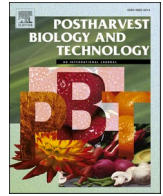




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Role of methyl jasmonate in enhancing pigmentation of Tarocco Sant'Alfio (*Citrus sinensis* [L.], Osbeck) oranges during cold storage

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ABSTRACT

The aim of study was to evaluate the effectiveness of postharvest methyl jasmonate (MeJA) treatment in enhancing flesh pigmentation and quality in the red orange cv. *Tarocco Sant'Alfio*, a cultivar known for its late ripening time and limited natural anthocyanin accumulation. Moreover, we tested an image analysis methodology to understand the dynamic of tissue pigmentation and, eventually, we investigate the specific role of individual anthocyanins. Oranges were treated with 0.1 mM MeJA and stored at 8 °C for 28 days. Weekly during storage, physical and organoleptic fruit traits as well as anthocyanins amount and composition, and bioactive compound, were measured. MeJA-treated fruit developed a significantly more intense and earlier red colouration than untreated fruit and exhibited a significantly lower weight loss. Image analysis of the flesh pigmentation gave a valuable insights into the effects of postharvest treatments and the dynamics of pigment accumulation, revealing a marked shift from low to high-intense red distributions in MeJA-treated fruit, in line with the spectrophotometric and HPLC-based anthocyanin quantification. Regression analyses showed that cyanidin 3-O-(6''-dioxalyl)-glucoside, peonidin 3-O-(6''-malonyl)-glucoside, cyanidin 3-O-glucoside, cyanidin 3-O-sophoroside and cyanidin derivatives, were the main contributors to colour development, with moderate to high correlation coefficients with R² ranging from 0.71 to 0.78, confirming the key role of specific anthocyanins in the observed pigmentation dynamics. These findings underscore the potential of MeJA as an effective postharvest treatment to enhance both the visual appeal and nutritional value of blood oranges with naturally low pigmentation.

1. Introduction

Most of commercial red oranges (*Citrus sinensis* [L.], Osbeck) have Mediterranean origins and are mostly grown in a few Mediterranean countries (Italy, Spain, Morocco, etc.) where favourable climatic conditions induce the synthesis of anthocyanins responsible of the red colour of both the flesh and the rind of the fruit (Rapisarda and Giuffrida, 1992; Butelli et al., 2012; Rapisarda et al., 2022; Legua et al., 2022). Indeed, consumers perceive the presence of anthocyanins as a major quality attribute of red oranges (Habibi et al., 2022). With more

than 35,000 ha in Sicily (Istat, 2020), *Tarocco* is the most cultivated red orange variety in Italy and several *Tarocco* lines with different morphological and qualitative features, including pigmentation, have been selected to widen the market calendar of red oranges from December until May (Tribulato and La Rosa, 1994). While the synthesis of anthocyanins depends mostly on genotype, other factors such as rootstock, environmental conditions, especially cold temperatures, significantly promote anthocyanin biosynthesis through transcriptional regulation of key genes in the flavonoid biosynthetic pathway (Lana et al., 2021, Modica et al., 2024). Light intensity, ripening period and

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irrigation management may also play a significant role in the expression of the anthocyanin synthesis (Stagno et al., 2015; Modica et al., 2022; Rapisarda et al., 2022). Several authors reported the role of the temperature in the activation of anthocyanin biosynthesis in both peel and juice (Pannitteri et al., 2017; Fabroni et al., 2016). Rapisarda et al. (2001) and Crifò et al. (2012) reported that storage at 8 °C or 4 °C, respectively, affected the increment of anthocyanin content in pigmented red oranges. Red oranges are non-climacteric fruit, but 9 °C temperatures more than 4 °C were more effective in increasing pigmentation during postharvest storage in *Moro* and *Sanguinelli* cultivars (Carmona et al., 2017, 2021). It has also been described that accumulation in anthocyanins in *Moro*, *Tarocco*, *Sanguinello* and *Sanguigno* cultivars is achieved at moderate temperatures, while temperatures below 3 °C have been found to be inefficient for the activation of anthocyanins (Habibi et al., 2020a). However, even if it is clear that postharvest fruit storage with constant temperatures induce pigmentation, the effect of fluctuant cold temperatures under field conditions is a topic that has been recently investigated (Modica et al., 2024).

Methyl jasmonate is a naturally occurring plant hormone derived from jasmonic acid (JA), involved in physiological processes such as plant defence mechanisms, fruit ripening and senescence and responses to biotic and abiotic stress factors (Wang et al., 2021). Postharvest treatment with MeJA has been reported to maintain quality and bioactive compounds during cold storage in pomegranate (Sayyari et al., 2011) reducing also CI symptoms in the rind of pigmented red orange cv. *Moro* (Habibi et al., 2019). Moreover, the postharvest dip treatment of 1.5 mM MeJA improved the juice colour and the levels of anthocyanins and flavonoids in fruit of *Tarocco Ippolito* after 45 days at 20 °C (Vithana et al., 2024).

Tarocco Sant'Alfio is the latest to ripe among *Tarocco* clones, however it usually shows a low degree of pigmentation and this may reduce its commercial value because the choice of the consumer is based on the relation between intensity of colouration, hence anthocyanins content, and nutritional value of the fruit. Eventually, differences in pigmentation among *Tarocco* clones may create misunderstandings in the market. Therefore, the objective of this study was to investigate whether MeJA treatments may enhance either flesh anthocyanin content during 28 days storage at 8 °C in this low pigmented *Tarocco* line, explaining the role of individual anthocyanins in developing juice pigmentation. Eventually, we also investigated the possible role of an image-based method to fastly understand the dynamic of colour distribution within the fruit flesh.

2. Materials and methods

2.1. Plant material, treatment and storage conditions

The experiment was carried out in a 10-year-old commercial orchard of *Tarocco Sant'Alfio* orange (*C. sinensis* L. Osbeck) grafted onto *Carrizo* citrange rootstock, grown in the plain of Catania (Italy) (37°16'59"N 14°53'14"E; 59 a.s.l.). Trees were spaced 5 m x 3 m apart. The texture components of the soil were 68.6% sand, 23.6% loam, and 7.8% clay, pH was 8.5, and the content of active lime was 3.0%. The orchard was subjected to standard cultural practices. Three hundred (300) uniform and without apparent damages fruit were randomly harvested at commercial maturity the first week of May 2024 and immediately transported in laboratory, without any fungicide or wax application. Sixty fruits were used to analyse their properties soon after they had reached the lab, at harvest time (day 0). The fruit were divided into two groups: one group (120 fruit) was immersed in a solution containing 0.1 mM MeJA, 1% Tween 20, and 1% ethanol for 10 min, while the other group (120 fruit) was soaked in distilled water for the same duration. All fruit were then stored at 8 °C and 90% relative humidity for 28 days. 10 fruits x 3 replicates for each treatment and sampling date was used for chemical and physical analysis.

