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HOT WATER DIP AND PRECONDITIONING TREATMENTS TO REDUCE CHILLING INJURY AND MAINTAIN POST-HARVEST QUALITY OF VALENCIA AND NAVEL ORANGES DURING COLD QUARANTINE

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ABSTRACT

The potential of hot water dipping (HWD) at 41±1°C for 20 min or at 50±1°C for 5 min and pre-storage conditioning (6 days at 16-18°C and 45-65% RH) treatments to control chilling injury (CI) and decay of W. Navel and Valencia Late oranges during cold quarantine at 1°C and 85–90% RH for 20 days, subsequent storage at 10°C and 85–90% RH for 20 days (*as a transit period*) and an additional 20 days of simulated marketing period (SMP) at 20±2°C and 40-65% RH was investigated over 2 harvest seasons (2006/2007 & 2007/2008). Untreated fruits were used as control. We studied their effects on various other postharvest quality parameters such as weight loss, juice %, soluble solids content (SSC), total acidity (TA), ascorbic acid (VC), and reducing sugars, free phenols, peroxidase (POX) and catalase (CAT) activities, and ascorbic acid oxidase (ASAO).

After SMP, HWD treatments reduced the incidence of CI in W. Navel (4.2-13% versus 29–33% in control and 12-21% in conditioning treatments) and Valencia (0–27% against 60-63% in control and 60–67% in conditioning treatments). HWD treatments effectively prevented the incidence of decay in both cultivars in all storage stages, while conditioning one reduced it in W. Navel to 17-13%, against 25% in the control, although in Valencia, a very little insignificant decay % was found (3-7%). HWD treatments reduced the rate of weight loss in both cultivars, while conditioning one reduced it in Valencia, only. In addition, HWD treatments mostly increased VC content, especially in W. Navel, while reduced ASAO activity.

HWD treatments increased free phenols content in both cultivars, as compared with control and conditioning treatments, although the last increased it as compared with control. HWD at 41°C for 20 min was more effective than at 50°C for 5 min in this respect.

All treatments significantly increased POX and CAT activities, whether in fruit peel or juice of both cultivars as compared with control. HWD at 41°C for 20 min treatment was more effective in this respect. Generally, the reduction in chilling injury % was paralleled to higher POX and CAT activities in fruit peel and juice, and free phenols in juice, this may explain how and why the treatments reduced CI incidence. Overall, it is concluded that short postharvest HWD treatment is preferable, since it effectively induced tolerance to cold temperatures in W. Navel and Valencia Late oranges without impairing any other postharvest qualities.

Keywords: *C.sinensis*; Cold quarantine; Chilling injury; Hot water; Conditioning; Catalase; Peroxidase

INTRODUCTION

Citrus is one of the most important fruit crops in Egypt. In 2006/2007 season, the cultivated area estimated by 382 027 fed., and produced 3 211 709 tons from which 796 000 tons (mainly, Valencia and Navel oranges) were exported (Statistics of Egyptian Ministry of Agric. 2007).

Various citrus-importing countries require quarantine security against Mediterranean fruit fly, and fruit must be certified free of Medfly. Cold quarantine treatments, which involve the exposure of fruit to near-freezing temperatures (1.1 - 2.2°C) for a period of 14–18 days (Powel, 2003), is a procedure accepted for Medfly disinfestation of citrus fruit by the regulatory agencies of most importing countries and is currently applied on a commercial scale (Schirra *et al.*, 2004). However, citrus fruit are susceptible to chilling injury (CI) when exposed to temperatures less than 2–5°C. Quarantine conditions may cause CI, especially when fruit are returned to warm temperature, predisposing them to decay (Underhill *et al.*, 1995).

Postharvest heat treatments can be used to induce fruit tolerance to cold temperatures and to reduce the development of CI symptoms during cold storage and cold quarantine treatments (Porat *et al.*, 2000; Schirra *et al.*, 2004). In citrus, these heat treatments include pre-storage conditioning for either 3 days at 21°C or 7 days at 16°C (Porat *et al.*, 2000). Pre-storage conditioning treatments of lemon fruit for 7 days at 16°C or orange for 3-4 days at 16°C (Cuquerella, 1998), or grapefruit for 7 days at 15°C (Biolatto *et al.*, 2005) eliminated or greatly reduced CI after cold quarantine treatment at 2°C for 18 days without adversely affecting its quality, and have an important commercial application.

Heat treatments, which reduced both decay and CI in different citrus fruits, could substitute the conditioning (16°C for 7 days) required as a pre-treatment before the cold disinfestations-quarantine treatment (1°C for 16 days) which would bring great saving in time of storage and cost of conditioning (Ben-Yehoshua, 2003).

Since previous studies on citrus fruit, it has been shown that post-harvest hot water dipping (HWD) treatments alleviate CI in sensitive cultivars and reduce storage decay (Rodov *et al.*, 2000; Porat *et al.*, 2000; Schirra *et al.*, 2000 and; 2004), it might be applied to overcome the risk of CI and decay of quarantined fruit (Porat *et al.*, 2000; Schirra *et al.*, 2002[a,b] and 2004; Biolatto *et al.*, 2005). HWD at 53°C for 3-6 min (Schirra *et al.*, 1997) or at 48°C for 12 min reduced CI and decay in Valencia orange fruits (Erkan *et al.*, 2005). In addition Schirra *et al.* (2004) reported that HWD at 50°C for 3 min also reduced CI and decay in quarantined blood orange cultivars at 1°C for 16 days.

However, the physiological responses of different cultivar fruits to heat treatments can vary by season and growing location. The reason for the variation in response among production regions may arise from differences in their climate, soil type, season, production practices, and fruit maturity stages at harvest (Fallik, 2004).

It is widely accepted that symptoms of CI are a consequence of oxidative stress in the tissues (Sala, 1998) occurring when active oxygen species (AOS) such as hydrogen peroxides, superoxides and hydroxyl radicals are in excess of the scavenging capacity of fresh tissue (Hodges *et al.*, 2004). Involvement of antioxidant enzymes in regulation of AOS can be followed by measuring Guaiacol Peroxidase (POX; EC 1.11.1.7) and Catalase (CAT; EC 1.11.1.6) activity during postharvest storage (Sala, 1998). Preconditioning treatments of fruit with hot water (Fallik, 2004; Schirra and D'hallewin, 1997) may induce chilling tolerance by modulating antioxidant systems that would prevent the accumulation of AOS (Martinez-Tellez and Lafuente, 1997; Sala and Lafuente, 2000).

Therefore, in this study, we aimed to examine the effects of preconditioning and hot water dipping (HWD) treatments on the induction of cold tolerance and maintaining quality of quarantined W. Navel and Valencia oranges that considered to be the main cultivars produced and exported in Egypt. We also assessed enzymes associated with oxidative stress and metabolic changes occurring in response to heat treatments and during induction of CI in the orange fruits.

