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Post-harvest Application of Methyl Jasmonate, 1-Methylcyclopropene and Salicylic Acid Elevates Health-promoting Compounds in Cold-stored 'Kinnow' Mandarin (*Citrus nobilis* Lour x *C. deliciosa* Tenora) Fruit

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ABSTRACT

An investigation was carried out to evaluate the influence of the post-harvest application of different ethylene inhibitors viz., 1-methylcyclopropene (1-MCP; 500, 1000 and 1500 ppb), methyl jasmonates (MeJA; 1, 2 and 3 mM) and salicylic acid (SA; 1, 2 and 3 mM) on health-promoting compounds such as total phenols, total antioxidants, flavonoids, protein, total free amino acids and sugars in cold-stored 'Kinnow' mandarin fruit. The fruit were stored at 6 ± 1°C and 90 ± 5% RH for 75 days. Fruit treated with MeJA (1 mM), 1-MCP (1500 ppb) and SA (2 mM) maintained significantly highest levels of total phenols, total antioxidants activity, flavonoids, protein, total free amino acids and sugars as compared with the control. In conclusion the post-harvest application of MeJA (1 mM), 1-MCP (1500 ppb) and SA (2 mM) can be used to maintain the highest levels of these bioactive compounds in cold-stored 'Kinnow' mandarin fruit for up to 60 days.

KEYWORDS
Total phenols; total antioxidant activity; cold storage; ethylene inhibitors; mandarin

Introduction

Horticultural crops are an ample source of health-promoting compounds including phenolic compounds and their antioxidative properties and are often cherished among consumers due to their health benefits as they are helpful in the cure of various ailments such as tumor and heart-stroke (Parr and Bolwell, 2000; Rautiainen et al., 2012). Thereby, to ensure better human health and environmental safety application of generally recognized as safe (GRAS) chemicals are receiving more worldwide attention to preserve these health-promoting compounds in fresh horticultural produce (Suleria et al., 2015).

Citrus fruits are a plentiful source of phenolics, antioxidants and flavonoids. The accumulation of these bioactive compounds in the fruit can be hampered due to unsafe usages of different post-harvest approaches and lack of appropriate storage facilities (Connor et al., 2002). Recently, the post-harvest application of various GRAS chemicals on 'Kinnow' mandarin resulted in significantly higher levels of ascorbic acid and total carotenoids (Baswal et al., 2020), and total phenols and antioxidant activity (Haider et al., 2020). The efficacy of different concentrations of ethylene inhibitors viz., 1-MCP, MeJA and SA on bioactive compounds including phenolics, flavonoids, antioxidants, protein and total free amino acids in 'Kinnow' mandarin fruit during cold-storage warrants to be investigated.

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Fruit softening and senescence can be delayed with the application of 1-methylcyclopropene (1-MCP) as it blocks the ethylene-dependent responses due to its ethylene inhibitory action (Sisler and Serek, 1997). The post-harvest application of 1-MCP has been reported to influence phenolic compounds in various fruit crops such as apple (Gago et al., 2015), pear (Arias et al., 2009), plum (Singh and Singh, 2012) and medlar fruit *(Mespilus germanica* L. cv. Istanbul) (Selcuk and Erkan, 2015). Earlier, the application of 1-MCP has been reported to maintain the post-harvest quality of 'Kinnow' mandarin (Asrey et al., 2012; Baswal et al., 2020; Tavallali and Moghadam, 2015) but no information is available on the dynamics of total phenols, flavonoids, total antioxidants, protein and total free amino acids content in 'Kinnow' mandarin fruit during long-term cold storage and demands to be investigated.

MeJA triggers miscellaneous defense signaling pathway and elicits the production of various secondary metabolites including phenolic compounds (Heredia and Cisneros-Zevallos, 2009; Kim et al., 2006). Previously, the application of MeJA has been reported to accumulate higher phenolic compounds in various fruit crops such as strawberry (Asghari and Hasanlooe, 2015), raspberry (Flores and Ruiz Del Castillo, 2014), chinese bayberries (Wang et al., 2009) and pomegranate (Sayyari et al., 2011). Recently, Baswal et al. (2020) also investigated the influence of post-harvest application of MeJA on cold-storage life and fruit quality of 'Kinnow' mandarin but lacked information on total phenols, flavonoids, total antioxidants, protein and total free amino acids content in cold-stored 'Kinnow' mandarin fruit thus deserves an investigation.