2.2. Postharvest quality parameters

Individual weight was recorded at day 0 and at each sampling date, after 7, 14, 21, 28 d of storage. Weight loss was expressed as the percentage reduction with respect to initial time, using the following equation: % Weight loss: [(Initial fruit weight-Final fruit weight) x 100]/Initial fruit weight. Fruit juice was extracted with a commercial juice extractor (Moulinex JU420D10.). Total soluble solids (TSS, expressed as °Brix), were determined from the juice of each fruit from each tray using a digital refractometer (model PR-101, Atago, Co., Tokyo, Japan) at 20 °C. Titratable acidity (TA, expressed as g L⁻¹) was determined by titration of 10 mL of juice with 0.1 M NaOH to an end of pH 8.1. The CieLab* values are valuable parameters for objectively describing and quantifying the colour characteristics of citrus fruits (Habibi et al., 2021). Juice colour (10 fruits x 3 replicates for each treatment and sampling date) was measured with a portable colourimeter (Minolta CR400, Minolta, Osaka, Japan), equipped with an 8-mm measuring head and a C illuminant (6774 K). The instrument was calibrated using the manufacturer's standard white plate. Juice orange colour changes were quantified in L*, a* and b* colour space and ΔE* was calculated as previously described (Allegra et al., 2022). Visual quality (colour, structural integrity and appearance) was evaluated on the 30 fruit after harvest (T0). Subsequently, at each sampling date, each male and female judge evaluated another 10 oranges per treatment. In order to carry out the sensory analysis, six panelists (3 males and 3 females), with an average age of 45 years, were selected from the University of Palermo campus according to Di Miceli et al., 2010. Panelists were selected based on having previously consumed orange fruit, having no known allergies to orange fruit, and being available to attend all training and tasting sessions. Visual appearance score resulted from the medium value of juice orange colour, and visual appearance. The different descriptors were quantified using a subjective 5–1 rating scale with 5 = very red, 4 = red, 3 = sufficient red, 2 = low red and 1 = yellow.

2.2.1. Image Analysis

The image acquisition setup was a DSLR camera (Canon EOS 400D DIGITAL, Tokyo, Japan) equipped with EF-S 18–55 mm, F3.5–5.6 IS lens. The camera settings included a focal length of 55 mm, a shutter speed of 1/4 sec. ISO of 100, F-stop of 7.1. For illumination, four OSRAM L 18 W/765 daylight lamps (Germany) were used, each with a luminous intensity of 1050 lm and a colour temperature of 6500 K. Obtained images presented a resolution of 1936 × 1288 pixels at 72 dpi. Image processing was conducted using self-developed algorithm in Python (version 3.11.5) to quantitatively track chromatic changes during storage. This analysis aimed to evaluate the intensity of red pigmentation in the internal flesh and to determine how closely this visual parameter reflected actual pigment accumulation (i.e., anthocyanins) in response to postharvest treatment with MeJA. Images were collected after 7, 14, 21, and 28 days, and analysed in the R, G, and B colour space. Morphological filtering was applied to isolate the central regions of the

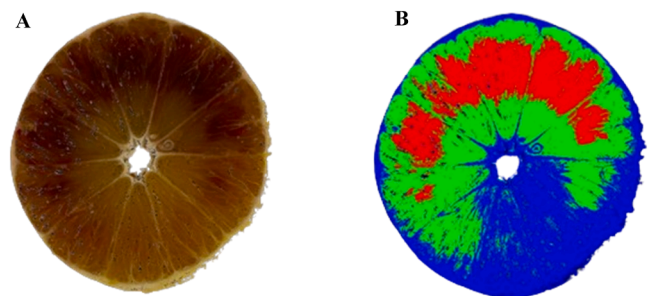


Fig. 1. Colour assessment of pigmentation in red orange cv. *Tarocco Sant'Alfio* (*Citrus sinensis* [L.], Osbeck). Original image (A) and segmented pixel intensity map (B) based on R channel (low = blue, medium = green, high = red).

fruit, and red colour intensity was quantified based on pixel distribution within the red channel (Fig. 1). Fruit's flesh areas were segmented through colour thresholding in combination with morphological operations, enabling the identification of the largest contours corresponding to individual fruit sections. Within these regions, pixel classification was performed based on red channel intensity (R), using empirically defined thresholds: $R \leq 70$ (Low-intensity red), $71 \leq R \leq 110$ (Medium-intensity red), and $R > 110$ (High-intensity red). These thresholds allowed for a quantitative assessment of anthocyanin-related pigmentation levels within the flesh. To facilitate visual interpretation, the classified red intensity levels were displayed using a false-colour RGB scheme, where R is red = high, G is green = medium, B is blue = low (Fig. 1). This approach enabled the detection of subtle chromatic gradients within the flesh, ranging from pale yellow to deep red. For each sample, the percentage distribution of pixels across the three intensity levels was calculated, providing a reproducible, image-based quantification of internal pigmentation.

2.2.2. Extraction and measurement of total anthocyanins, carotenoids, and phenolics

For each treatment, 10 fruits were divided in 10 replicates ($n = 10$) to extract and measure total anthocyanins in the frozen juice -18°C at every sample day. For that, 2.5 mL of red orange juice were mixed with 5 mL of extractant agent methanol/hydrochloric acid/water (25:1:24, v/v/v) and homogenized (Ultraturrax, T18 basic, IKA, Berlin, Germany) for 30 s. Then, the extract was cold centrifugated at 4°C and $10,000 \times g$ for 10 min, as previously described by García-Pastor, et al. 2020. Finally, total anthocyanin content in the juice was quantified by measuring the absorbance of the supernatant at 520 nm using a spectrophotometer (UNICAM Helios- α , Artisan Technology Group, Champaign, IL, USA). Results were expressed as mg kg^{-1} of cyanidin 3-O-glucoside equivalents, the predominant anthocyanin in red oranges. On the other hand, total carotenoids were extracted adapting the method used by Kneeb (1972). Total carotenoid content in juice was measured using 2.5 mL of juice in 10 mL of the extraction solution. Quantification was performed on the liposoluble phase extract reading the absorbance at 450 nm using a spectrophotometer (UNICAM Helios- α , Artisan Technology Group, Champaign, IL, USA). Results were expressed as $\mu\text{g g}^{-1}$ of β -carotene equivalent ($\epsilon = 2505 \text{ L mol}^{-1} \text{ cm}^{-1}$).

In a similar way, phenolic content in the juice was determined. 2.5 mL of frozen juice were mixed with 10 mL of the extractant water: methanol (2:8, v/v) added with 2 mM of sodium fluoride (NaF, 1.5 w/v). After homogenization (Ultraturrax T18 basic, IKA, Berlin, Germany) for 30 s, the sample was centrifugated at 4°C and $10,000 \times g$ for 10 min. Then, 200 μL of the supernatant were mixed with 300 μL of phosphate tampon (50 mM pH 7.8) and 2.5 mL of water-diluted 1:10 Folin-Ciocalteu reagent (Díaz-Mula, et al. 2009). The mixture was then incubated for 2.5 min at room temperature followed by the addition of 2 mL of NaCO_3 (53 g L^{-1}) to stop the colorimetric reaction. The final mixture was incubated in a water bath at 50°C for 5 min, after which absorbance was measured at 760 nm. Results were expressed as mean \pm SE in mg kg^{-1} gallic acid equivalents of fresh weight.

