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Alleviation of chilling injury induced by cold quarantine treatment in Midknight Valencia and Lane Late sweet orange fruit

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Abstract

Cold quarantine treatment (1°C for 21 days) induces chilling injury (CI) in sweet orange fruit. We investigated the effects of different treatments such as hot water dip (HWD, 50 °C) alone or combined with thiabendazole (TBZ) five-minute, different concentrations of salicylic acid (SA), methyl jasmonate (MJ) one-minute dip and fumigation of nitric oxide (NO) two-hour and ethylene (ET) (six-hour) on CI and fruit quality following 21 days cold quarantine treatment and 10-day at ambient temperature in 'Lane Late' and 'Midknight Valencia' fruit. The experiment was laid out by following completely randomised design included three replications. HWD alone or combined with TBZ, or MJ significantly reduced CI in both cultivars. NO (5 µL L^{-1}) fumigation significantly reduced weight loss in 'Lane Late' only as compared to all other treatments except SA (1, and 3 mM). SCC/TA ratio was significantly reduced with ethylene, HW alone or combined with TBZ or MJ (0.25 mM) as compared to all other treatments in 'Midknight Valencia', but not in 'Lane Late'. The NO (10 µL L^{-1}) fumigation resulted in the significantly highest level of vitamin C only in 'Midknight Valencia'. SA (3 mM) dip treatment resulted in the significantly highest levels of total antioxidants as compared to all other treatments in 'Lane Late' but not in 'Midknight Valencia'. In conclusion, HWD alone or in combination with TBZ (20 mg L^{-1}) or MJ (0.50 mM) effectively reduced CI caused after cold quarantine treatment without adversely affecting fruit quality.

Keywords: (*Citrus sinensis* L. Osbeck); Quarantine; Chilling injury; Fruit quality; Sweet orange.
Abbreviations: CI_ chilling injury; CI%_ chilling injury percentage; HW_ hot water; HWD_ hot water dipping; TBZ_ thiabendazole; MJ_ methyl jasmonate; SA_ salicylic acid; NO_ nitric oxide; ET_ ethylene; MFF_ Mediterranean fruit fly; QFF_ Queensland fruit fly; USDA_ United States Department of Agriculture; *h*°_ hue angle; CCI _ citrus colour index; POX _ peroxidase; CAT _ catalase; TP_ total phenolic content; HSPs_ heat shock proteins; ROS_ reactive oxygen species.

Introduction

Citrus is one of the most important fruit group in Australia with a total production of 470 MT in 2016-17 (USDA, 2017). Australia exports high quality fresh sweet oranges fruit (180 MT) during the off-season to markets of the Northern Hemisphere (USDA, 2017). In Australia, cold quarantine treatment against Mediterranean fruit fly [MFF; *Ceratitis capitata* (*Diptera*: *Tephritidae*)] and Queensland fruit fly [QFF; *Bactrocera tryoni* (Froggatt)] in the Western and Eastern regions respectively, is mandatory to comply with quarantine requirements of the importing countries (De Lima et al., 2007). Previously, fumigation with methyl bromide, irradiation and cold treatment were practised to disinfect the citrus fruit but currently the use of methyl bromide has been restricted in Australia because it shortens the storage life of citrus fruit due to its phytotoxic nature and also promotes ozone depletion (De Lima et al., 2007). Consumer demands, environmental factors and government regulations have led to research on adopting non-chemical methods for the post-harvest protection of horticulture commodities (Sharples, 1990). Cold quarantine treatment is a non-chemical, approved method for disinfestation of QFL and MFF. This involves the exposure of the sweet orange fruit to non-freezing temperatures (1.1 - 2.2°C) for a period of 14-18 days (d) (Powell, 2003). However, the application of this treatment leads to the development of chilling injury (CI) more or less in all citrus cultivars when kept at room temperature (Martinez-Téllez and Lafuente, 1997). The CI symptoms mainly manifest as scalding, rind pitting, watery breakdown, development of a woolly or leathery texture and decay (Reuther et al., 1989). CI negatively impacts the consumer preference and overall quality of the fruit. The sensitivity of the citrus fruit to low temperature causes serious economic implications for its export to the different fruit fly free zones of importing countries (US Department of Agriculture, Animal and Plant Protection Service, 1976).

Various approaches have been tested to minimise CI in different fruit crops such as post-harvest hot water dipping (HWD), application of MJ or NO fumigation. Previously, HWD alone or in combination with TBZ reduced CI in citrus fruit induced by cold quarantine in different cultivars of sweet oranges such as 'Washington Navel' and 'Valencia Late' (Bassal and El-Hamahmy, 2011), 'Tarocco', 'Moro',

