit had the lowest values with 57.1. The CCI of
267the other fruit was between 75.4 and 83.1. As harvest advanced, CCI tended to lower. The CT-fruit,
268with the lowest values, exhibited the minor reduction. Nevertheless, the decline in fruit from C35,
269M and VK was more accused and achieved similar values of CT-fruit at third harvest. CL fruit had
270the maximum internal color values. In previous studies, it was reported that CT in blood orange
271cultivars induced the lowest internal fruit color values compared to seven different rootstocks
272(Continella et al., 2018).
273In 'Tarocco Rosso' no significant effect of harvest was found in color juice although fruit from FA5
274and CL increased the CCI values at the second harvest. Nevertheless, the influence of rootstock on
275internal CCI was relevant. The fruit from C35, M and CT had the lowest CCI values, between 4.2
276and 11.0, while the highest values coming close to 30 for the FA5-fruit.
277
278**3.3. Anthocyanin content in juice**
279As previously mentioned, blood oranges are rich in anthocyanins, the water-soluble compounds
280responsible for their distinctive purple coloration. Anthocyanins in blood oranges depend strongly
281on variety. In this study, four anthocyanins were determined in the juice of 'Moro' and 'Tarocco
282Rosso': delphinidin 3-glucoside (Dp-3-glu), cyanidin 3-glucoside (Cy-3-glu), cyanidin 3-(6"-

283malonyl)-glucoside (Cy-3,6”mal-glu) and cyanidin 3-(6”-dioxalyl)-glucoside (Cy-3,6”diox-glu)
284(Fig. 2). As reported in most blood oranges, Cy-3-glu and Cy-3,6”mal-glu were identified as the
285major anthocyanins in the juice of both cultivars (Dugo, Mondello, Morabito & Dugo, 2003;
286Ballistreri, Fabroni, Romeo, Timpanaro, Amenta & Rapisarda, 2019; Cebadera-Miranda et al.,
2872019). Anthocyanin content was higher in ‘Moro’ than in ‘Tarocco Rosso’, as previously reported
288by Kafkas, Ercisli, Kemal, Baydar and Yilmaz (2009), who established that ‘Moro’ is the richest in
289anthocyanins among blood oranges. In each cultivar, anthocyanin evolution during harvests differed
290depending on each individual compound.
291In ‘Moro’, Cy-3,6”mal-glu content was affected by harvest and rootstock without significant
292interaction between these factors. Its content remained almost constant during the first two harvests.
293However, at the third one, the amount of this compound significantly dropped (Fig. 2). Cy-
2943,6”diox-glu content gradually decreased with harvest advance. Regarding the influence of
295rootstock on the content of these two anthocyanins, the fruit from CT and VK had the lowest
296values, which was specially observed during first and second harvest. After third harvest, when the
297content of these both compounds declined, minor differences among rootstocks appeared. It is
298important to remark that the smallest descent of both anthocyanins was observed in CL fruit, that
299exhibited the highest values of Cy-3,6”mal-glu in the third season. No significant effect of the
300harvest was found in Cy-3-glu, but an important influence of the rootstock was observed. The
301lowest values were found in fruit from CT and VK and the highest values was exhibited by CL-
302fruit. Finally, Dp-3-glu increased with the harvest advance and the rootstook also affect its content,
303without significant interaction between these factors. Similar to the other anthocyanins, the lowest
304content was for, CT and VK and the highest values for CL.
305Contrarily to observed in ‘Moro’, the harvest did not affect the content of Cy-3,6”mal-glu and Cy-
3063,6”diox-glu in ‘Tarocco Rosso’, nevertheless the rootstock had a significant influence. The fruit
307from C35, M and CT had the lowest content of these anthocyanins. Cy-3-glu and Dp-3-glu content
308increased throughout harvest. FA13 and FA5 were the rootstocks with more Cy-3-glu and Dp-3-glu
309content in fruit. Conversely, the fruit from C35, M and CT contained the smallest amount of both
310anthocyanins.
311The relation between coloration and anthocyanin content has been previously reported (Rapisarda et
312al., 2001; Carmona, Alquézar, Marques & Peña, 2017). Accordingly, in this study, the higher juice
313color of ‘Moro’ compared to ‘Tarocco Rosso’ was explained by the content of the all the individual
314determined anthocyanins. Likewise, the changes in internal coloration exhibited in both cultivars
315throughout harvests were corroborated with those recorded in anthocyanins. Therefore in ‘Moro’,
316the drastic drop in Cy-3,6”mal-glu and Cy-3,6”diox-glu content was concomitant with the reduction

317in internal color, which would indicate that these anthocyanins were the pigments that mostly
318contributed to the juice color intensity of ‘Moro’. Similarly, the least internal coloration of the fruit
319grafted onto CT and VK would be associated with the lower content of the four determined
320anthocyanins. In the case of ‘Tarocco Rosso’, the fruit with the least internal coloration was that
321grafted on to the rootstocks with the lowest content of the four anthocyanins (C35, M and CT), and
322increased juice color was exhibited only at the second harvest in the fruit from FA5 and CL, which
323coincides with the increment in Cy-3-glu and Dp-3-glu content.
324The descent of the Cy-3,6”mal-glu and Cy-3,6”diox-glu content observed at the third harvest in
325‘Moro’ indicated the degradation of these anthocyanins. This coincides with previous studies in
326which the degradation of pigments in both rind and flesh has been observed in late ripening stages
327(Lo Piero, 2015). The catabolic process could be due to either the chemical instability of pigments
328or specific enzymatic activity lowering the pigment concentration in plant tissues, which often
329occurs in parallel to the synthesis process Oren-Shamir (2009). Regarding enzymatic activity, it has
330been reported that enzyme β-glucosidase correlates with the degradation of anthocyanins in late
331ripening stages (Barbagallo et al., 2007).
332As mentioned above, an increase in the average maximum temperature from 18ºC to 22ºC after the
333second harvest, along with a drop in the average cold hours occurred (Fig. Supp. 1), which could
334cause the degradation of Cy-3,6”mal-glu and Cy-3,6”diox-glu in ‘Moro’. Nevertheless, Cy-3-glu
335did not alter as harvest advanced, and even an increase in Dp-3-glu was observed, which would
336indicate their lesser sensitivity to temperature changes. Previous studies on the thermal degradation
337of anthocyanins have shown a different stability to changes in environmental temperatures for each
338individual anthocyanin (Lo Piero, 2015).
339In ‘Tarocco Rosso’, no evidence for degradation of the studied individual anthocyanins was found
340with advancing harvests. Contrarily, Dp-3-glu and Cy-3-glu slightly increased, which would
341suggest that the influence of the environment on a specific pigment strongly depends on cultivar.
342
343**3.5 Sugars, Flavanones and Hydroxycinnamic Acids content in juice**
344Sugars are important components of the chemical composition of blood oranges. Apart from sugars
345having an effect on sensory properties, they are reported to be involved in anthocyanins
346biosynthesis (Solfanelli, Poggi, Loreti, Alpi & Perata, 2006; Li et al., 2014; Abdullah et al., 2018).
347The sugar profile of blood oranges depends on many factors, such as cultivar, harvest time and
348environment conditions (Kafkas et al., 2009). The effect of rootstock on the content of individual
349sugars has been addressed in different studies on citrus fruit (Legua, Forner, Hernández & Forner-
350Giner, 2014; Saini, Capalash, Kaur & Singh, 2019).

In the present study, as in most blood oranges, sucrose was the main sugar in both studied cultivars, followed by glucose and fructose (Fig. 3). In both cultivars the three sugars were influenced by harvest and rootstock and no significant interaction between these factors were detected.

In ‘Moro’, the sucrose content ranged from 36-37 g/L in the fruit from CT and VK to near 48.5 g/L in the fruit from CC and C35 at the first harvest. A gradual decrease during the following harvests were observed in all fruit. The rootstocks with the lowest mean of sucrose were M, CT and VK, and the highest ones were CC and C35. It is noteworthy that fruit from CC and C35 exhibited the most important decrease. The highest glucose and fructose content at the first harvest was detected in the CC-fruit with values of 27.3 g/L and 21.7 g/L, respectively, while the lowest values were for the CT-fruit, with 17.5 g/L of glucose and 14.5 g/L of fructose. In all cases, the content of both sugars gradually increased. Taking the mean of the three harvests, the CT and C35 fruit showed the lowest fructose and glucose content, with the highest values for the CC-fruit.

