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Pre- and Post-harvest Elicitation with Methyl Jasmonate and Salicylic Acid Followed by Cold Storage Synergistically Improves Red Colour Development and Health-Promoting Compounds in Blood Oranges

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Abstract
Red blush is one of the key quality markers of blood orange fruit (*Citrus sinensis* L. Osbeck). Therefore, the influence of pre- (1, 2, and 3 mM) and postharvest (0.5, 1, and 1.5 mM) methyl jasmonate (MeJA) and salicylic acid (SA) (1, 2, and 3 mM) treatments on redness, colouring pigments, and health-promoting compounds in cold stored 'Tarocco Ippolito' blood oranges was investigated. Preharvest application of 3 mM MeJA significantly increased rind citrus colour index (CCI) by 43.3% and colorimetric coordinate a* (redness) by 3.1% and decreased *h*° (hue angle, 0 = red), L* (lightness), and b* (yellowness) by 13.7%, 12.6%, and 23.4%, respectively. This treatment also enhanced CCI (57.4%), reduced *h*° (16.5%) and L* (36.4%), and increased total anthocyanins (88%), monomeric anthocyanins (117%), and flavonoids (77%) in the juice. Postharvest dip of 1.5 mM MeJA (5 min) improved CCI of juice (53.6%) and a* (5.5%), reduced *h*° (15.9%), L* (19.8%), and b* (19.4%), and increased total anthocyanins (66.7%), monomeric anthocyanins (74%), and flavonoids (23.4%) in the juice. Preharvest application of 1 mM SA increased rind CCI (50.8%) and reduced L* (13.6%), b* (16.4%), and *h*° (29.5%). All preharvest SA treatments significantly increased total phenolics in the juice. Lycopene was increased (61.7%) by 5 min postharvest dip of 3 mM SA. In conclusion, 3 mM preharvest spray application of MeJA four weeks before harvest and postharvest dip of 1.5 mM MeJA (5 min) are effective in improving red colour of rind and juice and health-promoting compounds in blood orange juice.

Keywords Blood orange · Red pigmentation · Methyl jasmonate · Salicylic acid · Anthocyanins · Cold storage

Introduction

Blood orange (*Citrus sinensis* L. Osbeck) is a unique fruit among sweet orange groups with a red blush on the rind and deep red juice. Consumers perceive this reddish stain on the rind as a major quality attribute of blood oranges (Habibi et al. 2022). Therefore, red colour development which is related to the presence of anthocyanins is of paramount importance in the trade of blood orange fruit. In addition to their attractive colour, anthocyanins that belong to the flavonoid family are well renowned for their health benefits (Fallico et al. 2017; Habibi et al. 2021a; Legua et al. 2022; Capetini et al. 2023). Due to the presence of high levels of anthocyanins and ascorbic acid, blood orange fruit is considered as a functional food (Legua et al. 2022). Accordingly, blood orange consumption improved endothelial function measured as flow-mediated dilation in overweight or obese people who were otherwise healthy (Li et al. 2020). Similarly, regular intake of blood orange juice regulated the gene expressions involved in the inflammatory process in overweight women indicating that blood orange juice is a potential dietary therapy, which could be useful in the reduction and management of non- communicable disease risk (Capetini et al. 2023). Furthermore, 'Moro' blood orange juice notably decreased the body weight gain in mice fed with a diet rich in lipids compared to blond-orange juice (Titta et al. 2010). Thus, levels of anthocyanins and other colouring pigments present in blood orange fruit are not only important for their visual appeal but also offer several health benefits to the consumers. Additionally, a close correlation has been identified between the bioactive pigment

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contents and colour in a range of food including citrus juice, suggesting 'colour' as a useful approach of quality control (Sant'Anna et al. 2013).

Even though blood oranges are classified under non-climacteric fruit, past investigations have reported an increase in pulp and rind pigmentation during postharvest cold storage possibly due to cold stress induced by low temperatures (Crifò et al. 2011; Habibi et al. 2021a). Moreover, several studies have reported that low temperature stress is required for the biosynthesis of anthocyanins (Lo Piero et al. 2005; Carmona et al. 2017, 2021; Chen et al. 2022) and flavonoids (Crifò et al. 2011) in blood orange fruit. Hence, the notably poor colour development at harvest maturity is a major constraint in blood oranges grown in warmer environments in the tropics and subtropics and Mediterranean climates (Lo Piero 2015; M. Kay, personal communication, August 4, 2022). Consequently, the postharvest improvement of colour through cold storage is currently employed as an option for poorly pigmented blood oranges grown under these climates (Habibi et al. 2022). Further to cold stress, elicitor treatments that induce stress in plants may also accelerate the biosynthesis of colouring pigments in blood orange fruit grown in warmer areas. The preharvest application of these elicitors may possibly stimulate the production of colouring pigments allowing sufficient pigmentation by the time of harvest. Besides, the pre- and postharvest application of different elicitors may aid in better development of red colour in comparison to the low temperature storage alone.

Methyl jasmonate (MeJA) and salicylic acid (SA) are such well recognised, elicitor compounds for their positive impacts on the levels of secondary metabolites in fruit (Vithana et al. 2019; Wang et al; 2021; Chen et al. 2023). MeJA is responsible for several important plant physiological and biochemical processes including the biosynthesis of different bioactive compounds by responding to external stresses (Khan et al. 2016; Wang et al. 2021). Thus, pre,—and postharvest applications of MeJA have proven to be promising strategies to improve antioxidant capacity and phenolics content in fruit while improving their colour (Vithana et al. 2019; Baswal et al. 2020; Rehman et al. 2021; Wang et al; 2021). SA is similarly known to regulate colour, ripening, and antioxidant systems in fruit (Vithana et al. 2019; Baswal et al. 2020; Chen et al. 2023). In agreement, the pre-harvest spray and postharvest fumigation of MeJA improved the colour of the rind of 'M7' Navel oranges (Rehman et al. 2021). Similarly, postharvest application of MeJA, 1-methylcyclopropene (1-MCP), and SA improved overall quality of cold stored 'Kinnow' mandarins (Baswal et al. 2020), while postharvest application of SA was able to reduce the loss of weight, firmness, and soluble solids concentration during storage of sweet oranges (Amiri et al. 2021). Moreover, the application of SA after harvesting improved bioactive compounds while inhibited the green mould development in 'Moro' blood orange fruit (Aminifard et al. 2013).

The effects of different postharvest applications including, γ-aminobutyric acid, MeJA or methyl salicylate (Habibi et al. 2019, 2020), and 24-epibrassinolide (Habibi et al. 2021b) on the antioxidant potential, chilling tolerance, aroma volatile compounds, phytonutrients, and overall quality of blood orange fruit have been previously reported. MeJA and SA improved the colour and carotenoids in mango and Navel sweet orange fruit (Vithana et al. 2019; Rehman et al. 2021). However, the effects of pre- harvest spray and postharvest dip treatments of MeJA and SA on red colour development of rind and juice along with the levels of colouring pigments such as anthocyanins, flavonoids, and lycopene in cold stored blood orange fruit is yet to be investigated. It was hypothesised that the pre- and postharvest application of MeJA and SA in combination with cold storage may enhance the colour development in the rind and juice of blood orange fruit by upregulating the production of colouring pigments including anthocyanins, flavonoids, and red carotenoids. The levels of other health-promoting compounds produced via the same biosynthetic pathways were also expected to increase along with these colouring pigments. Accordingly, this investigation was conducted to uncover a safe and feasible solution for the insufficient pigmentation in blood orange fruit grown under the Mediterranean climate in Western Australia. To the best of our knowledge, this report for the first time presents the effects of both pre- and postharvest application of different concentrations of MeJA and SA in combination with cold storage on red colour development of the rind and juice, the levels of major colouring pigments and health-promoting compounds in blood orange juice.

Materials and Methods

Four independent experiments were conducted to examine the effects of MeJA and SA on red colour development, levels of major colouring pigments and health-promoting compounds in cold stored blood oranges. The first two experiments were involved with preharvest spray application of different concentrations of MeJA and SA followed by cold storage. In the next two experiments, harvested fruit were dipped in various levels of MeJA and SA prior to cold storage.

