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Preharvest spray application of methyl jasmonate promotes fruit colour and regulates quality in M7 Navel orange grown in a Mediterranean climate

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Abstract

Poor rind colour in cv. M7 Navel (*Citrus sinensis* L. Osbeck) at harvest time severely affects the profits of the growers in Western Australia. The effects of the preharvest spray application of different concentrations (0, 1.25, 2.5, 5.0 and 7.5 mM) of methyl jasmonate (MJ) on the rind colour development and fruit quality of M7 Navel were investigated at 6 or 3 weeks before anticipated harvest (WBAH) during 2015 and 2016. The preharvest spray application of MJ (5.0 or 7.5 mM) resulted in enhanced rind colour, reduced *hue angle (h^o)* angle (55.7, 54.3) as well as increased citrus colour index (CCI) (11.0, 12.0) and total carotenoid levels (35.3, 58.3 mg kg^{-1}) of flavedo, respectively in M7 Navel, during 2015 and 2016. During 2015, comparatively higher levels of total carotenoid (40.4 mg kg^{-1}) were recorded when MJ was applied at 3 WBAH as a single spray. However, the time of MJ application did not influence hue angle (*h*°) and CCI. In 2015, all the preharvest MJ treatments except (1.25 mM) exhibited reduced fruit firmness. Furthermore, soluble solids concentration (SSC) in the fruit juice was reduced after MJ treatment. All the MJ treatments showed reduced levels of total sugars and organic acids in the juice, during 2015. In conclusion, MJ (5.0 or 7.5 mM) reduced *h*° but increased the total carotenoids levels and CCI in the flavedo of M7 Navel orange, when applied as a preharvest spray at 3 WBAH.

Keywords: citrus colour index; fruit quality; hue angle; methyl jasmonate; sweet orange; total carotenoids.
Abbreviations: JA_Jasmonic acid; MJ_methyl jasmonate; ACS: 1-aminocyclopropane-1-carboxylate synthase synthase; ACO: 1-aminocyclopropane-1-carboxylate oxidase, SSC_soluble solids content; TA_titratable acidity.

Introduction

The fruit colour is one of the major factors which influence the consumers' preference to purchase the citrus fruit. The M7 cultivar of sweet orange (*Citrus sinensis* L. Osbeck) is early maturing and in Western Australia, they are mainly harvested in the month of May. It is a Navel orange and is a bud mutation of Navelina. M7 has been introduced for commercial cultivation in Western Australia, during recent years, to extend the availability period of sweet orange fruit. The fruit is rounder in shape and contains comparatively more soluble solids concentration and acid ratio than Navelina (DAFWA, 2017). Usually M7 at fruit maturity attains excellent internal fruit quality but coupled with poor rind colour development in Western Australia, consequently affecting the profits of the fruit growers.

The orange colour development in the citrus flavedo is due to the degradation of chlorophyll and accumulation of carotenoid pigments (Gross, 2012). Various types of carotenoids and their isomers have been identified in citrus fruit (Goodner et al., 2001; Stewart and Wheaton, 1973). Alquezar et al. (2008) reported that these carotenoids are responsible for both internal and external colouration in Cara Cara sweet orange fruit. Apart from enhancing flavedo colour, carotenoids are also precursors of vitamin A and possess significant antioxidant activity to protect human beings against cardiovascular diseases and carcinogenesis (Sandmann, 2001).