MATERIALS AND METHODS

During two successive harvest seasons (2006/2007 & 2007/2008), W. Navel and Valencia late orange's (*C. sinensis* (L.) Osbeck) commercially mature fruits were harvested randomly from a private orchard at "Wady El-Mullak" region, Ismailia Governorate, Egypt. Fruits were directly transported to postharvest lab. in Hort. Dept., Fac., Agric., Suez Canal Univ., and then sorted to eliminate defects. Samples of fruits of uniform size and appearance were washed by chlorine solution (100 ppm), air dried and held for 24h at room temperature.

In the next day, fruits were randomly divided into four treatment groups, each of 48 fruits (Navel) or 60 fruits (Valencia). The first group was used as the control, without any treatment, the second group was left at shelf life conditions (16-18°C and 45-65% RH) for 6 days as a preconditioning treatment, the third group was subjected to hot water dip (HWD) at 41±1°C for 20 min, and the fourth group were subjected to HWD at 50±1°C for 5 min. HWD treatments were performed in a water bath constantly maintained within ±1°C of the required temperature by a thermostat.

Each treatment group was packed in 6 foam plates (8 or 10 fruits each) put in perforated polyethylene pages, and then divided into two subgroups each of three plates (replications). All treatments were exposed to cold quarantine at 1°C and 85–90% RH for 20 days (*a commercial cold quarantine treatment for Mediterranean fruit fly disinfestations*), and then stored for 20 days at 10°C and 85–90% RH (*as a transit period*). At the end of storage, fruit plates were maintained at shelf life conditions (18-20°C and 40-60% RH) for 20 days to simulate a marketing period (SMP). Fruit plates from the first subgroup were used for physical properties assessments (weight loss, decay and CI); while those of the second subgroup were used to determine the chemical characteristics (juice %, soluble solids content (SSC), total acidity (TA), ascorbic acid (VC), reducing sugars, free phenols, Peroxidase (POX) activity, Catalase (CAT) activity and Ascorbic acid oxidase (ASAO)).

Evaluation of chilling injury (CI), decay and fruit weight loss:

After quarantine, transit period and SMP, fruits of the two cultivars were inspected for CI and decay symptoms. Total number of fruits manifesting CI symptoms (peel pitting and brown staining) was determined and expressed as the CI %, additionally, in Valencia orange, the number of pits and brown stains per fruit were counted. Total number of fruits manifesting decay symptoms was determined and expressed as the decay %. Weight loss was calculated and expressed as a percentage of the original fresh weight of fruits.

Chemical analysis of juice:

At zero time, after conditioning treatment (Tables 1 & 2), quarantine, transit period and SMP, fruit samples were taken for determination of the chemical properties. Juice was extracted by a rotary extractor, and then juice % (w/w) was calculated. SSC was measured by refractometer; TA and VC were determined according to AOAC (1985), and then SSC:TA ratio was calcuated.

Ethanolic extract (96% ETOH) of juice were prepared according to Abdel-Rahman *et al.* (1975), then the free phenols were determined spectrophotometricaly (Beckman DK-2 Spectrophotometer) at 650 nm with Folin-Ciocalteu reagent according to AOAC (1985). Reducing sugars were determined with alkaline copper and arsenomolybdate reagents spectrophotometricaly at 540 nm according to Moore (1974).

Preparation of enzyme extract in juice and peel:

At zero time, after conditioning treatment (Tables 1 & 2), after quarantine, transit period and SMP, fruit samples were taken for the enzymes assay. 0.5g fresh juice or peel were homogenized by using a mortar and pestle with 0.1 M phosphate buffer (pH 6.5) at 4°C and stirred for 20 min. The suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 18000g for 15 min, 4°C. The supernatant was used to determine activity of antioxidant enzymes (Urbanek *et al.*, 1991) as follow:

Peroxidase (POX) [EC 1.11.1.7] assay:

The reaction mixture consisted of 3.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.3 ml of 0.1 % o-dianisidine solution, 0.2 ml of enzyme extract and 0.2 ml of 0.2 M hydrogen peroxide solution (Urbanek *et al.*, 1991). The reaction mixture was incubated at 30 °C for 10 min and the oxidation of o-dianisidine measured by changes in optical density at 430 nm (Beckman DK-2 Spectrophotometer). Corrections were done for the oxidation rate of o-dianisidine in the absence of H_2O_2 in the reaction mixture. The activity of POX was expressed as optical density per milligram of protein per minute. One unit of POX activity (AU) was taken as the change of 1.0 unit of optical density per minute.

Catalase (CAT) [EC 1.11.1.6] assay:

The reaction mixture consist of 0.01 ml enzyme extract and 2.99 ml hydrogen peroxide-phosphate buffer (pH 6.8) prepared after dilution of 0.16 ml of H_2O_2 (10% w/v) to 100 ml phosphate buffer. The oxidation of H_2O_2 was measured by changes in optical density at 240 nm in 30 sec. intervals for 5 min (Beckman DK-2 Spectrophotometer). The unit of CAT activity was defined as the amount of enzyme, which decomposes 1 mmol H_2O_2 per minute at 25°C (Bradford, 1976).

Ascorbic acid oxidase (ASAO) [E.C. 1.10.3.3] assay:

The reaction mixture consist of 0.1 ml enzyme extract and 2.9 ml ascorbic acid–phosphate buffer (pH 5.6) prepared as 8.8 mg ascorbic acid dissolved in 300 ml phosphate buffer. The oxidation of ascorbic acid was measured by changes in optical density at 265 nm in 30 sec. intervals for 5

min (Beckman DK-2 Spectrophotometer). The unit of ASAO activity was defined as the amount of enzyme, which decomposes 1 mmol ascorbic acid per minute at 25°C. Protein content of the extracts was determined according to Bradford (1976), using bovine albumin serum (BSA) as a standard.
Statistical analyses: All data were statistically analyzed as randomized complete blocks design (Steel *et al.,* 1997); using the MSTAT-C statistical package (M-STAT, 1990) and means were separated by LSD test, $P \leq 0.05$.

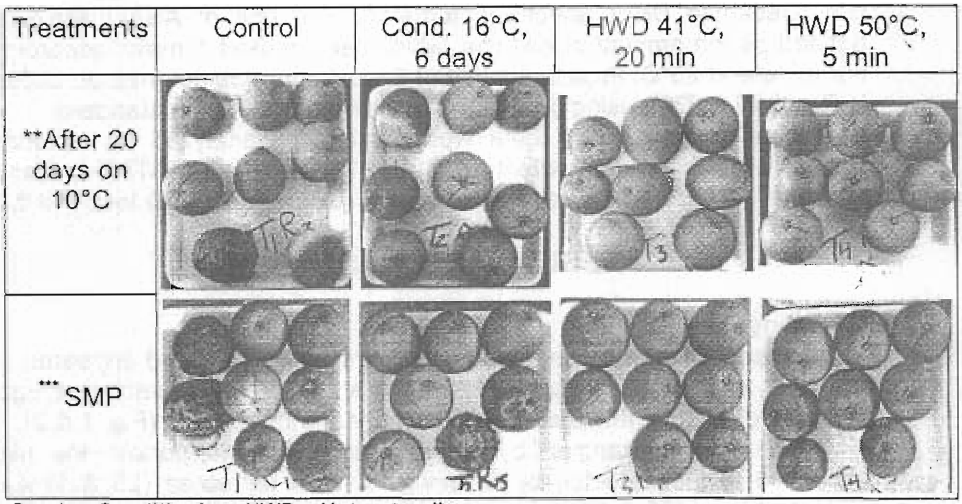
RESULTS AND DISCUSSION

I- Chilling injury (CI)

Generally, CI symptoms in both cultivars were slight; and appeared in the form of small pitted scattered areas and skin depressions irregularly distributed over the fruit surface, especially around the calyx (Fig. 1 & 2).

