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Reducing Chilling Injury and Decay in Stored 'Clementine' Mandarins with Hot Water and Curing Treatments

M. Erkan, M. Pekmezci, I. Karaşahin and H. Uslu
(Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey)

Summary

The effectiveness of postharvest hot water (at 53 °C for 3 and 6 min; at 48 °C for 12 min) and curing treatments (at 53 °C for 1 and 6 h; at 48 °C for 6 h) on chilling injury, decay and postharvest quality of 'Clementine' mandarins (*Citrus reticulata* (L.) Osbeck) during a two-month storage at 1 °C and subsequently one week of simulated shelf-life at 20 °C, was investigated. The fruits were not de-greened, waxed, or treated with any postharvest fungicides before and after storage. Chilling injury and decay were prevented by all hot water and curing treatments. Curing treatment at 53 °C for 6 h dramatically reduced decay compared to other treatment and chilling injury were not developed, but this treatment ranked last after 60 days of storage for overall quality. Weight loss, juice yield, total soluble solids, and total soluble solids/acid ratio were higher in cured fruit than in hot water dipped fruit. However, titratable acid, ascorbic acid, and overall quality were lower in cured fruit than control and hot water dipped fruit. There were no differences between heat-treated and control fruit in terms of peel colour. Detrimental effects on quality parameters of mandarins did not occur either in hot water or curing treatments. Overall, it was concluded that pre-storage hot water and curing effectively induce tolerance to chilling injury and they constitute an alternative to chemical treatment for 'Clementine' mandarins without impairing any other quality attributes.

Zusammenfassung

Reduktion von Kälteschäden und Fäulnis bei gelagerten Clementinen mittels Heißwasser- und trockener Wärmebehandlung. Die Wirksamkeit einer Heißwasser- (53 °C, 3 und 6 min lang; 48 °C, 12 min lang) bzw. einer trockenen Wärmebehandlung (53 °C, 1 und 6 h lang; 48 °C, 6 h lang) nach der Ernte hinsichtlich Kälteschäden, Fäulnis und Verbrauchsqualität wurde an Clementinen (*Citrus reticulata* (L.) Osbeck) untersucht, die anschließend zwei Monate bei 1 °C und zur Simulation der Verkaufssituation eine Woche bei 20 °C gelagert wurden. Die Früchte wurden vor und nach der Lagerung weder entgrünt, gewachst noch mit Lagerfungiziden behandelt. Alle Heißwasser- und trockene Wärmebehandlungen verhinderten Kälteschäden und Fäulnis. Im Vergleich zu den übrigen Behandlungen verminderte die Wärmebehandlung bei 53 °C über 6 h die Fäulnis deutlich und Kälteschäden blieben aus, doch lag diese Behandlungsmethode nach 60 Tagen Lagerung in der Gesamtqualität an letzter Stelle. Gewichtsverlust, Saftausbeute, Trockensubstanz und das Trockensubstanz/Säureverhälnis waren bei trockenbehandelten Früchten höher als bei den heißwasserbehandelten. Dagegen waren titrierbare Säure, Ascorbinsäure und Gesamtqualität bei trockenbehandelten Früchten geringer als bei Kontroll- und heißwasserbehandelten Früchten. Hinsichtlich Schalenfarben traten keinerlei Unterschiede zwischen den wärmebehandelten und der unbehandelten Kontrolle auf. Weder bei den Heißwasser- noch bei den Wärme-behandelten Mandarinen wurden nachteilige Effekte auf die Qualitätsparameter beobachtet. Zusammenfassend kann gesagt werden, dass eine vor der Lagerung durchgeführte Heißwasser- oder trockene Wärmebehandlung bei Clementinen eine Kühletoleranz induziert und eine Alternative zur chemischen Behandlung darstellt, ohne dass dadurch andere Qualitätsmerkmale beeinträchtigt würden.

Key words. *Citrus reticulata* – heat treatments – quality – postharvest – storage – deterioration

Introduction

Most fruits in subtropical regions are prone to physiological injury when exposed to low temperatures. Chilling injury and rots are primarily responsible for limiting postharvest life of mandarins similar to other citrus fruits. Rind pitting - also known as pox, chill spotting, brown spot, storage spot, and rind spotting - is the most common symptom of chilling injury in citrus (PAULL 1990). Mandarins and other citrus are sensitive to chilling injury and this sensitivity of mandarins to low temperatures affects negatively postharvest quality of mandarins during storage and marketing.

Postharvest green mould caused by *Penicillium digitatum* Sacc., and postharvest blue mould, caused by *Penicillium italicum* Wehmer, are the most economically important postharvest diseases of 'Clementine' mandarins (PALOU et al. 2002).