2.2.3. Metabolite profiles and content determined via HPLC/Uv-Vis /DAD and HPLC/ESI/Orbitrap MS analyses

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. High purity standards cyanidin 3-O-glucoside, rutin, chlorogenic acid, sinapic acid and p-coumaric acid were purchased from Sigma (VWR chemicals, Milan, Italy), whilst naringin, hesperidin and vitexin were from Extrasynthese (Lyon, France). Small portions (2 mL) of the juices were put in 15 mL plastic sample tubes and 100 μL of formic acid (98 %) were added. Samples were shaken during five minutes, then centrifuged at 4000 rpm for 10 min to separate the solid portion of the juices. 1 mL of the clear supernatants were transferred into 2 mL HPLC amber vials and immediately analysed.

Chromatographic analyses were carried out on an Ultimate 3000 UHPLC focussed 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 and DAD acquisitions were all performed according to Pannitteri et al. (2017). A series of HPLC/ESI/MS analyses were also performed on a selected number of representative samples to confirm spectrophotometry-based peak assignments. In this case, aliquots (5 mL) of previously centrifuged juices were freeze dried (Lyoquest-85, Telstar Italy, Legnano, Milan, Italy) then re-dissolved in 2 mL HPLC grade water and transferred into 2 mL HPLC amber vials ready to ESI/MS analyses. 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 performed according to our previous works (Modica et al., 2022; Pannitteri et al., 2017) All analyses were carried out in triplicate; results are reported in mg kg^{-1} of compound of juice.

2.2.4. Statistical analysis

Analysis of variance (ANOVA) was carried out using Systat 13.0 for Windows and used to test the significance of each variable. Significant differences ($P < 0.05$) between treatments were evaluated with Tukey's test. Linear regression analysis was conducted to correlate the concentration of individual anthocyanins with the percentage of high-intensity red area using R studio software (2025.09.1 +401, Posit software, PBC, Boston, US).

3. Results and discussion

3.1. Colour and weight loss

MeJA application significantly influenced fruit weight loss, sugar-acid balance, and especially external and internal colour attributes (Ezzat et al., 2017). MeJA-treated fruit consistently showed lower weight loss throughout storage, remaining below 1 % during the first two weeks, about half the value recorded in untreated fruit (Fig. 2). This reduced transpiration is likely to be due to the protective effect of MeJA on peel integrity and epicuticular waxes, as previously reported (Habibi et al., 2019).

Total soluble solids (TSS) increased progressively in untreated fruit during storage, from 13.3 ± 0.19 – $14.8 \pm 0.11^\circ\text{Brix}$, at day 0 and 28 respectively, with a rise averaging $\sim 11\%$, whereas MeJA-treated fruit maintained stable TSS levels ($13.4 \pm 0.24^\circ\text{Brix}$) during the first two weeks, to increase significantly at the end of the storage period ($14.3 \pm 0.20^\circ\text{Brix}$). No significant difference occurred between treatments (Table S1). Titratable acidity (TA) declined similarly in both treatments ($10.3 \pm 0.02 \text{ g L}^{-1}$ at day 0 and $9.8 \pm 0.05 \text{ g L}^{-1}$ at day 28 (Table S1).

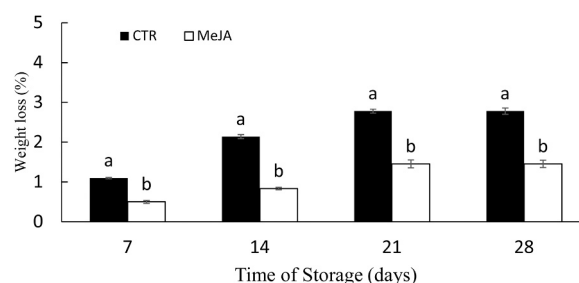


Fig. 2. Weight loss (%) of treated (0.1 mM MeJA) and untreated (CTR) orange fruit cv. *Tarocco Sant' Alfio* (*Citrus sinensis* [L.] Osbeck) just after being dipped (0) and after 7, 14, 21, 28 days of storage at 8°C . Different letters indicate significant differences between treatments at each sampling date. $p \leq 0.05$ was used in the Tukey's test. The data are provided as a mean \pm S.E. ($n = 30$).

As far as flesh colour is concerned, lightness (L^*) gradually decreased in both treatments, with no significant differences among them. This behavior, related to the reduction in L^* values, is likely to be related to the increase in anthocyanin content during storage in both treated and untreated samples. A similar result was observed by Habibi et al. (2024) in the juice and flesh of *Budd Blood* oranges stored at 10 °C and 12 °C. However, a^* values significantly increased in MeJA-treated fruit starting from day 14, indicating enhanced red pigmentation (Table 1). In fact, the increase in red ($+a^*$) components corresponds to the rise in anthocyanin pigments (Habibi et al., 2021), which were almost nihil at harvest, while in fruit treated with MeJA, the mean values significantly increased along with the storage time. The b^* values showed a general increasing trend in both treatments, consistent with typical ripening-associated yellowing, with significant higher in MeJA-treated samples occurring 7, 21, and 28 d after storage, suggesting that the treatment promoted a more intense yellow colourness. Overall, juice colour changes were further confirmed by ΔE^* values, which increased over time in both control and treated fruits, indicating visible colour changes as storage progressed. MeJA treatment showed significantly higher ΔE^* values at days 14 and 21, highlighting more pronounced visual changes, possibly due to alterations in pigment metabolism induced by the treatment (Table 1). Significant difference in visual score occurred only during the first two weeks of storage when panelists gave lower values to untreated fruit than for treated ones (Fig. 3). Different results showed by Carmona et al. (2017) on cv. *Moro* stored at 9 °C for 45 days where a colour change towards red occurred on day 30.