Salicylic acid (SA) is involved in different growth and development processes including accumulation of secondary metabolites during systemic acquired resistance (Chen et al., 2016). Earlier, the post-harvest application of SA exhibited higher content of phenolic compounds in peaches (Khademi and Ershadi, 2013), plum (Davarynejad et al., 2015) and guava (Amanullah et al., 2017). Recently, Baswal et al. (2020) reported the effect of post-harvest application of SA on cold-storage life and fruit quality of 'Kinnow' mandarin but lacked information on changes in phenolic compounds such as total phenols, flavonoids, total antioxidants, protein and total free amino acids content during cold storage. Haider et al. (2020) also reported that the post-harvest application of SA influenced total phenols and total antioxidant activity in cold-stored 'Kinnow' mandarin but lacked information on flavonoids, protein and total free amino acids content thus justifies to be investigated.

It is surmised that these bioactive compounds are the valuable contributors to the esthetic and nutritional properties of fruit and vegetables. The objective of the present investigation was therefore to evaluate the influence of the post-harvest application of different concentrations of 1-MCP, MeJA and SA on the dynamics of health-promoting compounds viz., total phenols, antioxidants, flavonoids, protein and total free amino acids content in cold-stored 'Kinnow' mandarin fruit.

Materials and Methods

Fruit Material

'Kinnow' mandarin fruit were harvested randomly at physiological maturity (SSC:TA ratio 12:1) from different trees grown in the same block by leaving a small protruding fruit stalk (button) at an experimental orchard at Regional Fruit Research Station, Abohar, Punjab, India (30°55"-N, 54°30" E) during 2016–17 and 2017–18. The fruit of uniform weight (250–300 g per fruit) and free from symptoms of diseases and bruises were used in the experiment. The fruit were treated with 1-MCP (500, 1000 and 1500 ppb), MeJA (1, 2 and 3 mM) and SA (1, 2 and 3 mM). 1-MCP was applied to the fruit as fumigation for 24 hours, whilst, MeJA and SA were applied as 5 min dip treatments by following methods recently detailed by Baswal et al. (2020). Each treatment was replicated three times and each replication comprised 12 fruits. Following the treatments, the fruit were stored at 6 ± 1°C and 90 ± 5% RH for 75 days. Fruit were analyzed for different quality attributes after 30, 45, 60 and 75 days of cold storage, respectively. Though fresh harvested fruit were evaluated for zero day.

Determination of Levels of Total Phenols, Total Antioxidant Activity and Flavonoids

Total phenols level was estimated by following procedure previously outlined by Meyers et al. (2003) with slight modifications earlier detailed by Baswal (2019) and expressed as mg gallic acid 100 ml^{-1} juice. For the estimation of total antioxidant activity 0.1 ml of 'Kinnow' juice extract was added in 3.9 ml aliquot of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.078 mM) made in 100 ml of 95% methanol. After incubation for 30 min in dark the absorbance of the sample was recorded using a micro-plate spectrophotometer (Epoch Biotech, USA) at 517 nm using 95% methanol as reagent blank and expressed in percentage (Brand-Williams et al., 1995). To estimate the flavonoids content, methanolic extract of 'Kinnow' juice (1.0 ml) was mixed with 1.5 ml methanol, 0.5 ml of 10% aluminum chloride ($AlCl_3 6H_2O$), 0.5 ml potassium acetate 1M and 2.0 ml of distilled water. The mixture was kept in the dark for 30 min incubation and the absorbance was recorded at 414 nm wavelength using a micro-plate spectrophotometer (Epoch Biotech, USA) and the results were expressed as mg quercetin 100 ml^{-1} juice (Chang et al., 2002).

Determination of Protein, Total Free Amino Acids and Sugars

The levels of protein were estimated by following the method previously detailed by Lowry et al. (1951) with slight modifications and expressed as mg 100 ml^{-1} juice. Total free amino acid content was determined following methods earlier described by Yemm and Cocking (1954) with slight modifications as detailed previously by Baswal (2019) and expressed as mg 100 ml^{-1} juice. From the fresh 'Kinnow' juice total and reducing sugars were estimated following the method detailed earlier by Hortwitz (1960) with slight modification recently described by Baswal (2019) and expressed in percentage.

The Experimental Design

This experiment was conducted by following two factors (treatments and storage time) factorial completely randomized design (CRD) with three replicates. The experimental data were analyzed by following a two-way analysis of variance (ANOVA) using SAS software (version 9.3). The least significant difference test ($P \leq 0.05$) was used to compare the effects of treatments, storage time and their interactions. The experimental data over two years (2016–17 and 2017–18) were pooled due to homogeneity of variance.