In ‘Tarocco Rosso’, sucrose content ranged from 37.5 g/L in C35 to 51.6 g/L in FA13 at the first harvest. Sucrose loss was exhibited in all fruit, overall in the third harvest. The highest sucrose mean was shown for the fruit from FA13 and FA5, and the M-fruit had the lowest value. Regarding glucose and fructose content in ‘Tarocco Rosso’, the C35-fruit had the lowest values, with 18.0 g/L and 16.9 g/L, respectively, for the first harvest. In the following harvests an increase in the content of these sugars were observed. The highest mean of both, glucose and fructose, was detected in FA13, while the fruit from C35 and CT had the lowest.

These results corroborate that the influence of rootstock on sugar content is cultivar-dependent. In blood oranges, a relation between sugar and anthocyanin accumulation has been reported as blood oranges require higher sugar metabolism to supply the need of carbon skeletons for anthocyanin biosynthesis (Muccilli et al., 2009; Carmona et al., 2019). Solfanelli et al. (2006) established that the sugar-dependent up-regulation of the anthocyanin synthesis pathway is sucrose-specific. In the same way, it was reported that sucrose induces anthocyanin biosynthesis (Li et al., 2014). Sucrose increases the stabilization of DELLA proteins and the degradation of gibberellins, and activates the structural genes of anthocyanin biosynthesis. So a positive correlation between sucrose concentration and anthocyanin accumulation has been found (Abdullah et al., 2018). In this study, a relation between sucrose content and anthocyanins was found in ‘Tarocco Rosso’. In this cultivar, M and C35 supplied the lowest sugar content and lower TSS in the fruit compared to the other rootstocks, which could cause the lower anthocyanin content observed in the fruit from these rootstocks. In ‘Moro’, this relation between sugars and anthocyanins was not as evident. Nonetheless, the rootstocks that led to fruit having the lowest anthocyanin content were CT and VK. These rootstocks presented the least sucrose.

385Hydroxycinnamic acids represent an important group of compounds that derive from the general
386phenylpropanoid pathway, which is the following step from individual sugars on the anthocyanins
387pathway. So blood oranges contain more hydroxycinnamic acids than blond ones, being proposed
388as varietal markers of blood orange juice (Rapisarda et al., 1998).
389In this study, the main hydroxycinnamic acids detected in both blood orange cultivars were
390chlorogenic, ferulic and sinapic acids. Traces of caffeic acid and ρ-coumaric, was also identified
391(Data not shown). In both cultivars, chlorogenic acid was the most dominant hydroxycinnamic acid,
392followed by ferulic and sinapic acid. Nevertheless, Kelebek et al. (2008) found in two blood orange
393cultivars that ferulic acid was the most dominant hydroxycinnamic acid, followed by chlorogenic
394and sinapic acids. The differences found is due to the distribution of individual hydroxycinnamic
395acids is variety-dependent (Rapisarda et al., 1998; Kelebek et al., 2008).
396In 'Moro', the content of three hydroxycinamic acids was affected by harvest and rootstock and
397only the interaction between these two factors was significant for chlorogenic acid. At the first
398harvest, chlorogenic acid ranged from 11 mg/L in the FA13-fruit to 15 mg/L in the FA5-fruit (Fig.
3994). During the following harvests, the content of this acid increased in all the fruit, except for the
400fruit from FA5. The lowest chlorogenic acid mean values were found in the FA13-fruit, with the
401highest ones in the fruit from FA5, C35 and M. The ferulic acid content in all the fruit came close to
40210-12 mg/L at the first harvest and, similarly to chlorogenic acid, it increased during the following
403harvests (Fig. 4). The lowest values were detected in M fruit. The amount of sinapic acid content
404was lower than the other acids, and ranged from 5.7 mg/L in the CT-fruit to 7 mg/L in both C35-
405fruit and CL-fruit at the first harvest with a significant increase during the following harvests. The
406lowest mean values were presented by the fruit from M and CT.
407In 'Tarocco Rosso', no significant effect of harvest was observed chlorogenic and ferulic acid
408content (Fig 4). Nevertheless, major differences among rootstocks were observed. The lowest
409chlorogenic acid content during the studied period was exhibited by the C35-fruit with values close
410to 14 mg/L, followed by FA13, CC and CT with values between 18 mg/L and 20 mg/L. The
411maximum content was detected in the fruit from FA5, CL, M and VK with 22-23 mg/L. The lowest
412mean of ferulic acid was found for M-fruit, close to 8 mg/L. Finally, sinapic acid was significant
413increased with the harvest advance and the lowest amount was also shown by M-fruit, with mean
414value of 6.28 mg/L.
415The increase in chlorogenic and ferulic acids observed in 'Moro', overall at the third harvest, was
416concomitant to anthocyanin Cy-3,6"mal-glu and Cy-3,6"diox-glu degradation. This could be
417explained by the general phenylpropanoid pathway. Phenylalanine is a direct precursor of
418anthocyanin synthesis through complex reactions. The first step is the transformation from

419phenylalanine to trans-cinnamic acid. In the following steps, cinnamic acid is hydroxylated by
420generating p-coumaric acid. Then p-coumaric is condensed by the specific enzyme of the
421anthocyanin biosynthetic pathway, chalcone synthase, to produce naringenin chalcone, which is
422implicated in anthocyanin biosynthesis. In 'Moro', anthocyanin degradation was observed at third
423harvest, which would stop the biosynthesis anthocyanin process, while the p-coumaric formed in
424the first steps of the phenylpropanoid pathway would be used to synthesize hydroxycinnamic acids,
425specifically chlorogenic and fumaric acids.
426Flavanones are major flavonoids in citrus fruit. In both studied cultivars, hesperidin (HES) was the
427predominant flavanone, followed by narirutin (NAR) and didymin (DID), which agrees with what
428has been previously reported for other blood orange cultivars (Barreca, Bellocco, Leuzzi & Gattuso,
4292014; Cebadera-Miranda et al., 2019).
430In 'Moro', the three flavanones were affected by rootstock and harvest with significant interaction
431between these two factors. HES content at first harvest varied from 104 mg/L in the CT-fruit to 142
432mg/L in the FA5-fruit (Fig. 5). In all cases, a significant increase during the following harvests was
433observed, except in the FA5-fruit, that exhibited the maximum HES values during first and second
434season to then dropped in the third until achieve the lowest amount of 134 mg/L. C35 was one of
435the rootstock that displayed higher HES content in the three harvest. NAR content also rose in all
436the fruit while harvest advanced. At third harvest, the maximum level was also achieved by the
437C35-fruit (42 mg/L) and the minimum by the FA13-fruit (27 mg/L). An increase in DID content
438was also recorded as harvest advanced, except for the FA5-fruit, which maintained the same values
439during all three harvests. Although the differences among rootstocks depended on the harvest,
440similar to other flavanones, C35-fruit exhibited one of the highest DID content and the lowest was
441for both CT-fruit and FA13-fruit.
442In 'Tarocco Rosso', the content of the three studied flavanones fell within a lower range than
443'Moro'. Similar to 'Moro', HES and NAR content was affect by harvest and rootstock with
444significant interaction between them. The amount of HES varied from 51 mg/L in the FA13-fruit to
44595 mg/L in the CT-fruit at the first harvest. A drop was observed in all the fruit during the following
446harvests. In the third harvest lower differences among rootstocks were found. NAR content ranged
447from close to 13 mg/L in FA13 and C35-fruit to 21 mg/L in CL and CT-fruit at first harvest. All
448fruit exhibited an increase the second harvest to then decrease in the third, except C35 that did not
449change with harvest advance and had the lowest values. In 'Tarocco Rosso', harvest time had no
450affect DID content. Nevertheless, the differences among rootstocks were evident. C35-fruit had the
451lowest values with a mean of 3.37 mg/L. The maximum values were displayed by the CC, CT and
452CL fruit with values close to 5-6 mg/L.