'Sanguinello' and 'Doppio Sanguigno' (Schirra et al., 2004), and 'Tarocco' (Palma et al., 2013). MJ has been previously reported to reduce CI in different fruit crops such as lemon (*Citrus limon* Burm.f.) (Siboza at al., 2014), mangoes (*Mangifera indica* cv. 'Kent') (Gonzalez- Aguilar et al., 2000,), guava (*Psidium guajava*) (Gonzalez-Aguilar et al., 2004), peach (*Prunus persica* Batsch. cv. 'Baifeng') (Meng et al., 2009), loquat (*Eriobotrya japonica* Lindl.) (Cao et al., 2009), pomegranate (*Punica granatum* L.) (Mirdehghan and Ghotbi, 2014), pineapple (*Ananas comosus* L. Merr) (Nilprapruck et al., 2013), bell pepper (*Capsicum annuum* L.), avocado (*Persea americana* Mill.), and grapefruit (*Citrus paradisi* Macf.) (Meir et al., 1996). No research work has been reported on the efficacy of exogenously applied MJ in reducing CI induced by cold quarantine treatment in sweet orange fruit. The application of salicylic acid alone and combined application of MJ and salicylic acid enhanced chilling tolerance in cold-stored pomegranate (Mirdehghan and Ghotbi, 2014), lemon fruit (Siboza and Bertling, 2013) and tomato (Ding et al., 2002), Nitric oxide (NO) is a free radical and highly reactive gas, acting as a multifunctional signalling molecule in various physiological responses (Wendehenne et al., 2001). NO has been reported to reduce chilling injury and maintain fruit quality in climacteric fruits such as Japanese plum cv 'Amber Jewel' (Singh et al., 2009), banana (*Musa* spp., AAA group cv. 'Cavendish') (Wang et al., 2013), peach cv. 'Feicheng') (Zhu et al., 2010) and mango cv. 'Kensington Pride') (Zaharah and Singh, 2011). Recently, Ghorbani et al. (2017) reported that 0.5mM SNP (sodium nitroprusside) 5 minute (min) dip treatment reduce CI in 'Washington Navel' orange stored for five months at 3°C. However, the efficacy of NO in reducing CI induced by cold quarantine treatment in sweet oranges yet to be investigated. No research work has been reported on the effects of exogenous application of MJ, NO and SA on inducing chilling tolerance during cold quarantine treatment (1 °C for 21 days) in 'Lane Late' and 'Midknight Valencia' sweet orange fruit. It was hypothesised that exogenous postharvest application of MJ, NO and SA may induce chilling tolerance in sweet orange fruit when exposed to cold quarantine at 1 °C for 21 days. These observations prompted to investigate the effects of exogenous application of MJ, SA, NO, TBZ, and HW on the incidence of chilling injury induced by cold quarantine treatment (1 °C for 21 days) and fruit quality in sweet orange.

Results

Chilling injury percentage

HW dip at 50°C alone and combined with TBZ (20 mg L^{-1}) 5 min dip and MJ (0.50 mM) 1 min dip have significantly ($P \leq 0.05$) reduced the incidence of CI (8.9, 8.9 and 15.6 % respectively) as compared to the control (40.5 %) in 'Lane Late' sweet orange (Fig. 1). In 'Midknight Valencia', HW at 50°C alone or combined with TBZ for 5 min dip and MJ (0.50 mM) significantly reduced CI (8.8 to 16.6 %) as compared to the control (28.8 %) (Fig 2). Fruit dipped in MJ (0.50 mM) for 1 min exhibited the lowest percentage of CI incidence as compared to all other treatments (Fig 2).

Fruit Colour

Citrus colour index (CCI) was significantly affected by all treatments in 'Lane Late' sweet orange but not in 'Midknight Valencia'. In 'Lane Late', MJ (0.10 mM) 1 min dip treatment resulted in significantly reduced *h*° (60.7) and increased CCI (8.7) as compared to the control and all other treatments (Table 1). In 'Midknight Valencia', TBZ (20 mg L^{-1}) combined with HW 5 min dip treatment showed significantly reduced *h*° (61.2) as compared to the control and all other treatments (Table 1).

Weight loss (%) and fruit firmness (N)

All the treatments significantly ($P \leq 0.05$) affected percentage weight loss in 'Lane Late' sweet orange fruit but not in 'Midknight Valencia'. In 'Lane Late', NO fumigation (5 µL L^{-1}) for 2 h significantly reduced weight loss (2.9 %) as compared to the control (6.0 %) and all other treatments (Table 2). Fruit firmness (N) was not significantly affected by any of the treatments in both the cultivars (Table 2).

SSC, TA and SCC/TA ratio

All the treatments except MJ (0.50 mM) 1 min dip resulted in significantly reduced SSC (%) in the juice of 'Lane Late' sweet orange as compared to the control. However, in 'Midknight Valencia', SSC (%) was reduced as compared to the control and all the treatments applied except ethylene (10 µL L^{-1}) six h fumigation, TBZ combined with HWD 5 min and NO (10 and 20 µL L^{-1}) two h fumigation (Table 3). In 'Lane Late', TA (%) and SSC/TA ratio were not significantly affected by any of the treatments applied (Table 3). Moreover, TA in 'Midknight Valencia' juice was significantly ($P \leq 0.05$) highest (0.91 and 0.92 %) when the fruit were fumigated with ethylene (10 µL L^{-1}) for six h and MJ (0.25 mM) one min dip treatment, respectively. SSC/TA ratio in the juice of 'Midknight Valencia' was significantly higher in all the treatments except ethylene (10 µL L^{-1}) six h fumigation, HW alone 5 min dip, TBZ combined with HW 1 min dip and MJ (0.25 mM) 1 min dip.

Vitamin C and total antioxidants

The concentrations of vitamin C in the juice were significantly ($P \leq 0.05$) affected by all treatments in 'Midknight Valencia' but not in 'Lane Late'. In 'Midknight Valencia', all the treatments except ethylene (10 µL L^{-1}) six h fumigation, MJ (0.50 mM) 1 min dip and NO (5 and 10 µL L^{-1}) 2 h fumigation significantly reduced the concentrations of vitamin C in the juice as compared to the control (295.9 mg L^{-1}) (Table 3). Meanwhile, all the treatments significantly affected the level of total antioxidants in 'Lane Late' sweet orange fruit but not in 'Midknight Valencia'. 'Lane Late' fruit treated with SA (3 mM) 1 min dip resulted in the significantly highest level of antioxidants (569.5 µM Trolox L^{-1}) as compared to the control and all other treatments (Table 3).