Results

Influence of Different Concentration of 1-MCP, MeJA and SA on Health-promoting Compounds

Total Phenols

Total phenols exhibited a significant decline with extension in cold-storage period irrespective of the treatments. Amongst all, the fruit treated with MeJA 1 mM recorded significantly highest mean total phenols content (132.94 mg 100 ml^{-1} juice) as compared to control (98.78 mg 100 ml^{-1} juice) and all other treatments except 1-MCP 1500 ppb and SA 2 mM (131.73 mg 100 ml^{-1} juice and 131.27 mg 100 ml^{-1} juice, respectively). A significant interaction was noticed between all the treatments and storage period for total phenol content. Following 30 and 45 days of cold storage, the fruit treated with 1-MCP (1500 ppb) and SA (2 mM) resulted in a significantly higher content of total phenols (222.47 mg 100 ml^{-1} juice and 157.86 mg 100 ml^{-1} juice, respectively) as compared with the control and all the other treatments. Whereas preceding 60 and 75 days of cold storage, the fruit treated with MeJA (1 mM) exhibited significantly higher content of total phenols (124.23 mg 100 ml^{-1} juice and 68.04 mg 100 ml^{-1} juice, respectively) as compared with all other treatments and control (Table 1).

Table 1. Influence of different concentrations of 1-MCP, MeJA and SA and cold storage periods on total phenol, total antioxidant activity and flavonoids content of 'Kinnow' fruits stored at 6 ± 1°C and 90 ± 5% RH.