On the flavonoid pathway, it is noteworthy that naringenin chalcone, besides being involved in anthocyanins biosynthesis, is implicated in the formation of other phenolic compounds like flavanones. This could explain the increase in flavanones in the 'Moro' fruit that parallel the stop of the anthocyanins synthesis process which occurs when they are degraded. Contrary to that observed in 'Moro', the 'Tarocco Rosso' fruit did not exhibit anthocyanin degradation during the study period. Consequently, no relevant changes in the content of hydroxycinnamic acids or flavanones took place.

### 3.6 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) representing both cultivars, rootstocks and the internal parameters previously detailed are shown in Fig. 6 (A,B). The first two components explain 85.4% of the total variance data (Principal Component 1 (PC1): 63.2%; Principal Component 2 (PC2): 22.2%). PC1 are correlated positively with flavonoids and individual anthocyanins content, juice color, acidity and ferulic acid and separates the set of samples into two groups: 'Tarocco Rosso' and 'Moro'.

In order to further explore the effect of rootstock and harvest moment on the internal quality parameters a PCA was independently performed for each cultivar. In 'Moro' the two first components explain 69.9% of the total variance Fig. 6 (C,D). The first component (PC1) is correlated positively with TA and negatively with individual flavonoids, hydroxycinnamic acids and sugars. This component explains the effect of the harvest moment, since separate the three maturity stages for all rootstocks. So, while the fruit from first harvest was related with the acidity and the anthocyanins Cy-3,6"mal-glu and Cy-3,6"diox-glu, as harvest advance the fruit was correlated with flavonoids, hydroxycinnamic acids and sugars. Moreover, M, CT and VK are located in Q3 and Q4, separated from the other cultivars by PC2, inversely correlated with juice color and individual anthocyanins.

In 'Tarocco Rosso', PCA shows that the internal quality was more influenced by rootstock effect than the harvest moment (Fig. 6. E,F). The two first components explain 65.9% of the total variance. PC1 is positively correlated with sugars, anthocyanins, juice color, ferulic and sinapic acids. The second component is positively correlated mainly with the content of the three flavonoids. PC1 corroborates that the lowest content of sugars and anthocyanins are shown by C35, M and CT (located in Q1 and Q3) while the highest content is shown by FA5 and FA13 (located in Q2 and Q4). Moreover, C35 is characterized by the low content of flavonoids and chlorogenic and sinapic acids. FA13 clearly is separated by PC2 also by the low flavonoids content.

In conclusion, the results herein obtained point out the strong influence of rootstock on the quality parameters herein studied, which was highly scion-dependent. Furthermore, the changes that fruit undergoes during harvest are also affected by the rootstock onto which it was grafted.

Taking into account that in blood oranges, fruit firmness is currently one of the most important quality parameters from the commercial point of view, CT and C35 in 'Moro' and CT, FA13 and CC in 'Tarocco Rosso' were the rootstocks that induced the higher firmness values in fruit. The effect on the content of sugars and acids also depends on the rootstock/scion interaction. The evaluation of the four main anthocyanins revealed their relation to internal fruit color. In 'Moro', the fruit from M, CT and VK displayed the lowest juice color and the lowest content for all four anthocyanins. In 'Tarocco Rosso', C35, M and CT were the rootstocks that induced less color in fruit. The differences in anthocyanin content detected among rootstocks were also related to the supplied sucrose content. In addition, the degradation of Cy-3,6"mal-glu and Cy-3,6"diox-glu that took place in 'Moro' during the last harvest, as a response of increased temperature, contributed to increase the synthesis of hydroxycinnamic acids and flavanones. In 'Tarocco Rosso', no anthocyanin degradation was shown, which suggests that this cultivar is less sensitive to environmental changes.

## 4. Acknowledgments

The authors thank Anecoop S. Coop. for supplying the fruit herein used and for its technical support. This study was co-financed by FEDER and European Social Funds.

## 5. References

Abdullah, M., Cheng, X., Shakoor, A., Cao, Y., Lin, Y., Cai, Y., & Shangao, J. (2018). New Opinion of Sugar and Light Crosstalk in the Induction of Anthocyanins Biosynthesis in Fruits. *International Journal of Agriculture and Biology*, *20*(11), 2465-2474. https://doi.org/10.17957/IJAB/15.0790

Ballistreri, G., Fabroni, S., Romeo, F. V., Timpanaro, N., Amenta, M., & Rapisarda, P. (2019). Anthocyanins and Other Polyphenols in Citrus Genus: Biosynthesis, Chemical Profile, and Biological Activity. In *Polyphenols in Plants* (pp. 191-215). Academic Press. https://doi.org/10.1016/B978-0-12-813768-0.00014-1

Barbagallo, R.N., Palmeri, R., Fabiano, S., Rapisarda, P., & Spagna, G. (2007). Characteristic of β-glucosidase from Sicilian blood oranges in relation to anthocyanin degradation. *Enzyme and microbial technology*, *41*(5), 570-575. https://doi.org/10.1016/j.enzmictec.2007.05.006

518Barreca, D., Bellocco, E., Leuzzi, U., & Gattuso, G. (2014). First evidence of C-and O-glycosyl
519 flavone in blood orange (Citrus sinensis (L.) Osbeck) juice and their influence on antioxidant
520 properties. *Food Chemistry*, 149, 244-252.
521 https://doi.org/10.1016/j.foodchem.2013.10.096
522Barry, G.H., Castle, W.S., & Davies, F.S. (2004). Rootstocks and plant water relations affect sugar
523 accumulation of citrus fruit via osmotic adjustment. *Journal of the American Society for*
524 *Horticultural Science, 129*(6), 881-889.
525 https://doi.org/10.21273/JASHS.129.6.0881
526Barry, G.H., Caruso, M., & Gmitter Jr, F.G. (2020). Commercial scion varieties. In *The Genus*
527 *Citrus* (pp. 83-104). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-812163-
528 4.00005-X
529Bermejo, A., Pardo, J.L., Morales, J., & Cano, A. (2016). Comparative study of bioactive
530 components and quality from juices of different mandarins: discriminant multivariate analysis
531 of their primary and secondary metabolites. *Agricultural Sciences*, 7(6), 341-351.
532 https://doi.org/10.4236/as.2016.76035
533Butelli, E., Licciardello, C., Zhang, Y., Liu, J., Mackay, S., Bailey, P. Reforgiato Recupero, G., &
534 Martin, C. (2012). Retrotransposons control fruit-specific, cold-dependent accumulation of
535 anthocyanins in blood oranges. *Plant Cell, 24*(3), 1242-1255.
536 https://doi.org/10.1105/tpc.111.095232
537Carmona, L., Alquézar, B., Marques, V. V., & Peña, L. (2017). Anthocyanin biosynthesis and
538 accumulation in blood oranges during postharvest storage at different low temperatures. *Food*
539 *chemistry*, 237, 7-14.
540 https://doi.org/10.1016/j.foodchem.2017.05.076
541Castle, W.S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New*
542 *Zealand Journal of Crop and Horticultural Science, 23*(4), 383-394.
543 https://doi.org/10.1080/01140671.1995.9513914
544Cebadera-Miranda, L., Domínguez, L., Dias, M. I., Barros, L., Ferreira, I. C., Igual, M., Martínez-
545 Navarrete, N., Fernández-Ruiz, V., Morales, P., & Cámara, M. (2019). Sanguinello and
546 Tarocco (Citrus sinensis [L.] Osbeck): Bioactive compounds and colour appearance of blood
547 oranges. *Food Chemistry, 270*, 395-402.
548 https://doi.org/10.1016/j.foodchem.2018.07.094
549Continella, A., Pannitteri, C., La Malfa, S., Legua, P., Distefano, G., Nicolosi, E., & Gentile, A.
550 (2018). Influence of different rootstocks on yield precocity and fruit quality of 'Tarocco Scirè'
551 pigmented sweet orange. *Scientia horticulturae*, *230*, 62-67.