Preharvest Experiments: Experimental Site, Plant Materials, and the Experimental Design

The two preharvest experiments were conducted in a citrus orchard at Bindoon, Western Australia (Latitude 31°23′8" S, Longitude 116°5′49" E) during the winter season of 2022.

Ten-year old 'Tarocco Ippolito' blood orange trees of uniform size grafted on Troyer Citrange root stocks were used in this experiment. The tree spacing was maintained at 8 m between rows and 6 m between trees with north–south row orientation. All the trees of the orchard were supplied with similar nutrition, water, and crop protection methods excluding the experimental treatments. The preharvest experiments were set out using Randomised Block Design (RBD). An experimental unit comprised of a single tree and replicated four times.

Experiment 1: Effect of Preharvest Spray Application of MeJA on Fruit Colour Development, Colouring Pigments, and Health-Promoting Compounds in Cold Stored bLood Orange 'Tarocco Ippolito'

The experimental trees were sprayed with aqueous emulsions of methyl jasmonate (MeJA, 1 mM, 2 mM, and 3 mM) until runoff, four weeks before the anticipated harvest date. The concentrations and time of application were decided based on the findings of our former investigations (Vithana et al. 2019; Rehman et al. 2021). Tween®—20 (0.25%) was added as a surfactant in all emulsions. Unsprayed trees were treated as the control. At harvest maturity, 20 fruit from each tree without any visible deformities, pest attacks or disease symptoms were arbitrarily harvested around the tree canopy and transported to the Horticulture Laboratory, School of Science, Edith Cowan University, Joondalup in an airconditioned vehicle within two hours after harvesting. Afterwards, to simulate commercial practice, the fruit were stored at 6 ± 1 °C and 80 ± 5% RH (Habibi et al. 2022) for 45 days. After 45 days of cold storage, the colour parameters of the rind and freshly extracted (Citrus PressTM Pro, Breville Citrus Juicer) composite juice samples were analysed. The soluble solids content (SSC) and the levels of ascorbic acid were also estimated using the same fresh juice composites from 20 fruit just after extraction. The remaining juice composites were kept at -80 °C until the levels of different colouring pigments and other health-promoting compounds in the juice were determined.

Experiment 2: Effect of Preharvest Spray Application of SA on Fruit Colour Development, Colouring Pigments, and Health-Promoting Compounds in Cold Stored Blood Orange 'Tarocco Ippolito'

In the second experiment, the blood orange trees in the same orchard were sprayed with aqueous solutions of diverse levels of salicylic acid (SA, 1 mM, 2 mM, and 3 mM) and Tween®—20 (0.25%), four weeks before the anticipated harvest date. The whole trees were sprayed until runoff while untreated trees served as the control. The concentrations and time of application of SA were decided based on our earlier work (Vithana et al. 2019; Baswal et al. 2020). The observations on fruit colour development and quality were the same as detailed in experiment 1.

Postharvest Experiments: Plant Materials, and the Experimental Design

'Tarocco Ippolito' blood oranges at harvest maturity, free from any visual defects were harvested from the same commercial orchard for the two post-harvest experiments. The fruit were transported to the Horticulture Laboratory, School of Science, Edith Cowan University, Joondalup under similar transportation conditions as with the pre-harvest experiments. The postharvest experiments were set out using Completely Randomised Design (CRD) with four replications. Twenty blood oranges were used in each replication.

Experiment 3. Effect of Postharvest Dip Treatment of MeJA on Fruit Colour Development, Colouring Pigments, and Health-Promoting Compounds in Cold Stored Blood Orange 'Tarocco Ippolito'

The experimental fruit were dipped in aqueous emulsions of MeJA (0.5 mM, 1 mM, and 1.5 mM) with Tween®—20 (0.25%) for 5 min. The concentrations and dipping duration of MeJA were decided based on our earlier experiments on mango and sweet oranges (Vithana et al. 2019; Baswal et al. 2020; Rehman et al. 2021). The control fruit were dipped in water containing Tween 20® (0.25%) for 5 min. All fruit were air-dried at 21 ± 1 °C to remove surface moisture and stored at 6 ± 1 °C and 80 ± 5% RH (Habibi et al. 2022) for 45 days. Following 45 days of cold storage, the colour parameters of the rind and juice as well as the levels of different colouring pigments and other health-promoting compounds in the juice were determined in the same way as with preharvest experiments.

Experiment 4. Effect of Postharvest Dip Treatment of SA on Fruit Colour Development, Colouring Pigments, and Health-Promoting Compounds in Cold Stored Blood Orange 'Tarocco Ippolito'

The fourth experiment was conducted in the same way as the third postharvest experiment except for the treatments. In this experiment, fruit were dipped in SA solutions (1 mM, 2 mM, and 3 mM) with Tween®—20 (0.25%) for 5 min. The storage conditions, duration and the recorded colour and other parameters were the same as in the third experiment.

Chemicals

All reagents, SA, Tween®—20 and other chemicals except for MeJA and analytical standards were purchased from

ROWE Scientific Pty Ltd, Wangara, Western Australia. All analytical standards and MeJA were acquired from Sigma Aldrich, St Louis, SG, USA.

Colour Profile

The colour parameters of rind and juice of blood orange samples were determined using ColorFlex EC HunterLab colour spectrophotometer with 45°/0° geometry (Hunter Associates Laboratory Inc, 11,491 Sunset Hills Road, Reston, Virginia, 20,190, USA). The colorimetric coordinates L* (L* = 0 is black, L* = 100 is white), a* (+a* = reddish, − a* = greenish), and b* (+b* yellowish, − b* = bluish) were determined at two opposite cheeks of the fruit along the equatorial axis (20 fruit per replicate) and composite juice samples per replicate (juice of 20 fruit). The hue angle ($h°$) (0° = red, 90° = yellow, 180° = green, and 270° = blue), chroma (vividness or brightness), and citrus colour index (CCI) defined based on HunterLab colour coordinates were calculated using following formulae as previously reported by Rehman et al. (2021) and Sant'Anna et al. (2013).

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}}$$

$$\text{Hue angle}(h°) = \arctan(b^*/a^*)$$

$$\text{CCI} = (1000a^*)/(L^*.b^*)$$

Determination of Major Colouring Pigments in Blood Orange Juice

The analysis of all pigments was conducted under subdued light levels to avoid photodegradation.

Total Monomeric Anthocyanin Pigments

The level of total monomeric anthocyanin pigments in blood orange juice was quantified using pH differential method (AOAC official method) as detailed earlier by Lee et al. (2005). In brief, pH 1.0 buffer solution (0.025 M potassium chloride) and pH 4.5 buffer solution (0.4 M sodium acetate) were prepared for the analysis and the final pH was adjusted by adding hydrochloric acid (HCl) cautiously. The centrifuged juice samples (3000 × g for 5 min at 4 °C) were diluted with each buffer solution separately (juice: buffer, 1/4, v/v). The absorbances of samples diluted with each buffer were recorded at 520 nm (A_{520nm}) and 700 nm (A_{700nm}) against a blank (distilled water) within 30 min (Jenway UV/Visible spectrophotometer, Model 7205, Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, UK). The reading at 700 nm corrected haze. Then the level of total monomeric anthocyanin pigments was calculated using the following formula and recorded as mg cyanidin-3-glucoside equivalents L^{-1}.

Total monomeric anthocyanin pigments

$$\left(\text{cyanidin} - 3 - \text{glucoside equivalents in mgL}^{-1}\right) = (A \times MW \times DF \times 10^3)/(\varepsilon \times L).$$

$A = (A_{520nm}—A_{700nm})$ pH 1.0—$(A_{520nm}—A_{700nm})$ pH 4.5; MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside; DF = dilution factor; L = path length (cm); $\mathcal{E}$ = 26,900 (molar extinction coefficient in L mol^{-1} cm^{-1} for cyanidin-3-glucoside); 10^3 = conversion from g to mg.