	Total phenols (mg 100 ml^{-1} juice)						Total antioxidant activity (%)						Flavonoids (mg quercetin 100 ml^{-1} juice)					
	Storage time (Days)						Storage time (Days)						Storage time (Days)					
Treatment (T)	0	30	45	60	75	Mean ± S.E. (T)	0	30	45	60	75	Mean± S.E. (T)	0	30	45	60	75	Mean± S.E. (T)
1-MCP (500 ppb)	129.98 ± 1.07	159.59 ± 18.97	97.52 ± 9.71	87.60 ± 7.90	35.99 ± 0.93	102.14^{f} ± 8.42	26.60 ± 1.05	23.30 ± 0.17	16.68 ± 0.46	11.41 ± 0.34	10.91 ± 0.14	17.78^{g} ± 1.18	57.95 ± 1.47	70.99 ± 0.41	66.44 ± 1.24	49.05 ± 1.07	35.19 ± 0.21	55.92^{g} ± 2.42
1-MCP (1000 ppb)	129.98 ± 1.07	183.12 ± 20.52	113.55 ± 10.93	107.73 ± 13.55	32.78 ± 0.34	113.44^{e}± 9.79	26.60 ± 1.05	22.56 ± 0.36	18.49 ± 1.38	12.53 ± 0.12	10.29 ± 1.10	18.10^{fg}± 1.17	57.95 ± 1.47	79.93 ± 3.34	63.82 ± 0.84	50.33 ± 0.07	38.53 ± 1.53	58.11^{f} ± 2.65
1-MCP (1500 ppb)	129.98 ± 1.07	222.47 ± 1.24	140.15 ± 10.78	115.03 ± 2.10	51.01 ± 0.43	131.73^{a}± 10.36	26.60 ± 1.05	25.06 ± 0.64	22.38 ± 1.06	20.14 ± 0.82	11.57 ± 0.65	21.15^{ab}± 1.03	57.95 ± 1.47	83.30 ± 1.13	78.88 ± 2.30	71.00 ± 2.57	41.18 ± 0.63	66.46^{b}± 2.92
MeJA (1 mM)	129.98 ± 1.07	197.60 ± 0.46	144.82 ± 6.75	124.23 ± 6.75	68.04 ± 0.29	132.94^{a}± 7.87	26.60 ± 1.05	25.05 ± 1.01	23.80 ± 1.11	22.01 ± 0.72	12.98 ± 0.36	22.09^{a}± 0.94	57.95 ± 1.47	89.42 ± 0.95	86.09 ± 1.53	83.68 ± 1.74	31.88 ± 0.93	69.81^{a}± 4.13
MeJA (2 mM)	129.98 ± 1.07	184.44 ± 4.68	126.28 ± 17.46	107.54 ± 13.66	57.85 ± 4.10	121.22^{c} ± 8.29	26.60 ± 1.05	22.43 ± 0.74	18.67 ± 0.30	16.36 ± 0.19	9.70 ± 0.64	18.75^{e}± 1.09	57.95 ± 1.47	79.24 ± 0.34	70.50 ± 1.82	59.75 ± 4.83	30.68 ± 1.27	59.63^{e}± 3.17
MeJA (3 mM)	129.98 ± 1.07	182.29 ± 0.77	125.51 ± 28.61	84.04 ± 8.43	36.64 ± 3.44	111.69^{e}± 10.06	26.60 ± 1.05	23.71 ± 0.03	19.24 ± 0.49	11.19 ± 0.12	9.47 ± 0.48	18.04^{fg}± 1.27	57.95 ± 1.47	80.92 ± 1.05	65.38 ± 2.30	47.96 ± 0.56	37.11 ± 0.53	57.87^{f} ± 2.83
SA (1 mM)	129.98 ± 1.07	186.51 ± 12.00	150.32 ± 5.06	111.83 ± 2.10	51.87 ± 1.23	126.10^{b}± 8.54	26.60 ± 1.05	25.33 ± 1.12	21.54 ± 0.61	15.28 ± 0.24	9.49 ± 0.61	19.65^{d}± 1.23	57.95 ± 1.47	86.06 ± 1.50	71.22 ± 0.84	68.17 ± 1.52	34.64 ± 0.88	63.61^{d}± 3.21
SA (2 mM)	129.98 ± 1.07	205.24 ± 2.36	156.26 ± 4.43	106.14 ± 3.91	58.73 ± 4.75	131.27^{a}± 9.20	26.60 ± 1.05	25.72 ± 1.41	19.61 ± 0.62	18.44 ± 0.09	10.88 ± 0.18	20.25^{c} ± 1.10	57.95 ± 1.47	87.90 ± 0.75	70.92 ± 1.69	65.93 ± 2.35	43.32 ± 0.88	65.20^{c} ± 2.80
SA (3 mM)	129.98 ± 1.07	173.91 ± 11.15	157.86 ± 12.44	103.45 ± 8.49	28.86 ± 1.09	118.82^{d}±9.87	26.60 ± 1.05	23.11 ± 0.33	18.74 ± 0.04	12.73 ± 0.75	10.64 ± 0.11	18.37^{ef}± 1.14	57.95 ± 1.47	74.59 ± 0.13	70.40 ± 0.23	59.26 ± 3.11	32.57 ± 0.68	58.96^{ef}± 2.79
Control	129.98 ± 1.07	175.42 ± 8.87	100.80 ± 17.55	60.79 ± 5.71	26.87 ± 1.58	118.82^{d}± 10.10	26.60 ± 1.05	21.84 ± 0.28	16.94 ± 0.41	10.63 ± 0.37	7.73 ± 0.59	16.75^{h} ± 1.31	57.95 ± 1.47	80.42 ± 5.79	59.40 ± 4.04	38.84 ± 1.19	24.05 ± 1.50	52.13^{h} ± 3.74
Mean± S.E.(ST)	129.98^{b}± 0.54	187.06^{a}± 3.33	131.31^{b}± 4.23	100.84^{c} ± 2.96	44.87^{d}± 1.87		26.60^{a}± 0.26	23.81^{b}± 0.26	19.61^{c} ± 0.34	15.07^{d}± 0.51	10.37^{e}± 0.22		57.95^{d}± 0.40	81.28^{a}± 0.93	70.31^{b}± 1.08	59.40^{c} ± 1.74	34.92^{e}± 0.74	
LSD ($P \le 0.05$)	Treatment (T) = 2.29 Days (ST) = 1.62 SIST × T = 5.12						Treatment (T) = 0.40 Days (ST) = 0.28 ST × T = 0.89						Treatment (T) = 1.21 Days (ST) = 0.86 ST × T = 2.72					

Each value represents a pooled mean of two years data (2017 and 2018)

Total Antioxidant Activity

All the treatments and control fruit exhibited a linear decline in total antioxidant activity with extension in cold-storage period. The fruit treated with MeJA (1 mM) showed significantly highest mean total antioxidant activity (22.09%) as compared to the control (17.75%) and all other treatments except 1-MCP (1500 ppb). A significant interaction was detected between all the treatments and storage period for total antioxidant activity. The fruit treated with MeJA (1 mM) resulted in significantly higher total antioxidant activity following 45, 60, and 75 days after cold storage as compared to control and all other treatments. Whilst, 30 days after cold storage, the fruit treated with SA (2 mM) resulted in significantly higher total antioxidant activity as compared to control and all other treatments (Table 1).