https://doi.org/10.1016/j.scienta.2017.11.006

Crifò, T., Puglisi, I., Petrone, G., Reforgiato Recupero, G., & Lo Piero, A.R. (2011). Expression analysis in response to low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. *Gene*, *476*(1-2), 1-9.
https://doi.org/10.1016/j.gene.2011.02.005

Dugo, P., Mondello, L., Morabito, D., & Dugo, G. (2003). Characterization of the anthocyanin fraction of sicilian blood orange juice by micro-HPLC-ESI/MS. *Journal of Agricultural and Food Chemistry*, *51*(5), 1173-1176.
https://doi.org/10.1021/jf026078b

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 Research International*, 131, 108916.
https://doi.org/10.1016/j.foodres.2019.108916

Filho, F.D.A.A., Espinoza-Núñez, E., Stuchi, E.S., & Ortega, E.M.M. (2007). Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarins on four rootstocks. *Scientia Horticultura.* 114(1), 45-49.
https://doi.org/10.1016/j.scienta.2007.05.007

Habibi, F., Ramezanian, A., Guillén, F., Serrano, M., & Valero, D. (2020). Blood oranges maintain bioactive compounds and nutritional quality by postharvest treatments with γ-aminobutyric acid, methyl jasmonate or methyl salicylate during cold storage. *Food Chemistry*, 306, 125634.
https://doi.org/10.1016/j.foodchem.2019.125634

Hou, D. X. (2003). Potential mechanisms of cancer chemoprevention by anthocyanins. *Current Molecular Medicine*, 2003, 3(2), 149–159.
https://doi.org/10.2174/1566524033361555

Incesu, M., Çimen, B., Yesiloglu, T., & Yilmaz, B. (2013). Rootstock effects on yield, fruit quality, rind and juice color of Moro blood orange. *Journal of food, Agriculture & Environment*, *11*(3&4), 867-871.

Jimenez-Cuesta M., Cuquerella J., & Martínez-Jávega J. (1981) Determination of a color index for citrus fruit degreening. *Proceedings of the International Society of Citriculture*, 2: 750–753.

Kafkas, E., Ercisli, S., Kemal, K.N., Baydar, K., & Yilmaz, H. (2009). Chemical composition of blood orange varieties from Turkey: A comparative study. *Pharmacognosy Magazine*, *5*(20), 329. https://doi.org/10.4103/0973-1296.58155

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.)

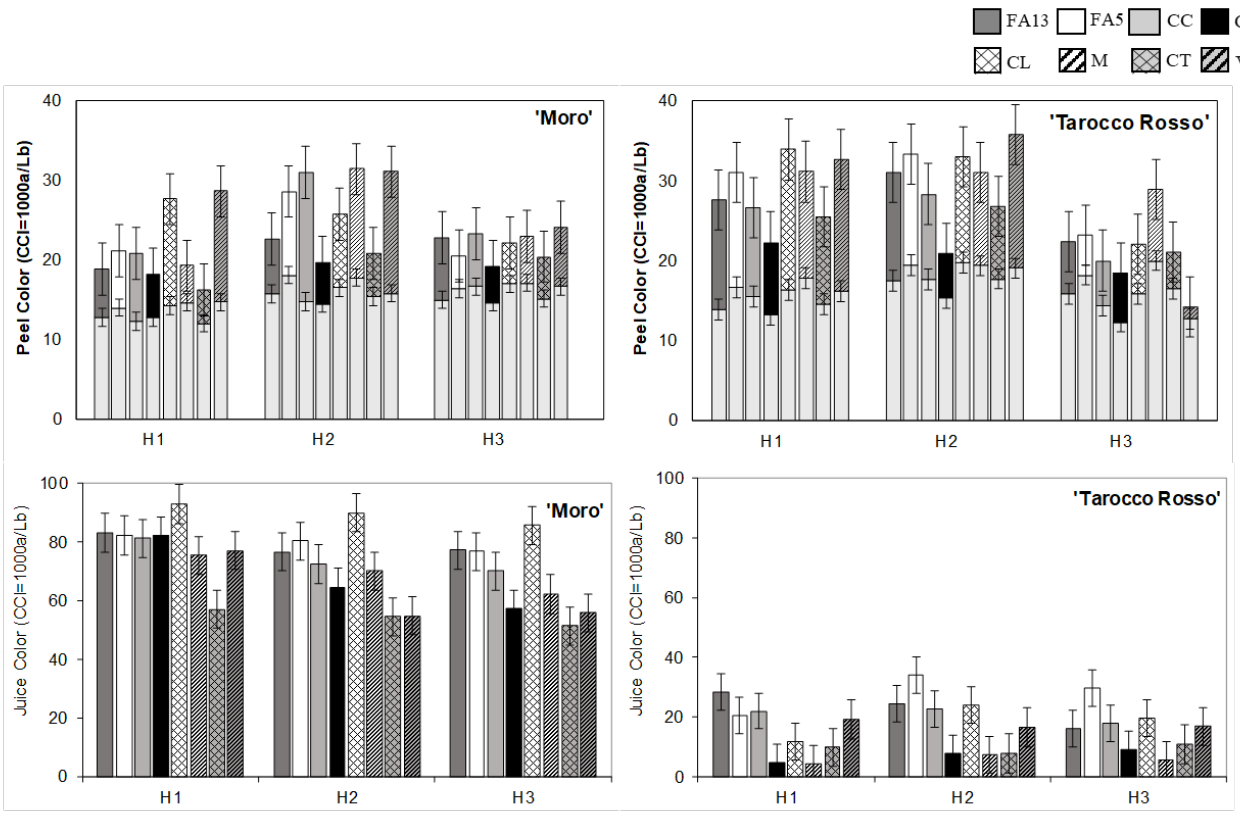
586 Osbeck) grown in Turkey. *Food Chemistry*, *107*(4), 1710-1716.
587 https://doi.org/10.1016/j.foodchem.2007.10.004
588Laribi, A.I., Palou, L., Intrigliolo, D.S., Nortes, P.A., Rojas-Argudo, C., Taberner, V., Bartual, J., &
589 Pérez-Gago, M.B. (2013). Effect of sustained and regulated deficit irrigation on fruit quality of
590 pomegranate cv. 'Mollar de Elche' at harvest and during cold storage. *Agricultural Water*
591 *Management*, 125, 61-70.
592 https://doi.org/10.1016/j.agwat.2013.04.009
593Legua, P., Forner, J. B., Hernández, F., & Forner-Giner, M.A. (2014). Total phenolics, organic
594 acids, sugars and antioxidant activity of mandarin (Citrus clementina Hort. ex Tan.): Variation
595 from rootstock. *Scientia Horticulturae*, *174*, 60-64.
596 https://doi.org/10.1016/j.scienta.2014.05.004
597Li, Y., Van den Ende, W., & Rolland, F. (2014). Sucrose induction of anthocyanin biosynthesis is
598 mediated by DELLA. *Molecular Plant, 7*(3), 570-572.
599Lo Piero, A.R. (2015). The state of the art in biosynthesis of anthocyanins and its regulation in
600 pigmented sweet oranges [(Citrus sinensis) L. Osbeck]. *Journal of agricultural and food*
601 *chemistry*, *63*(16), 4031-4041.
602 https://doi.org/10.1021/acs.jafc.5b01123
603Mondello, L., Cotroneo, A., Errante, G., Dugo, G., & Dugo, P. (2000). Determination of
604 anthocyanins in blood orange juices by HPLC analysis. *Journal of pharmaceutical and*
605 *biomedical analysis*, *23*(1), 191-195.
606 https://doi.org/10.1016/S0731-7085(00)00269-7
607Muccilli, V., Licciardello, C., Fontanini, D., Russo, M.P., Cunsolo, V., Saletti, R., Reforgiato
608 Recupero, G., & Foti, S. (2009). Proteome analysis of Citrus sinensis L. (Osbeck) flesh at
609 ripening time. *Journal of Proteomics*, *73*(1), 134-152.
610 https://doi.org/10.1016/j.jprot.2009.09.005
611Oren-Shamir, M. (2009). Does anthocyanin degradation play a significant role in determining
612 pigment concentration in plants? *Plant Science, 177*(4), 310-316.
613 https://doi.org/10.1016/j.plantsci.2009.06.015
614Pallottino, F., Menesatti, P., Lanza, M. C., Strano, M. C., Antonucci, F., & Moresi, M. (2012).
615 Assessment of quality-assured Tarocco orange fruit sorting rules by combined physicochemical
616 and sensory testing. *Journal of the Science of Food and Agriculture,* 93(5), 1176-1183.
617 https://doi.org/10.1002/jsfa.5871