3.2. Total carotenoids, phenolics and anthocyanins content

Total carotenoids did not change with treatments during most of the storage period, but at the last sampling date, when treated fruit had the highest values (Fig. 4A). Similar results occurred in *Navelina* orange fruit stored at 12 °C, after 14 days of storage, showing also an accumulation of B-cryptoxanthin, a carotenoid with pro-vitamin (Carmona et al., 2012). Polyphenols content (Fig. 4B) increased approximately 25 % during storage in both treatments. However, a significant difference between treatments was observed only during the first week post-storage. The increase in phenolic content is the response of plants to biotic and abiotic stresses, such as a pathogen attack, wounding, high levels of visible light, and cold stress (Amodio et al., 2014). It is likely that MeJA treatment retarded a few days the increase of polyphenols content due to temperature induced stress. MeJA is well known to effectively promote the accumulation of total phenolic content as a fruit disease resistance mechanism (Wang et al. 2021), but our results support the hypothesis that storage temperatures higher than 6 °C may enhance phenolic biosynthesis in red orange fruit by activating the phenylpropanoid more efficiently than MeJA (Carmona et al., 2017). It has been widely shown that anthocyanin content in red orange fruits may increase drastically through cold postharvest storage (Lo Piero et al., 2005; Rapisarda et al., 2001). Our results suggest that orange fruit treated with MeJA had a higher anthocyanin content than the control throughout the storage period and at each of the sampling dates (Fig. 4C). The increase in anthocyanin content induced by MeJA became

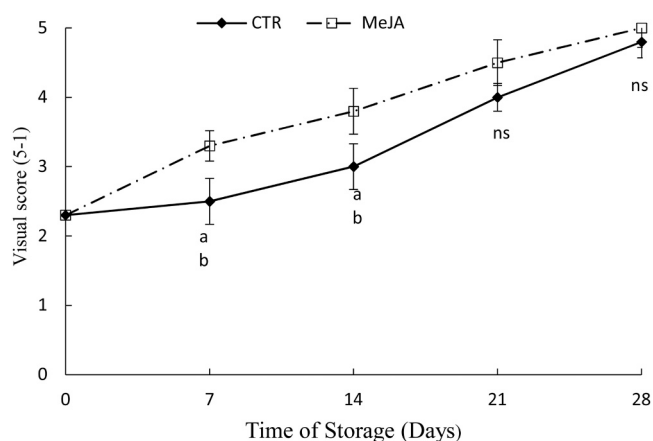


Fig. 3. Visual score (5–1) of treated (0.1 mM MeJA) and untreated (CTR) orange fruit cv. *Tarocco Sant' Alfio* (*Citrus sinensis* [L.] Osbeck) just after being dipped (0) and after 7, 14, 21, 28 days of storage at 8 °C. The different descriptors were quantified using a subjective 5–1 rating scale with 5 = very red, 4 = red, 3 = sufficient red, 2 = low red and 1 = yellow. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as a mean \pm S.E.

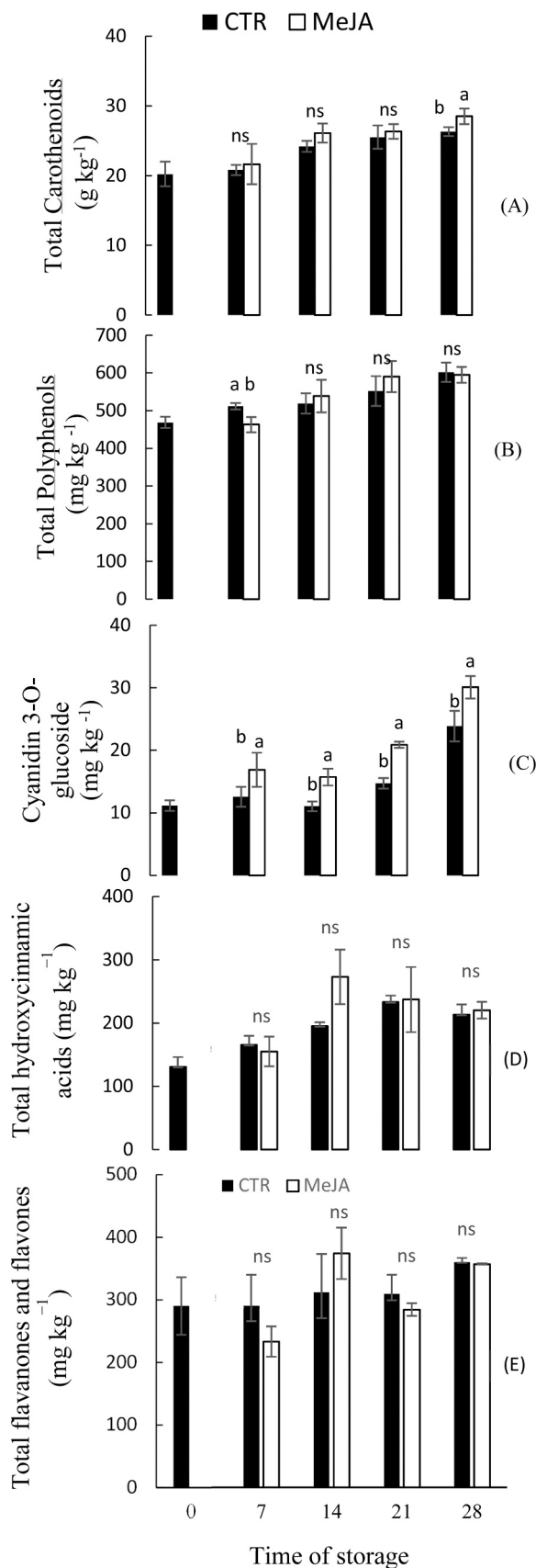
significant one week soon after the treatment was applied; at this stage and until two weeks after storage the anthocyanin content in untreated fruit did not significantly change from T0 values. Anthocyanin production takes place in the cytosol, after which the molecules are transported into the vacuole. This compartmentalization helps to protect anthocyanins from oxidation and allowing them to function as pigments (Lo Piero, 2015). The biosynthesis process can be activated also with cold temperatures which can boost the activity of several key enzymes such as phenylalanine ammonia-lyase, anthocyanidin synthase, and UDP-glucose flavonoid glucosyltransferase (Carmona et al., 2017). This is consistent with what reported by Rapisarda et al., (2001) who showed that the genotype effect (*Tarocco* and *Moro*) on anthocyanin biosynthesis may differ when fruit are stored at 8 °C for a long period. Flavonol synthase and dihydroflavonol 4-reductase genes (DFR) may be involved in this process, as demonstrated in a previous study on *Moro* oranges stored at two different temperatures. In that study, DFR expression was significantly higher in fruits stored at 9 °C than at 4 °C, showing a strong correlation with the red-purple pigmentation of the flesh (Carmona et al., 2017). Therefore, it is reasonable to suggest that stress induced by MeJA treatment might also enhance DFR expression in a similar way.

As already discussed, several factors influence the accumulation of bioactive compounds (specialized metabolites) and, specifically, temperatures play a fundamental role in the biosynthesis and accumulation of citrus metabolites, particularly anthocyanins. It is broadly reported that red orange pigmentation depends on low temperatures, able to stimulate the biosynthesis of anthocyanins (Modica et al., 2022; Modica et al., 2024; Modica et al., 2025). Additionally, previous studies have focussed on the effect of post-harvest temperatures and on the

Table 1

Juice color change (CieLab* and ΔE^*) of treated (0.1 mM MeJA) and untreated (CTR) orange fruit cv. *Tarocco Sant' Alfio* (*Citrus sinensis* [L.] Osbeck) just after being dipped (0) and after 7, 14, 21, 28 days of storage at 8 °C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as a mean \pm S.E. (n = 30).