Flavonoids

Flavonoids content decreased significantly with the advancement in cold-storage period. The fruit treated with MeJA (1 mM) exhibited significantly highest mean flavonoids content (69.81 mg quercetin 100 ml^{-1} juice) followed by 1-MCP 1500 ppb and SA 2 mM (66.46 mg quercetin 100 ml^{-1} juice and 65.20 mg quercetin 100 ml^{-1} juice, respectively) compared with the control (52.13 mg quercetin 100 ml^{-1} juice) and all the other treatments. There was a significant interaction between all the treatments and storage period for flavonoids content. The fruit treated with MeJA (1 mM) recorded a significantly higher level of flavonoids content in 30, 45 and 60 days cold-stored fruit as compared to all other treatments and control. Whilst, 75 days cold-stored fruit which were treated with SA (2 mM) exhibited significantly higher level of flavonoids as compared to the control and all other treatments (Table 1).

Protein

The fruit treated with MeJA 1 mM recorded significantly highest mean protein content (135.79 mg 100 ml^{-1} juice) followed by 1-MCP 1500 ppb and SA 2 mM (135.51 mg 100 ml^{-1} juice and 135.07 mg 100 ml^{-1} juice, respectively) as compared with the control (117.25 mg 100 ml^{-1} juice) and all other treatments. All the treatments resulted in a significant rise in protein content up to 45 days of cold-storage period thereafter showed a sharp and steady decline till 75 days of cold storage. A significant interaction was observed between all the treatments and storage period for protein content. The fruit treated with SA (3 mM and 2 mM) recorded significantly higher protein content in 30 days of cold-stored fruit. Whilst, the fruit treated with 1-MCP (1500 ppb) resulted in significantly higher protein content in 45 days cold-stored fruit. Following 60 days and 75 days of cold-storage period fruit treated with SA (3 mM) and SA (2 mM) manifested significantly higher protein content, respectively (Table 2).

Total Free Amino Acids

The fruit treated with MeJA 1 mM resulted significantly highest mean total free amino acids content (83.28 mg 100 ml^{-1} juice) followed by 1-MCP 1500 ppb (81.95 mg 100 ml^{-1} juice, respectively) compared with the control (53.22 mg 100 ml^{-1} juice) and all the other treatments (Table 2). The level of mean total free amino acids was significantly highest in 75 days cold-stored fruit as compared to all other storage periods. All the treatments and storage period exhibited a significant interaction for total free amino acids level. Following 45 and 75 days cold-storage period fruit treated with MeJA (1 mM) showed significantly higher total free amino acids content, whereas, 1-MCP (1500 ppb) treated fruit exhibited significantly higher total free amino acids content in 30 and 60 days cold-stored fruit, respectively (Table 2).

Total Sugars

The fruit treated with MeJA (1 mM) showed significantly highest mean total sugars (7.65%) followed by 1-MCP (1500 ppb) fumigated fruit (7.51%) as compared to all other treatments and control (6.51%) (Table 3). Irrespective of the treatments, the mean total sugars were significantly highest in 60 days cold-stored fruit as compared to all other cold-storage periods. There was a significant interaction

Table 2. Influence of different concentrations of 1-MCP, MeJA and SA and cold-storage periods on protein and total free amino acids content of 'Kinnow' fruits stored at 6 ± 1°C and 90 ± 5% RH.