618Rapisarda, P., Carollo, G., Fallico, B., Tomaselli, F., & Maccarone, E. (1998). Hydroxycinnamic
619 acids as markers of Italian blood orange juices. *Journal of Agricultural and Food Chemistry*,
620 *46*(2), 464-470. https://doi.org/10.1021/jf9603700
621Rapisarda, P., Bellomo, S.E., & Intelisano S. (2001). Storage temperature effects on blood orange
622 fruit quality. *Journal of Agricultural and Food Chemistry, 49*(7), 3230-3235.
623 https://doi.org/10.1021/jf010032l
624Reforgiato Recupero, G.R., Russo, G., Recupero, S., Zurru, R., Deidda, B., & Mulas, M. (2009)
625 Horticultural evaluation of new citrus latipes hybrids as rootstocks for citrus. *HortScience*,
626 44(3), 595-598. https://doi.org/10.21273/HORTSCI.44.3.595
627Rodrigo, M.J., Alquézar, B., Alós, E., Lado, J., & Zacarías, L. (2013). Biochemical bases and
628 molecular regulation of pigmentation in the peel of Citrus fruit. *Scientia Horticulturae*, *163*, 46-
629 62. https://doi.org/10.1016/j.scienta.2013.08.014
630Saini, M.K., Capalash, N., Kaur, C., & Singh, S.P. (2019). Comprehensive metabolic profiling to
631 decipher the influence of rootstocks on fruit juice metabolome of Kinnow (C. nobilis× C.
632 deliciosa). *Scientia Horticulturae*, *257*, 108673.
633 https://doi.org/10.1016/j.scienta.2019.108673
634Solfanelli, C., Poggi, A., Loreti, E., Alpi, A., & Perata, P. (2006). Sucrose-specific induction of the
635 anthocyanin biosynthetic pathway in Arabidopsis. *Plant physiology*, *140*(2), 637-646.
636 https://doi.org/10.1104/pp.105.072579
637Steyn, W.J. (2008). Prevalence and functions of anthocyanins in fruits. In *Anthocyanins* (pp. 86-
638 105). Springer, New York, NY.
639 https://doi.org/10.1007/978-0-387-77335-3_4
640Zhang, Y., Butelli, E., & Martin, C. (2014). Engineering anthocyanin biosynthesis in plants.
641 *Current opinion in plant biology,* 19, 81-90.
642 https://doi.org/10.1016/j.pbi.2014.05.011

643**Table 1.** Firmness, total soluble solids (TSS) and acidity (TA) of blood oranges 'Moro' and 644'Tarocco Rosso' grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from 645early February to late March. The lowercase letters in each column represent the least significant 646difference (LSD) intervals ($p \leq 0.05$) when comparing the effect of rootstocks for each harvest time. 647The capital letters in each row represent the least significant difference (LSD) intervals ($p \leq 0.05$) 648when comparing the effect of harvest time per each rootstock.

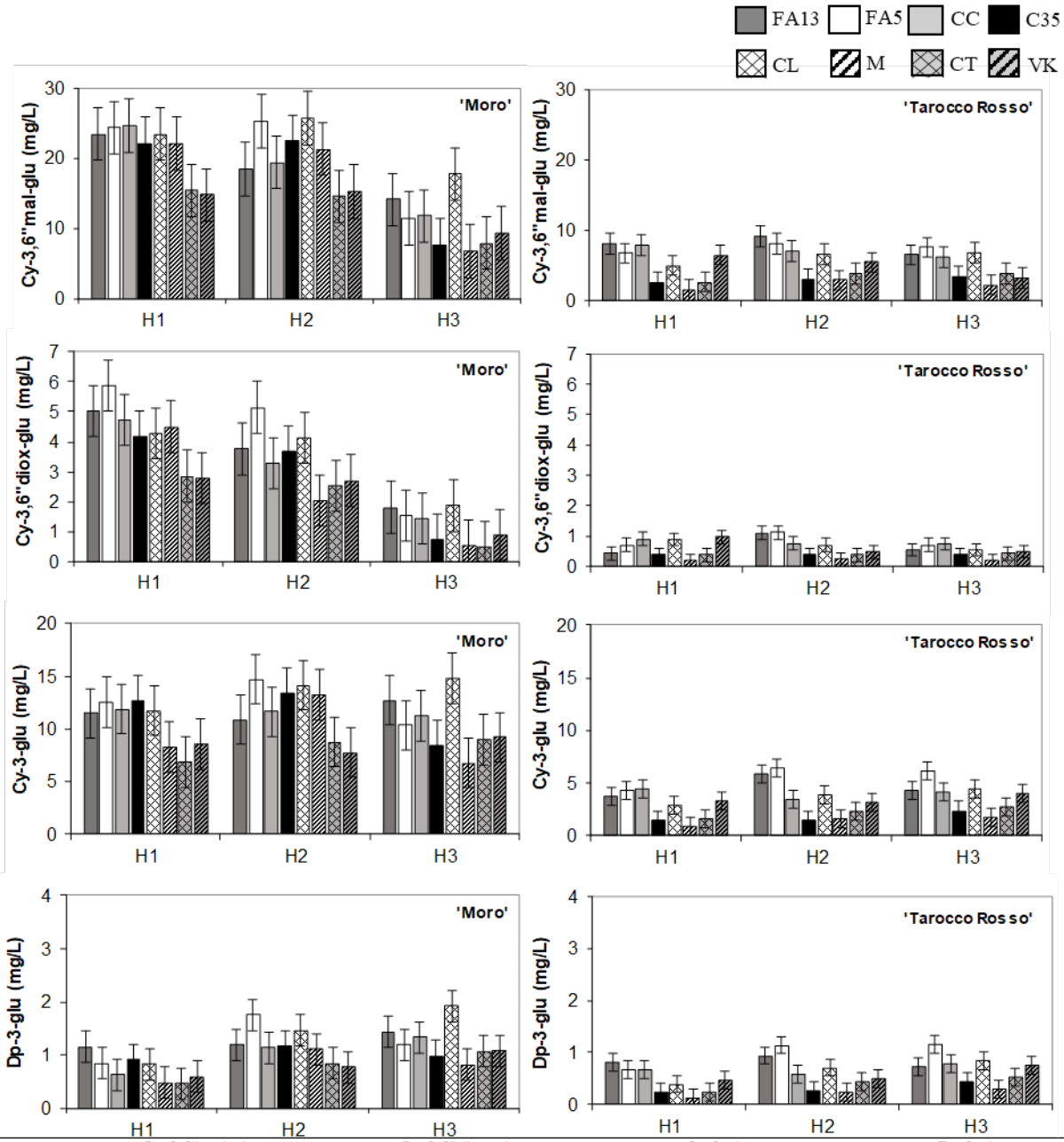
| | | Moro | | | Tarocco Rosso | | |
|---|---|---|---|---|---|---|---|
| | | H1 | H2 | H3 | H1 | H2 | H3 |
| Firmness (% Def.) | FA13 | 1,8 a A | 2,1 b AB | 2,3 c B | 1,9 bcd A | 1,9 bcd A | 1,9 abc A |
| | FA5 | 1,9 a A | 2,1 bc A | 2,0 bc A | 1,6 ab A | 1,8 abc A | 1,6 a A |
| | CC | 2,2 b A | 2,0 ab A | 2,0 bc A | 1,9 bcd A | 2,0 cd A | 1,9 ab A |
| | C35 | 1,8 a A | 1,9 ab A | 1,7 ab A | 1,7 abc A | 1,7 ab A | 2,3 bc B |
| | CL | 2,2 b A | 2,2 bc A | 2,0 c A | 2,0 cd A | 2,0 bcd A | 2,1 abc A |
| | M | 2,2 b A | 2,4 c A | 2,1 c A | 2,2 de A | 2,4 e A | 3,3 d B |
| | CT | 1,6 a A | 1,8 a A | 1,6 a A | 1,4 a A | 1,5 a A | 1,6 a A |
| | VK | 2,2 b A | 2,1 bc A | 2,0 bc A | 2,6 e A | 2,4 de A | 2,5 c A |
| TSS (ºBrix) | FA13 | 9,3 a A | 9,8 a AB | 10,5 ab B | 11,2 c A | 11,4 cd A | 11,3 bc A |
| | FA5 | 10,3 cd A | 10,6 de AB | 10,8 b B | 11,0 bc A | 11,8 d B | 11,7 c B |
| | CC | 10,4 d A | 10,8 e A | 10,8 b A | 10,4 b A | 11,1 cd B | 11,1 bc B |
| | C35 | 10,0 bcd A | 10,5 cde B | 10,7 b B | 10,2 ab A | 10,3 ab A | 10,5 b A |
| | CL | 10,1 cd A | 10,4 cde AB | 10,8 b B | 10,7 bc A | 11,1 cd B | 11,1 bc B |
| | M | 9,5 ab A | 9,9 ab AB | 10,1 a B | 9,9 a A | 10,0 a A | 9,9 a A |
| | CT | 9,8 bc A | 10,3 bcd B | 10,4 ab B | 10,8 bc A | 11,2 cd A | 11,2 bc A |
| | VK | 9,9 bc A | 10,0 abc A | 10,3 ab A | 10,7 bc A | 10,8 bc A | 10,8 b A |
| TA (g citric acid/ L juice) | FA13 | 1,36 a B | 1,31 a B | 1,14 a A | 1,21 b B | 1,11 bc A | 0,85 a A |
| | FA5 | 1,48 bcd B | 1,29 a B | 1,24 a A | 1,13 ab B | 1,08 bc A | 0,90 abc A |
| | CC | 1,44 abc B | 1,34 a AB | 1,21 a A | 1,14 ab B | 1,04 ab AB | 0,90 abc A |
| | C35 | 1,54 cd B | 1,49 c B | 1,26 ab A | 1,11 ab A | 1,11 bc A | 0,99 c A |
| | CL | 1,48 bcd B | 1,46 bc A | 1,26 ab A | 1,10 ab B | 1,08 bc AB | 0,94 abc A |
| | M | 1,49 bcd A | 1,41 abc A | 1,40 c A | 1,00 a A | 0,92 a A | 0,85 a A |
| | CT | 1,60 d C | 1,51 c B | 1,37 bc A | 1,15 ab B | 1,17 c B | 0,97 c A |
| | VK | 1,35 a B | 1,34 a A | 1,14 a A | 1,09 ab B | 1,01 ab AB | 0,87 a A |