Days	L^*		a^*		b^*		ΔE^*	
	CTR	MeJA (0.1 mM)	CTR	MeJA (0.1 mM)	CTR	MeJA (0.1 mM)	CTR	MeJA (0.1 mM)
T0	44.9 \pm 0.14		-0.47 \pm 0.12		11.85 \pm 0.45		-	
T7	41.2 \pm 0.38	41.9 \pm 0.37 ns	4.4 \pm 0.37 b	3.5 \pm 0.27 a	9.6 \pm 0.44 b	11.5 \pm 0.32 a	6.6 \pm 0.59	5.1 \pm 0.42 ns
T14	39.6 \pm 0.43	40.3 \pm 0.51	4.1 \pm 0.27 b	6.2 \pm 0.18 a	8.7 \pm 0.63 b	16.5 \pm 0.48 a	8.4 \pm 0.67	9.5 \pm 0.55 ns
T21	39.6 \pm 0.32	40.1 \pm 0.61	7.1 \pm 0.53 b	9.1 \pm 0.29 a	15.6 \pm 0.53	17.0 \pm 1.04 ns	9.5 \pm 0.56 b	11.8 \pm 0.90
T28	37.3 \pm 0.37	36.7 \pm 0.86	12.3 \pm 0.44 b	14.9 \pm 0.54 a	14.5 \pm 0.68 b	16.4 \pm 1.15 a	16.6 \pm 0.95	16.5 \pm 0.64 ns



(caption on next column)

Fig. 4. A-E. Total carotenoids (g kg^{-1}) (A) and polyphenols (gallic acid) (B), cyanidin 3-O-glucoside (C) (D), and flavones (D), hydroxycinnamic acid (E) (mg kg^{-1}), in the frozen juice of untreated (CTR) and treated (0.1 mM MeJA) orange fruit cv. *Tarocco Sant'Alfio* (*Citrus sinensis* [L.] Osbeck) just after being dipped (0) and after 7, 14, 21, 28 days of storage at 8 °C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as a mean \pm S.E. ($n = 10$).

application of plant growth regulators or elicitors on the biosynthesis of individual metabolites on blood oranges (Pannitteri et al., 2017; Habibi et al., 2019; Fabroni et al., 2020; Vithana et al., 2024). The total content of colourless flavonoids in the juice did not undergo significant changes along the storage period (Fig. 4D). At T28 it was noticed that the values ranged from 360.1 mg L^{-1} (CTR) to 357.0 mg L^{-1} (MeJA). Regarding the biochemical class of hydroxycinnamic acids (Fig. 4E) at T14 an increase was recorded in MeJA-treated fruits, although no statistical difference was noted during the storage periods.

Anthocyanins are water-soluble pigments belonging to the flavonoid group, responsible for vibrant colours that vary from red to purple and blue in fruits and vegetables (Strack & Wray, 2017). Their chemical structures usually consist of a flavan-3-ol backbone, with variations in hydroxyl and methoxyl replacements strongly influencing colour nuances and stability (Nassour et al., 2020). Individual anthocyanins give rise to different colours, e.g. cyanidin and its derivatives transmit a red colour commonly seen in strawberries, while delphinidin and its derivatives contribute to blue tones found in blueberries (Martín-Gómez et al., 2020).

The colour of blood orange and juice tissues is significantly influenced by individual anthocyanins, which have been the subject of several studies emphasizing their statistical correlations and optical properties. Anthocyanin pigments are essential to define the characteristics of the colour of the blood orange juice, contributing significantly to its visual charm and nutritional properties. Rapisarda et al. (2022) elucidated the distribution and bioavailability of these pigments in different citrus species, highlighting their impact on antioxidant capacity. Similarly, Liu et al. (2025) employed hyperspectral image techniques to predict anthocyanin content, revealing correlations between pigment identity and concentration and juice quality. Several anthocyanins, such as derivatives of cyanidin and peonidin, show unique structural configurations that influence their intensity of colour and shades (Rapisarda et al., 2022).

Nine different anthocyanins contributed to the colour of juice: four cyanidin-based pigments, 2 delphinidin derivatives, 2 peonidin derivatives and finally one petunidin-based metabolite (Table S2). All of them showed a trend similar to that of total anthocyanin content along the storage period (Table 2). Cyanidin-based compounds were the most abundant in the juice, as previously reported by Habibi et al. (2020b). At T28, MeJA-treated fruits registered the highest value of these pigments (35.37 mg L^{-1}), although no significant difference were recorded. Peonidin derivatives were the second most abundant compounds, followed by delphinidin and petunidin derivatives, respectively. Previous studies observed that individual and total anthocyanin amount in *Tarocco Sant'Alfio* fruit stored at 1 °C (Pannitteri et al., 2017) and in *Sanguinello* and *Moro* fruit stored at 2 °C (Habibi et al., 2020a) were lower at the end of the post-harvest period than fruit stored at 4 °C and 5 °C. This suggests an inhibition of anthocyanin accumulation due to the very low temperatures. Storing *Tarocco Sant'Alfio* fruit at 4 °C resulted in a higher accumulation of cyanidin, delphinidin and peonidin than at 1 °C (Pannitteri et al., 2017). In our study, in addition to the higher values of the other anthocyanins, also petunidin was quantified during the storage at 8 °C. This difference can be related to the role of temperature storage that may affect the accumulation of individual anthocyanins in pigmented citrus. A total of four metabolites belonging to the biochemical subclass of flavanones and a single flavone (Table S2) were quantified in the juice of *Tarocco Sant'Alfio*; three flavanones (ericiotrin, hesperetin

Table 2

Individual anthocyanins (delphinidin, cyanidin, peonidin and petunidin), flavanones and flavones (eriocitrin, narirutin, hesperidin and vicenin-2) and hydroxycinnamic acids (p-coumaric acid, caffeic acid, ferulic acid and sinapic acid derivatives) contents in the juice of orange fruit cv. *Tarocco Sant' Alfio* (*Citrus sinensis* [L.] Osbeck) just after being dipped (0) and after 7, 14, 21, 28 days of storage at 8 °C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as a mean \pm S.E. (n = 10).