	Protein (mg 100 ml^{-1} juice)						Total free amino acids (mg 100 ml^{-1} juice)					
	Storage time (Days)						Storage time (Days)					
Treatment (T)	0	30	45	60	75	Mean ± S.E. (T)	0	30	45	60	75	Mean ± S.E. (T)
1-MCP (500 ppb)	136.09 ± 2.63	138.41 ± 1.62	141.86 ± 1.24	130.60 ± 1.11	90.80 ± 0.92	127.55^{e}± 3.60	83.89 ± 1.41	35.35 ± 0.53	24.15 ± 0.60	54.33 ± 1.08	91.31 ± 3.14	57.81^{g} ± 4.92
1-MCP (1000 ppb)	136.09 ± 2.63	147.82 ± 0.24	144.63 ± 3.87	130.38 ± 3.58	102.44 ± 5.19	132.28^{cd}± 3.30	83.89 ± 1.41	50.09 ± 0.14	33.42 ± 7.53	58.90 ± 4.44	81.07 ± 7.02	61.48^{f} ± 3.91
1-MCP (1500 ppb)	136.09 ± 2.63	144.03 ± 5.53	162.95 ± 3.20	131.64 ± 0.27	102.84 ± 3.11	135.51^{a}± 3.87	83.89 ± 1.41	71.30 ± 0.47	51.98 ± 0.21	74.54 ± 1.16	128.04 ± 1.43	81.95^{a}± 4.73
MeJA (1 mM)	136.09 ± 2.63	144.67 ± 1.55	161.62 ± 0.78	131.37 ± 2.91	105.19 ± 4.15	135.79^{a}± 3.62	83.89 ± 1.41	68.17 ± 0.47	60.52 ± 0.63	70.90 ± 0.19	132.89 ± 2.31	83.28^{a}± 4.86
MeJA (2 mM)	136.09 ± 2.63	144.60 ± 3.44	160.41 ± 1.15	130.79 ± 3.60	97.81 ± 1.97	133.94^{abc}± 4.01	83.89 ± 1.41	60.76 ± 1.17	47.16 ± 0.96	64.44 ± 0.45	100.81 ± 0.87	71.41^{d}± 3.53
MeJA (3 mM)	136.09 ± 2.63	141.01 ± 1.93	143.94 ± 2.00	125.36 ± 1.13	104.35 ± 1.16	130.15^{de}± 2.84	83.89 ± 1.41	40.26 ± 0.53	32.49 ± 0.75	50.55 ± 1.03	98.20 ± 4.36	61.08^{f} ± 4.81
SA (1 mM)	136.09 ± 2.63	143.44 ± 1.70	158.99 ± 2.49	131.48 ± 0.54	100.04 ± 1.37	134.01^{abc}± 3.74	83.89 ± 1.41	51.86 ± 0.26	48.44 ± 0.41	71.69 ± 0.33	122.12 ± 1.23	75.60^{c} ± 4.98
SA (2 mM)	136.09 ± 2.63	148.17 ± 2.88	154.69 ± 2.00	129.49 ± 0.57	106.89 ± 1.38	135.07^{ab}± 3.26	83.89 ± 1.41	61.82 ± 0.89	57.13 ± 0.41	66.14 ± 0.33	129.55 ± 1.67	79.71^{b}± 4.96
SA (3 mM)	136.09 ± 2.63	148.19 ± 4.67	147.30 ± 6.42	132.68 ± 1.07	99.33 ± 5.45	132.72^{bcd}± 3.69	83.89 ± 1.41	52.48 ± 0.09	32.48 ± 8.73	58.65 ± 2.69	103.14 ± 3.82	66.13^{e}± 4.83
Control	136.09 ± 2.63	137.65 ± 1.79	128.74 ± 3.09	104.27 ± 8.04	79.48 ± 0.72	117.25^{f} ± 4.41	83.89 ± 1.41	43.36 ± 0.72	27.14 ± 0.66	61.16 ± 0.67	50.53 ± 0.89	53.22^{h} ± 3.54
Mean ± S.E. (ST)	136.09^{c} ± 0.81	143.80^{b}± 1.01	150.52^{a}± 1.65	127.81^{d}±1.38	98.92^{e}± 1.29		83.89^{b}± 0.47	53.55^{d}± 1.49	41.49^{e}± 1.82	63.13^{c} ± 1.08	103.77^{a}± 3.30	
LSD ($P \le 0.05$)	Treatment (T) = 2.62 Days (ST) = 1.85 ST × T = 5.86						Treatment (T) = 1.46 Days (ST) = 1.03 ST × T = 3.27					

Each value represents a pooled mean of two years data (2017 and 2018)

Table 3. Influence of different concentrations of 1-MCP, MeJA and SA and cold-storage periods on total and reducing sugars content of 'Kinnow' fruits stored at 6 ± 1°C and 90 ± 5% RH.