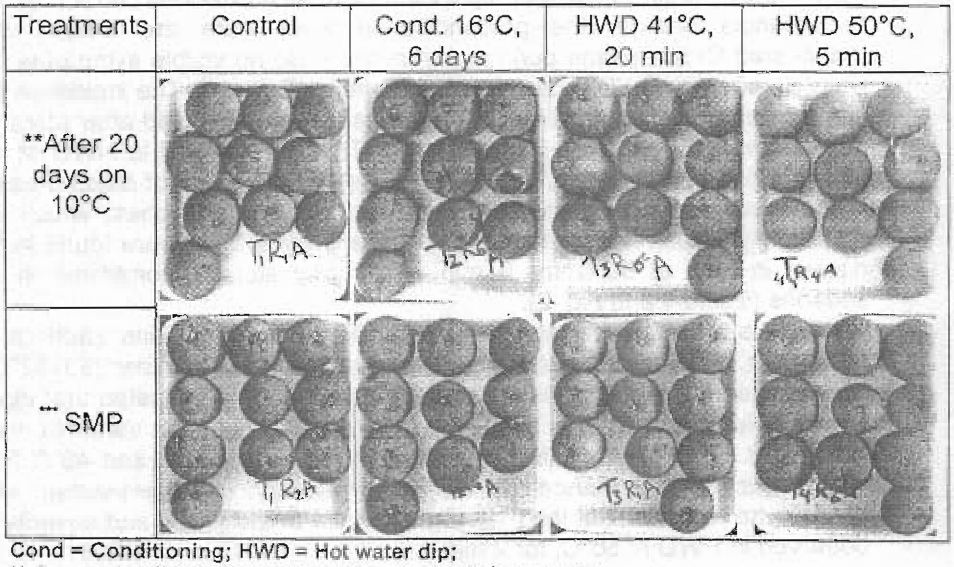
In W. Navel oranges, by the end of quarantine period, the highest incidence of fruit affected by CI being found in untreated (25 & 17%) and preconditioned (21 & 17%) fruits in both seasons (Table 3). HWD treatments significantly reduced the incidence of CI (0–4%). On the other hand, the incidence of CI in untreated fruits increased remarkably, reaching the maximum rates after simulated marketing period (SMP) (29–33%), while in HWD treatments, the maximum percentage of fruits affected by CI after SMP was low (4.2-13%). The preconditioning treatment showed lower affected fruits than control, but without significant differences between both (Table 3). In Valencia orange, the preconditioned fruits were the unique which manifested CI symptoms during quarantine, while no visible symptoms of CI were detected in HWD treaded or control fruits (Table 4). The incidence of CI in the control and preconditioned fruits was clearly increased after storage at 10°C, reaching the maximum after SMP. The fruits dipped in HWD at 41°C for 20 min had significantly low CI percentage and number of pitted areas per fruit as compared with those preconditioned or control ones, which were statistically similar in this respect. No visible CI symptoms were found in fruits dipped in HW at 50°C for 5 min, under any storage conditions in both seasons (Table 4 and Fig. 2).

This supports previous findings on 'Fortune' mandarins (Schirra and D'hallewin, 1997) which showed a protective effect of hot water (50–52°C for 3 min) against CI, and (Sala and Lafuente, 2000), who indicated that dipping the fruits for 3 min at 53°C increased the tolerance to CI; on Valencia orange (Erkan, *et al.*, 2005), who found that HWD (53°C for 6 min and 48°C for 12 min) reduced CI incidence; on Satsuma mandarin (Ghasemnezhad *et al.*, 2008), who reported that the major reduction in CI incidence and severity was observed in HWD at 50°C, for 2 min.



Cond. = Conditioning; HWD = Hot water dip;
** Quarantine (20 days at 1°C) + Storage (20 days at 10°C);
*** Quarantine + storage + 20 days shelf life (20±2°C and 40-65% RH)

Figure (1): Chilling injury and decay symptoms on peel surface of W. Navel orange under different treatments and different storage conditions



Cond = Conditioning; HWD = Hot water dip;
** Quarantine (20 days at 1°C) + Storage (20 days at 10°C);
*** Quarantine + storage + 20 days shelf life (20±2°C and 40-65% RH)

Figure (2): Chilling injury and decay symptoms on peel surface of Valencia orange under different treatments and different storage conditions

Table (1): Chemical composition of W. Navel orange fruit at zero time and after conditioning (6 days under lab. conditions)

Seasons	Juice %	SSC %	TA %	SSC/TA ratio	V. C mg/100 ml juice	W. loss %	Reducing sugars mg/g FW	Free phenols mg/g FW	POX activity units/mg protein/min		CAT activity units/mg protein/min		ASAO units/mg protein/min
									Peel	Juice	Peel	Juice	
At zero time													
2006/2007	54.9	11.4	0.63	18.1	49.5	--------	66.9	49.4	0.08	0.13	0.11	0.37	0.47
2007/2008	55.7	11.2	0.61	18.4	48.4	--------	60.6	35.7	0.10	0.12	0.19	0.21	0.28
After pre-conditioning (6 days under lab conditions, 16°C)													
2006/2007	56.9	11.6	0.65	17.8	49.0	0.95	126.6	57.1	0.10	0.26	0.14	0.56	1.23
2007/2008	56.7	11.6	0.62	18.7	45.1	1.00	132.9	59.4	0.08	0.30	0.18	0.57	1.31

ASAO = Ascorbic acid oxidase; POX = Peroxidase; CAT = Catalase

Table (2): Chemical composition of Valencia orange fruit at zero time and after conditioning (6 days under lab. conditions)

Seasons	Juice %	SSC %	TA %	SSC/TA ratio	V.C mg/100 ml juice	W. loss %	Reducing sugars mg/g FW	Free phenols mg/g FW	POX activity units/mg protein/min		CAT activity units/mg protein/min		ASAO units/mg protein/min
									Peel	Juice	Peel	Juice	
At zero time													
2006/2007	57.2	11.0	1.20	9.17	68.2	----	32.2	39.5	0.11	0.07	0.18	0.37	0.32
2007/2008	56.7	11.4	1.22	9.34	67.1	----	26.7	33.6	0.21	1.10	0.13	0.22	0.18
After pre-conditioning (6 days under lab conditions, 16°C)													
2006/2007	57.1	10.3	1.20	8.59	51.2	2.24	50.38	66.46	0.14	0.35	0.22	0.69	1.10
2007/2008	54.7	10.5	1.23	8.54	51.7	2.19	52.89	69.14	0.17	0.40	0.15	0.70	1.17

ASAO = Ascorbic acid oxidase; POX = Peroxidase; CAT = Catalase

Table (3): Effect of conditioning and HWD treatments on chilling injury, decay, and weight loss of W. Navel orange fruits under different storage conditions.