| p-value | Firmness | | TSS | | TA | |
|---|---|---|---|---|---|---|
| | Moro | Tarocco Rosso | Moro | Tarocco Rosso | Moro | Tarocco Rosso |
| A:Rootstock | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,000 * |
| B: Harvest | 0,160 | 0,001 * | 0,000 * | 0,054 | 0,000 * | 0,000 * |
| AB | 0,056 | 0,010 * | 0,763 | 0,999 | 0,349 | 0,473 |



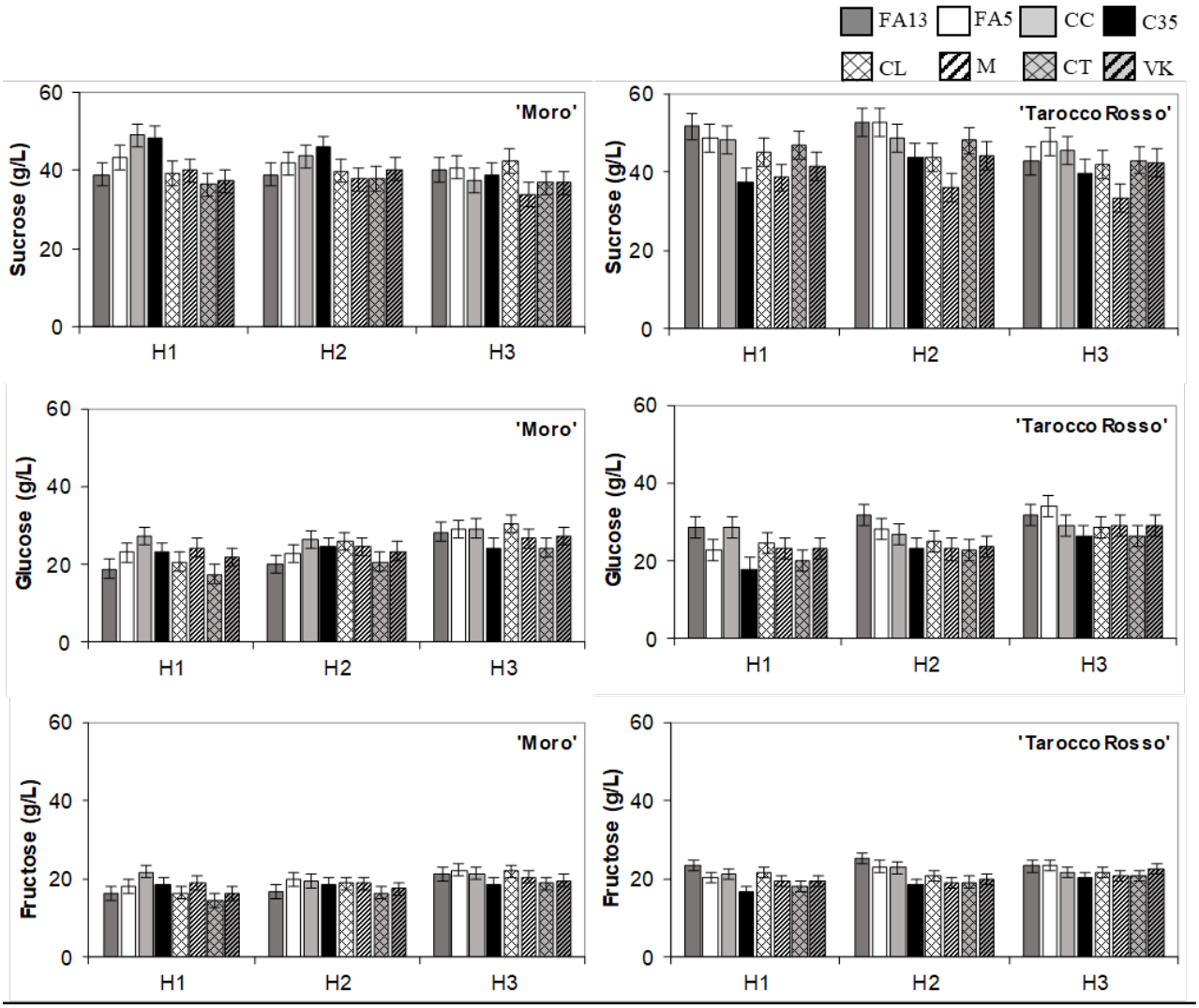

| p-value | Darkest Area Color | | Bright Area Color | | Juice Color | |
|---|---|---|---|---|---|---|
| | Moro | Tarocco Rosso | Moro | Tarocco Rosso | Moro | Tarocco Rosso |
| A:Rootstock | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,000 * |
| B:Harvest | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,022 * | 0,364 |
| AB | 0,159 | 0,115 | 0,638 | 0,173 | 0,887 | 0,544 |

649**Fig. 1.** Peel (dark and bright areas) and juice color of blood oranges ‘Moro’ and ‘Tarocco Rosso’ 650grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to 651late March. Vertical bars represent the LSD test ($p \leq 0.05$).