Days of storage	Anthocyanins - 520 nm		Days of storage	Flavanones and flavones - 280 nm		Days of storage	Hydroxycinnamic acids - 330 nm	
	Cyanidin			Eriocitrin			Caffeic	
	CTR	MeJA		CTR	MeJA		CTR	MeJA
0	4.84 \pm 1.5 ns	4.84 \pm 1.5	0	7.65 \pm 0.5 ns	7.65 \pm 0.5	0	16.59 \pm 4.0 ns	16.59 \pm 4.0
7	7.09 \pm 3.6 ns	3.28 \pm 1.0	7	7.37 b \pm 2.7 ns	9.27 a \pm 0.4	7	13.99 \pm 2.5 ns	12.74 \pm 2.2
14	9.34 \pm 5.7 ns	14.40 \pm 2.9	14	7.00 \pm 1.5 b	10.27 \pm 0.6 a	14	15.55 \pm 3.0 ns	13.04 \pm 1.4
21	15.81 \pm 2.2 ns	18.67 \pm 1.9	21	10.49 \pm 0.5 ns	8.96 \pm 0.8	21	15.63 \pm 1.5 ns	15.06 \pm 0.4
28	34.81 \pm 4.9 ns	35.37 \pm 7.9	28	7.75 \pm 1.8 ns	7.54 \pm 0.8	28	14.20 \pm 1.5 ns	14.10 \pm 0.7
	Delphinidin			Narirutin			Ferulic	
	CTR			CTR			CTR	
0	0.24 \pm 0.1 ns	0.24 \pm 0.1	0	30.78 \pm 2.7 ns	30.78 \pm 2.7	0	50.90 \pm 9.0 ns	50.90 \pm 9.0
7	0.29 \pm 0.1 ns	0.13 \pm 0.1	7	28.86 \pm 4.0 ns	28.93 \pm 4.6	7	60.25 \pm 23.1 ns	48.94 \pm 14.5
14	0.34 \pm 0.1 ns	0.54 \pm 0.1	14	30.13 \pm 6.7 ns	40.43 \pm 4.9	14	64.09 \pm 11.4 ns	141.18 \pm 71.5
21	0.47 \pm 0.1 ns	0.55 \pm 0.1	21	37.39 \pm 4.2 a	29.28 \pm 0.8 b	21	64.31 a \pm 4.7 ns	54.49 b \pm 2.2
28	1.17 \pm 0.3 ns	2.02 \pm 0.6	28	29.79 \pm 4.5 ns	29.42 \pm 0.3	28	49.97 \pm 4.4 ns	48.45 \pm 1.8
	Peonidin			Hesperidin			P-coumaric	
	CTR			CTR			CTR	
0	0.50 \pm 0.1 ns	0.50 \pm 0.1	0	243.45 \pm 44.7 ns	243.45 \pm 44.7	0	60.27 \pm 14.3 ns	60.27 \pm 14.3
7	0.67 \pm 0.2 ns	0.32 \pm 0.1	7	247.01 \pm 51.3 ns	187.61 \pm 20.9	7	74.42 \pm 10.4 ns	69.81 \pm 6.8
14	0.85 \pm 0.4 ns	1.20 \pm 0.2	14	266.68 \pm 56.0 ns	311.62 \pm 45.2	14	107.02 \pm 9.4 ns	108.98 \pm 19.0
21	1.26 \pm 0.3 ns	1.37 \pm 0.1	21	251.45 \pm 25.2 ns	238.30 \pm 10.0	21	150.05 \pm 4.8 ns	146.55 \pm 14.9
28	2.47 \pm 0.2 ns	2.50 \pm 0.5	28	313.52 \pm 11.4 ns	310.92 \pm 0.8	28	130.12 \pm 8.1 ns	137.19 \pm 11.0
	Petunidin			Vicenin-2			Sinapic	
	CTR			CTR			CTR	
0	0.21 \pm 0.2 ns	0.21 \pm 0.2	0	8.25 \pm 0.7 ns	8.25 \pm 0.7	0	3.06 \pm 0.4 ns	3.06 \pm 0.4
7	0.42 \pm 0.2 ns	0.30 \pm 0.2	7	6.65 \pm 0.7 ns	7.14 \pm 1.0	7	16.86 \pm 13.6 ns	23.64 \pm 10.3
14	0.63 \pm 0.2 ns	0.55 \pm 0.2	14	7.71 \pm 1.7 a	11.81 \pm 0.4 b	14	8.45 \pm 9.6 ns	9.72 \pm 0.3
21	0.73 \pm 0.2 ns	0.90 \pm 0.5	21	9.74 \pm 1.0 a	7.73 \pm 0.5 b	21	3.20 \pm 0.3 a	22.01 \pm 1.2 b
28	1.04 \pm 0.2 ns	1.08 \pm 0.3	28	9.02 \pm 1.5 ns	9.09 \pm 0.4	28	19.15 \pm 2.2 ns	20.64 \pm 1.8

and narirutin) and the flavone vicenin-2. Hesperidin was found to be the most abundant compound in all treatments from the beginning of the observation period (Table 2), as previously reported by Modica et al. (2025). Concerning eriocitrin and vicenin-2, statistically significant differences were observed between treatments only at T7 and T14.

Eleven different hydroxycinnamic acid derivatives were identified and quantified in *Tarocco Sant' Alfio* juices: caffeic acid derivatives (caffeoyl-hexose), five p-coumaric acid derivatives (p-coumaroyl hexose and four p-coumaroylquinic acid isomers, four ferulic acid derivatives (feruloyl hexose, two feruloylquinic acid isomers and ferulic acid), and sinapic acid (Table S2). P-coumaric and ferulic acids were the most abundant compounds quantified in pigmented red orange fruit juice (Table 2). The same trend observed for total hydroxycinnamic acids was observed in the individual compounds. Specifically, at T21 a significant increase in ferulic and sinapic acids was recorded in the control sample.

3.3. Colour image assessment

RGB image analysis was used to describe and quantify the effect of MeJA treatment on the dynamics of red colour development in blood oranges during postharvest storage, as assessed through R-channel intensity (Fig. 5A). Fruit treated with MeJA consistently showed a faster and more pronounced accumulation of red pigmentation compared to untreated controls (Fig. 5B). This was evident from the sharper decline in the proportion of pixels with low-intensity red and a concurrent increase of pixel with medium and high-intensity red. In MeJA-treated fruit, the percentage of pixels in the low-intensity red class dropped from 63.94 % at day 7–14.10 % at day 14. At the same time, a marked increase was observed in the proportion of pixels with high red intensity, particularly at days 14), when MeJA-treated fruit reached 21.36 % and 34.04 %, respectively, compared with 1.26 %. After 7 days, MeJA-treated fruit also showed a significantly higher proportion of medium-intensity red pixels (35.33 %) compared with controls (16.85 %),

indicating an earlier shift from low to medium red coloration, as can be observed in Fig. 11. Over longer storage (T21, T28), control fruits retained a higher proportion of medium-intensity red, reaching 63.49 % at day 28. In contrast, MeJA-treated fruit underwent a stronger transition from both low (14.48 % at T28) and medium (51.48 % at T28) intensity levels toward high-intensity pigmentation, ultimately achieving 34.04 % compared with 17.9 % in controls.

Overall, these results demonstrate that MeJA not only accelerated red colour development, but also promoted a more complete transition to the most intense pigmentation stage.