Jasmonates (JA_S) are cyclopentanone forms, which are naturally present in plants as jasmonic acid (JA) and methyl jasmonate (MJ). These compounds regulate a variety of responses in plants (Sembdner and Parthier, 1993), including the degradation of chlorophyll (Hung and Kao, 1996) and accumulation of anthocyanins pigment (Shafiq et al., 2013). Jasmonates are also reported to delay postharvest decay, inhibit green mould growth, enhance fruit colour and reduce of chilling injury incidence during cold storage (Reyes-Díaz et al., 2016). It has been found that MJ promoted chlorophyll degradation as well as anthocyanin biosynthesis in the apple (Perez et al. 1993), tomato (Saniewski and Czapski, 1983), lychee (Yang et al., 2011), papaya (Gonzalez-Aguilar et al., 2003) and mango fruits (Gonzalez-Aguilar et al. 2001). MJ has also been reported to regulate aroma development in mango (Lalel et al., 2003) and apple fruit (Olias et al., 1992). The 'La France' pear fruit dipped in 0.39 mM n-propyl dihydro jasmonate (PDJ), a derivative of jasmonic acid, exhibited upregulated expression and thus resulting in increased levels of ethylene production, following storage at 4 °C for 15 days (Kondo et al., 2007).

Levels of JA in the cell, vary with the fruit developmental stage. The concentrations of MJ in the apple fruit were higher at a mature stage in comparison with the early fruit development stage (Kondo et al., 2000). While in case of the fruits such as grapes and sweet cherry, the MJ levels were higher at early fruit growth stage and decreased gradually towards harvest maturity (Kondo et al., 2000, Kondo and Fukuda, 2001). Exogenous application of MJ has stimulated accumulation of β-carotene in tomato fruit while inhibiting lycopene, during the ripening process (Saniewski and Czapski, 1983). MJ has enhanced the red blush and flavonoid accumulation in apple fruit when applied as a preharvest spray (Shafiq et al. 2013). Kondo et al. (2001) reported that MJ application enhanced the apple fruit colour, independent of ethylene action. This signifies that the colour development in apple fruit could be partly independent of ethylene action (Kondo et al. 2001). As a prelude, MJ application as preharvest spray has been reported to accelerate the chlorophyll degradation and accumulation of anthocyanin in various fruit. Recently Rehman et al. (2018 a and b) have reported that application of paclobutrazol (PBZ), abscisic acid (S-ABA) or prohexadione-calcium (Pro-Ca) as preharvest spray 3 to 6 weeks before harvest has enhanced fruit rind colour in M7 sweet orange. In the meantime, no other research has been reported on the effects of MJ application on the colour development and levels of carotenoids in the flavedo as well as fruit quality of M7 sweet orange. The objective of the present study was to explicate the effects of preharvest application of MJ in enhancing deep orange colour development and total carotenoids accumulation in the flavedo while maintaining the fruit quality of M7 Navel orange, grown in the Mediterranean climate of Western Australia.

Results

Effect of MJ on Hue angle (h°)

During 2015, the mean *h*° was reduced significantly ($P \leq 0.05$) with the preharvest spray application of MJ (5.0 mM) (55.7) when compared to that of control (61.1) and all other treatments (Table 1). But the mean *h*° value was not significantly affected by the spray application time. The interaction effect of MJ treatments and time of spray application was not significant for *h*°. In 2016, MJ (7.5 mM) spray application at 3 WBAH showed significantly lower *h*° (54.3) in comparison with control (62.9) and other MJ treatments except 2.5 and 5.0 mM (Table 2).

Effect of MJ on the citrus colour index (CCI)

MJ (5.0 mM) treatment significantly increased mean CCI (11.0) during 2015, when compared to all other treatments (Table 1), while, the spray application time did not show any significant effect on the mean CCI values. The interaction effect between MJ treatments and spray time was non-significant for CCI in 2015. During 2016, MJ spray significantly enhanced the CCI values, regardless of concentration applied (Table 2).