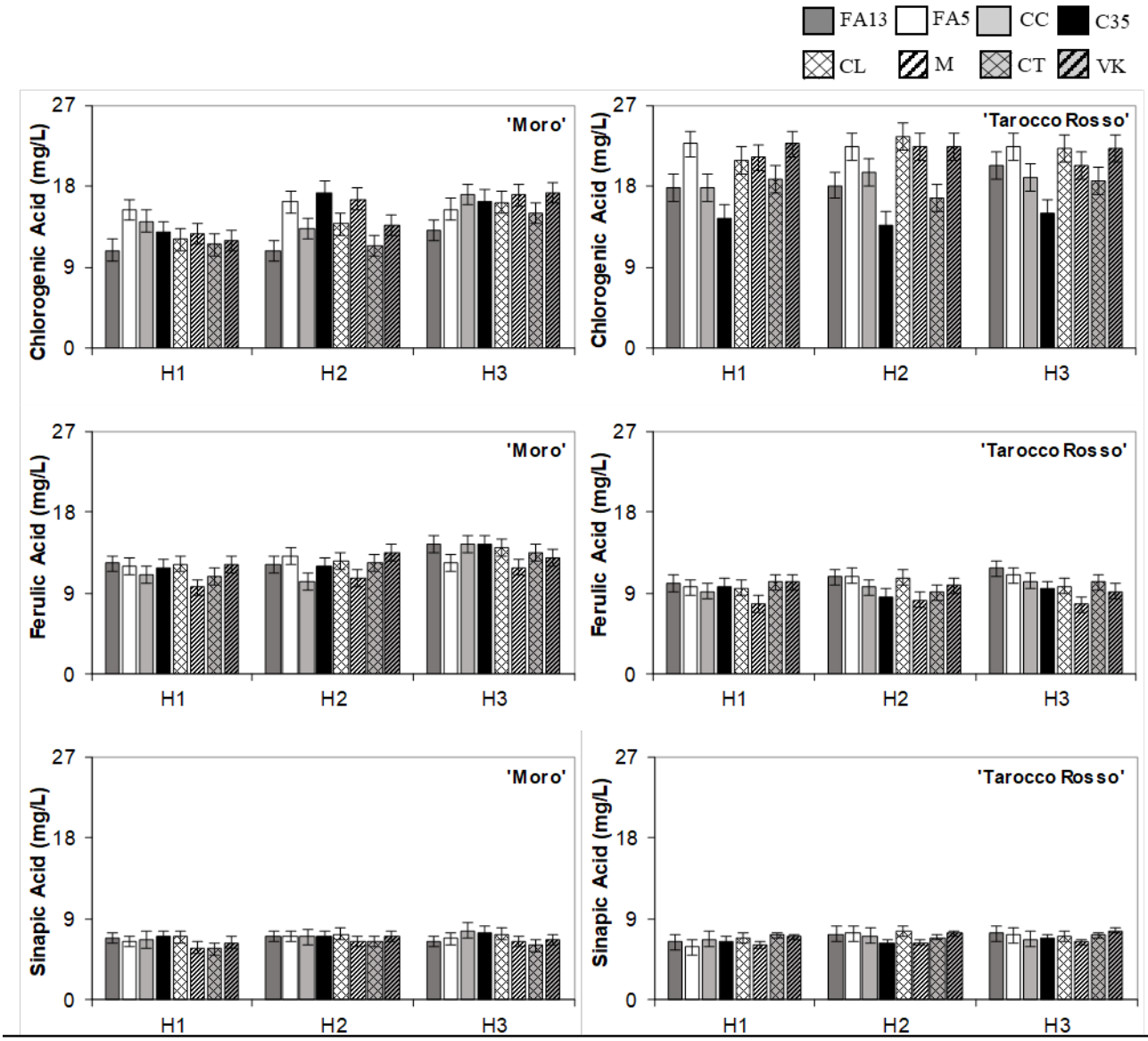

| p-value | Cy-3,6"mal-glu | | Cy-3,6"diox-glu | | Cy-3-glu | | Dp-3-glu | |
|---|---|---|---|---|---|---|---|---|
| | Moro | Tarocco Rosso | Moro | Tarocco Rosso | Moro | Tarocco Rosso | Moro | Tarocco Rosso |
| A:Rootstock | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,004 * | 0,000 * | 0,002 * | 0,000 * |
| B:Harvest | 0,000 * | 0,427 | 0,000 * | 0,113 | 0,296 | 0,011 * | 0,000 * | 0,001 * |
| AB | 0,188 | 0,830 | 0,350 | 0,053 | 0,345 | 0,440 | 0,213 | 0,362 |

652**Fig. 2.** Individual anthocyanins (delphinidin 3-glucoside (Dp-3-glu), cyanidin 3-glucoside (Cy-3-
653glu), cyanidin 3-(6”malonyl)-glucoside (Cy-3,6”mal-glu) and cyanidin 3-(6”-dioxalyl)-glucoside
654(Cy-3,6”diox-glu)) of blood oranges ‘Moro’ and ‘Tarocco Rosso’ grafted onto eight different
655rootstocks at three harvest times (H1, H2, H3) from early February to late March. Vertical bars
656represent the LSD test ($p \leq 0.05$).



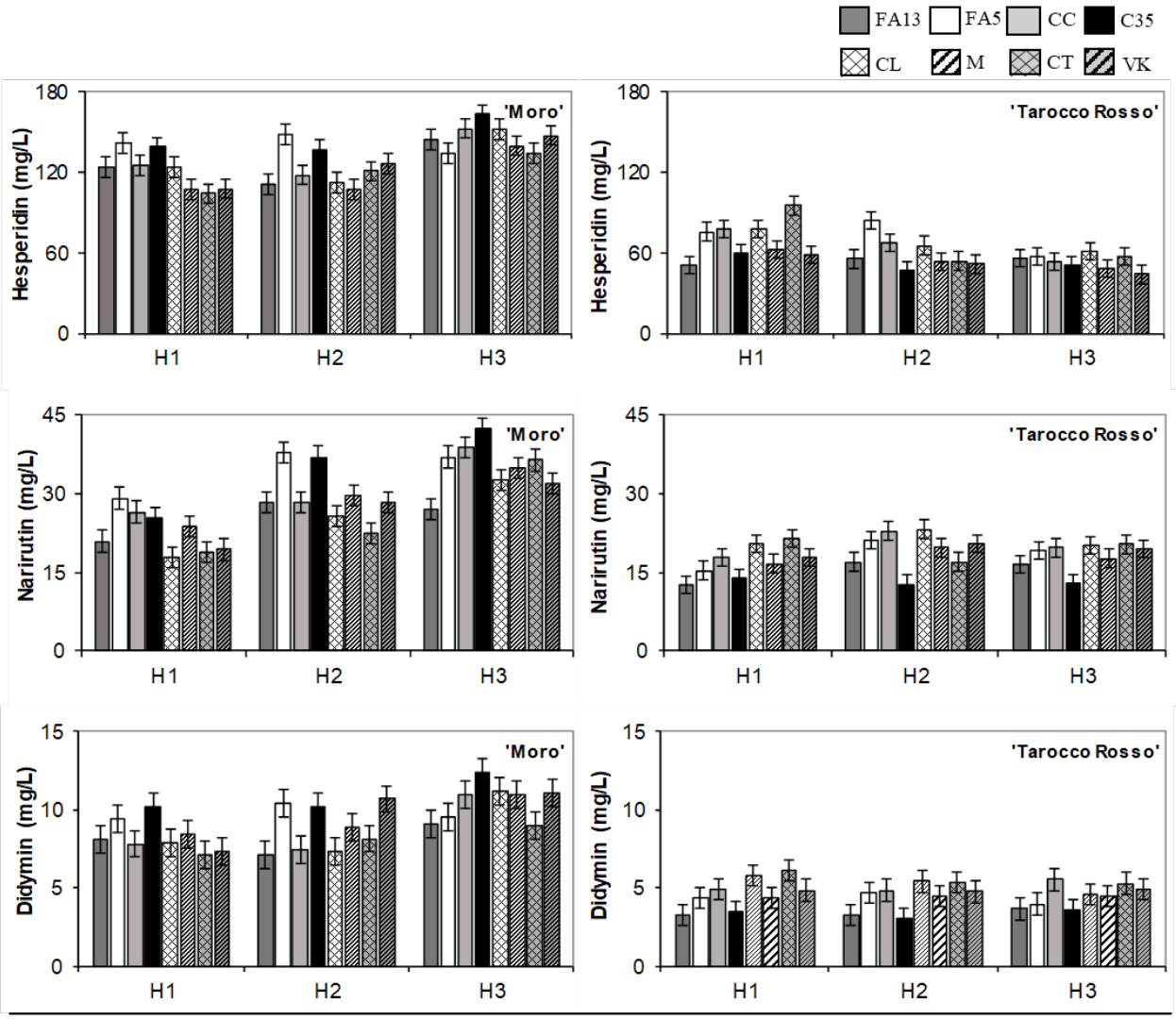

| p-value | Sucrose | | Glucose | | Fructose | |
|---|---|---|---|---|---|---|
| | Moro | Tarocco Rosso | Moro | Tarocco Rosso | Moro | Tarocco Rosso |
| A:Rootstock | 0,000 * | 0,000 * | 0,001 * | 0,000 * | 0,000 * | 0,000 * |
| B:Harvest | 0,011 * | 0,003 * | 0,000 * | 0,000 * | 0,000 * | 0,002 * |
| AB | 0,067 | 0,723 | 0,274 | 0,301 | 0,094 | 0,198 |