Individual and total sugars

All the treatments significantly affected the level of glucose in the juice of 'Lane Late' and 'Midknight Valencia'. In 'Lane Late', all the NO treatments, SA (3 mM) and MJ (0.10 and 0.50 mM) 1 min dip, showed a significantly ($P \leq 0.05$) reduced concentration of glucose in juice as compared to the control (19.3 g L^{-1}) and all other treatments (Table 4). Moreover, 'Midknight Valencia' fruit fumigated with NO (20 µL L^{-1}) for two h and MJ (0.1 or 0.50 mM) 1 min dip exhibited

Table 1. Effects of HW alone and combined with TBZ, different concentrations of MJ, SA dips and NO fumigation on hue angle (*h*°) and citrus colour index (CCI) in sweet orange cv. ''Lane Late'' and ''Midknight Valencia'' following the cold quarantine treatment (1°C for 21 d) and 10 d at simulated shelf-life conditions.

	Fruit colour	
	'Lane Late'	
Treatments	*h*°	CCI
Control	62.0a	8.1b
HW 50°C	62.1a	8.0b
TBZ (20 mgL^{-1}) + HW	62.1a	8.1b
MJ (0.10 mM)	60.7b	8.7a
MJ (0.25 mM)	61.8a	8.2b
MJ (0.50 mM)	61.8a	8.2b
SA (1 mM)	62.2a	8.0b
SA (2 mM)	62.5a	7.9b
SA (3 mM)	62.4a	7.9b
NO (5 µL L^{-1})	62.6a	7.9b
NO (10 µL L^{-1})	62.5a	7.9b
NO (20 µL L^{-1})	62.5a	7.8b
	'Midknight Valencia'	
Control	62.2ab	8.0
ET (10 µL L^{-1})	61.7abc	8.3
HW 50°C	62.2ab	8.0
TBZ (20 mgL^{-1}) + HW	61.2c	8.4
MJ (0.10 mM)	61.6abc	8.3
MJ (0.25 mM)	62.4a	8.0
MJ (0.50 mM)	62.3a	8.1
NO (5 µL L^{-1})	62.0abc	8.1
NO (10 µL L^{-1})	62.1ab	8.2
NO (20 µL L^{-1})	61.4bc	8.5

Data represent means of 3 replicate samples of 30 units for 'Lane Late' and 'Midknight Valencia'. Mean separation for significant analysis of variance within the columns and rows was tested using Duncan's multiple range test at ($P < 0.05$). Mean followed by the same letter was not significantly different within the columns. HW (hot water) 5-min dip, TBZ (thiabendazole) combined with HW 5 min dip, ET (ethylene) 6 h fumigation, MJ (methyl jasmonate) one min dip, SA (salicylic acid) one min dip and NO (nitric oxide) fumigation for 2 h.

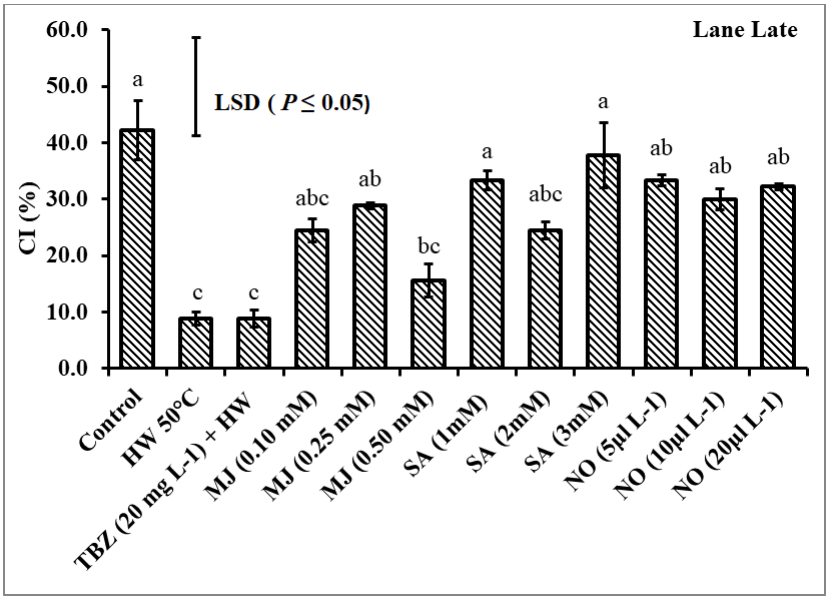

Fig 1. Incidence of CI (percentage chill injured fruit) affected by hot water alone and combined with TBZ and different concentrations of MJ, SA dips and fumigation of NO in sweet orange cv. 'Lane Late' following the cold quarantine treatment (1°C for 21 d) and 10 d at simulated shelf-life conditions. Vertical bars represent SE, n = three replicates, thirty fruit per replication. Any two means with different lower case letters represent significant differences at ($P \leq 0.05$). HW (hot water) 5-min dip, TBZ (thiabendazole) combined with HW 5 min dip, MJ (methyl jasmonate) one min dip, SA (salicylic acid) 1 min dip and NO (nitric oxide) fumigation for 2 h.

Table 2. Effect of HW alone and combined with TBZ, different concentration of MJ, SA dips and fumigation of NO on weight loss (%) and fruit firmness in sweet orange cv. 'Lane Late' and 'Midknight Valencia' following the cold quarantine treatment (1°C for 21 days) and 10 days at simulated shelf-life conditions.