Effect of MJ on total carotenoids levels

The mean level of total carotenoids in the flavedo was significantly higher at the 5.0 mM MJ (35.3 mg kg^{-1}) application as compared to the control (18.2 mg kg^{-1}) and all other treatments during the year 2015 (Table 1). Whilst, MJ application at 3 WBAH exhibited the maximum mean total carotenoid levels (40.4 mg kg^{-1}) as related to the other spray timings. During 2015, there was a significant interaction effect of MJ application and spray timings on total carotenoid levels and MJ (1.25 to 5.0 mM) single spray application applied at 3 WBAH exhibited the highest levels of total carotenoids (45.0 to 49.2 mg kg^{-1}) (Table 1). In 2016, MJ (5.0 and 7.5) spray at 3 WBAH significantly enhanced the total carotenoid levels (47.6 and 58.9 mg kg^{-1}), respectively, as compared to other treatments as well as control (18.0 mg kg^{-1}) (Table 2).

Effect of MJ on fruit firmness

All MJ treatments except MJ (1.25 mM) showed significantly lower fruit firmness as compared to the control (398.9 N) during 2015 (Table 3). The time of spray application also significantly affected the mean fruit firmness during 2015. The double spray application at 6 WBAH followed by 3 WBAH, exhibited significantly higher fruit firmness (395.5 N) when compared to other spray times (Table 3). The interaction effect between MJ treatments and spray timings on the fruit firmness was not significant. During 2016, when compared to control (366.1 N), all the MJ treatments applied at 3 WBAH reduced fruit firmness (Table 4).

Effect of MJ on SSC, TA and SSC: TA in juice

The MJ spray treatments showed significantly lowered the mean SSC values in the fruit juice when compared to the control. Whilst their effect on mean TA and SSC: TA were found to be non-significant in the year 2015 (Table 3). The MJ application at 3 WBAH significantly reduced the mean values of SSC and TA in the juice, when compared to the other spray timings. Furthermore, the mean values of SSC: TA were found to be highest (13.5) with the single MJ spray application at 3 WBAH (Table 3). A significant interaction effect was found between different MJ treatments and spray timings on the SSC values but not on TA and SSC: TA during 2015. In the year 2016, all the MJ treatments applied at 3 WBAH did not show any significant effect on SSC values. However, the MJ spray application (1.25 mM) increased the TA (1.3 %) values when compared to that of the control fruit and all other MJ treatments (Table 4). The SSC: TA values were highest in the MJ (5.0 mM) treated fruit.

Table 1. Hue angle (*h*°), citrus colour index (CCI) and levels of total carotenoids in flavedo of M7 Navel influenced by different treatments (Tr) of MJ (methyl jasmonate) and time of application (Tm) applied at 6, 3 WBAH (weeks before anticipated harvest) as single spray and 6 followed by (fb) 3 WBAH as double spray in 2015 growing seasons.

Treatments (mM)	6 WBAH	3 WBAH	6 fb 3 WBAH	Mean (Tr)
		Hue angle (*h*°)		
Control	61.2±0.49	61.7±0.26	60.5±0.36	61.1a
MJ (1.25)	58.7±0.23	56.7±0.06	57.8±0.15	57.7b
MJ (2.5)	57.7±0.25	56.0±0.19	57.2±0.21	57.0b
MJ (5.0)	55.3±0.14	55.8±0.09	56.1±0.13	55.7c
Mean (Tm)	58.2	57.5	57.9	
LSD ($P \leq 0.05$)	Tr = 0.98	Tm = ns	Tr xTm = ns	
		Citrus colour index (CCI)		
Control	8.5±0.22	8.2±0.08	8.7±0.16	8.5c
MJ (1.25)	9.6±0.10	10.3±0.07	10.1±0.09	10.1b
MJ (2.5)	10.0±0.12	10.9±0.10	10.2±0.11	10.4b
MJ (5.0)	11.3±0.07	10.9±0.04	10.8±0.08	11.0a
Mean	9.9	10.2	10.0	
LSD ($P \leq 0.05$)	Tr = 0.46	Tm = ns	Tr xTm = ns	
		Total carotenoids (mg kg^{-1})		
Control	16.2 ±0.25d	20.5±1.4cd	18.1±0.44cd	18.2c
MJ (1.25)	22.3±0.50c	45.0±0.62a	19.0±0.24cd	28.8b
MJ (2.5)	21.5±0.68c	47.1±0.43a	20.7±0.62cd	29.8b
MJ (5.0)	38.9±0.25 b	49.2±0.52a	17.7±0.38cd	35.3a
Mean	24.7b	40.4a	18.8c	
LSD ($P \leq 0.05$)	Tr = 0.46	Tm = 2.2	Tr xTm = 4.5	