Total Anthocyanin Pigments

The levels of total anthocyanin pigments were estimated using the procedure earlier reported by Zheng and Tian (2006) with a few modifications. Frozen composite juice (1 g) was mixed with 10 mL of HCl: methanol (15/85, v/v). Then the mixture was centrifuged (4000 × g) for 5 min at 4 °C and the absorbance of the supernatant was noted at 530, 620, and 650 nm, respectively (Jenway UV/Visible spectrophotometer, Model 7205, Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, UK). The total anthocyanin content was calculated based on the following formula and expressed as ΔA g^{-1}.

$$\Delta A g^{-1} = \left(A_{530} - A_{620}\right) - 0.1(A_{650} - A_{620})$$

Total Flavonoids

The determination of the levels of total flavonoids in blood orange juice was carried out using the procedure described by Guerrouj et al. (2016). Firstly, composite juice samples were centrifuged at 3000 × g for 10 min at 4 °C. Then the supernatant (250 µL) was mixed with deionised water (1.25 mL) and 5% sodium nitrite (75 µL) solution and vortexed for 30 s. After 6 min, a 10% aluminium chloride solution (150 µL) was added, shaken gently and allowed the mixture to stand for another 5 min. By the end of 5 min, 1 M sodium hydroxide (0.5 mL) and deionised water (275 µL) were added and mixed well. Soon after mixing, the absorbance was recorded at 510 nm (Jenway UV/Visible spectrophotometer, Model 7205, Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, UK). A catechin standard curve was

used to calculate the total flavonoid contents. The results were reported as mg catechin equivalent L^{-1}.

Lycopene

The lycopene content in blood orange juice was estimated using the method previously reported by Suwanaruang (2016) with some modifications. The composite juice samples were centrifuged at 3000×*g* for 5 min at 4 °C. Then the supernatant (1 mL) was mixed with deionised water (1 mL). The diluted samples were placed in a water bath for 1 h (30 °C). After 1 h, each sample was diluted with (8.0 mL) a mixture of hexane: ethanol: acetone (2/1/1, v/v/v) and kept for 10 min. Then the samples were vortexed and left for a further 10 min for phase separation. After 10 min, the absorbance of the coloured portion was measured at 503 nm (Jenway UV/Visible spectrophotometer, Model 7205, Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, UK). The lycopene content was recorded as mg L^{-1} based on a lycopene standard curve.

Chlorophyll Pigments and Total Carotenoids

The levels of chlorophyll a, chlorophyll b, and total chlorophylls as well as carotenoids were quantified following the procedure reported by Guo et al. (2022) with following modifications. One g of frozen juice was extracted for 3 h in acetone (6 mL) and vortexed for phase separation. Then the pigment portion (1.5 mL) was mixed with 80% acetone (1.5 mL) and the absorbance was recorded at 470, 645, and 663 nm, respectively (Jenway UV/Visible spectrophotometer, Model 7205, Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, UK). The levels of chlorophyll pigments and carotenoids were calculated based on the following formulae and expressed as mg L^{-1}.

$$Chlorophyll\ a\ (C_a gL^{-1}) = (12.7 \times A_{663nm} - 2.69 \times A_{645nm}) \times V/(w)$$

$$Chlorophyll\ b\left(C_b gL^{-1}\right) = (22.9 \times A_{645nm} - 4.68 \times A_{663nm}) \times V/(w)$$

$$Chlorophyll\ content\left(gL^{-1}\right) = (20.0 \times A_{645nm} + 8.02 \times A_{663nm}) \times V/(w)$$

$$Carotenoid\ content\left(gL^{-1}\right) = (A_{470nm} - 2.05 \times C_a - 114.8 \times C_b)/245$$

V = the total volume of pigment extract in L; w = the initial weight in g.

Determination of the Levels of Other Health-Promoting Compounds

All analyses were conducted under subdued light levels to avoid photodegradation.

Total Antioxidant Capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Brand-Williams et al. 1995) was employed to determine the total antioxidant capacity of blood orange juice. A few modifications were made as follows. The composite orange juice sample (5 mL) was mixed with 10 mL of extraction buffer (2 mL deionised water + 8 mL methanol + 0.8 mg sodium fluoride) and mixed well. The diluted sample was then centrifuged (3220 × g) for 15 min at 4 °C. Afterwards, the supernatant (50 µL) was mixed with 950 µL of DPPH working solution (DPPH stock solution: methanol 1/4, v/v) and left for 15 min. After 15 min, the reading at 515 nm was recorded (Jenway UV/Visible spectrophotometer, Model 7205, Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, UK) and the total antioxidant capacity was computed using the below formula.

$$\%\text{Inhibition of radical DPPH} = \{1 - (A_1 - A_0)\} \times 100$$

A_1 = sample absorption; A_0 = blank absorption.

Total Phenolics Content

Folin-Ciocalteu method was followed to quantify the levels of total phenolics in blood orange juice as previously detailed by Vithana et al. (2018) with a few modifications.

Composite blood orange juice (5 mL) was mixed with 80% methanol (5 mL) and centrifuged (4000 × g) for 25 min at

4 °C. Deionised water (3 mL) and two-fold diluted Folin reagent (250 µL) was added to the supernatant (50 µL) and left in the dark for 5 min. After 5 min, 7% sodium carbonate solution (250 µL) and deionised water (1.45 mL) were added to each sample. Then the samples were allowed to stand in the dark for a further 90 min before absorbance values were recorded at 750 nm (Jenway UV/Visible spectrophotometer, Model 7205, Cole-Parmer Ltd., Beacon Road, Stone, Staffordshire, UK). A gallic acid standard curve was used to quantify the total phenolics content in blood orange juice and reported as g gallic acid equivalent (GAE) L^{-1}.

Ascorbic Acid

The level of ascorbic acid in blood orange juice was quantified following the procedure reported by Rehman (2018). An ascorbic acid standard curve was used to compute the concentration of ascorbic acid in blood orange juice and reported as mg L^{-1}.

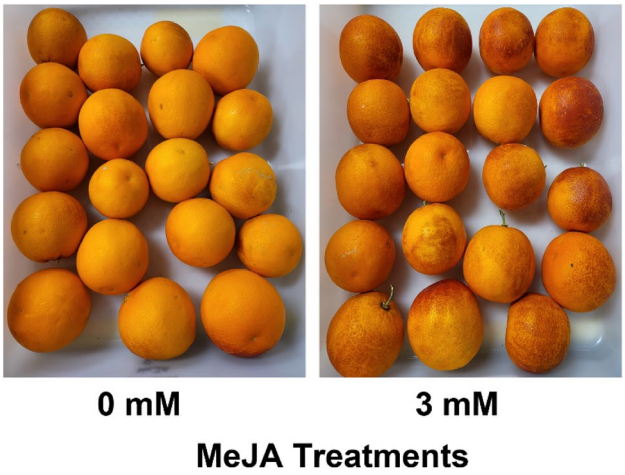

Fig. 1 Rind pigmentation in cold stored 'Tarocco Ippolito' fruit treated with 3 mM MeJA four weeks before harvest compared to untreated control

Soluble Solids Content (SSC)

The SSC of fresh composite juice samples were determined in % with the use of a digital refractometer

Table 1 Effects of pre-harvest spray application of different concentrations of MeJA and SA four weeks before harvest on colour parameters of the rind and juice of cold stored (45 days) 'Tarocco Ippolito' blood orange fruit