'Lane Late'		
Treatment	Weight loss (%)	Firmness (N)
Control	6.0abc	262.2
HW 50°C	7.6ab	302.4
TBZ (20 mgL^{-1}) + HW	7.1abc	286.7
MJ (0.10 mM)	6.3abc	291.8
MJ (0.25 mM)	7.6ab	274.1
MJ (0.50 mM)	4.3abc	269.0
SA (1 mM)	4.9bcd	266.5
SA (2 mM)	7.9ab	272.0
SA (3 mM)	5.1bcd	284.1
NO (5 µL L^{-1})	2.9d	277.4
NO (10 µL L^{-1})	8.3a	253.1
NO (20 µL L^{-1})	9.0a	267.9
'Midknight Valencia'		
Control	1.8	371.2
ET (10 µL L^{-1})	1.3	358.8
HW 50°C	1.4	414.4
TBZ (20 mgL^{-1}) + HW	1.5	380.9
MJ (0.10 mM)	1.4	405.4
MJ (0.25 mM)	1.5	393.1
MJ (0.50 mM)	1.6	394.5
NO (5 µL L^{-1})	1.4	368.8
NO (10 µL L^{-1})	1.4	355.9
NO (20 µL L^{-1})	1.3	336.3

Weight loss data represent means of 3 replicate samples of 90 units for 'Lane Late' and 'Midknight Valencia'. Fruit firmness data represent means of 3 replicates simple of 30 units for both cultivars. Mean separation for significant analysis of variance within the columns and rows was tested using Duncan's multiple range test at ($P < 0.05$). Mean followed by the same letter was not significantly different within the columns. HW (hot water) 5-min dip, TBZ (thiabendazole) combined with HW 5 min dip, ET (ethylene) 6 h fumigation, MJ (methyl jasmonate) one min dip, SA (salicylic acid) one min dip and NO (nitric oxide) fumigation for 2 h.

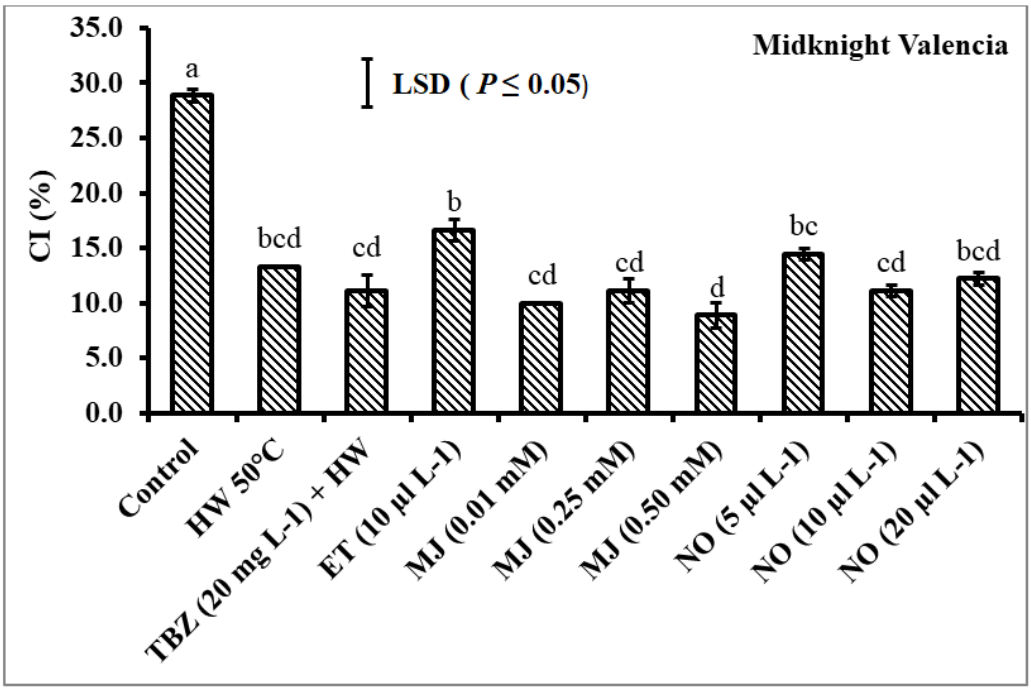

Fig 2. Incidence of CI (percentage chill injured fruit) affected by hot water alone and combined with TBZ and different concentrations of MJ, SA dips and fumigation of NO in sweet orange cv. 'Midknight Valencia' following the cold quarantine treatment (1 °C for 21 d) and 10 d at simulated shelf-life conditions. Vertical bars represent SE, n = three replicates, thirty fruit per replication. Vertical bars represent SE, n = three replicates, thirty fruit per replication. Any two means with different lower case letters represent significant differences at ($P \leq 0.05$). HW (hot water) 5-min dip, TBZ (thiabendazole) combined with HW 5 min dip, ET (ethylene) 6 h fumigation, MJ (methyl jasmonate) one min dip, SA (salicylic acid) 1 min dip and NO (nitric oxide) fumigation for 2 h.

Table 3. Effect of HW alone and combined with TBZ, different concentrations of MJ, SA dips and fumigation of NO on SSC, TA, SSC/TA ratio, vitamin C and total antioxidants in the juice of sweet orange cv. 'Lane Late' and 'Midknight Valencia' following the cold quarantine treatment (1°C for 21 days) and 10 days at simulated shelf-life conditions.

	'Lane Late'				
Treatment	SSC (%)	TA (%)	SSC/TA ratio	Vitamin C (mg L^{-1})	Antioxidants (µM Trolox L^{-1})
Control	12.1a	0.61	19.7	253.2	460.3cd
HW 50°C	11.1b	0.61	18.2	261.4	476.0bc
TBZ (20 mgL^{-1}) + HW	11.4b	0.59	19.3	253.6	501.7b
MJ (0.10 mM)	11.4b	0.63	18.2	244.1	442.6d
MJ (0.25 mM)	11.0b	0.56	19.5	255.4	478.6bc
MJ (0.50 mM)	11.6ab	0.55	21.0	231.2	436.9d
SA (1 mM)	11.2b	0.60	18.7	225.1	439.5d
SA (2 mM)	10.4c	0.58	18.0	261.8	478.8bc
SA (3 mM)	9.9c	0.55	18.1	270.9	569.5a
NO (5 µL L^{-1})	11.3b	0.59	19.2	237.2	453.7cd
NO (10 µL L^{-1})	11.3b	0.56	20.1	225.6	406.7e
NO (20 µL L^{-1})	11.3b	0.58	19.6	240.7	463.4cd
	'Midknight Valencia'				
Control	13.1a	0.78c	16.8a	295.9abc	482.3
ET (10 µL L^{-1})	13.0ab	0.91a	14.3d	324.0ab	490.0
HW 50°C	12.7bcd	0.81bc	15.7bc	244.6cd	492.5
TBZ (20 mgL^{-1}) + HW	12.8abcd	0.83b	15.4c	215.2d	507.9
MJ (0.10 mM)	12.5cd	0.77c	16.3ab	247.6bcd	474.6
MJ (0.25 mM)	12.7bcd	0.92a	13.8d	222.6cd	454.9
MJ (0.50 mM)	12.5d	0.78c	16.0abc	297.2abc	466.0
NO (5 µL L^{-1})	12.6cd	0.78c	16.1abc	295.5abc	484.0
NO (10 µL L^{-1})	12.9abc	0.78c	16.5ab	359.4a	485.7
NO (20 µL L^{-1})	12.8abcd	0.78c	16.4ab	213.5d	465.7