Parameters	Chilling injury %			Decay %				Weight loss %		
Storage conditions	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	Total	*20 d 1°C	**20 d 10°C	***SMP
Treatments	2006/2007 season									
Control	25.0	29.2	33.3	0.0	16.7	8.3	25.0	1.3	3.7	10.0
Cond. 16°C 6 days	20.8	20.8	20.8	0.0	12.5	4.2	16.7	2.1	4.3	10.5
HWD 41°C 20 min	8.3	12.5	12.5	0.0	0.0	0.0	0.0	2.0	4.3	8.4
HWD 50°C 5 min	4.2	4.2	8.3	0.0	0.0	0.0	0.0	1.7	4.4	7.7
LSD 0.05	8.37	13.8	17.2	----	7.21	NS	7.21	0.24	0.35	0.82
2007/2008 season										
Control	16.7	16.7	29.2	8.3	8.3	8.3	25	1.7	4.9	11.3
Con. 16°C 6 days	16.7	16.7	20.8	0.0	8.3	4.2	12.5	2.2	4.8	11.0
HWD 41°C 20 min	4.2	4.2	12.5	0.0	0.0	0.0	0.0	2.3	5.0	9.1
HWD 50°C 5 min	0.0	0.0	4.2	0.0	0.0	0.0	0.0	1.9	4.6	8.1
LSD 0.05	12.49	12.5	13.8	NS	8.32	NS	12.49	0.28	0.20	0.62

Cond. = Conditioning; HWD = Hot water dip;

*** Quarantine, 20 days at 1°C;**

**** Quarantine + storage, 20 days at 10°C;**

***** Quarantine + storage, 20 days at 10°C + 20 days shelf life (20±2°C, 40-65% RH)**

Table (4): Effect of conditioning and HWD treatments on chilling injury, decay, and weight loss of Valencia orange fruits under different storage conditions.

Parameters	Chilling injury %			Chilling injury pit no /fruit	Decay %				Weight loss %		
Storage conditions	*20 d 1°C	**20 d 10°C	***SMP	***SMP	*20 d 1°C	**20 d 10°C	***SMP	Total	*20 d 1°C	**20 d 10°C	***SMP
Treatments	2006/2007 season										
Control	0.0	46.7	60.0	2.2	0.0	0.0	0.0	0.0	3.7	6.7	11.4
Cond. 16°C6 days	36.7	36.7	60.0	2.5	3.3	3.3	0.0	6.67	3.9	6.1	10.0
HWD 41°C 20 min	0.0	16.7	20.0	1.2	0.0	0.0	0.0	0.0	3.6	6.4	10.7
HWD 50°C 5 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	5.9	10.4
LSD 0.05	5.8	15.3	19.1	0.91	NS	NS	---	NS	0.29	0.50	0.61
	2007/2008 season										
Control	0.0	53.3	63.3	2.1	0.0	0.0	0.0	0.0	4.2	7.2	12.0
Con. 16°C 6 days	36.7	36.7	66.7	2.0	0.0	3.3	0.0	3.3	4.4	6.6	11.2
HWD 41°C 20 min	0.0	16.7	26.7	1.2	0.0	0.0	0.0	0.0	3.9	7.0	10.9
HWD 50°C 5 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8	6.6	11.2
LSD 0.05	5.8	16.0	19.7	0.71	---	NS	---	NS	0.27	0.51	0.73

Cond. = Conditioning; HWD = Hot water dip;

* Quarantine, 20 days at 1°C;

** Quarantine + storage, 20 days at 10°C;

*** Quarantine + storage, 20 days at 10°C + 20 days shelf life (20±2°C, 40-65% RH)

II- Decay percentage

No decay occurred either in untreated or treated fruits of W. Navel and Valencia oranges during quarantine, in both seasons, except control fruits of W. Navel in the second season and Valencia in the first one which showed little decay percentage (8 and 3%), without significant differences among treatments (Tables 3 & 4). Both HWD tratments effectively prevented the incidence of rotten fruits, whereas no decay was observed in HWD treated W. Navel and Valencia fruits in any storage stage, in both seasons (Tables 3 & 4 and Fig. 1 & 2). On the other hand, conditioned fruits for 6 days at 16°C significantly reduced total decay percentage in W. Navel in both seasons to 17-13%, versus the control, which had 25% total decayed fruits (Table 3), while in Valencia orange, a very little total decay percentage was found (3-7%) in this treatment, without significant differences among treatments and control (Table 4).

These results are in line with those obtained by previous studies on citrus fruits (Rodov *et al.*, 2000; Porat *et al.*, 2000; Schirra *et al.*, 2000; Mohamed *et al.* 2002; Erkan *et al.*, 2005; Hong *et al.*, 2007). They mentioned that postharvest HWD significantly reduced decay incidence during storage. In addition Schirra *et al.* (2004) reported that HWD at 50°C for 3 min also reduced decay in quarantined blood orange cultivars at 1°C for 16 days. On the other hand, Porat *et al.* (2000) on grapefruit, found that conditioning for 7 days at 16 °C reduced decay development somewhat, but their effect was not significant.

The mod of action of heat treatment in reducing decay of fresh produce is not clearly understood (Erkan *et al.*, 2005), however heat treatment could enhance activity of anti-fungal substances (Ben-Yehoshua *et al.*, 1995), rendering the heat-treated tissues more resistant to decay.

III- Weight loss percentage

During quarantine, treatments by preconditioning and HWD at 41°C for 20 min increased the weight loss in W. Navel (Table 3), but did not affect it in Valencia (Table 4) as compared with control, while HWD at 50°C for 5 min reduced it in Valencia. After SMP, HWD treatments significantly reduced the rate of weight loss in both cultivars, during both seasons, while preconditioning one reduced it in Valencia orange, only. On the other hand, it was noticed that W. Navel orange control fruits were softer than those in other treatments (data not shown). If the fruit transpire too much water, they lose turgidity, and hence firmness, and may even appear slightly shriveled (Hong *et al.*, 2007).

Heat treatment can either increase or decrease water loss of fruit, depending on the treatment and the commodity. The influence of hot water treatments (HWT) on citrus fruit during storage was an increased weight loss in 'Fortune' mandarins (Schirra and D'hallewin, 1997), navel oranges (Birla *et al.*, 2005) and blood oranges (Schirra *et al.*, 2004) but a decreased weight loss in 'Valencia' oranges & grapefruit (Mohamed *et al.*, 2002) and kumquat & 'Marsh' grapefruit (Rodov *et al.*, 1995). 'Valencia' oranges hot water dipped at 45°C for 42 min became firmer, whereas the fruit at 53°C for 12 min showed an increased weight loss and decreased firmness (Williams *et al.*, 1994). This inconsistency in the HWT effect on weight loss may be attributed to different fruit responses to the heat treatment. The response of a particular fruit to the heat treatment results from a combination of factors including the host, physiological age of the commodity, time and temperature of exposure, treatment-methods, and storage temperature (Lydakis and Aked, 2003). In addition, Porat *et al.* (2000) on 'star ruby' grapefruit and Hong *et al.* (2007) on 'Satsuma' mandarin reported that HWD treatments did not affect fruit weight loss. On the other hand, preconditioning treatment for 7 days at 16°C significantly increased fruit weight loss of 'star ruby' grapefruit (Porat *et al.*, 2000) or decreased it (McDonald *et al.*, 1993).