This increase in pigmentation is consistent with the reported effect of MeJA on anthocyanin accumulation in pigmented red oranges (Habibi et al., 2020b; Liu et al., 2022). It is known that MeJA increases the redness level in pigmented red oranges primarily by stimulating the activity of the enzyme Phenylalanine Ammonia-Lyase (PAL). Higher PAL activity leads to increased production of precursors for anthocyanin biosynthesis (such as cyanidin-3-glucoside), the main anthocyanins identified in the present study. Additionally, MeJA might contribute to controlling anthocyanin degradation by modulating factors like Polyphenol Oxidase (PPO) activity, leading to a higher accumulation of these red pigments (Catalano et al., 2009; Lo Piero, 2015; Habibi et al., 2020b). These findings suggest that exogenous MeJA application acts as a potent elicitor of anthocyanin biosynthesis or accumulation, the primary pigments responsible for the characteristic red colour in pigmented oranges. The observed shifts between low, medium, and high intensity red colour classes indicated a more efficient anthocyanin synthesis in MeJA-treated fruit. This agrees with recent research on the *Tarocco Ippolito* cultivar, where postharvest MeJA application was also shown to significantly improve flesh colour and increase the synthesis of anthocyanin levels and the inhibition of its degradation (Vithana et al., 2024; Habibi et al., 2020b). While control orange fruit also underwent to natural colour development, the MeJA treatment markedly amplified this process, leading to a greater overall proportion of highly pigmented areas by the end of the storage period. This highlights the potential of

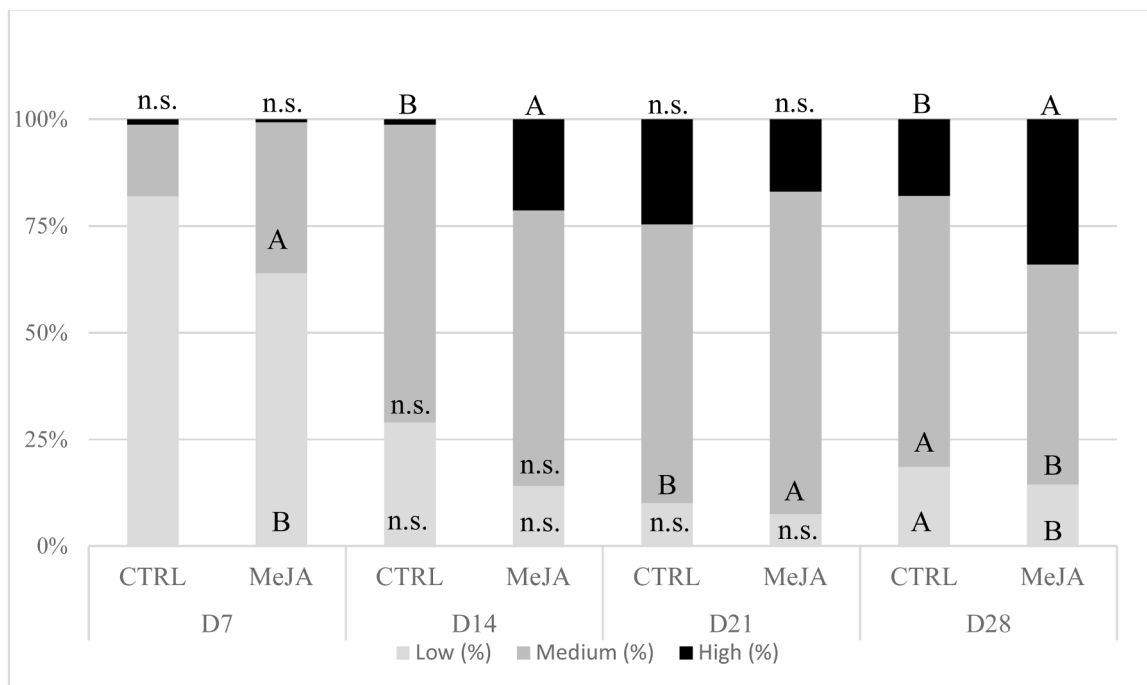
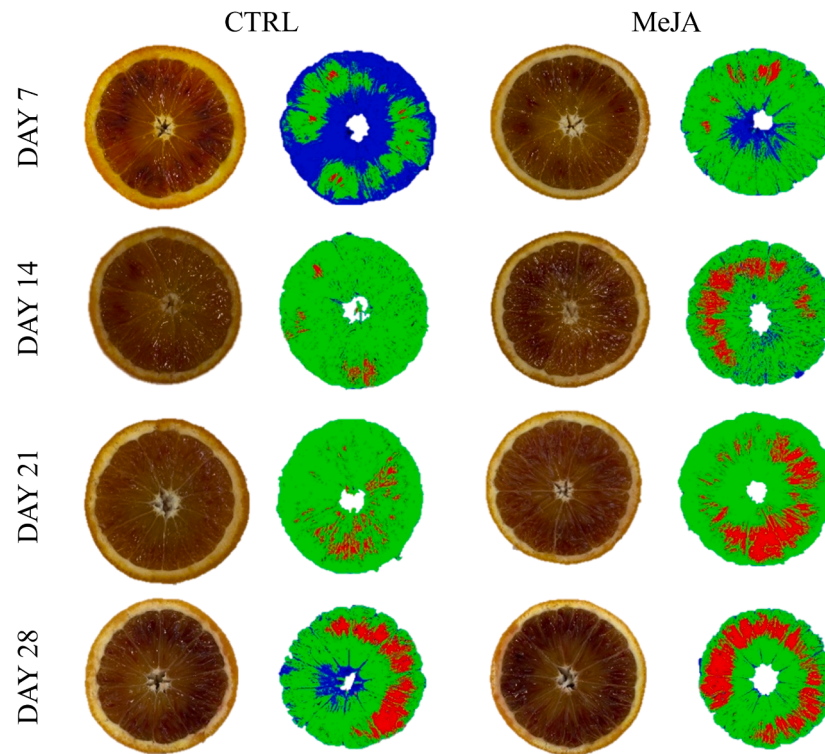


Fig. 5. Example of distribution of red pixel (A) and percentage distribution of red intensity classes (Low, Medium, High) as resulted by the segmentation method proposed based on R channel on pigmented orange cv. *Tarocco Sant' Alfio* (*Citrus sinensis* [L.], Osbeck) after 7, 14, 21, 28 days of storage at 8 °C (B). Different letters indicate significant differences between treatments at each sampling day. ns indicate no changes between treatments. $p \leq 0.05$ was used in the Tukey's test.