Mean separation within the column (Mean Tr) was tested with LSD *(P<0.05)*; while mean separation within the row (Mean Tm) was tested with LSD *(P<0.05)*. Mean separation for the interaction effects (Tr x Tm) was tested with LSD *(P<0.05)* at the same level of Tm across Tr. ns = not significant, n = three replicates (25 fruit per replication). Standard error SE (±).

Table 2. Effect of preharvest single spray application of different concentrations of MJ applied at 3 WBAH on (*h*°), (CCI) and level of total carotenoids in the flavedo of M7 Navel in 2016.

Treatments (MJ mM)	*h*°	CCI	Total carotenoids (mg kg^{-1})
Control	62.9±0.25a	7.8±0.09c	18.0±1.5c
MJ (1.25)	57.0±0.33b	10.5±0.18b	38.1±1.0b
MJ (2.5)	55.4±0.22bc	11.4±0.12ab	43.3±1.6b
MJ (5.0)	55.4±0.18bc	11.4±0.10ab	47.6±2.5ab
MJ (7.5)	54.3±0.26c	12.0±0.18a	58.9±1.2a
LSD ($P \leq 0.05$)	1.8	0.9	12.9

Mean separation within the column (Mean Tr) was tested with LSD *(P<0.05)*. ns = not significant, n = four replicates (25 fruit per replication). Standard error SE (±).

Table 3. Fruit firmness (N), SSC (%), TA (%) and SSC/TA ratio in M7 Navel influenced by different treatments (Tr) of MJ (methyl jasmonate) and time of application (Tm) applied at 6, 3 WBAH (weeks before anticipated harvest) as single spray and 6 followed by (fb) 3 WBAH as double spray in 2015 growing seasons.

Treatments (MJ mM)	6 WBAH	3 WBAH	6 fb 3 WBAH	Mean (Tr)
		Fruit firmness (N)		
Control	389.3±4.4	389.3±5.4	418.0±3.2	398.9a
MJ (1.25)	394.3±2.2	386.9±1.7	407.1±1.6	396.1a
MJ (2.5)	356.6±2.2	354.3±5.9	364.1±2.4	358.3b
MJ (5.0)	365.6±3.0	369.3±10.0	392.7±2.0	375.7b
Mean (Tm)	376.3b	374.9b	395.5a	
LSD ($P \leq 0.05$)	Tr = 18.6	Tm = 16.1	Tr xTm = ns	
		SSC (%)		
Control	12.8±0.11cd	13.4±0.11a	13.2±0.02abc	13.1a
MJ (1.25)	13.1±0.07abc	12.3±0.04ef	12.1±0.04f	12.5b
MJ (2.5)	12.8±0.11bcd	11.5±0.01g	13.2±0.04ab	12.5b
MJ (5.0)	12.5±0.07de	12.4±0.02ef	12.2±0.02ef	12.3b
Mean	12.8a	12.4b	12.8a	
LSD ($P \leq 0.05$)	Tr = 0.22	Tm = 0.19	Tr xTm = 0.39	
		TA (%)		
Control	1.01±0.02	0.93±0.04	1.15±0.01	1.03
MJ (1.25)	1.02±0.02	0.93±0.01	0.99±0.01	0.98
MJ (2.5)	1.02±0.02	0.88±0.02	0.96±0.01	0.96
MJ (5.0)	1.05±0.02	0.95±0.01	0.95±0.01	0.98
Mean	1.03a	0.92b	1.01a	
LSD ($P \leq 0.05$)	Tr = ns	Tm = 0.06	Tr xTm = ns	
SSC/TA				
Control	12.7±0.13	14.7±0.73	11.4±0.08	12.9
MJ (1.25)	12.8±0.16	13.2±0.12	12.2±0.06	12.7
MJ (2.5)	12.5±0.19	13.1±0.23	13.8±0.11	13.1
MJ (5.0)	12.0±0.21	13.1±0.08	12.8±0.09	12.6
Mean	12.5b	13.5a	12.6b	
LSD ($P \leq 0.05$)	Tr = ns	Tm = 0.6	Tr xTm = ns	