IV- Internal quality characteristics

Juice percentage: In W. Navel orange, the effect of treatments on fruit juice content had no clear trend, whereas during quarantine period, the highest juice % was evident in HWD at 41°C treated fruits as compared with those in other treatments, in both seasons, and control fruits in the first one (Table 5). After storage period, conditioning for 6 days at 16°C reduced fruit juice content, while HWD did not affect it in comparison with untreated fruits, in both seasons. After SMP, HWD at 41°C treatment significantly increased fruit juice content as compared to control and conditioning ones in the first season, while in thesecond one, conditioned and HWD at 41°C treated fruits had highest juice content compared with untreated one.

Concerning Valencia orange, HWD and conditioning treatments did not affect the juice % after quarantine, in the first season and after SMP period in both seasons (Table 6). HWD at 41°C treatment significantly increased fruit juice content after storage period as compared with control and conditioning treatments in both seasons. In addition, this treatment had the significant highest juice % after quarantine in the second one, only.

Soluble solids content (SSC): For W. Navel orange, SSC did not affect by HWD or conditioning treatments in any stage, in both seasons, except HWD at 41°C treatment, which significantly reduced it during quarantine period in the first season, only (Table 5). Concerning Valencia orange, SSC was not affected by any treatment after SMP in both seasons, while no obvious trend was found during quarantine and storage periods in both seasons.

Total acidity (TA): Generally, no significant differences were found among treatments in most cases in both cultivars during both seasons, except HWD at 41°C treatment during storage period in the first season and HWD at 50°C after SMP period in the second one, which significantly reduced TA content in W. Navel as compared with control (Table 5), also conditioning treatment, which increased it in Valencia after SMP in both seasons, but this effect was significant in the first season only (Table 6).

Maturity index: HWD and conditioning treatments show no significant effect on SSC/ acid ratio during quarantine period in W. Navel in both seasons (Table 5), and in Valencia in the first one only (Table 6). After storage period, HWD at 41°C significantly increased the maturity index in W. Navel orange in both seasons as compared with control, while in Valencia, the conditioned fruits had significantly higher maturity index in the second season only (Table 6).

Table (8): Effect of conditioning and HWD treatments on reducing sugars, free phenols and POX & CAT activity in peel and juice fruit of Valencia orange under different storage conditions.

Parameters	Reducing sugars mg/g FW			Free phenols mg/g FW			POX activity units/mg protein/min						CAT activity units/mg protein/min					
							Peel			Juice			Peel			Juice		
Storage conditions	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP
Treatments	2006/2007 season																	
Control	69.4	69.9	93.2	53.8	43.8	30.1	0.28	0.39	0.43	0.36	0.40	0.44	0.82	0.92	0.97	0.95	1.04	1.07
Cond. 16°C 6 days	67.8	68.8	92.1	51.7	41.3	53.9	0.74	0.82	1.26	0.78	0.90	1.21	0.89	1.27	1.51	1.33	1.41	1.42
HWD 41°C 20 min	65.7	65.9	81.4	76.4	64.4	56.9	1.42	1.78	2.78	1.66	1.93	2.32	2.21	3.43	4.78	3.44	3.70	4.86
HWD 50°C 5 min	59.5	58.8	94.4	68.1	55.8	47.9	0.53	0.79	0.93	0.50	0.60	0.79	2.14	2.14	4.14	2.09	2.21	3.56
LSD 0.05	6.75	5.5	5.9	3.6	2.9	1.6	0.13	0.19	0.12	0.12	0.16	0.71	0.15	0.27	0.22	0.19	0.19	0.14
	2007/2008 season																	
Control	67.9	69.1	93.3	55.4	43.9	27.4	0.39	0.46	0.76	0.43	0.49	0.71	0.90	0.94	1.08	1.01	1.09	1.11
Cond. 16°C 6 days	67.5	68.1	91.8	49.0	39.8	35.5	0.86	0.89	1.46	0.83	1.01	1.31	1.19	1.39	1.39	1.34	1.46	1.29
HWD 41°C 20 min	68.4	68.0	87.1	78.5	64.6	56.9	1.42	1.78	2.91	1.68	1.99	2.81	2.41	3.80	5.41	3.85	4.14	5.34
HWD 50°C 5 min	61.5	61.2	90.7	65.6	53.6	45.9	0.53	0.79	1.17	0.47	0.76	1.12	2.26	2.18	3.95	2.11	2.49	3.36
LSD 0.05	1.7	2.6	NS	3.4	2.6	2.7	0.10	0.16	0.24	0.11	0.16	0.29	0.21	0.16	0.47	0.28	0.28	0.38

Cond. = Conditioning; HWD = Hot water dip; POX = Peroxidase; CAT = Catalase

* Quarantine, 20 days at 1°C;

** Quarantine + storage, 20 days at 10°C;

*** Quarantine + storage, 20 days at 10°C + 20 days shelf life (20±2°C, 40-65% RH)

Table (6): Effect of conditioning and HWD treatments on fruit quality of Valencia orange under different storage conditions.

Parameters	Juice %			SSC %			Total acidity %			SSC/TA ratio			V.C mg/100 ml juice			ASAO units/mg protein/min		
Storage conditions	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20 d 10°C	***SMP	*20 d 1°C	**20d 10°C	***SMP
Treatments	2006/2007 season																	
Control	55.8	54.1	55.8	10.5	10.9	10.6	1.08	1.13	0.89	9.75	9.72	12.0	52.4	51.3	50.6	2.29	2.61	4.22
Cond. 16°C 6 days	54.6	55.7	53.3	10.7	10.4	10.5	1.08	1.07	1.05	9.88	9.75	10.0	51.3	48.6	49.7	2.09	2.22	2.80
HWD 41°C 20 min	56.6	58.5	54.2	10.8	10.8	10.5	1.08	1.10	0.89	10.0	9.81	11.9	51.7	51.3	50.9	0.92	1.01	1.43
HWD 50°C 5 min	56.5	57.2	54.0	10.3	10.6	10.8	1.08	1.04	0.85	9.54	10.2	12.7	58.3	56.3	51.3	1.47	1.67	1.81
LSD 0.05	NS	1.64	NS	0.46	0.38	NS	NS	NS	0.11	NS	NS	1.02	2.76	3.40	NS	0.18	0.31	0.38
	2007/2008 season																	
Control	57.1	58.6	57.9	11.4	11.3	11.3	1.14	1.19	1.09	10.1	9.49	10.4	56.5	55.7	54.4	2.41	2.60	3.87
Cond. 16°C 6 days	56.8	58.2	55.9	11.3	11.3	11.1	1.18	1.12	1.13	9.6	10.1	9.77	57.9	53.5	55.9	1.80	1.86	2.32
HWD 41°C 20 min	60.3	59.8	56.8	11.1	11.3	11.1	1.15	1.18	1.03	9.61	9.63	10.8	54.1	54.6	56.3	1.02	1.14	1.35
HWD 50°C 5 min	58.1	59.3	57.1	10.7	10.9	11.2	1.16	1.14	0.98	9.23	9.56	11.5	60.0	59.2	55.7	1.49	1.67	2.18
LSD 0.05	1.84	0.89	NS	0.37	0.33	NS	NS	NS	0.12	0.38	0.26	0.89	2.71	NS	NS	0.27	0.23	0.75

Cond. = Conditioning; HWD = Hot water dip; ASAO = Ascorbic acid oxidase

* Quarantine, 20 days at 1°C;

** Quarantine + storage, 20 days at 10°C;

*** Quarantine + storage, 20 days at 10°C + 20 days shelf life (20±2°C, 40-65% RH)

After SMP, all treatments did not affect the maturity index of W. Navel in the first season, while in the second one, HWD at 50°C significantly increased it as compared to control and other treatments. While, in Valencia, the conditioned fruits had significantly lower maturity index in both seasons compared with HWD treatments (Table 6).