Treatment	Rind colour parameters					
	L*	a*	b*	Chroma	Hue angle ($h°$)	CCI
MeJA						
Control	57.1 ± 1.1c	38.1 ± 0.6ab	55.8 ± 1.1b	67.6 ± 0.9b	55.7 ± 0.8c	12.0 ± 0.6a
1 mM	53.4 ± 2.3b	37.5 ± 0.8a	48.8 ± 4.2a	61.5 ± 3.7a	52.3 ± 2.1b	14.6 ± 1.8b
2 mM	51.2 ± 1.0ab	38.3 ± 0.7ab	45.9 ± 1.9a	59.8 ± 1.8a	50.1 ± 0.9ab	16.4 ± 0.8bc
3 mM	50.6 ± 0.8a	39.3 ± 0.6b	45.2 ± 1.1a	59.9 ± 0.9a	49.0 ± 0.8a	17.2 ± 0.7c
SA						
Control	56.8 ± 3.2b	36.5 ± 1.5a	54.4 ± 5.1b	65.6 ± 4.7b	56.0 ± 2.1c	12.0 ± 1.5a
1 mM	48.8 ± 4.4a	35.7 ± 1.1a	42.0 ± 7.2a	55.3 ± 6.2a	49.3 ± 3.6a	18.1 ± 3.4b
2 mM	52.5 ± 2.3ab	39.3 ± 1.0b	48.6 ± 3.6ab	62.5 ± 3.3ab	51.0 ± 1.5ab	15.5 ± 1.4ab
3 mM	55.5 ± 2.1b	37.4 ± 0.6ab	53.5 ± 2.8b	65.3 ± 2.4b	55.0 ± 1.4bc	12.7 ± 1.2a
Juice colour parameters						
MeJA						
Control	21.0 ± 2.8b	31.10 ± 1.20	16.7 ± 2.3	35.3 ± 1.8	28.2 ± 3.1b	93.0 ± 23.8a
1 mM	19.6 ± 2.8ab	30.8 ± 0.58	15.6 ± 1.8	34.6 ± 1.3	38.6 ± 1.1c	110.1 ± 31.5ab
2 mM	19.4 ± 2.1ab	30.4 ± 0.72	15.1 ± 1.5	33.9 ± 1.1	26.3 ± 2.2ab	106.7 ± 19.6ab
3 mM	15.4 ± 1.2a	30.7 ± 0.64	13.8 ± 1.1	33.7 ± 1.0	24.2 ± 1.4a	146.4 ± 19.3b
SA						
Control	20.0 ± 2.6	32.5 ± 1.7b	17.5 ± 1.4	36.9 ± 0.9b	28.3 ± 3.1	96.2 ± 20.4
1 mM	21.6 ± 2.1	31.8 ± 0.1ab	17.5 ± 1.1	36.3 ± 0.5b	28.9 ± 1.6	85.7 ± 15.7
2 mM	21.1 ± 1.5	30.3 ± 0.9a	16.8 ± 0.8	34.6 ± 0.8a	29.0 ± 1.5	86.9 ± 11.5
3 mM	19.4 ± 0.2	30.5 ± 0.2a	15.6 ± 0.2	34.2 ± 0.3a	27.1 ± 0.2	100.8 ± 1.7

N = 4 replicates with 20 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by different lower-case letters. No lettering denotes non-significance. Lettering of MeJA and SA and lettering of rind and juice are independent of each other. Results are presented as mean ± SD

(Atago-Palette PR 101, Atago CO. Ltd, Itabashi-Ku, and Tokyo, Japan).

Statistical Analysis

The Genstat statistical system (23rd edition, VSN International, Hemel Hempstead, UK) was used for the statistical analysis of recorded data. One-way Analysis of Variance (ANOVA) and the Duncan's Multiple Range Test were used to determine the statistical significance ($P \leq 0.05$). The statistical significance is denoted by lowercase letters. The results were reported as means ± standard deviation (SD).

Results

Preharvest Spray Application of MeJA and SA

Rind Colour

The redness of the rind was significantly ($P \leq 0.05$) improved with the preharvest spraying of MeJA (Fig. 1). The highest CCI and a*, and the lowest $h°$ values ($P \leq 0.05$) denoting a dark reddish shade were recorded under 3 mM MeJA treatment compared to the untreated control (Table 1). The values of L*, b*, chroma, and $h°$ were the highest ($P \leq 0.05$) in the control fruit indicating an orangish-yellow colour saturation (Table 1). Preharvest spray of 1 mM SA exhibited the highest ($P \leq 0.05$) CCI and the lowest values of L*, b*, chroma, and $h°$ of the rind. However, a* was significantly ($P \leq 0.05$) higher in the rind of 2 mM and 3 mM SA-sprayed fruit compared to the untreated blood oranges (Table 1).

Juice Colour

The highest ($P \leq 0.05$) CCI and the lowest L* and $h°$ values were observed in the juice of 3 mM MeJA-sprayed fruit (Table 1, Fig. 2a and 2b). The highest chroma ($P \leq 0.05$) was recorded in both control and 1 mM SA preharvest treatment, while the highest a* was noted in the juice of the control fruit (Table 1). The preharvest spray application of MeJA or SA was not significantly effective ($P \leq 0.05$) in improving other colour parameters of the juice of cold-stored blood orange fruit.

Total Anthocyanins, Total Monomeric Anthocyanins, and Total Flavonoids

Preharvest spraying of MeJA significantly ($P \leq 0.05$) improved the levels of total anthocyanins, total monomeric anthocyanins, and total flavonoids in blood orange juice (Table 2). The highest total anthocyanins content was recorded in the fruit sprayed with 1 mM and 3 mM MeJA, while the maximum levels of total monomeric anthocyanins and total flavonoids were observed in 3 mM MeJA-sprayed fruit compared to the control (Table 2). The preharvest spray application of SA did not affect the level of total anthocyanins significantly, but the total flavonoids were markedly higher in the fruit sprayed with 2- and 3-mM SA as compared to the untreated fruit (Table 2).

Lycopene, Chlorophyll Pigments, and Total Carotenoids

The lycopene content was not affected by the preharvest spray application of MeJA and SA substantially (Table 2). The level of chlorophyll a in juice of cold stored blood orange fruit was not affected significantly by preharvest spray application of MeJA, while the levels of total chlorophylls and chlorophyll b were significantly lower in 1 mM MeJA-sprayed fruit when compared with the other MeJA treatments and untreated control (Table 2). The level of chlorophyll a was significantly higher in 1 mM SA-sprayed fruit, while 3 mM SA spray treatment reduced the level of chlorophyll a significantly compared to the control and the other SA treatments. The content of chlorophyll b was significantly higher in 3 mM SA-sprayed fruit compared to the other SA treatments but was not statistically different from the control. However, total chlorophyll content was not affected notably by the preharvest spray application of SA (Table 2). Carotenoids were not at detectable levels (mg L^{-1}) in any of the samples.

Total Antioxidant Capacity, Total Phenolics, and Ascorbic Acid

The highest ($P \leq 0.05$) total antioxidant capacity and the total phenolics content were noted in the juice of blood orange fruit sprayed with 1 mM MeJA (Table 3). All preharvest SA spray treatments significantly ($P \leq 0.05$) increased the level of total phenolics in comparison to the control fruit (Table 3). However, the total antioxidant capacity was not significantly influenced with preharvest spray application of SA (Table 3). The level of ascorbic acid in blood orange juice was not considerably ($P \leq 0.05$) influenced by preharvest spraying of either MeJA or SA (Table 3).

Soluble Solids Content

The SSC values in preharvest MeJA-sprayed fruit were 12.6% (control), 13.3% (1 mM), 13.0% (2 mM), and 13.0% (3 mM) and were not affected ($P \leq 0.05$) by preharvest spraying of MeJA. The preharvest application of 2 mM (12.07%) and 3 mM (12.3%) SA reduced SSC significantly ($P \leq 0.05$) compared to 1 mM SA spray application (12.7%) and the control (13.1%).