Mean separation within the column (Mean Tr) was tested with LSD *(P<0.05)*; while mean separation within the row (Mean Tm) was tested with LSD *(P<0.05)*. Mean separation for the interaction effects (Tr x Tm) was tested with LSD *(P<0.05)* at the same level of Tm across Tr. ns = not significant, n = three replicates (25 fruit per replication). Standard error SE (±).

Table 4. Effect of preharvest single spray application of different concentrations of MJ applied at 3 WBAH on fruit firmness (N), SSC (%), TA (%) and SSC/TA in M7 Navel during 2016.

Treatment (MJ mM)	Firmness (N)	SSC (%)	TA (%)	SSC/TA ratio
Control	366.1±5.0	11.9±0.15	1.1±0.0	10.5±0.13
MJ (1.25)	361.5±2.3	11.9±0.12	1.3±0.01	9.5±0.03
MJ (2.5)	358.3±3.8	12.2±0.04	1.2±0.02	10.2±0.13
MJ (5.0)	327.9±6.1	11.7±0.15	1.1±0.01	11.1±0.19
MJ (7.5)	323.8±3.7	11.7±0.07	1.2±0.01	9.9±0.11
LSD ($P \le 0.05$)	33.20	ns	0.08	1.02

Mean separation within the column (Mean Tr) was tested with LSD *(P<0.05)*. ns = not significant, n = four replicates (25 fruit per replication). Standard error SE (±).

Table 5. Levels of ascorbic acid and total antioxidants in the juice of M7 Navel influenced by different treatments (Tr) of MJ (methyl jasmonate) and time of application (Tm) applied at 6, 3 WBAH (weeks before anticipated harvest) as single spray and 6 followed by (fb) 3 WBAH as a double spray in 2015 growing season.

Treatment (MJ mM)	6 WBAH	3 WBAH	6 fb 3 WBAH	Mean (Tr)
		Ascorbic acid (mg L^{-1})		
Control	541.1±4.8bcd	564.8±5.6 abc	570.9±8.1abc	558.9
MJ (1.25)	607.6±4.2a	577.8±3.4abc	505.3±9.4 d	563.5
MJ (2.5)	547.6±10.0bcd	586.0±5.6ab	560.1±11.6abcd	564.5
MJ (5.0)	549.7±8.3bcd	592.9±6.3ab	524.7±3.2cd	555.8
Mean	561.5ab	580.4a	540.2b	
	LSD ($P \le 0.05$) Tr = ns	Tm = 25.0	Tr x Tm = 50.0	
		Total antioxidants (μM Trolox L^{-1})		
Control	497.8±4.0	553.8±26.1	532.6±5.1	528.1
MJ (1.25)	450.4±8.2	493.4±18.7	496.4±13.9	480.0
MJ (2.5)	402.5±0.18	505.4±5.0	456.8±14.9	513.9
MJ (5.0)	452.6±11.1	604.8±15.6	503.2±12.9	520.2
Mean	450.8b	539.4a	497.2b	
LSD ($P \le 0.05$)	Tr = ns	Tm = 81.2	Tr x Tm = ns	

Mean separation within the column (Mean Tr) was tested with LSD *(P<0.05)*; while mean separation within the row (Mean Tm) was tested with LSD *(P<0.05)*. Mean separation for the interaction effects (Tr x Tm) was tested with LSD *(P<0.05)* at the same level of Tm across Tr. ns = not significant, n = three replicates (25 fruit per replication). Standard error SE (±).