Ascorbic acid content: The effect of HWD and conditioning treatments did not show an evident trend. In W. Navel (Table 5), all treatments significantly increased ascorbic acid content after quarantine period, while in Valencia (Table 6), only, HWD at 50°C treatment, significantly increased it as compared with control in both seasons. After storage and SMP, W. Navel fruits conditioned or HWD treated had significantly higher ascorbic acid content as compared with control in both seasons, except conditioned one after storage (in the second season) and SMP (in the first season). In Valencia orange (Table 6), there was no significant difference among treatments in the ascorbic acid content after storage period or SMP in both seasons, except after storage in the first one, whereas HWD at 50°C treatment significantly increased it.

In this respect, Mohamed *et al.* (2002) on Valencia orange and March grapefruit, found that HWD at 50°C or 55°C for 5 min treatments had no effect on juice %, TSS, but maintained fruits with high TA content, while had no obvious trend on ascorbic acid content. In addition, Schirra *et al.* (2004) reported that HWD did not affect the internal fruit quality attributes (juice yield, SSC, TA, and ascorbic acid) of blood oranges. Also, HWT at 50°C for 2-6 min did not affect the juice TSS and TA content in Navel and Valencia oranges (Birla *et al.*, 2005), in 'Satsuma' mandarin (Hong *et al.* 2007) and TSS/acid ratio in grapefruit (Porat *et al.*, 2000 and Hong *et al.* 2007). While, HWD treatments had no consistent effects on TA, SSC and ascorbic acid content of Valencia orange (Erkan *et al.*, 2005). On the other hand, conditioning treatment at 15-16°C for 7 days had no significant effect on SSC and acid grapefruit content after SMP (Porat *et al.*, 2000 and Biolatto *et al.*, 2005), but increased the juice TSS/acid ratio (Porat *et al.*, 2000).

V- Ascorbic acid oxidase (ASAO)

From data presented in Tables 5 & 6, HWD and conditioning treatments significantly reduced ASAO activity in fruit juice of Navel and Valencia oranges during quarantine, after storage period and SMP in both seasons, as compared with control one. HWD at 41°C for 20 min treatment was more effective in this respect.

In this study, HWD treatments mostly increased ascorbic acid content, especially in W. Navel oranges, while reduced ASAO activity (Fig. 3). This may explains the lower ascorbic acid content of untreated fruits due to its oxidation. Schirra *et al.* (2004) stated that "we are unable to explain whether the loss of ascorbic acid during SMP in hot air treatment (HAT) fruit was due to its oxidation to dehydroascorbic acid (known to have antiscorbutic activity similar to ascorbic acid) or biological transformation to compounds with no activity".

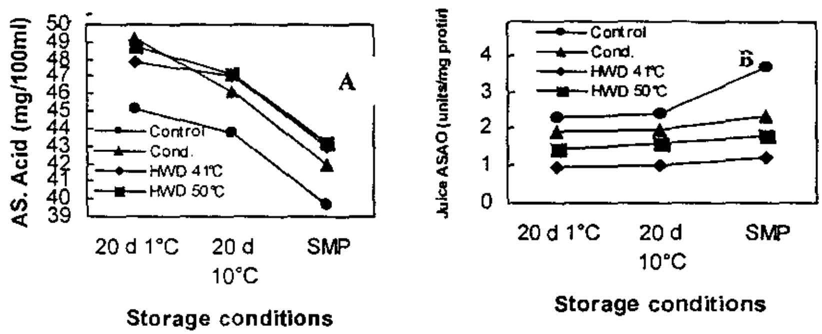


Fig. 3: Effect of conditioning and HWD treatments on ascorbic (As.) acid content (A) and ascorbic acid oxidase (ASAO) (B) of W. Navel orange fruit under different storage conditions. Each value is the mean of two seasons.

VI- Reducing sugars

HWD at 50°C for 5 min significantly reduced fruit juice content of reducing sugars during quarantine and after storage period in comparison with control and other treatments in both cultivars and seasons (Tables 7 & 8). No significant difference was found between both HWD treatments during quarantine in the first season, only. After SMP, reducing sugars juice content did not significantly affected by any treatment in both cultivars and seasons, except HWD at 41°C for 20 min, which reduced it in the first season, only.

VII- Free phenols

Conditioning treatment did not affect the free phenols content in any time, except after SMP, which significantly increased it as compared with control.

HWD treatments significantly increased free phenols content for fruits of both orange cultivars during quarantine, after storage period and SMP in both seasons, as compared with control and conditioning ones (Tables 7 & 8). HWD at 41°C for 20 min was more effective than at 50°C for 5 min in this respect. Overall, the highest free phenols content (as antioxidant) in HWD treated fruits was associated with the lowest peel CI symptoms (Tables 3 & 4), this may explain why and how HWD treatments reduced CI incidence.

Rapisarda *et al.*, (2008) reported that during storage, there was a decrease in total phenolics in Valencia orange. In our study, the same trend can be notice with free phenols, especially in untreated fruits.

VIII- Peroxidase (POX) activity

HWD and conditioning treatments significantly increased POX activity, whether in fruit peel or juice of W. Navel and Valencia oranges during quarantine, after storage period and SMP as compared with control, in both seasons, except HWD at 50°C for Valencia fruit juice after SMP in the first season and during quarantine, for both orange cultivars in the second one only, where this increment was insignificant (Tables 7 & 8).

Table (7): Effect of conditioning and HWD treatments on reducing sugars, free phenols and POX & CAT activity in peel and juice fruit of W. Navel orange under different storage conditions.