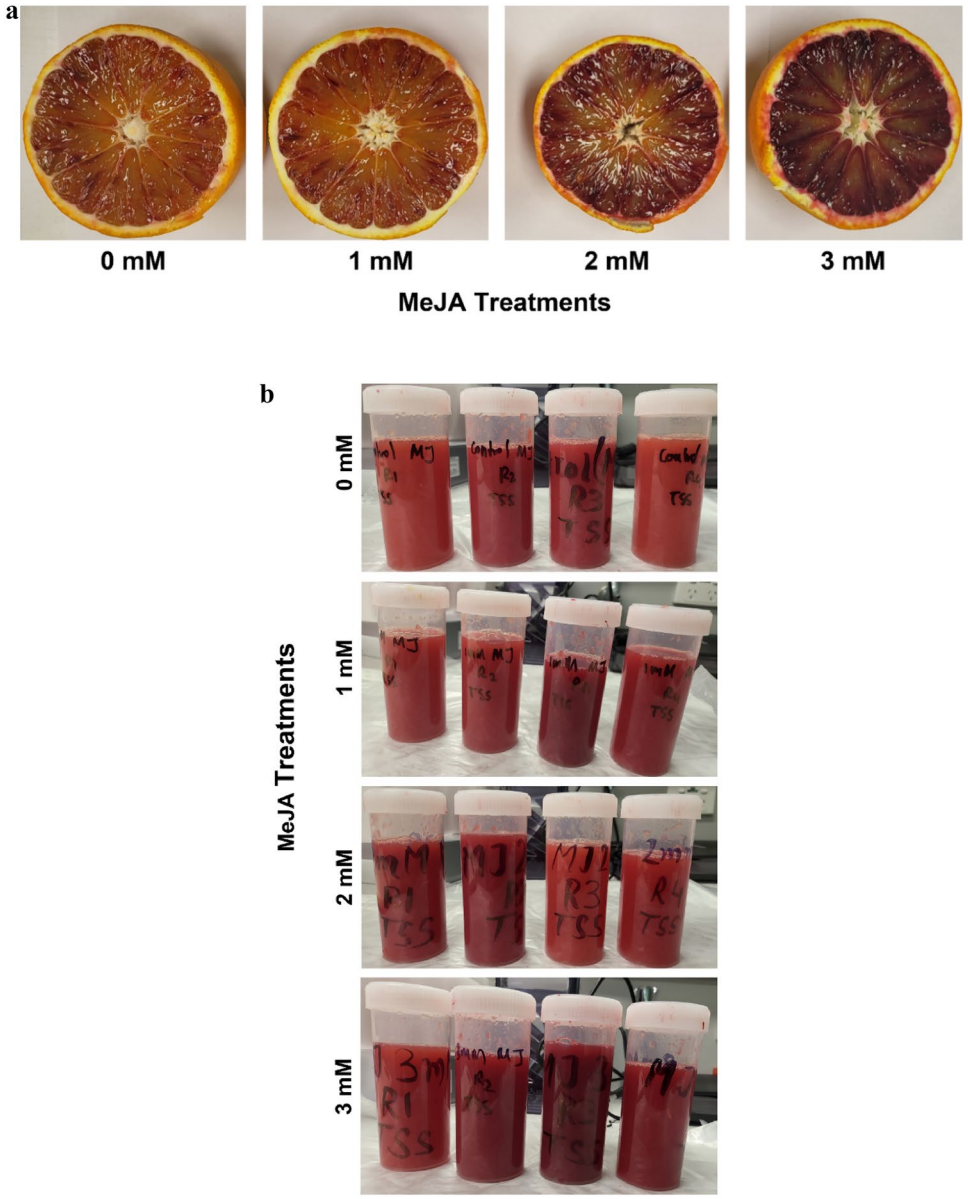

Fig. 2 **A** Pulp pigmentation in cold stored 'Tarocco Ippolito' blood orange fruit treated with different concentrations of MeJA four weeks before harvest compared to untreated control (0 mM). **b** Juice pigmentation of cold stored 'Tarocco Ippolito' blood orange fruit treated with different concentrations of MeJA four weeks before harvest compared to untreated control (0 mM)

Postharvest Dip Treatments of MeJA and SA

Rind Colour

The colorimetric coordinate a* was the only colour parameter in the rind which was notably influenced ($P \leq 0.05$) by the postharvest dipping of MeJA and SA (Table 4). The highest a* was recorded in the rind of 1 mM MeJA treated fruit denoting a darker red colour compared to 1.5 mM MeJA treated fruit and the untreated control, while 1 mM SA treatment recorded the highest a* in comparison to the other SA treatments and untreated fruit (Table 4).

Juice Colour

Conversely, the colour of the juice was significantly ($P \leq 0.05$) influenced by the dip treatment of MeJA (Table 4). The value of a* of juice was significantly high in both 0.5 and 1.5 mM MeJA-dipped fruit compared to untreated control. The b* and $h°$ were the highest in control fruit denoting a yellowish tone (Table 4). The highest CCI was exhibited by 1.5 mM MeJA dip treatment, whereas L* and chroma were not significantly influenced by the postharvest dip treatment of MeJA (Table 4). However, the postharvest dip treatment of SA (1 and 2 mM) significantly increased the chroma of the juice (Table 4).

Table 2 Effects of pre-harvest spray application of different concentrations of MeJA and SA 4 weeks before harvest on the levels of major colouring pigments in the juice of cold stored (45 days) 'Tarocco Ippolito' blood orange fruit (Units: Total anthocyanins = ΔA g^{-1}, total monomeric anthocyanins = mg cyanidin-3-glucoside equivalents L^{-1}, total flavonoids = mg catechin equivalent L^{-1}, lycopene, chlorophyll a, chlorophyll b, and total chlorophylls = mg L^{-1})

Treatment	Colouring pigments						
	Total anthocyanins	Total monomeric anthocyanins	Total flavonoids	Lycopene	Chlorophyll a	Chlorophyll b	Total chlorophylls
MeJA							
Control	0.25 ± 0.03a	28.3 ± 3.2a	61.3 ± 4.0a	22.36 ± 12.9	10.9 ± 0.9	20.6 ± 3.0b	31.2 ± 3.8b
1 mM	0.45 ± 0.2b	48.8 ± 16.5ab	85.8 ± 18.0ab	15.79 ± 12.8	6.5 ± 3.2	10.2 ± 4.3a	17.9 ± 6.2a
2 mM	0.43 ± 0.1ab	44.5 ± 5.9ab	82.9 ± 10.0ab	23.06 ± 5.7	9.2 ± 1.4	16.8 ± 3.2b	25.6 ± 4.5b
3 mM	0.47 ± 0.1b	61.5 ± 15.5b	108.0 ± 21.0b	23.92 ± 7.3	10.7 ± 1.7	20.2 ± 3.4b	30.7 ± 4.6b
SA							
Control	0.5 ± 0.2	38.8 ± 15.1b	57.0 ± 11.0a	37.9 ± 12.4	11.3 ± 0.6b	27.1 ± 1.8ab	38.1 ± 1.9
1 mM	0.4 ± 0.1	27.1 ± 2.7a	59.0 ± 5.0a	42.0 ± 8.9	12.4 ± 0.2c	24.6 ± 1.3a	36.8 ± 1.2
2 mM	0.3 ± 0.1	23.9 ± 5.1a	79.0 ± 10.0b	43.3 ± 14.8	11.4 ± 0. b	24.8 ± 0.2a	36.0 ± 0.8
3 mM	0.4 ± 0.05	32.2 ± 2.2ab	116.0 ± 9.0c	42.6 ± 10.4	10.3 ± 0.7a	28.5 ± 2.8b	38.6 ± 2.2

N = 4 replicates with 20 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by different lower-case letters. No lettering denotes non-significance. Lettering of MeJA and SA are independent of each other. Results are presented as mean ± SD

Table 3 Effects of pre-harvest spray application of different concentrations of MeJA and SA 4 weeks before harvest on other health-promoting phytochemicals in the juice of cold stored (45 days) 'Tarocco Ippolito' blood orange fruit

Treatment	Health promoting phytochemicals		
	Total antioxidant capacity (% inhibition of radical DPPH)	Total phenolics (g/L)	Ascorbic acid (mg/L)
MeJA			
Control	70.39 ± 5.2 ab	2.73 ± 0.5 a	363.39 ± 17.7
1 mM	74.18 ± 4.9 b	5.11 ± 1.0 b	365.26 ± 37.6
2 mM	68.26 ± 4.9 ab	4.15 ± 1.0 ab	358.79 ± 33.8
3 mM	63.75 ± 4.6 a	3.45 ± 0.5 a	367.42 ± 66.7
SA			
Control	72.4 ± 3.7	2.7 ± 0.5a	356.5 ± 48.7
1 mM	66.0 ± 6.7	4.4 ± 0.6b	296.0 ± 25.0
2 mM	74.1 ± 9.9	3.9 ± 0.8b	311.7 ± 31.3
3 mM	73.7 ± 5.0	4.6 ± 0.3b	331.1 ± 53.5

N = 4 replicates with 20 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by different lower-case letters. No lettering denotes non-significance. Lettering of MeJA and SA are independent of each other. Results are presented as mean ± SD

Total Anthocyanins, Total Monomeric Anthocyanins, and Total Flavonoids

The levels of total anthocyanins, total monomeric anthocyanins, and total flavonoids were significantly ($P \leq 0.05$) improved with the postharvest dip treatment of MeJA in comparison with the untreated blood oranges (Table 5). The highest total anthocyanin content was recorded in 1.5 mM MeJA-dipped fruit compared to the untreated control. The MeJA (0.5 mM and 1.5 mM) dip treatments significantly increased the total monomeric anthocyanins and total flavonoids contents in comparison to the control. However, the levels of total anthocyanins and total flavonoids were not influenced significantly ($P \leq 0.05$) by postharvest dip treatment of SA (Table 5).