Table 6. Effect of spray application of different concentrations of MJ applied at 3 weeks before anticipated harvest on levels of ascorbic acid and total antioxidants in the juice of M7 Navel in 2016.

Treatment (MJ mM)	Ascorbic acid (mg L^{-1})	Total antioxidant capacity (μM Trolox L^{-1})
Control	511.6±14.9bc	473.9±6.3b
MJ (1.25)	504.5±14.3bc	590.6±8.3a
MJ (2.5)	577.7±6.0a	446.8±7.0b
MJ (5.0)	496.4±9.1c	590.7±6.1a
MJ (7.5)	563.4±6.8ab	588.2±3.5a
LSD ($P \le 0.05$)	57.9	46.6

Mean separation within the column (Mean Tr) was tested with LSD *(P<0.05)*. ns = not significant, n = four replicates (25 fruit per replication). Standard error SE (±).

Effect of MJ on ascorbic acid and total antioxidant capacity

Regardless of the concentrations, all the MJ treatments did not show any significant ($P \le 0.05$) effect on the mean ascorbic acid as well as total antioxidants levels in 2015 (Table 5). However, mean levels of ascorbic acid and total antioxidant capacity in the juice were significantly higher (580.4 mg L^{-1} and 539.4 μM Trolox L^{-1} respectively) with the MJ spray applied at 3 WBAH. There was a significant interaction effect between MJ treatments and spray timings on the levels of ascorbic acid but not for total antioxidant capacity. The single spray application of MJ (1.25 mM) at 6 WBAH exhibited a higher level of ascorbic acid (607.6 mg L^{-1}) in the juice as compared to all other treatments. During 2016, MJ application (2.5 mM) showed the highest mean ascorbic acid levels in the juice (577.7 mg L^{-1}) when compared to all other treatments (Table 6). The MJ treatments except 2.5 mM MJ spray substantially enhanced the levels of total antioxidant capacity in the fruit juice.

Discussion

The plant pigment groups such as carotenoids, anthocyanins, chlorophyll and flavonoids play a major role in developing the fruit colour. The development of orange colour in the sweet orange flavedo is due to degradation of chlorophyll and accumulation of carotenoid pigments (Gross, 2012). Alquezar et al. (2008) earlier reported that both the internal and external colouration in the orange fruit is due to carotenoid pigments.

Application of MJ as preharvest spray significantly reduced *h*° angle and enhanced CCI in two consecutive years 2015 and 2016 (Table 1). An enhanced colour in M7 Navel orange with an MJ application could be attributed to increased levels of total carotenoids in the fruit flavedo (Table 1). The mechanism by which MJ promotes flavedo colour through the accumulation of carotenoids and anthocyanin is still unclear. Possibly, MJ enhances the flavedo colour independently or through the upregulation of ethylene production in M7 Navel. The increased carotenoid accumulation in the flavedo with the MJ spray is due to the enhanced β-carotene levels. Application of MJ has been previously reported to increase β-carotene levels in the tomato (Saniewski and Czapski, 1983) and the apple (Perez et al., 1993) peel and thus enhance the fruit colour. MJ stimulates chlorophyll degradation independently or through enhanced ethylene production (Hung and Kao, 1996). Previously, the application of MJ decreased the content of chlorophyll in *the Arabidopsis thaliana* plant (Jung 2004).

Furthermore, the ratio of chlorophyll a and b was reduced with the MJ application in the Fuji apple fruit (Rudell and Mattheis, 2008). Jung et al. (2007) have reported that the application of 100 µM MJ or higher concentration, down-regulated the genes involved in chlorophyll *a*/*b*-binding protein. Application of MJ degraded chlorophyll-*a* more rapidly than chlorophyll *b* in barley (*Hordeum vulgare* L.) as reported by (Cuello, 1997) and chlorophyll a/b ratio decreased more quickly in Golden Delicious apple fruit peel with increased MJ exposure (Perez et al., 1993).