MeJA as a postharvest treatment to improve the visual quality and marketability of pigmented red oranges by promoting a more desirable, uniform, and intense red colouration. A strong positive linear correlation between the percentage of high-intensity red area, derived from image analysis of *Tarocco Sant' Alfio* oranges, with total and individual anthocyanins was found (Fig. 6 and Table S3). In particular, total anthocyanins either measured spectrophotometrically (data not shown) or

via HPLC (Fig. 6A) gave a regression coefficient of 0.78 and 0.79, indicating that the percentage of the area with high-intensity red colour increased with the increasing of anthocyanins content. To further describe the contribute of specialized pigments on visual colour, a regression analysis was performed for each compound (Table S3). This analysis revealed that individual cyanidin and peonidin derivatives were the primary drivers of the intense red colouration. Cyanidin

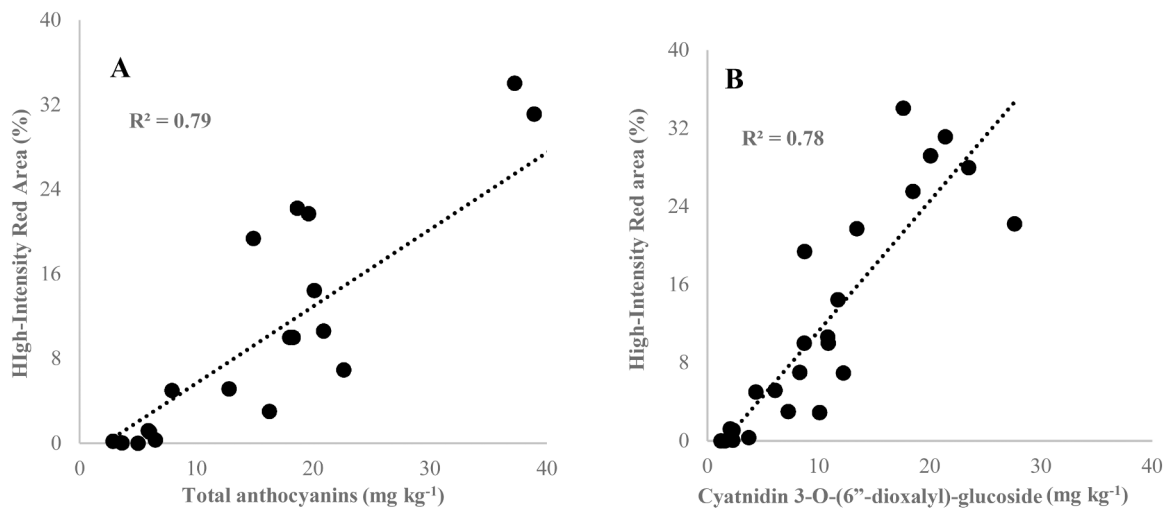


Fig. 6. Regression analysis of percentage of high-intensity red pixels and Total Anthocyanin Content measured with HPLC (A), and the individual anthocyanin cyanidin 3-O-(6''-dioxalyl)-glucoside (B) quantified in red orange cv. *Tarocco Sant'Alfio* (*Citrus sinensis* [L.], Osbeck) after 7, 14, 21, 28 days of storage at 8 °C.

3-O-(6''-dioxalyl)-glucoside which is the main anthocyanin found for this orange variety, was also exhibiting the strongest correlation ($R^2 = 0.78$) (Fig. 6B), but not much different from correlation coefficients found by peonidin 3-O-(6''-malonyl)-glucoside ($R^2 = 0.77$), cyanidin 3-O-glucoside ($R^2 = 0.75$), and cyanidin 3-O-sophoroside ($R^2 = 0.75$). The highest errors reported for cyanidin 3-O-(6''-dioxalyl)-glucoside and cyanidin 3-O-glucoside are justified with the higher concentration of these compounds with an average ratio from 10 to 20 times higher than minor compounds, and to the variability among replicates and storage times. Several other cyanidin derivatives also showed high correlation coefficients ($R^2 > 0.71$) with high-intensity red pixels. In contrast, compounds like delphinidin 3-O-(6''-malonyl)-glucoside showed a very weak correlation ($R^2 = 0.23$), indicating their minimal contribution to the most intensely pigmented areas. These results precisely identify the key pigment responsible for the red colour observed in *Tarocco Sant'Alfio* oranges and confirm the direct link between the accumulation of these specialized metabolites. On the other side, no significant correlation was found between high-intensity red area and CIEL*a*b* red index a*, suggesting that MeJA affected colour development dynamic of *Tarocco Sant'Alfio* red oranges, more in term of spatial colour distribution than of enhancement of red chromaticity (a* values). These consistent, strong correlations further validate the use of image-derived method for high-intensity red area as a reliable indicator of anthocyanin presence, which in *Tarocco Sant'Alfio* genotype is not homogeneously distributed over the fruit flesh.

4. Conclusions

Tarocco Sant'Alfio is a late-ripening clone characterized by a genetically lower potential for anthocyanin accumulation compared to other pigmented red orange cultivars (Caruso et al., 2016; Seminara et al., 2023). This inherent limitation may contribute to irregular internal pigmentation and, consequently, may reduce visual appeal for

consumers. The application of exogenous MeJA modulated the pigmentation pattern during postharvest storage and notable differences in pigmentation between control and MeJA-treated samples were observed after 28 days of storage (Fig. 7). These differences, clearly visible in the representative images, further highlight the potential of MeJA to enhance and normalize internal colour development, even in cultivars like *Tarocco Sant'Alfio* with inherently low and inconsistent pigmentation.

This study demonstrates that postharvest application of methyl jasmonate (MeJA) significantly enhanced pigmentation in flesh of *Tarocco Sant'Alfio* red orange during storage at 8 °C. Treated orange fruit showed improved visual quality, with accelerated and more intense red colour development supported by higher anthocyanin content. The RGB image analysis proved to be effective in tracking pigment distribution and intensity, showing a clear transition from low to high red pigmentation in MeJA treatment groups. Besides, the key pigment responsible for pigmentation was correlated with image analysis by the analysis of the individual specialized metabolites. Carotenoids also responded to MeJA effect, but anthocyanins were the most sensitive indicators of treatment efficacy. These findings suggest that MeJA can serve as an effective tool to improve rapidly internal coloration and consumer appeal in red orange cultivars with limited natural pigmentation.

Author agreement statement

Author Agreement Statement We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process.

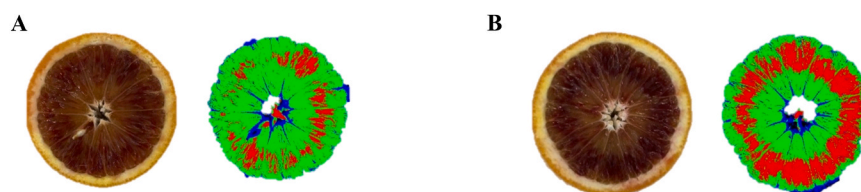


Fig. 7. Visual appearance of internal cross-section of red orange cv. *Tarocco Sant'Alfio* (*Citrus sinensis* [L.] Osbeck) fruit after 28 days of storage at 8 °C. Control (A), MeJA (B).

Declaration of Competing Interest

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.postharvbio.2025.113988](https://doi.org/10.1016/j.postharvbio.2025.113988).

Data availability

Data will be made available on request.

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