	Total sugars (%)						Reducing sugars (%)					
	Storage time (Days)						Storage time (Days)					
Treatment (T)	0	30	45	60	75	Mean ± S.E. (T)	0	30	45	60	75	Mean ± S.E. (T)
1-MCP (500 ppb)	5.15 ± 0.29	5.39 ± 0.35	6.14 ± 0.46	8.50 ± 0.05	7.43 ± 0.31	6.52^{d}± 0.26	2.39 ± 0.42	2.59 ± 0.33	2.85 ± 0.24	4.21 ± 0.15	3.88 ± 0.13	3.18^{f} ± 0.16
1-MCP (1000 ppb)	5.15 ± 0.29	5.98 ± 0.18	6.89 ± 0.18	7.70 ± 0.43	7.35 ± 0.32	6.61^{d}± 0.20	2.39 ± 0.42	2.59 ± 0.35	2.83 ± 0.32	4.54 ± 0.19	4.06 ± 0.19	3.29^{de}± 0.19
1-MCP (1500 ppb)	5.15 ± 0.29	7.35 ± 0.13	7.60 ± 0.11	9.35 ± 0.29	8.11 ± 0.15	7.51^{a}± 0.27	2.39 ± 0.42	3.07 ± 0.27	3.33 ± 0.20	4.73 ± 0.00	3.97 ± 0.09	3.50^{b}± 0.17
MeJA (1 mM)	5.15 ± 0.29	6.63 ± 0.08	8.21 ± 0.20	9.30 ± 0.33	8.93 ± 0.16	7.65^{a}± 0.30	2.39 ± 0.42	3.02 ± 0.36	3.44 ± 0.44	5.29 ± 0.26	4.22 ± 0.16	3.68^{a}± 0.22
MeJA (2 mM)	5.15 ± 0.29	5.45 ± 0.29	6.04 ± 0.53	9.31 ± 0.12	8.38 ± 0.14	6.87^{c} ± 0.33	2.39 ± 0.42	2.84 ± 0.25	3.01 ± 0.34	4.58 ± 0.20	4.01 ± 0.07	3.37^{c} ± 0.18
MeJA (3 mM)	5.15 ± 0.29	5.90 ± 0.42	6.81 ± 0.33	7.87 ± 0.13	7.27 ± 0.27	6.60^{d}± 0.21	2.39 ± 0.42	2.62 ± 0.44	3.13 ± 0.22	4.82 ± 0.40	3.21 ± 0.01	3.23^{ef}± 0.19
SA (1 mM)	5.15 ± 0.29	7.13 ± 0.08	7.26 ± 0.07	8.05 ± 0.27	7.87 ± 0.28	7.09^{b}± 0.21	2.39 ± 0.42	2.49 ± 0.45	3.02 ± 0.20	4.84 ± 0.27	4.22 ± 0.02	3.39^{c} ± 0.21
SA (2 mM)	5.15 ± 0.29	6.06 ± 0.35	7.23 ± 0.07	9.41 ± 0.31	8.05 ± 0.22	7.18^{b}± 0.29	2.39 ± 0.42	3.01 ± 0.37	3.29 ± 0.22	4.79 ± 0.11	3.93 ± 0.08	3.48^{b}± 0.18
SA (3 mM)	5.15 ± 0.29	5.84 ± 0.04	6.77 ± 0.16	8.94 ± 0.20	7.20 ± 0.34	6.78^{c} ± 0.25	2.39 ± 0.42	2.56 ± 0.44	3.23 ± 0.48	4.58 ± 0.24	3.84 ± 0.35	3.32^{cd}± 0.20
Control	5.15 ± 0.29	6.33 ± 0.48	6.60 ± 0.40	7.42 ± 0.19	7.05 ± 0.09	6.51^{d}± 0.18	2.39 ± 0.42	2.77 ± 0.28	3.31 ± 0.27	4.08 ± 0.01	2.55 ± 0.09	3.02^{g} ± 0.14
Mean ± S.E. (ST)	5.15^{e}± 0.06	6.21^{d}± 0.10	6.96^{c} ± 0.11	8.59^{a}± 0.11	7.77^{b}± 0.09		2.39^{d}± 0.09	2.76^{cd}± 0.08	3.15^{c} ± 0.07	4.65^{a}± 0.06	3.79^{b}± 0.07	
LSD ($P \leq 0.05$)	Treatment (T) = 0.13 Days (ST) = 0.095 ST × T = 0.30						Treatment (T) = 0.075 Days (ST) = 0.053 ST × T = 0.168					

Each value represents a pooled mean of two years data (2017 and 2018)

between the treatments and storage period for the levels of total sugars in the fruit. The fruit fumigated with 1-MCP (1500 ppb) showed significantly higher total sugar levels in 30 days cold-stored fruit as compared to all other treatments and control. Whilst in 45-day and 75-day cold-stored fruit treated with MeJA (1 mM) exhibited significantly higher total sugar levels as compared to control and all other treatments. However, following 60 days cold-stored period fruit treated with SA (2 mM) exhibited significantly higher total sugar levels as compared to control and all other treatments (Table 3).