The interest in alternative methods for postharvest decay control of citrus and other crops has been continuously growing in order to minimize pre - or postharvest treatments with agrochemicals. During the past few years, heat treatments have attracted increasing interest as a result of the growing demand to reduce the postharvest use of chemical fungicides (PORAT et al. 2000a). Hot water treatment is one of the most easily applied and environmentally safe fruit treatments for decay control (GONZALES-AGUILAR et al. 1997; SCHIRRA and BEN-YEHOSHUA 1999; SCHIRRA et al. 2000). According to BEN-YEHOSHUA et al. (1995) the mode of action of heat treatment in reducing decay of citrus fruits might be explained in part by the enhanced activity of pre-formed anti-fungal substances. WILD and HOOD (1989) demonstrated that very short application of hot water (2 min, 53 °C) treatment reduces significantly sensitivity of oranges to chilling injury. The hot water for 2 min at 52–53 °C inhibited the development of decay in lemons (NAFUSSI et al. 2001).

Curing treatment is generally applied at 34–36 °C for 48–72 h to control postharvest decay of citrus fruits and well described by LURIE (1998). However, no information is available on the effect of curing at high temperatures (48–53 °C) for a short period on mandarins. The exact mode of action of curing in reducing decay of citrus fruit is not clear, although heat treatments induce accumulation of antifungal compounds (PLAZA et al. 2003). According to BEN-YEHOSHUA et al. (1987) maintenance of high relative humidity for curing treatment is essential for the development of the defence mechanism. Below 75 % RH, cells surrounding the injury were damaged by desiccation and were not capable of lignin synthesis.

In this study, our purpose was to compare the effects of hot water and curing treatments at high temperatures for short durations on chilling injury, decay and postharvest quality of 'Clementine' mandarins.

Materials and Methods

Plant material and fruit sampling

'Clementine' mandarins were harvested randomly from the Research Orchard of Faculty of Agriculture, Akdeniz University in Antalya, Turkey and fruit was delivered to the laboratory on the day of harvest, sorted for uniform size and lack of defects, placed in plastic boxes (45 fruit per box) and divided into three treatment groups. The first group of fruit samples was dipped in hot water at 48 °C for 12 min and at 53 °C for 3 and 6 min. The second group of fruit samples was cured at 48 °C for 6 h and at 53 °C for 1 and 6 h. The third group of fruit samples was considered as control group without any treatment.

Postharvest heat treatments

Hot water treatments were performed in a bath tank containing 500 litre of water and fitted with heating elements and an electronic re-circulation pump. Three boxes of fruit were dipped at a time. Water bath tank temperature was constantly maintained within ±0.5 °C of the programmed temperature by an electronic thermostat and probe. Following treatment the fruit was left to dry at 20 °C for 2 h.

Curing treatments were performed by means of an electric heater and the room temperature was constantly, monitored during curing. During curing the relative humidity of the rooms was kept at around 90–92 % by using a humidifier in order to prevent water loss. There were no treatments for the control fruits. Each treatment group was then divided into two sub-groups. The fruit in the first sub-group was numbered, individually weighed and used for the evaluation of chilling injury, decay incidence, heat damage, peel colour, weight losses and overall quality. The second sub-group was used for chemical analyses. Following hot water and curing treatments, 'Clementine' mandarins were stored at 1 °C and 90–92 % RH for 2 months. After 20, 40 and 60 days in storage the fruit was transferred to 20 °C for an additional week for monitoring shelf-life conditions. They were evaluated for chilling injury, decays, heat damage, weight loss, juice yield, total soluble solids (TSS), titratable acids, TSS/acid ratio, ascorbic acid, peel colour and overall quality. Each treatment included three replications with one box in each replicate containing 45 fruit. The experiment was conducted in 2001 and 2002.

Evaluation of chilling injury

After 20, 40 and 60 days of cold storage at 1 °C and an additional week at 20 °C, fruit was sorted into four categories according to their chilling injury severity: none (score 0, no pitting), slight (score 1, a few scattered pits), moderate (score 2, pitting covering up to 30 % of the fruit surface), and severe (score 3, extensive pitting covering >30 % of the fruit surface). The chilling injury index was determined for each treatment by multiplying the number of fruit in each category by their score and dividing the product by the total number of fruit assessed (PORAT et al. 2000b).

Evaluation of decay

Evaluation of decay incidence was performed after 20, 40 and 60 days of storage at 1 °C and an additional week of shelf life at 20 °C. The total number of fruit manifesting decay symptoms (mainly green and blue mould) was determined in each treatment and expressed as the decay percentage (PORAT et al. 2000b).