Parameters	Reducing sugars mg/g FW			Free phenols mg/g FW			POX activity units/mg protein/min						CAT activity units/mg protein/min					
							Peel			Juice			Peel			Juice		
Storage conditions	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP
Treatments	2006/2007 season																	
Control	175.3	179.3	202.9	45.7	44.2	34.2	0.21	0.23	0.27	0.27	0.28	0.29	0.34	0.42	0.72	0.66	0.69	0.71
Cond. 16°C 6 days	171.2	176.5	200.7	44.4	41.7	40.8	0.46	0.52	0.70	0.58	0.63	0.81	0.49	0.64	0.90	0.93	0.94	0.95
HWD 41°C 20 min	165.8	169.1	177.4	65.6	65.1	64.7	1.28	1.32	1.65	1.23	1.34	1.77	1.77	2.32	2.73	2.41	2.47	3.24
HWD 50°C 5 min	150.1	150.9	205.6	58.5	56.3	54.4	0.55	0.59	0.61	0.37	0.42	0.53	1.19	1.32	1.70	1.46	1.48	2.37
LSD 0.05	17.04	14.13	12.84	3.00	2.93	1.74	0.13	0.10	0.14	0.10	0.11	0.10	0.17	0.21	0.46	0.13	0.13	0.09
	2007/2008 season																	
Control	171.6	177.1	203.3	47.6	44.4	31.2	0.24	0.26	0.42	0.32	0.34	0.49	0.38	0.49	0.72	0.71	0.73	0.74
Cond.16°C 6 days	170.4	174.6	199.9	42.1	40.2	40.3	0.48	0.62	0.69	0.62	0.71	0.88	0.54	0.76	0.94	0.94	0.97	0.87
HWD 41°C 20 min	172.8	174.4	197.6	67.5	65.3	64.6	1.12	1.30	1.76	1.25	1.39	1.88	1.76	2.56	2.79	2.69	2.76	3.56
HWD 50°C 5 min	155.3	156.8	189.8	56.4	54.2	52.2	0.52	0.64	0.73	0.36	0.53	0.75	1.19	1.33	1.85	1.48	1.53	2.24
LSD 0.05	4.3	6.59	NS	2.94	2.6	3.08	0.14	0.10	0.14	0.08	0.11	0.19	0.15	0.19	0.31	0.19	0.18	0.25

Cond. = Conditioning; HWD = Hot water dip; POX = Peroxidase; CAT = Catalase

* Quarantine, 20 days at 1°C; ** Quarantine + storage, 20 days at 10°C;

*** Quarantine + storage, 20 days at 10°C + 20 days shelf life (20±2°C, 40-65% RH)

Table (8): Effect of conditioning and HWD treatments on reducing sugars, free phenols and POX & CAT activity in peel and juice fruit of Valencia orange under different storage conditions.

Parameters	Reducing sugars mg/g FW			Free phenols mg/g FW			POX activity units/mg protein/min						CAT activity units/mg protein/min					
							Peel			Juice			Peel			Juice		
Storage conditions	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP	*20 d 1°C	**20 d 10°C	*** SMP
Treatments	2006/2007 season																	
Control	69.4	69.9	93.2	53.8	43.8	30.1	0.28	0.39	0.43	0.36	0.40	0.44	0.82	0.92	0.97	0.95	1.04	1.07
Cond. 16°C 6 days	67.8	68.8	92.1	51.7	41.3	53.9	0.74	0.82	1.26	0.78	0.90	1.21	0.89	1.27	1.51	1.33	1.41	1.42
HWD 41°C 20 min	65.7	65.9	81.4	76.4	64.4	56.9	1.42	1.78	2.78	1.66	1.93	2.32	2.21	3.43	4.78	3.44	3.70	4.86
HWD 50°C 5 min	59.5	58.8	94.4	68.1	55.8	47.9	0.53	0.79	0.93	0.50	0.60	0.79	2.14	2.14	4.14	2.09	2.21	3.56
LSD 0.05	6.75	5.5	5.9	3.6	2.9	1.6	0.13	0.19	0.12	0.12	0.16	0.71	0.15	0.27	0.22	0.19	0.19	0.14
	2007/2008 season																	
Control	67.9	69.1	93.3	55.4	43.9	27.4	0.39	0.46	0.76	0.43	0.49	0.71	0.90	0.94	1.08	1.01	1.09	1.11
Cond. 16°C 6 days	67.5	68.1	91.8	49.0	39.8	35.5	0.86	0.89	1.46	0.83	1.01	1.31	1.19	1.39	1.39	1.34	1.46	1.29
HWD 41°C 20 min	68.4	68.0	87.1	78.5	64.6	56.9	1.42	1.78	2.91	1.68	1.99	2.81	2.41	3.80	5.41	3.85	4.14	5.34
HWD 50°C 5 min	61.5	61.2	90.7	65.6	53.6	45.9	0.53	0.79	1.17	0.47	0.76	1.12	2.26	2.18	3.95	2.11	2.49	3.36
LSD 0.05	1.7	2.6	NS	3.4	2.6	2.7	0.10	0.16	0.24	0.11	0.16	0.29	0.21	0.16	0.47	0.28	0.28	0.38

Cond. = Conditioning; HWD = Hot water dip; POX = Peroxidase; CAT = Catalase

* Quarantine, 20 days at 1°C;

** Quarantine + storage, 20 days at 10°C;

*** Quarantine + storage, 20 days at 10°C + 20 days shelf life (20±2°C, 40-65% RH)

The highest significant increment in POX activity, whether in fruit peel or juice was induced by HWD at 41°C in both cultivars and seasons. Mostly, no significant differences were found between conditioning and HWD at 50°C treatments with respect to POX activity in fruit peel, contrary to in fruit juice, whereas POX activity in conditioned fruits was significantly higher than HWD at 50°C treated fruits.

In this study, generally, the reduction in chilling injury percentage (Tables 3 & 4) was paralleled by higher POX activity (Tables 7 & 8), contrary to those of Ghasemnezhad *et al.* (2008), who reported that decreasing chilling injury in HWD treatments was correlated with decreased POX activity, and high activity of POX in most treatments was associated with severe peel damage, especially at 55°C for 5 min.

VIII- Catalase (CAT) activity

HWD treatments significantly increased CAT activity in fruit peel and juice during quarantine, after storage period and SMP as compared with control and conditioning ones in both seasons, whether in Navel or in Valencia (Tables 7 and 8). HWD at 41°C for 20 min treatment was significantly more effective in this respect. Conditioning treatment increased CAT activity in fruit peel and juice under all storage conditions compared with control one in both seasons, but this increment was not significant in some cases.

Overall, the reduction in CI percentage was paralleled with higher CAT activity (Fig. 4 & 5), supporting the finding of Ghasemnezhad *et al.* (2008) that elevated levels of CAT in hot water treated mandarins showed suppressed CI. In addition, Sala and Lafuente (2000) reported that CAT activity in the flavedo of 'Fortune' mandarins quickly increased after the HWD treatment at 53°C for 3 min. CAT may be a major antioxidant enzyme involved in the defense mechanism of mandarin fruits against chilling stress. Also the different effectiveness of the heat-conditioning treatments in increasing chilling tolerance of 'Fortune' mandarins may be related to induction of CAT activity during heating and on its persistence during cold storage.