Data represent means of 3 replicate samples of 30 units for 'Lane Late' and 'Midknight Valencia'. Mean separation for significant analysis of variance within the columns and rows was tested using Duncan's multiple range test at ($P < 0.05$). Mean followed by the same letter was not significantly different within the columns. HW (hot water) 5-min dip, TBZ (thiabendazole) combined with HW 5 min dip, ET (ethylene) 6 h fumigation, MJ (methyl jasmonate) one min dip, SA (salicylic acid) one min dip and NO (nitric oxide) fumigation for 2 h.

Table 4. Effect of HW alone and combined with TBZ, different concentrations of MJ, SA dips and fumigation of NO on the levels of individual and total sugars in sweet orange cv. 'Lane Late' and 'Midknight Valencia' following the cold quarantine treatment (1°C for 21 days) and 10 days at simulated shelf-life conditions.

	'Lane Late'			
	Individual and total sugars (gL^{-1})			
Treatment	Glucose	Fructose	Sucrose	Total Sugars
Control	19.3a	25.4a	59.8a	104.5a
HW 50°C	17.9abcde	22.8bc	50.8bc	91.7bc
TBZ (20 mgL^{-1}) + HW	19.2ab	24.5ab	52.4bc	96.3b
MJ (0.10 mM)	17.7cde	22.7bc	51.6bc	92.1bc
MJ (0.25 mM)	18.4abcd	23.2bc	48.9cd	90.7bc
MJ (0.50 mM)	17.7cde	23.2bc	55.1ab	96.1b
SA (1 mM)	18.7abc	23.5bc	51.4bc	93.6b
SA (2 mM)	17.9abcde	22.5c	45.4d	85.5c
SA (3 mM)	17.9bcde	22.5c	44.9d	85.3c
NO (5 µL L^{-1})	17.5cde	22.7bc	51.8bc	92.1bc
NO (10 µL L^{-1})	17.0e	22.3c	51.0bc	90.4bc
NO (20 µL L^{-1})	17.3de	22.6c	54.6abc	94.6b
	'Midknight Valencia'			
Control	19.9ab	26.0a	53.4a	99.3a
ET (10 µL L^{-1})	20.1a	26.1a	53.4a	99.7a
HW 50°C	18.9bc	24.6bc	50.8c	94.4bcd
TBZ (20 mgL^{-1}) + HW	19.2abc	25.1ab	52.1b	96.5b
MJ (0.10 mM)	17.9d	23.8c	52.1b	93.9cd
MJ (0.25 mM)	19.2abc	25.1ab	51.7bc	96.0bc
MJ (0.50 mM)	18.4cd	24.3bc	51.1bc	93.8d
NO (5 µL L^{-1})	19.3abc	25.2ab	50.9c	95.4bcd
NO (10 µL L^{-1})	19.7ab	26.0a	54.2a	100.0a
NO (20 µL L^{-1})	18.5cd	24.4bc	51.3bc	94.1cd

Data represent means of 3 replicate samples of 30 units for 'Lane Late' and 'Midknight Valencia'. Mean separation for significant analysis of variance within the columns and rows was tested using Duncan's multiple range test at ($P < 0.05$). Mean followed by the same letter not significantly different within the columns. HW (hot water) 5-min dip, TBZ (thiabendazole) combined with HW 5 min dip, ET (ethylene) 6 h fumigation, MJ (methyl jasmonate) one min dip, SA (salicylic acid) one min dip and NO (nitric oxide) fumigation for 2 h.

Table 5. Effect of HW alone and combined with TBZ, different concentrations of MJ, SA dips and fumigation of NO on the levels of individual and total organic acids in sweet orange cv. 'Lane Late' and 'Midknight Valencia' following the cold quarantine treatment (1°C for 21 days) and 10 days at simulated shelf-life conditions.