Chemical analyses, peel colour determination and overall quality

Three replicates of 10 healthy fruit were randomly selected prior to treatments, after cold storage, and after shelf-life condition for assessment of internal quality attributes. The TSS content in the juice was determined with a refractometer, and the titratable acidy percentage was measured by titration with 0.1 N NaOH to pH 8.1, the results being expressed as citric acid. Ascorbic acid content was determined by the 2, 6-dichlorophenolindophenol titration method (ERKAN 1997; RAPISARDA et al. 2001) and juice was extracted by using an electric rotary juicer. Percent juice yield,, was determined by dividing the weight of extracted juice by pre-

treatment fruit weight and multiplying by 100 (SHELLIE and MANGAN 1994; ERKAN 1997). Peel colour was determined by measuring the fruit skin with a Chromo Meter (model CR-200; Minolta Corp., Ramsey, N.J.) at the three marked sites on the surface of each fruit (MCGUIRE 1992). Overall visual and eating quality was rated subjectively into one of five categories: 4 (excellent), 3 (good), 2 (fair), 1 (poor) and 0 (very poor), by an informal panel of 5 people familiar with mandarins.

Data analyses

Analysis of variance (ANOVA) was performed by MSTAT-C software (1991) and means separated by Duncan's multiple range test at P=0.05 level.

Results and Discussion

Influence of different heat treatments on chilling injury

All of the postharvest hot water and curing treatments reduced chilling injury incidence compared to control fruit (Table 1). Between the various hot water and curing treatments tested, curing fruit at 53 °C for 6 h was the most effective in reducing the development of chilling injury incidence. At the end of the 60-day storage period chilling injury index was 0.0 in cured fruit at 53 °C for 6 h compared to 1.23 of control fruit.

Table 1. Influence of different postharvest heat treatments on chilling injury and decayed fruit of 'Clementine' mandarins after 20, 40 and 60 days of storage at 1 °C plus one week at 20 °C.

Storage time (days)	Heat Treatments	Chilling injury index[c]	Decay (%)
20	Control	0.46±0.17 e[d]	0.44±0.06 g
	HWD[a] (48 °C/12 min)	0.00±0.00 h	0.00±0.00 g
	HWD (53 °C/3 min)	0.00±0.00 h	0.00±0.00 g
	HWD (53 °C/6 min)	0.00±0.00 h	0.00±0.00 g
	C[b] (48 °C/6 h)	0.00±0.00 h	0.00±0.00 g
	C (53 °C/1 h)	0.00±0.00 h	0.00±0.00 g
	C (53 °C/6 h)	0.00±0.00 h	0.00±0.00 g
40	Control	1.04±0.07 b	8.24±0.79 b
	HWD (48 °C/12 min)	0.64±0.05 d	6.20±0.26 c
	HWD (53 °C/3 min)	0.48±0.06 e	4.86±0.94 d
	HWD (53 °C/6 min)	0.32±0.04 fg	3.12±0.54 de
	C (48 °C/6 h)	0.28±0.06 g	3.64±1.34 de
	C (53 °C/1 h)	0.00±0.00 h	2.88±0.85 e
	C (53 °C/6 h)	0.00±0.00 h	1.34±0.25 f
60	Control	1.23±0.10 a	14.78±2.60 a
	HWD (48 °C/12 min)	1.09±0.10 ab	8.80±2.11 b
	HWD (53 °C/3 min)	0.99±0.02 bc	5.20±1.06 cd
	HWD (53 °C/6 min)	0.84±0.06 c	4.24±0.35 d
	C (48 °C/6 h)	0.26±0.04 g	3.80±0.69 de
	C (53 °C/1 h)	0.38±0.09 f	3.34±1.12 de
	C (53 °C/6 h)	0.00±0.00 h	1.96±0.49 ef

[a]Hot water; [b]Curing; [c]Chilling injury index : 0 = normal, 1 = slight, 2 = moderate, 3 = severe
[d]Mean separation by Duncan's multiple range test. Values followed by different letters within a column are significantly different at the 5 % level.

Our findings regarding the effects of various postharvest treatments in reducing chilling injury in 'Clementine' mandarins are consistent with those of previous studies of the effects of each of these treatments separately on other citrus cultivars (RODOV et al. 1995; SCHIRRA and MULAS 1995a; SCHIRRA et al. 1997; HOLLAND et al. 2002).