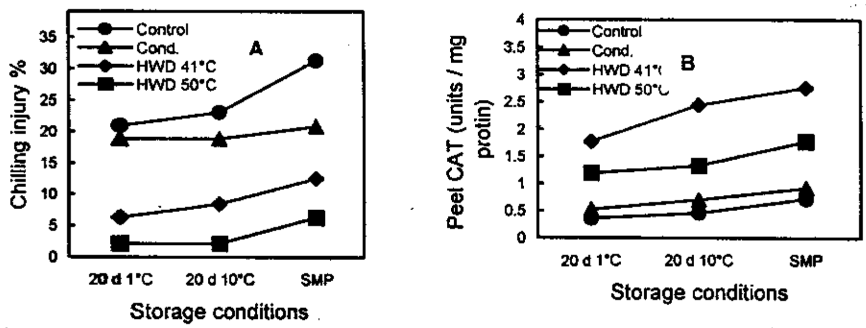


Fig. 4: Effect of conditioning and HWD treatments on CI % (A) and CAT activity (B) of W. Navel orange fruit peel under different storage conditions. Each value is the mean of two seasons.

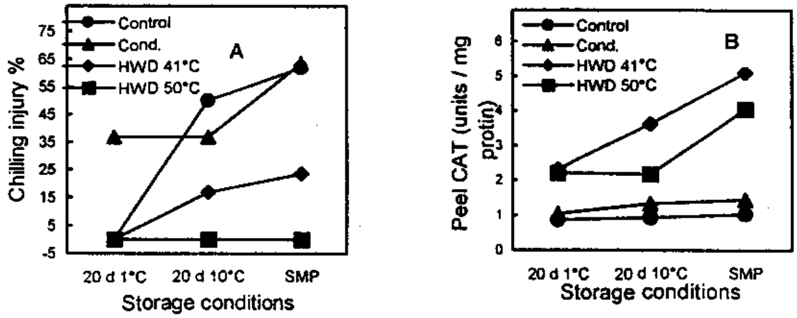


Fig. 5: Effect of conditioning and HWD treatments on CI % (A) and CAT activity (B) of Valencia orange fruit peel under different storage conditions. Each value is the mean of two seasons.

Conclusion

Generally, the reduction in CI % was paralleled by higher POX and CAT activities in fruit peel and juice, and free phenols in juice, this may explain why the treatments reduced CI incidence. Overall, it is concluded that short postharvest HWD treatment is preferable, since it effectively induce tolerance to cold temperatures in W. Navel and Valencia Late oranges without impairing any other postharvest qualities. Another advantage of the short HWD treatments is that they are simple to apply, as they can be easily incorporated into the packinghouse sorting line and do not require any special handling, whereas conditioning requires the use of different storage rooms. On the other hand, we did not observe any damaging effects with any of the hot water treatments examined in this study.

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معاملات الغمر في الماء الساخن والتكييف الحراري قبل معاملة الحجر البارد لتقليل أضرار البرودة والمحافظة على جودة ثمار البرتقال بسره والفالنشيا بعد الجمع

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أجريت هذه الدراسة لمعرفة كفاءة المعاملة بالغمر في الماء الساخن (٤١±١°م لمدة ٢٠ دقيقة أو ٥٠±١°م لمدة ٥ دقائق) أو التكييف الحراري (٦ أيام على ١٦-١٨°م + ٤٥-٦٠% رطوبة نسبية) قبل معاملة الحجر البارد على ١°م + ٨٥-٩٠% رطوبة نسبية لمدة ٢٠ يوما (معاملة تشترطها بعض الدول المستوردة للموالح) على تقليل أضرار البرودة والأعفان والمحافظة على جودة ثمار البرتقال بسره والفالنشيا. والتي تم تخزينها بعد ذلك على ١٠°م لمدة ٢٠ يوما أخرى (تماثل درجة حرارة الشحن) بالإضافة إلى ٢٠ يوم أخرى على درجة حرارة الغرفة (٢٠±٢°م +٤٥-٦٠% رطوبة نسبية) تماثل فترة التسويق. وقد تم تقييم تأثير هذه المعاملات على عناصر جودة الثمار الأخرى مثل الفقد في الوزن – نسبة العصير– نسبة المواد الصلبة الذائبة – الحموضة الكلية – فيتامين ج. أيضا محتوى العصير من السكريات المختزلة والفينولات الحرة ونشاط كل من إنزيم البيروكسيديز و الكتاليز في كل من قشرة وعصير الثمار وأيضا الأسكوربيك أسد أكسيديز في العصير.

أوضحت النتائج أنه في نهاية فترة عرض وتسويق الثمار ما يلي:

- المعاملة بالماء الساخن قللت أضرار البرودة في ثمار البرتقال بسره (٤,٢ – ١٣% مقابل ٢٩-٣٣% في المقارنة و ١٢-٢١% في معاملة التكييف الحراري) وفى ثمار البرتقال الفالنشيا (صفر – ٢٧% مقابل ٦٠-٦٣% في المقارنة و ٦٠-٦٧% في معاملة التكييف الحراري).
- المعاملة بالماء الساخن كانت فعالة في منع أعفان الثمار في كلا الصنفين، بينما معاملة التكييف الحراري قللت نسبة الأعفان في ثمار البرتقال بسره (١٣-١٧% مقابل ٢٥% في المقارنة) وفى ثمار البرتقال الفالنشيا كانت هذه النسبة غير معنوية (٣-٧%).
- المعاملة بالماء الساخن خفضت معدل الفقد في وزن الثمار في كلا الصنفين، بينما معاملة التكييف الحراري قللته في ثمار البرتقال الفالنشيا فقط.
- غمر الثمار في الماء الساخن أدى إلى زيادة محتواها من فيتامين ج وخاصة في ثمار البرتقال بسره وكان ذلك مصاحبا لإنخفاض في نشاط إنزيم الأسكوربيك أسد أكسيديز في العصير.
- المعاملة بالماء الساخن أدت إلى زيادة محتوى العصير من الفينولات الحرة في كلا الصنفين بالمقارنة بكل من معاملة المقارنة والتكييف الحراري، بالرغم أن المعاملة الأخيرة أدت إلى زيادتها بالمقارنة بمعاملة المقارنة. وقد كانت معاملة الغمر في الماء الساخن ٤١°م لمدة ٢٠ دقيقة أكثر فعالية في هذا الشأن.
- جميع المعاملات أدت إلى زيادة نشاط كل من إنزيم البيروكسيديز والكتاليز سواء في قشرة أو عصير الثمار في كلا الصنفين. وقد كانت معاملة الغمر في الماء الساخن ٤١°م لمدة ٢٠ دقيقة أكثر فعالية في هذا الشأن. وإن كانت هذه الزيادة غير معنوية في بعض الحالات.
- بصفة عامة، فإن الانخفاض في نسبة أضرار البرودة كان مصحوب بزيادة في نشاط كل من إنزيم البيروكسيديز والكتاليز في قشرة وعصير الثمار وكذلك محتوى العصير من الفينولات الحرة، وهذا ربما يفسر كيف أو لماذا تؤدى المعاملة بالماء الساخن إلى تقليل أضرار البرودة في ثمار البرتقال المعاملة.

الخلاصة: على ضوء النتائج المتحصل عليها يمكن أن نستخلص أنه من المفضل غمر ثمار البرتقال بعد الجمع في الماء الساخن لمدة قصيرة، حيث أنها فعالة في إحداث مقاومة لدرجات الحرارة المنخفضة ودون أن تؤثر سلبا على صفات جودة الثمار.