Lycopene, Chlorophyll Pigments, and Total Carotenoids

The levels of lycopene, chlorophyll a, chlorophyll b, and total chlorophyll in cold stored blood orange juice were not influenced by postharvest dip treatments of MeJA (Table 5). However, the postharvest dip treatment of 3 mM SA significantly improved ($P \leq 0.05$) the level of lycopene and decreased the contents of chlorophyll b and total chlorophyll compared to the untreated fruit (Table 5). The total carotenoids were not at detectable levels (mg L^{-1}) in control and SA or MeJA-dipped fruit.

Total Antioxidant Capacity, total Phenolics, and Ascorbic Acid

The total antioxidant capacity and the level of ascorbic acid in cold stored blood orange juice were not influenced by the postharvest dip treatments of MeJA or SA (Table 6). However, the total phenolics content in juice was notably decreased ($P \leq 0.05$) by 1 mM SA dip treatment compared to 2 mM and 3 mM SA-dipped fruit and control (Table 6).

Table 4 Effects of post-harvest dip treatment of different concentrations of MeJA and SA for 5 min on colour parameters of the rind and juice of cold stored (45 days) 'Tarocco Ippolito' blood orange fruit

Treatment	Rind colour parameter					
	L*	a*	b*	Chroma	Hue angle ($h°$)	CCI
MeJA						
Control	53.1 ± 2.6	32.8 ± 0.4a	39.5 ± 3.8	51.3 ± 2.9	50.2 ± 2.8	15.9 ± 2.5
0.5 mM	52.4 ± 2.7	33.1 ± 0.7ab	36.0 ± 5.0	49.1 ± 3.3	47.1 ± 4.5	18.5 ± 3.8
1 mM	52.1 ± 2.8	33.9 ± 0.5b	39.6 ± 4.5	52.2 ± 3.4	49.2 ± 3.2	16.8 ± 2.7
1.5 mM	50.8 ± 1.8	33.0 ± 0.1a	35.6 ± 2.3	48.6 ± 1.7	47.2 ± 1.8	18.4 ± 1.8
SA						
Control	50.3 ± 1.3	35.6 ± 1.1a	36.6 ± 1.9	51.1 ± 0.9	45.7 ± 2.3	19.4 ± 1.7
1 mM SA	51.3 ± 1.8	38.7 ± 2.8b	38.6 ± 2.8	52.0 ± 2.8	48.0 ± 1.2	17.6 ± 1.3
2 mM SA	50.9 ± 2.7	35.9 ± 1.4a	37.9 ± 4.9	52.3 ± 4.3	46.4 ± 3.1	19.0 ± 2.9
3 mM SA	51.6 ± 1.5	35.1 ± 1.1a	38.1 ± 1.8	51.8 ± 2.3	47.3 ± 0.6	17.9 ± 0.9
Juice Colour Parameters						
MeJA						
Control	30.3 ± 1.1	45.5 ± 0.9a	22.2 ± 1.8b	50.7 ± 0.8	26.0 ± 1.5b	67.0 ± 2.7a
0.5 mM	28.7 ± 3.4	47.7 ± 1.2b	19.4 ± 5.9ab	51.5 ± 3.5	22.0 ± 4.1ab	85.7 ± 9.5ab
1 mM	28.9 ± 2.9	46.6 ± 1.3ab	19.8 ± 5.0ab	50.6 ± 2.8	23.0 ± 3.7ab	81.4 ± 8.4ab
1.5 mM	25.3 ± 2.3	48.0 ± 0.4b	13.7 ± 4.0a	49.9 ± 2.9	15.0 ± 2.5a	138.5 ± 7.8b
SA						
Control	24.7 ± 0.7	29.2 ± 0.6	18.9 ± 0.6a	34.8 ± 0.5a	33.0 ± 0.8	62.5 ± 3.8
1 mM SA	24.0 ± 0.7	29.4 ± 0.9	20.3 ± 0.5b	35.8 ± 0.5b	34.6 ± 1.5	60.8 ± 5.2
2 mM SA	25.0 ± 0.9	28.8 ± 0.5	20.8 ± 0.8b	35.6 ± 0.6b	35.9 ± 1.2	55.5 ± 4.5
3 mM SA	24.8 ± 1.0	28.1 ± 0.4	19.8 ± 1.0ab	34.3 ± 0.7a	35.1 ± 1.4	57.6 ± 5.2

N = 4 replicates with 20 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by different lower-case letters. No lettering denotes non-significance. Lettering of MeJA and SA and lettering of rind and juice are independent of each other. Results are presented as mean ± SD

Table 5 Effects of post-harvest dip treatment of different concentrations of MeJA and SA for 5 min on the levels of major colouring pigments in the juice of cold stored (45 days) 'Tarocco Ippolito' blood orange fruit (Units: Total anthocyanins = $\Delta A\ g^{-1}$, total monomeric anthocyanins = mg cyanidin-3-glucoside equivalents L^{-1}, total flavonoids = mg catechin equivalent L^{-1}, lycopene, chlorophyll a, chlorophyll b, and total chlorophylls = mg L^{-1})

Treatment	Colouring pigments						
	Total anthocyanins	Total monomeric anthocyanins	Total flavonoids	Lycopene	Chlorophyll a	Chlorophyll b	Total chlorophylls
MeJA							
Control	0.24 ± 0.03a	23.46 ± 3.0a	62.4 ± 2.0a	25.01 ± 10.8	10.3 ± 1.3	23.1 ± 2.6	33.2 ± 2.8
0.5 mM	0.34 ± 0.1ab	35.29 ± 7.0b	78.3 ± 5.0b	26.27 ± 24.7	10.3 ± 1.4	19.6 ± 5.8	29.7 ± 7.0
1 mM	0.30 ± 0.08ab	31.33 ± 6.3ab	71.2 ± 7.0ab	19.31 ± 2.9	10.6 ± 1.1	20.5 ± 2.7	30.9 ± 3.2
1.5 mM	0.40 ± 0.08b	40.89 ± 6.3b	77.3 ± 7.0b	24.23 ± 9.8	8.3 ± 3.1	20.3 ± 9.7	28.4 ± 12.6
SA							
Control	0.3 ± 0.01	25.3 ± 2.3b	73.6 ± 3.6	33.2 ± 3.9a	10.4 ± 0.6	27.8 ± 0.7	37.8 ± 0.4c
1 mM	0.3 ± 0.01	21.5 ± 3.3ab	71.8 ± 5.2	39.4 ± 5.2b	11.2 ± 0.5	27.2 ± 0.4	38.1 ± 0.5c
2 mM	0.3 ± 0.03	20.7 ± 1.3a	69.1 ± 4.1	42.1 ± 4.1b	11.1 ± 0.5	25.4 ± 0.2	36.3 ± 0.4b
3 mM	0.3 ± 0.04	19.2 ± 1.9a	66.3 ± 4.0	53.7 ± 10.7c	10.8 ± 0.5	23.3 ± 1.2	33.9 ± 1.2a

N = 4 replicates with 20 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by different lower-case letters. No lettering denotes non-significance. Lettering of MeJA and SA are independent of each other. Results are presented as mean ± SD

Table 6 Effects of post-harvest dip treatment of different concentrations of MeJA and SA for five min on the levels of other health-promoting phytochemicals in the juice of cold stored (45 days) 'Tarocco Ippolito' blood orange fruit