**Fig. 3.** Individual sugars (sucrose, glucose and fructose) of blood oranges 'Moro' and 'Tarocco Rosso' grafted onto eight different rootstocks at three harvest times (H1, H2, H3) from early February to late March. Vertical bars represent the LSD test ($p \leq 0.05$).



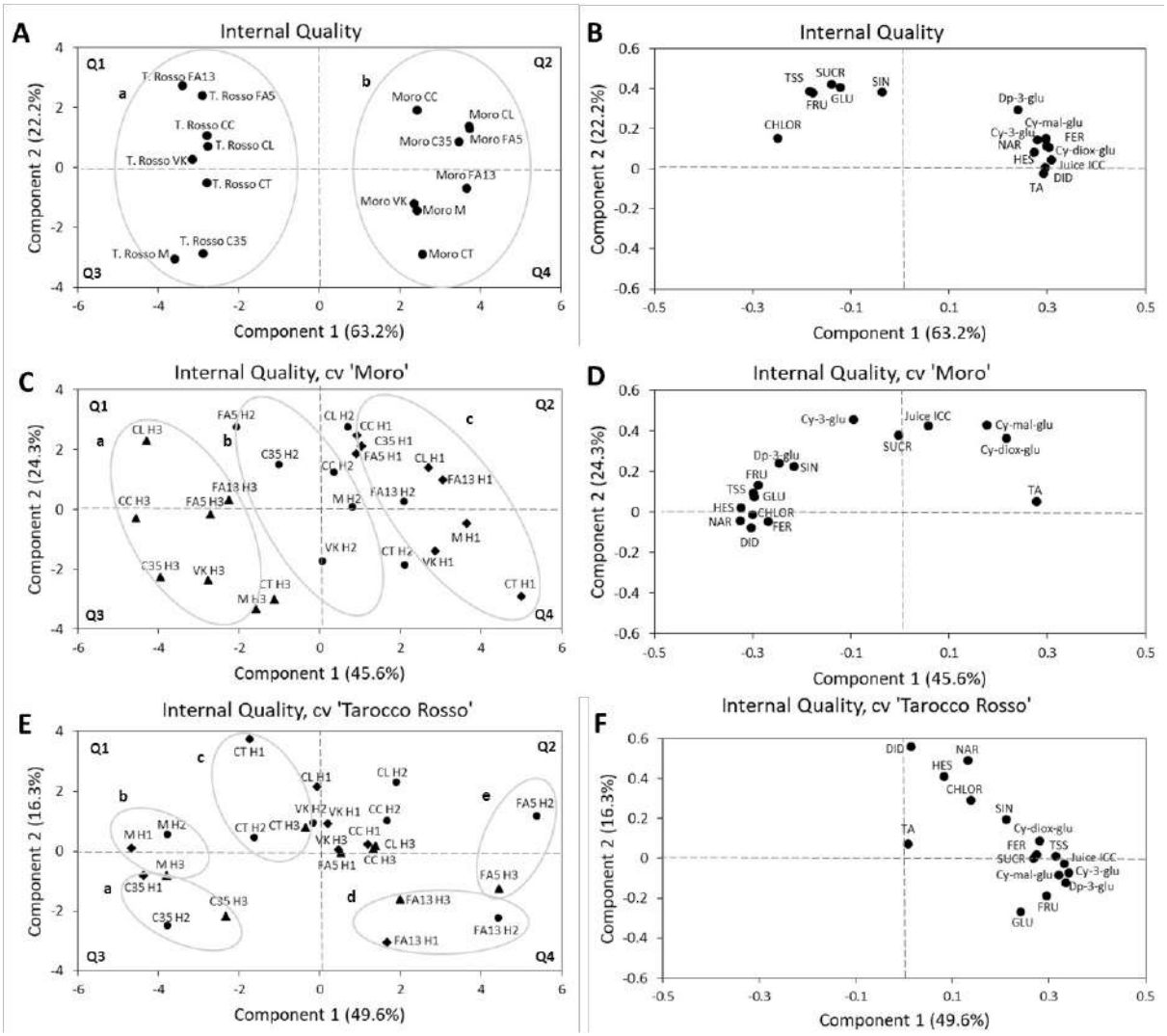

| p-value | Chlorogenic | | Ferulic | | Sinapic | |
|---|---|---|---|---|---|---|
| | Moro | Tarocco Rosso | Moro | Tarocco Rosso | Moro | Tarocco Rosso |
| A:Rootstock | 0,000 * | 0,000 * | 0,003 * | 0,000 * | 0,012 * | 0,015 * |
| B:Harvest | 0,000 * | 0,680 | 0,000 * | 0,434 | 0,032 * | 0,016 * |
| AB | 0,011 * | 0,389 | 0,100 | 0,371 | 0,924 | 0,581 |

661**Fig. 4.** Individual hydroxycinnamic acids (chlorogenic, ferulic and sinapic acid) of blood oranges 662'Moro' and 'Tarocco Rosso' grafted onto eight different rootstocks at three harvest times (H1, H2, 663H3) from early February to late March. Vertical bars represent the LSD test ($p \leq 0.05$).




| p-value | Hesperidin | | Narirutin | | Didymin | |
|---|---|---|---|---|---|---|
| | Moro | Tarocco Rosso | Moro | Tarocco Rosso | Moro | Tarocco Rosso |
| A:Rootstock | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,000 * | 0,000 * |
| B:Harvest | 0,000 * | 0,000 * | 0,000 * | 0,003 * | 0,000 * | 0,791 |
| AB | 0,000 * | 0,000 * | 0,000 * | 0,021 * | 0,009 * | 0,906 |

664**Fig. 5** Individual flavanone (hesperidin (HES), narirutin (NAR) and didymin (DID)) of blood 665oranges 'Moro' and 'Tarocco Rosso' grafted onto eight different rootstocks at three harvest times 666(H1, H2, H3) from early February to late March. Vertical bars represent the LSD test ($p \leq 0.05$).




667**Fig. 6** Principal component analysis of the internal quality parameters of ‘Moro’ and ‘Tarocco 668Rosso’ blood oranges grafted onto eight rootstocks. The score and loading plots for the first and 669second principal components are shown. A, B: Data set (two cultivars, eight rootstocks). C, D: Data 670set (‘Moro’ cultivar, eight rootstocks and three maturity stages). E, F: Data set (‘Tarocco Rosso’ 671cultivar, eight rootstocks and three maturity stages).

672(Flavonoids: HES=hesperidin, NAR=narirutin, DID=didymin; individual sugars: SUCR=sucrose, GLU=glucose; 673FRU=fructose; hydroxycinnamic acids: CHLOR=chlorogenic, FER=ferulic, SIN=sinapic; individual anthocyanins: Cy-674mal-glu= cyanidin 3-(6”malonyl)-glucoside, Cy-diox-glu= cyanidin 3-(6”-dioxalyl)-glucoside, Cy-3-glu= cyanidin 3-675glucoside, Dp-3-glu= delphinidin 3-glucoside).