	'Lane Late'					
	Individual and total organic acids (g L^{-1})					
Treatment	Citric	Malic	Tartaric	Fumaric	Succinic	Total organic acids
Control	2.1abc	0.68	0.63	0.41	0.76	4.6
HW 50°C	2.3a	0.59	0.63	0.39	0.79	4.7
TBZ (20 mgL^{-1}) + HW	2.0abc	0.53	0.64	0.39	0.83	4.4
MJ (0.10 mM)	1.7bc	0.61	0.63	0.39	0.69	4.1
MJ (0.25 mM)	1.6c	0.49	0.63	0.37	0.90	4.0
MJ (0.50 mM)	1.7bc	0.71	0.62	0.42	0.77	4.2
SA (1 mM)	2.2ab	0.61	0.63	0.39	0.72	4.6
SA (2 mM)	1.8abc	0.49	0.62	0.36	0.82	4.6
SA (3 mM)	2.1abc	0.48	0.63	0.36	0.80	4.1
NO (5 µL L^{-1})	1.7bc	0.57	0.63	0.37	0.79	4.1
NO (10 µL L^{-1})	1.7bc	0.53	0.62	0.38	0.73	4.0
NO (20 µL L^{-1})	1.8abc	0.51	0.63	0.37	0.75	4.1
	'Midknight Valencia'					
Control	1.8	0.51	0.63	0.36	0.64	4.0
ET (10 µL L^{-1})	2.7	0.76	0.63	0.43	0.67	5.2
HW 50°C	2.1	0.58	0.63	0.39	0.61	4.3
TBZ (20 mgL^{-1}) + HW	2.7	0.75	0.63	0.43	0.63	5.1
MJ (0.10 mM)	2.1	0.76	0.63	0.42	0.66	4.5
MJ (0.25 mM)	2.2	0.82	0.62	0.39	0.66	4.3
MJ (0.50 mM)	2.0	0.60	0.62	0.44	0.64	4.7
NO (5 µL L^{-1})	2.3	0.64	0.63	0.40	0.70	4.7
NO (10 µL L^{-1})	2.3	0.80	0.63	0.43	0.67	4.8
NO (20 µL L^{-1})	2.1	0.52	0.64	0.37	0.66	4.3

Data represent means of 3 replicate samples of 30 units for 'Lane Late' and 'Midknight Valencia'. Mean separation for significant analysis of variance within the columns and rows was tested using Duncan's multiple range test at ($P < 0.05$). Mean followed by the same letters or no letters was not significantly different within the columns. HW (hot water) 5-min dip, TBZ (thiabendazole) combined with HW 5 min dip, ET (ethylene) 6 h fumigation, MJ (methyl jasmonate) one min dip, SA (salicylic acid) one min dip and NO (nitric oxide) fumigation for 2 h.

a reduced level of glucose in the juice as compared to the control and all other treatments. In 'Lane Late', all the treatments except TBZ (20 mg L^{-1}) combined with HWD 5 min dip show significantly reduced concentrations of fructose in the juice as compared to the control (25.4 g L^{-1}). On the other hand, in 'Midknight Valencia' HWD alone for 5 min, MJ (0.1 and 0.50 mM) 1 min dip, and NO (20 µL L^{-1}) two h fumigation resulted in significantly reduced levels of fructose in the juice as compared to the control (26.0 g L^{-1}) and all other treatments (Table 4). The concentrations of sucrose in the juice of 'Lane Late' were significantly reduced by all treatments except MJ (0.50 mM) 1 min dip and NO (20 µL L^{-1}) 2 h fumigation as compared to the control (59.8 g L^{-1}). In 'Midknight Valencia', all the treatments except ethylene (10 µl L^{-1}) six h fumigation and NO (10 µL L^{-1}) 2 h fumigation exhibited reduced concentrations of sucrose in the juice as compared to the control (53.4 g L^{-1}). The concentrations of total sugars in the juice of 'Lane Late' were significantly reduced by all the treatments as compared to the control (104.5 g L^{-1}). Meanwhile, in 'Midknight Valencia', the concentrations of total sugars in the juice were significantly reduced with all the treatments except ethylene (10 µL L^{-1}) six h fumigation and NO (10 µL L^{-1}) 2 h fumigation as compared to the control (99.3 g L^{-1}).

Individual and total organic acids

Amongst various organic acids, citric, malic, tartaric, fumaric and succinic acid were identified and quantified in the juice of both cultivars (Table 5). The concentrations of all individual and total organic acids in the juice of 'Lane Late' and 'Midknight Valencia' sweet orange fruit (except citric acid in 'Lane Late') were not significantly affected by any of the treatments applied (Table 5). The concentration of citric acid in the juice of 'Lane Late' was significantly reduced when the fruit were treated with MJ (0.1, 0.25 or 0.50 mM) 1 min dip and NO (5 and 10 µL L^{-1}) two h fumigation as compared to the control and all other treatments.

Discussion

Postharvest disinfection of citrus fruit by employing quarantine treatments is mandatory to meet the requirements outlined by importing countries. During the past few decades, heat treatments have been used to control postharvest fungal disease and insect disinfestation (Barkai-Golan and Phillips 1991). The heat treatment can also be commercially used to enhance chilling tolerance during cold storage (Wang, 1993). The symptoms of CI on citrus fruit is expressed as rind staining, pitting, red blotches, scalding, watery breakdown, sunken tissues, damage to the styler end of lemons and necrosis on the flavedo (Reuther et al., 1989).

The experimental results indicate that HWD at 50 °C for 5 min is effective in reducing CI caused by cold quarantine treatment (1°C for 21 d) in sweet orange cv. 'Lane Late' and 'Midknight Valencia'. Possibly, HWD treatment might enhance the natural defence system of the fruit against CI by changing the arrangement, morphology and assembly of

epicuticular wax, known to play a role in CI development (McDonald et al., 1993). Secondly, heat treatment may alter the enzyme system responsible for tissue degradation during the development of CI (Parkin et al., 1989; Martinez-Tellez and Lafunte, 1997). Thirdly, HWD treatment probably enhanced chilling tolerance by the upregulation of POX, CAT and total phenolic content (TP) as reported earlier in 'Valencia' and 'Navel' oranges (Bassal and El- Hamahmy 2011). Previously, HWD (2-3 min) has been reported to enhance the chilling tolerance in 'Valencia' sweet orange fruit (Wild and Hood, 1989) and HWD (53 ºC for 6 min and 48 ºC for 12 min) also reduced CI incidence in 'Satsuma' mandarins (Ghasemnezhad et al., 2008).