Treatment	Health promoting phytochemicals		
	Total antioxidant capacity (% inhibition of radical DPPH)	Total phenolics (g/L)	Ascorbic acid (mg/L)
MeJA			
Control	63.10 ± 5.5	2.27 ± 0.1	315.82 ± 32.2
0.5 mM	69.70 ± 4.6	2.87 ± 0.3	340.54 ± 24.9
1 mM	72.20 ± 7.7	2.1 ± 0.6	333.36 ± 10.2
1.5 mM	69.51 ± 2.5	2.64 ± 0.2	345.86 ± 13.2
SA			
Control	64.2 ± 7.0	2.9 ± 0.1b	346.0 ± 27.5
1 mM	53.0 ± 9.3	2.3 ± 0.3a	315.8 ± 32.2
2 mM	68.2 ± 4.8	2.7 ± 0.2ab	324.9 ± 24.3
3 mM	46.8 ± 13.4	3.0 ± 0.3b	335.5 ± 17.5

N = 4 replicates with 20 fruit per replication. Mean values significantly different at $P \leq 0.05$ are indicated by different lower-case letters. No lettering denotes non-significance. Lettering of MeJA and SA are independent of each other. Results are presented as mean ± SD

Soluble Solids Content

All postharvest MeJA dip treatments significantly increased SSC (11.9, 11.7, 11.7%, respectively) compared to control (11.3%). However, the SSC values recorded in SA dip treatments {11.4% (3 mM), 11.8% (1 mM), 11.6% (2 mM)} were not significantly different compared to the untreated fruit (11.7%).

Discussion

Poor red colour development in blood orange fruit grown under Mediterranean climatic conditions leads to significant economic losses (Lo Piero 2005; M. Kay, personal communication, August 4, 2022). Chemical elicitation is considered as a feasible method to increase the production of secondary metabolites including a range of colouring pigments in fruit (Vithana et al. 2019; Rehman et al. 2021). Due to pronounced increase in consumer concern on food and environmental safety, the influence of safe natural chemical elicitors on red colour development in blood oranges were investigated in this study. Both MeJA (Asghari and Hasanlooe 2015) and SA (Hewajulige and Wijesundera 2014) are classified as "generally recognised as safe" (GRAS) compounds by the United States Food and Drug Administration (FDA). MeJA is also declared as a safe food additive by Food and Agriculture Organisation and the World Health Organisation (FAO/WHO 2007) and has exhibited anti-cancer and anti-inflammatory effects in several animal and human in vitro and in vivo studies (Fingrut and Flescher 2002; Jarocka-Karpowicz and Markowska 2021).

As hypothesised, both preharvest spray application four weeks before harvest and postharvest dip treatment (5 min) of MeJA and SA significantly influenced the colour parameters of rind and juice of the cold stored blood orange fruit compared to the cold stored untreated control (Table 1 and 3, Fig. 1, 2a and 2b). The preharvest spray application of 3 mM MeJA four weeks before harvest was the most effective treatment in improving the reddish colour of the rind and juice in cold stored blood oranges (Table 1, Fig. 1, 2a and 2b). This treatment increased the CCI of blood orange rind by 43.3% and a* by 3.1% and decreased $h°$, L*, and b* by 13.7%, 12.6%, and 23.4%, respectively, compared to the untreated fruit. The same treatment increased the CCI of blood orange juice by 57.4% and reduced $h°$ and L* by 16.5% and 36.4% correspondingly in comparison to the control. Even though the postharvest dip treatments of MeJA for 5 min were less effective on rind colour improvement, the juice colour was significantly improved with 1.5 mM MeJA dip treatment (Table 4). This treatment increased the CCI of blood orange juice by 53.6% and a* by 5.5% and reduced $h°$, L*, and b* by 15.9%, 19.8%, and 19.4% correspondingly compared to the control. The level of anthocyanins is one of the key determinants of red colour in blood orange fruit (Habibi et al. 2022). Thus, the significant improvement in red colour of the rind and juice observed in this study is possibly ascribed to the increased levels of anthocyanins and other pigments such as flavonoids and lycopene, the red carotenoid (Table 2 and 5). The reduced levels of chlorophyll pigments (Table 2 and 5) in blood orange juice may also have influenced the rind and juice colour. In accordance, the preharvest spray application of 3 mM MeJA which resulted in the highest red colour development recorded the maximum increase in the levels of total anthocyanins, total monomeric anthocyanins, and total flavonoids (88%, 117%, and 77% correspondingly) in comparison to the untreated fruit (Table 2). The postharvest dip treatment of 1.5 mM MeJA also enhanced the total anthocyanins, total monomeric anthocyanins, and total flavonoids by 66.7%, 74%, and 23.4% correspondingly compared to the control (Table 5). Anthocyanins, the major red pigments in blood oranges, are biosynthesised via phenylalanine ammonia-lyase (PAL) in the phenylpropanoid pathway (Fig. 3), along with flavanols and phenolic acids synthesis (Holton and Cornish 1995; Carmona et al. 2017). MeJA has proven its ability to increase anthocyanin biosynthesis in different fruit by stimulating the action of the enzyme PAL. The biosynthesis of anthocyanins was induced in harvested grape berries (Flores et al. 2015), cold stored blue berries

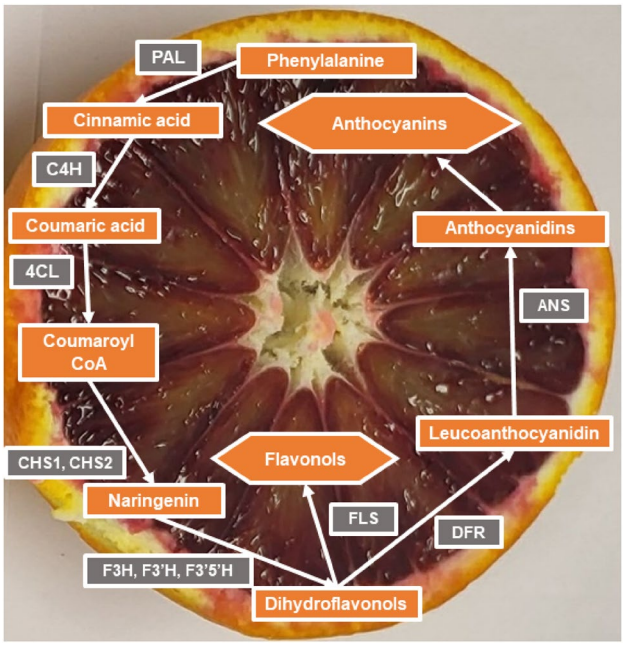

Fig. 3 Anthocyanin biosynthetic pathway (Holton and Cornish 1995). *PAL* phenylalanine ammonia-lyase, *C4H* cinnamate 4-hydroxylase, *4CL* 4-hydroxy-cynnamoyl CoA ligase, *CHS* chalcone synthase, *CHI* chalcone isomerase, *F3H* flavanone 3-hydroxylase, *F3'H* flavonoid 3'-hydroxylase, *F3'5'H* flavonoid 3′5'-hydroxylase, *FLS* flavonol synthase, *DFR* dihydroflavonol 4-reductase, *ANS* anthocyanidin synthase