Reducing Sugars

The fruit treated with MeJA (1 mM) exhibited significantly highest mean reducing sugar level (3.68%) as compared to all other treatments and control (3.02%). The levels of mean reducing sugars were significantly highest (4.65%) in 60 days cold-stored fruit as compared to all other cold-storage periods (Table 3). A significant interaction was noticed between all the treatments and storage period for reducing sugar levels in the fruit. MeJA (1 mM) exhibited significantly higher reducing sugar content in all the cold-stored fruit except days 30 where fruit treated with 1-MCP (1500 ppb) showed significantly higher reducing sugar levels (Table 3).

Discussion

The post-harvest application of MeJA (1 mM), 1-MCP (1500 ppb) and SA (2 mM) delayed the loss of polyphenols including total phenols possibly due to the stimulation in the activity of phenylalanine ammonia lyase (PAL) by these compounds which assists in the accumulation of higher level of total phenols (Yao and Tian, 2005). Likewise, the post-harvest application of 1-MCP in 'Kinnow' mandarin and kiwi fruit (Park et al., 2015; Tavallali and Moghadam, 2015), MeJA in Blood oranges and Chinese bayberries (Habibi et al., 2020; Wang et al., 2009) and SA in 'Kinnow' mandarin, peaches and pineapple cv. Sawi (Haider et al., 2020; Khademi and Ershadi, 2013; Sangpyayoon et al., 2019) have been reported to elevate the content of total phenols. These ethylene inhibitors exhibited an initial increase in total antioxidant activity may be due to stimulation in the activity of alternative oxidase (AOX) enzyme (Qin et al., 2003). Low-temperature storage results in an initial rise in antioxidant activity which increases the level of oxidative stress in the form of ROS at the transcript, protein and activity level which consequently manipulates the cellular homeostasis (Suzuki and Mittler, 2006). Similarly, the post-harvest application of 1-MCP in 'Kinnow' mandarin (Tavallali and Moghadam, 2015), MeJA in Blood oranges and Chinese bayberries (Habibi et al., 2020; Wang et al., 2009) and SA in 'Kinnow' mandarin and peaches (Haider et al., 2020; Khademi and Ershadi, 2013) exhibited significantly enhanced activity of total antioxidants. A significantly higher level of flavonoids in the fruit with these treatments ascribed to the reflection of higher total antioxidant activity (Bocco et al., 1998). Likewise, the post-harvest application of 1-MCP in 'Medlar' fruit (Selcuk and Erkan, 2015), MeJA in Chinese bayberries (Wang et al., 2009) and SA in turnip (Thiruvengadam et al., 2016) exhibited significantly highest content of flavonoids. Higher protein content in MeJA (1 mM), 1-MCP 1500 ppb and SA (2 mM) treated fruit may be attributed to restrained ethylene-induced electrolyte leakage which might have contributed to the accumulation of protein content (Jiang et al., 2002). However, the mechanism of ethylene inhibitors affecting the protein content during storage is not known and needs further investigations. Earlier, the application of 1-MCP in coriander (Jiang et al., 2002) and SA in radish (Canakci, 2008) maintained significantly highest protein content. These treatments have accumulated significantly higher free amino acid content may be due to its negative reflection on protein content as amino acids increases as a result of the breakdown of protein at senescence (Tulio et al., 2002). Though the exact mechanism on how these ethylene inhibitors influenced the total free amino acids content during cold storage is not fully understood hence warrants to be investigated. These treatments have registered an initial increase in sugar content may be due to the action of the key enzyme, sucrose-phosphate synthase (SPS) involved in sucrose biosynthesis and sugars during the ripening process (Hubbard et al., 1991). Likewise, the post-harvest application of 1-MCP in 'Kinnow (Baswal et al., 2020; Tavallali and Moghadam, 2015), MeJA in Blood orange (Habaibi et al., 2020) and SA in 'Kinnow' and apricot (Ezzat et al., 2017; Haider et al., 2020) exhibited significantly sugar content. However, in previous studies the post-harvest application of 1-MCP and SA did not influence the sugar content in medlar, peach and pomegranate fruit (Park et al., 2015; Sayyari et al., 2011; Selcuk and Erkan, 2015) may be due to their anti-senescent and antiripening actions may have delayed the ripening process (Asghari and Aghdam, 2010).

Conclusion

In conclusion, the postharvest application of MeJA (1 mM), 1-MCP (1500 ppb) and SA (2 mM) maintained significantly higher levels of total phenols, total antioxidant activity, flavonoids, protein and total free amino acids content up to 60 days in cold-stored 'Kinnow' mandarin fruit.

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