The experimental results exhibit that TBZ (20 mg L^{-1}) combined with hot water (HW) (50°C for 5 min) dip treatment significantly reduced CI in 'Lane Late' and 'Midknight Valencia' sweet orange. Presently, the exact mode of action of TBZ in enhancing chilling tolerance in sweet orange fruit is unclear. However, it may be argued that TBZ may have induced chilling tolerance by acting indirectly to suppress latent infections that might develop due to low-temperature storage as reported earlier in grapefruit, as result weaken fruit resistance to CI (Schiffman-Nadel et al., 1972). Regarding the efficacy of TBZ in controlling CI, it has been reported that due to increased fungicide concentrations and deposits in fruit; its physiological effect has been attributed to a decreased rate of peel senescence (Schiffman-Nadel et al., 1972). It has also been speculated that the HW fungicide treatment enriched infiltration of the fungicide through the epicuticular wax (Hordijk et al., 2013). The effectiveness of TBZ with HW to reduce CI in various citrus fruits has been reported in 'Marsh' and 'Redblush' grapefruit (*Citrus paradisi* Macf.) (McDonald et al., 1991), 'Tarocco' oranges (Schirra and Mulas, 1995) and 'Valencia' oranges (Wild and Hood, 1989). The synergistic effect of TBZ with HW on reducing CI in fruits has also been previously reported by Hordijk et al. (2013).

MJ (0.5 mM) 1-min dip treatment reduced CI in both cultivars. However, MJ (0.1 or 0.25) treatments induced chilling tolerance only in 'Midknight Valencia'. Possibly, MJ has reduced CI by the activation of defence mechanisms, such as heat shock proteins (HSPs) and phenolic compounds (Meir et al., 1996; Meng et al., 2009). MJ has also been reported to stimulate the build-up of HSPs which reduced chilling injury in tomato fruit (Ding et al., 2002). Meir et al. (1996) suggested that MJ probably acts together with single transduction cascade of the chemical changes involved in the reduction of CI. Previously, MJ has been applied to control CI in many fruits such as guava, tomato, papaya, mango and pomegranate (González-Aguilar et al., 2004; Ding et al., 2002; Mirdehghan and Ghotbi, 2014).

All NO (5, 10 or 20 µL L^{-1}) fumigation treatments for two h showed significantly reduced CI only in 'Midknight Valencia' not 'Lane Late' fruit. The mode of action through which NO induces chilling tolerance in citrus fruit is yet to be explored. Possibly, NO fumigation may have protected the membrane from damage through the reduction of reactive oxygen species (ROS) and enhanced levels of antioxidants in 'Midknight Valencia' sweet orange. Earlier, Zhu et al. (2008) reported that treatments with (1µmol L^{-1}) NO aqueous solution could protect kiwi fruit from oxidative damage caused by ROS through an enhanced activity of antioxidant enzymes. It may also be argued that endogenous NO production plays an important role in alleviating chilling injury by affecting the antioxidant defence system as reported earlier by Xu et al. (2012).

Fumigation with NO (5µL L^{-1}) exhibited reduced (2.9 %) water loss in 'Lane Late' but not in 'Midknight Valencia' after cold quarantine treatment (1°C for 21 d), which may be ascribed to the genetic difference between two cultivars. Possibly, the reduced water loss in horticultural commodities with NO treatment may be ascribed to a reduced transpiration rate as reported earlier (Ku et al., 2000).

In 'Lane Late' all the treatments except MJ (0.01 mM) showed significantly reduced SSC (%) and enhanced CCI as compared to the control but not in 'Midknight Valencia'. Previously, an increased SSC (%) in 'Tommy Atkin' MJ treated mangoes has been reported (Gonzalez-Anguilar et al. 2000). Fruit firmness was not significantly affected by any of the treatments in both the cultivars. Significantly reduced total sugars were recorded in both cultivars except NO and ET (10 µL L^{-1}) in 'Midknight Valencia'. Contrarily, Deng et al. (2013) reported earlier that pre-harvest treatment with (50 µM) SNP efficiently maintained a higher content of sucrose and lower content of glucose in Golden Delicious apples. Furthermore, Li et al. (2014) claimed the levels of glucose; fructose and sucrose during fruit ripening of papaya fruit were significantly influenced by NO fumigation. It appears that the effects of NO fumigation are not only limited to SSC but also influence post-harvest sugar metabolism.

Materials and methods

Plant materials

Mature fruit of cv. 'Lane Late' and 'Midknight Valencia' (*Citrus sinensis* (L.) Osbeck) were harvested randomly around the tree canopy from a commercial orchard at Moora (30° 35' S/115° 55' E), Western Australia during 2015. 'Lane Late' and 'Midknight Valencia' sweet orange trees (seven and nine years old respectively) earlier grafted on Carrizo citrange (*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* Raf.) rootstock. The trees were spaced at 2.7 × 7.5 m, row direction north-south. Following the harvest, the fruit were brought to the Horticulture Research Laboratory, Curtin University, Perth, WA. The fruit used in this experiment were free from symptoms of diseases, pest damage, blemishes and physical injuries.

Experiment 1: Effects of HW, MJ, TBZ and NO treatments on CI incidence and fruit quality in ''Lane Late'' sweet orange

'Lane Late' sweet orange fruit were dipped in HW (50°C for 5 min) alone and HW combined with TBZ (20 mg L^{-1}), MJ (0.10, 0.25 or 0.50 mM) and SA (1, 2 or 3 mM) 1 min dip and untreated fruit were kept as control. Fruit were fumigated with different concentrations of NO (5, 10 or 20 µL L^{-1}) for 2 h in a 60 L container. Following the treatments, the fruit were dried for 6 h at room temperature (20 ± 1 °C) and relative humidity (60 ± 5%). The fruit were transferred to the cold storage (1°C) for 21 days with (85-90 %) relative humidity. The experiment was laid out by following completely randomised design and included three replications. Each replication included 30 fruit. The observations recorded were incidence of chilling injury (%), colour coordinates [(*h*° and citrus colour index (CCI)], percentage weight loss, fruit firmness, soluble solids

concentration (SSC), titratable acidity (TA), SSC: TA, vitamin C and total antioxidants, individual and total sugars and organic acids, were determined from the fruit stored at 1ºC for 21 days and followed by 10 days in simulated shelf conditions (21 ± 1°C). However, the fruit percentage weight loss was recorded only at 22 days after cold storage.