It has been well documented that ethylene enhances the process of chlorophyll degradation as well as carotenoids synthesis in the fruit (Stewart and Wheaton, 1973). MJ vapour application to Golden Delicious apples accelerated the ethylene production (2.5 and 4.6 folds in the tissues of cortex and peel, respectively) (Olias et al., 1992). Moreover, it has been well acknowledged that application of MJ exogenous relatively increases the endogenous production of ethylene in fruit. It also promoted the colour development in the non-climacteric fruit like strawberry (Mukkun and Singh, 2009) as well as in the climacteric fruit like mango, plum and apple (Khan and Singh, 2007; Lalel et al., 2003). Previously, it was proved that preharvest MJ application enhanced the peel colour in Fuji apple, by increasing accumulation of pigments causing red colour (Rudell and Mattheis, 2008; Shafiq et al., 2013).

The findings from the present experiment showed that MJ treatments reduced SSC (%) and TA (%), but the SSC: TA remained unaffected by any of the MJ treatments (Table 3). Previously, it was reported that preharvest MJ application increased SSC (%) levels and decreased TA (%) in plum varieties such as Fortune and Friar (Ozturk et al., 2015), Amber Jewel, Angelino and Black Amber (Khan and Singh, 2007) as well as in Jewel (blackberry) Autumn Bliss (red blackberry) (Wang and Zheng, 2005) and blackberry cultivars (Hull Thornless, Chester Thornless and Triple Crown (Wang et al., 2008). These findings are in contrary to our results, but the effect of MJ on sugar content in the literature is debatable.

In the present study, preharvest spray application of MJ (1.25, 5.0 and 7.0 mM) exhibited enhanced antioxidant capacity in the fruit juice when compared to that of control. Similarly, Khan and Singh (2007) also found a rise in the antioxidant capacity of various plum cultivars due to MJ application. Furthermore, MJ treatments increased the total phenolic levels and subsequently enhanced the antioxidant capacity in various fruits (Rudell et al., 2002; Wang and Zheng, 2005; Cao et al., 2009).

Materials and methods

Plant material

M7 Navel orange fruit was obtained from the commercial citrus orchard in Moora (30° 35' S/115° 55' E), Western Australia (WA). The fruit was hand-harvested from five-year-old trees, which were budded on the rootstock Carrizo citrange (*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliate* Raf.). M7 trees were planted in the North-South direction with the spacing of 5.0 m between the rows and 2.5 m within the rows. Similar cultural practices such as fertilisers, plant protection and irrigation were provided to all the trees in the orchard. Two independent experiments were conducted during 2015 and 2016.

Experiment 1: *Application of MJ as preharvest spray at 6 or 3 WBAH in form of single sprays and double spray at 6 WBAH followed by 3 WBAH in M7 Navel orange during the year 2015.*

Aqueous emulsion of MJ (1.25, 2.5 or 5.0 mM) (Sigma-Aldrich, Saint Louis, USA) and Tween® 20 (0.25 %) were sprayed onto the M7 trees until runoff. Tween® 20 was used as a surfactant in the experiment. A single spray application at 6 WBAH (8 April) or 3 WBAH (30 April) and two sprays at 6 WBAH followed by 3 WBAH were applied in the year 2015. The trees left unsprayed were considered as control. Two-factor factorial (MJ treatments and times of application) randomised block design replicated 4 times was used to conduct the experiment. Trees with uniform canopy, free from any diseases or pests were selected for experiment and each tree was used as an experimental unit with three replicates. At harvest maturity, 25 fruit per tree free from any blemishes or damages were randomly harvested around the tree canopy. The harvested fruit was immediately transported to Curtin Horticulture Research Laboratory using an air-conditioned vehicle. The colour parameters such as *h°* and CCI; total carotenoid levels in the flavedo and fruit firmness were determined. The fruit quality parameters such as SSC, titratable acidity (TA), SSC: TA, total antioxidant capacity as well as levels of ascorbic acid, individual sugars and organic acids were estimated from the juice samples extracted.