(Huang et al. 2015), and red raspberries (Flores and del Castillo. 2014) by MeJA treatment due to the enhanced activity of PAL. MeJA also promotes anthocyanin accumulation by promoting the gene expressions in the phenylpropanoid pathway (Tang et al. 2022). In agreement, the overexpression of MdMYB24L gene due to MeJA treatment elevated anthocyanin accumulation in apple fruit (Wang et al. 2019). Moreover, the anthocyanin production in 'Fuji' apple skin was increased as a response to stress and increased ethylene production resulted by the exogenous application of MeJA (Rudell et al. 2002). Thus, the noted increase in the levels of anthocyanins and flavonoids with MeJA treatments in this study may possibly attributed to this combined stimulation of PAL activity, upregulation of expression of relevant genes and/or ethylene production. Besides, the preharvest spray application of 1 mM MeJA significantly reduced the total chlorophyll (125%) and chlorophyll b (102%) levels in comparison to the untreated fruit (Table 2). Perhaps, the ability of MeJA to stimulate chlorophyll degradation either alone or synergistically with increased ethylene production could be the reason for this observation (Rudell et al. 2002). The degradation of chlorophyll probably has been contributed to improved redness of blood orange fruit. The notably higher concentrations of SSC in MeJA-dipped blood oranges compared to the untreated fruit probably implies enhanced ripening due to increased ethylene production. The lycopene content in sweet orange (*Citrus reticulata*) pulp was earlier reported as 13.2 mg kg^{-1} (Suwanaruang 2016), while the lycopene concentration was comparatively higher in blood orange juice as expected (Table 2 and 5). Nevertheless, the lycopene content of cold stored blood oranges was not influenced by pre- and postharvest application of MeJA in the present study. Similar improvements in different colour parameters and colouring pigments with exogenous application of MeJA were reported previously. For instance, Rehman et al. (2021) reported an increase in CCI and a reduction in *h*° in 'M7' Navel orange fruit with pre-harvest spray application of MeJA, probably due to the increased concentration of total carotenoids. Also, exogenous MeJA could decrease L*, b*, and *h*° in the skins of 'Fuji' apple with a significant increase in a* (Altuntas et al. 2012). In agreement with current study, the red blush of 'Cripps pink' apple was reportedly improved with the preharvest spray application of MeJA due to the increased levels of anthocyanins and flavonoids (Shafiq et al. 2013).

The influence of SA on red colour development also varied with the concentrations and time of application (Table 1 and 4). The 1 mM SA preharvest spray application improved the rind colour significantly by increasing CCI by 50.8% and reducing *h*°, L*, and b* by 13.6%, 16.4%, and 29.5% correspondingly, compared to the untreated blood oranges. However, the colorimetric coordinate a* of the rind was best improved with the preharvest spray application of 2 mM SA (7.7%) and postharvest 1 mM SA dip treatment (8.7%) compared to the untreated control. Similarly, postharvest SA treatment could significantly reduce L* and b* values in blond-orange rind (Amiri et al. 2021). Nevertheless, the red colour of cold stored blood orange juice was not improved by either pre- or postharvest treatments of SA. Hence, the highest a* of the juice was recorded in control fruit compared to the postharvest SA dip treatments. In accordance, the highest monomeric anthocyanin content was also present in the control fruit (Table 2 and 5). The significant increase observed in the levels of total anthocyanins, total monomeric anthocyanins, and total flavonoids with MeJA treatments was less evident under SA treatments. Similarly, in a previous study, a comparatively higher PAL activity was observed in sweet cherry fruit treated with MeJA compared to SA, even though the preharvest application of both SA and MeJA demonstrated increased activity of this enzyme than the control (Yao and Tian 2005). Besides, the SA treatment was found to reduce ethylene production, while MeJA stimulated the process in carrot callus cultures (Sudha and Ravishankar 2003). The significantly lower concentrations of SSC in SA-sprayed blood orange fruit compared to the untreated fruit possibly indicates delayed ripening caused by lowered production of ethylene. The synergistic effect of higher PAL activity and ethylene production may be

ascribed to the better effectiveness of MeJA compared to SA in the production of anthocyanins and flavonoids in blood orange fruit. The significant reduction in the levels of total chlorophylls and chlorophyll b with 3 mM SA dip treatment (11.5% and 19.4%, respectively) also possibly have affected the final juice colour (Table 5). However, Kumar et al. (2021), earlier reported a delay in the degradation of chlorophylls in SA treated tomatoes due to delayed ripening. Being non-climacteric, the reduction in ethylene biosynthesis and the rate of respiration due to SA treatment could be less impactful in cold stored blood orange fruit compared to tomato. Conversely, 3 mM SA dip and preharvest spray treatments significantly improved the levels of lycopene (61.7%) and total flavonoids (105%), respectively, compared to the untreated fruit (Table 5). In agreement, SA could significantly increase the production of red pigments in tomato compared to MeJA (Baek et al. 2023).

The influence of MeJA on the levels of health-promoting phytochemicals was dependent on the dose and time of application. The preharvest spray application of 1 mM MeJA significantly increased the total antioxidant capacity (5.4% higher than control) (Table 3) as well as total phenolics (88.9% higher than control) (Table 3) in blood orange juice, while the ascorbic acid content was not influenced by MeJA treatments (Table 3). Nevertheless, postharvest dip treatment of MeJA was not significantly effective on any of these parameters probably due to shorter exposure (5 min) and shorter time for the changes to occur in metabolic activities before cold storage compared to preharvest spray (Table 6). All preharvest SA treatments (1, 2, and 3 mM) significantly improved the total phenolics content in comparison to the untreated fruit (63%, 44%, and 70%, respectively) (Table 6) possibly due to an increase in PAL activity. However, the total antioxidant capacity and levels of ascorbic acids were not influenced by either pre- or postharvest SA treatments (Table 3 and 6). Thus, the modulation of the concentrations of different secondary compounds followed by the signal transduction network initiated due an elicitor treatment is possibly concentration, elicitor, (MeJA and SA), tissue (rind, pulp), and species dependent.

Based on the information gathered from several studies, Sant'Anna et al. (2013) summarised that the colour parameters are highly corelated with the pigment and other bioactive compound contents of food. For instance, the presence of anthocyanins was highly corelated with higher a* values and lower L* values representing darker shades of red. Moreover, they testified a high correlation between colorimetric coordinate a*, total phenolics and total flavonoids indicating darker red food having higher concentrations of these compounds. However, the total monomeric anthocyanins were better correlated with $h°$, where lower $h°$ values denoting higher levels of total monomeric anthocyanins. The lower L* values were also associated with higher contents of polyphenols and antioxidant activity. Due to these clear relationships, it is recommended that colorimetric techniques as an appropriate quality index of beverages including fruit juice. The findings of this study also confirm the above observations. Overall, blood orange juice with the highest ($P \leq 0.05$) L* b* chroma and $h°$ values representing yellowish tones of colour recorded the lowest ($P \leq 0.05$) levels of major colouring pigments including total anthocyanins, total monomeric anthocyanins, and total flavonoids (Tables 1, 2, 4 and 5). The total phenols were also significantly lower in these samples (Table 3 and 6). Blood orange juice with the highest a* and CCI values and the lowest $h°$ indicating dark reddish tones of colour recorded the highest ($P \leq 0.05$) levels of total anthocyanins, total monomeric anthocyanins, and total flavonoids contents. Thus, the current study also confirms that 'colour' can be used as a simple, yet a reliable tool to assess the levels of colouring pigments and overall health benefits of cold stored blood orange juice. The influence of prior- and postharvest application of MeJA and SA on the levels of individual anthocyanins, flavonoids, phenolic acids, and carotenoids warrants to be investigated in cold stored blood orange juice.

In summary, this investigation for the first time testified that the spray application of 3 mM MeJA four weeks before harvest was the most effective treatment in enhancing red colour development of the rind and juice and the levels of anthocyanins and flavonoids in the juice of cold stored blood orange fruit in comparison to the control. The postharvest dip treatment of 1.5 mM MeJA also markedly improved the juice colour and the levels of anthocyanins and flavonoids. Preharvest spray application of 1 mM SA was the most effective treatment among SA treatment on improving CCI while reducing L*, b*, and $h°$, whereas only 2- and 3-mM SA treatments improved a* which represents red colour of the rind of cold stored blood oranges. While improving the colour parameters, both MeJA and SA increased the levels of colouring pigments and other health-promoting phytochemicals in cold stored blood orange fruit. Thus, the exogenous application of plant growth regulators facilitates better red colour development and higher production of anthocyanins and flavonoids and other bioactive compounds compared to cold storage alone. Overall, the colour is a reliable index to assess the levels of colouring pigments and other -health-promoting compounds in cold stored blood orange juice.

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for experiments, project administration, writing—reviewing and editing original draft, reviewing and improvement of grant application, MUH: investigation, writing—reviewing and editing original draft, preparation of images and graphs.

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Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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