Experiment 2: Effects of HW, MJ, TBZ, ET and NO treatments on CI incidence and fruit quality in 'Midknight Valencia' sweet orange

In this experiment, the 'Midknight Valencia' sweet orange fruit were treated with HW (50°C for 5 min) dip alone and combined with TBZ (20 mg L^{-1}), MJ (0.10, 0.25 or 0.50 mM) 1 min dip as well as fruit fumigated with different concentrations of NO (5, 10 or 20 µL L^{-1}) for 2 hrs and ethylene (ET) (10 µL L^{-1}) for 6 h in 60 L containers. The fruit were dried for 6 h at room temperature (20 ± 1 °C) and relative humidity (60 ± 5%) and then transferred to the cold storage (1°C) for 21 days with (85-90 %) relative humidity. The experiment was designed by following completely randomised with one factor including treatments with three replications. Each replication has 30 fruit. All the observations mentioned in Experiment 1 were also recorded in this experiment.

Observations recorded

Chilling Injury incidence (%)

All the fruit were visually examined for the symptoms of CI following 90 days cold storage and 10 days simulated shelf conditions (21 ± 1°C). Chill injured fruit were counted from the total fruit in each replication. Percentage chilling injury incidence (as percentage of chill injured fruit) was calculated using the following formula:

$$\text{Chilling injury incidence (\%)} = \frac{\text{Number of chill injured fruit}}{\text{Total number of fruit}} \times 100$$

Determination of the fruit colour

Citrus rind colour was recorded by using colour flex EZ (45°/0° design) spectrocolorimeter (Hunter Lab, Hunter Associates Laboratory Inc., Reston, VA, USA) on three positions around the equatorial plane of the fruit. Ten fruit were randomly selected from each replication. While using the head 15 mm diameter the colour coordinates (L*, a* and b*) values define a uniform three-dimensional colour space, where *L** is the vertical axis and describes the lightness of the fruit colour (0 to 100, black to white) and *a** and *b** are the horizontal axis defining the redness (+a*) or greenness (-a*), and yellow (+b*) or blue (-b*), respectively. For colour explanation, red was an angle of 0^{o} to 360^{o}, yellow at 90^{o}, green at 180^{o}, and blue at 270^{o}. The hue angle (h^{o}) value was calculated as $h^{o} = tan^{-1}\ b^{*}/a^{*}$ McGuire (1992). Citrus colour index (CCI) was calculated by using the following formula reported by Jiménez -Cuesta *et al.* (1981):

$$\mathrm{CCI} = (1000.\mathrm{a})/\mathrm{Lxb}$$

Determination of loss of fruit weight

The weight of the fruit at the commencement of storage (initial fruit weight) and following the 22 days cold storage (final fruit weight) was recorded by using a digital weighing balance. Fruit weight loss was calculated using the following formula and expressed as percent:

$$\text{Weight loss (\%)} = \frac{\text{(Initial weight} - \text{Final weight)} \times 100}{\text{Initial weight}}$$

Fruit firmness

Fruit firmness was determined using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Fareham, UK) interfaced with Nexygen® 4.6 software by employing a previously detailed method by Hussain (2014). Fruit firmness was expressed in newtons (N).

Soluble solids concentration (SSC) and Titratable acidity (TA)

A digital refractometer (Atago-Palette PR 101, Atago CO. Ltd, Itabashi-Ku, and Tokyo, Japan) was used to estimate SSC in the fresh juice of 'Midknight Valencia' and 'Lane Late'. SSC was expressed as a percentage. The juice was titrated against 0.1N NaOH to a pink colour end point. Phenolphthalein (2-3 drops) was used as an indicator. TA was calculated as percentage equivalent of citric acid.

Determination of sugars and organic acids

The levels of individual sugars and organic acids in the juice of Midknight' Valencia' (ten fruit) and 'Lane Late' (ten fruit) were determined by following the method including conditions of analysis reported (Hussain, 2014) by using reverse-phase high-performance liquid chromatography system (RP-HPLC; Waters, Milford, MA, USA) fitted with refractive index detector (sugars) and dual wavelength UV detector (organic acids). All the individual sugars and organic acids were expressed as (g L^{-1}).

Determination of vitamin C and total antioxidants

Ten randomly selected fruit from each cultivar were used to extract the juice, which was used for the determination of vitamin C and total antioxidants by using the method reported earlier by Hussain, (2014) and Brand-Williams et al. (1995) respectively, using a UV/VIS spectrometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). The standard curve of L-ascorbic acid was used to calculate vitamin C concentration in the juice and expressed as (mg L^{-1}) of fresh juice. However, standard curve of 6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox) was used to calculate total antioxidant and expressed as µM Trolox equivalent antioxidant activity (TEAC) (L^{-1}) FJ basis.

Statistical analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) using GenStat 14th edition (release 14.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). The influences of treatments on different variables were evaluated by ANOVA. The least significant differences (LSD) were tested following the Duncan multiple range test method at probability ($P \leq 0.05$).

Conclusion

HW dip (50°C for 5 min) alone or combined with thiabendazole (20 mg L^{-1}) or MJ (0.05 mM) 1 min dip were

effective in mitigating chilling injury in 'Lane Late' and 'Midknight Valencia' caused during cold quarantine treatment (1°C for 21 d). NO (5 µL L^{-1}) fumigation for 2 h significantly reduces percentage weight loss in 'Lane Late' only. MJ (0.1 mM) dip treatment significantly enhanced CCI only in 'Lane Late'.

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