Experiment 2: *Application of MJ as a preharvest spray at 3 WBAH in M7 Navel orange during 2016.*

During 2016, an emulsion of MJ (1.25, 2.5, 5.0, 7.5 mM) treatments and Tween® 20 (0.25 %) as a surfactant was sprayed on the tree canopy at 3 WBAH (30 April) till runoff. The unsprayed trees were considered as control. The experiment was designed as one-factor (MJ treatments) factorial randomised block design with four replications. Each tree was considered as an experimental unit. 25 fruit per tree were harvested randomly from around the canopy of the tree. The harvested fruit was then immediately transported to the Curtin Horticulture Research Laboratory, Perth, WA within four hours using an air-conditioned vehicle. The fruit colour parameters such as *h°*, CCI and total carotenoids levels in the flavedo were estimated. Quality variables (except levels of organic acids and individual sugars) were recorded and analysed similarly to that of Experiment 1.

Fruit colour, carotenoids and fruit quality variables

Fruit colour

The colour coordinates (L*, a* and b*) of the fruit flavedo were determined from three positions at the equatorial plane of the fruit using Colorflex EZ (45°/0° design) spectrocolorimeter (Hunter Lab, Hunter Associates Laboratory Inc., Reston, VA, 20190, USA) by the method earlier described by Rehman et al. (2018a). The hue angle (h^o) and CCI were then calculated using the formulae $h^o = tan^{-1}\ b^*/a^*$ and $\mathrm{CCI} = \frac{1000.\mathrm{a}}{\mathrm{L.b}}$ respectively.

Total carotenoid content

A small portion of flavedo from ten randomly selected M7 Navel fruit was excised in each replication. The levels of total carotenoids from the pooled flavedo samples were determined using the method followed by Lee and Castle (2001) and described in detail by Rehman et al. (2018a).

Fruit firmness

The firmness of the ten randomly selected fruit per replication was determined using the texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Fareham, UK) connected with Nexygen® 4.6 software following the method previously mentioned by Rehman et al. (2018a). The calculated fruit firmness was expressed in newtons (N).

SSC, TA and SSC: TA

The SSC (%) of pooled fruit juice samples was determined using the digital refractometer (Atago-Palette PR 101, Atago CO. Ltd, Tokyo, Japan). The TA was determined by titrating the pooled sample of fruit juice using 2-3 drops of phenolphthalein against 0.1N NaOH until the pink colour endpoint is achieved. The calculated TA was expressed as percentage citric acid. The SSC: TA was obtained by dividing the values of SCC by TA.

Ascorbic acid and total antioxidant activity

The ascorbic acid (ascorbic acid) and total antioxidant capacity in the pooled fruit juice was estimated using UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). The detailed method for the calculation of levels of ascorbic acid and total antioxidant capacity has been previously described by Rehman et al. (2018a).

Statistical analysis

All the data were subjected to one-way or two-way analysis of variance (ANOVA) with *GenStat* software (edition 14.1, Lawes Agricultural Trust, Rothamsted, UK). Fisher's least significant differences (LSD) were calculated when F-test was significant ($P \leq 0.05$). Within ANOVA, the effects of MJ treatments, as well as the time of the spray application and their interaction effect on the variables, were evaluated.

Conclusion

In conclusion, application of 5.0 or 7.5 mM MJ as spray application at 3 WBAH promoted the colour change from yellow toward deep orange, while reducing $h°$ and enhancing CCI and total carotenoids levels in the flavedo, without any adverse effects on the fruit quality.

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