Influence of different heat treatments on decayed fruit

Hot water and curing treatment of 'Clementine' mandarins markedly reduced their decay. After 60 days of storage at 1 °C, hot water treated fruit (48 °C for12 min, 53 °C for 3 min and 53 °C for 6 min) had only 8.80, 5.20 and 4.24 % decay, respectively. The amount of decay was 14.78 % in control fruit at the end of 60 days storage period. Curing treatment especially at 53 °C for 6 h gave the best results in controlling decay and decay percentage was only 1.96 % in cured fruit at 53 °C for 6 h. Decay percentage was 3.80 % and 3.34 % in 48 °C 6 h and 53 °C 1 h cured fruit, respectively. In our study, no decayed fruit was monitored during the first 20 days storage in hot water dipped and cured fruit at 1 °C storage plus additional week at 20 °C. Decay percentage was 0.44 % in control fruit. Curing inoculated oranges at 33 °C for 65 h resulted in a significant reduction in green mould at low temperature (PLAZA et al. 2003). Similar results, were also obtained by SCHIRRA and MULAS (1995b) and MILLER and MCDONALD (1997). In contrast to our results curing treatments in 'Star Ruby' cultivar did not reduce decayed fruit (PORAT et al. 2000b). In this experiment curing treatment was excessive and resulted in heat damage that made the fruit more accessible to pathogens.

Influence of different heat treatments on weight loss, juice yield, ascorbic acid, citric acid, total soluble solids, TSS/acid ratio and overall quality

Weight loss of the hot water dipped fruit after 60 days storage at 1 °C plus an additional week at 20 °C reached to 3.88 %, 3.24 % and 3.41 % in hot water dipped fruit at 48 °C for 12 min, at 53 °C for 3 min and at 53 °C for 6 min, respectively (Table 2). Weight loss of the cured fruit after 60 days storage at 1 °C plus an additional week at 20 °C reached to 3.98 %, 3.56 % and 4.12 % in cured fruit at 48 °C for 6 h, at 53 °C for 1 h and at 53 °C for 6 h, respectively (Table 2).

Juice yield increased during the first 20 days of the storage, then decreased during the rest of the storage period. At the end of the 60-day storage period, except hot water dipped fruit at 53 °C for 3 min, juice yield of the cured and hot water dipped fruit was higher than the initial value (40.87 %).

Ascorbic acid and citric acid content of mandarins decreased during storage and significant differences were found among different heat treatments (Table 2). Similar results were obtained by PORAT et al. (2000b).

Table 2. Influence of different postharvest heat treatments on the postharvest quality of 'Clementine' mandarins after 20, 40 and 60 days of storage at 1 °C plus one week at 20 °C[a].

Storage time (days)	Heat Treatments	Weight loss (%)	Juice yield (%)	Ascorbic acid (mg 100ml^{-1})	Citric acid (%)	TSS (%)	TSS/acid ratio	Overall quality[d]
0			40.87 f	50.23 a	1.43 a	11.40 a	7.97 e	4.0 a
20	Control	1.38 g	42.91 cd	48.19 ab	1.41 a	11.36 a	8.05 e	4.0 a
	HWD[y] (48 °C/12 min)	1.03 h	44.27 b	46.89 b	1.43 a	11.34 a	7.93 e	4.0 a
	HWD (53 °C/3 min)	0.99 h	43.38 c	48.65 ab	1.42 a	11.40 a	8.02 e	4.0 a
	HWD (53 °C/6 min)	1.04 h	44.87 ab	49.23 a	1.44 a	11.38 a	7.90 e	4.0 a
	C[z] (48 °C/6 h)	1.42 g	45.98 a	44.24 c	1.36 b	11.54 a	8.48 d	4.0 a
	C (53 °C/1 h)	1.23 gh	44.69 ab	45.34 bc	1.38 b	11.62 a	8.42 d	4.0 a
	C (53 °C/6 h)	1.38 g	46.34 a	43.45 cd	1.34 c	11.84 a	8.83 d	4.0 a
40	Control	2.16 e	42.52 d	43.67 cd	1.33 c	10.94 b	8.22 de	3.5 b
	HWD (48 °C/12 min)	1.92 f	42.13 de	45.34 bc	1.29 d	11.00 b	8.52 d	3.8 ab
	HWD (53 °C/3 min)	2.03 ef	40.90 f	46.23 b	1.31 cd	11.20 a	8.54 d	4.0 a
	HWD (53 °C/6 min)	1.94 f	43.34 c	45.67 bc	1.30 cd	11.20 a	8.61 d	3.8 ab
	C (48 °C/6 h)	2.12 e	43.29 b	44.64 c	1.23 e	11.72 a	9.52 b	3.3 bc
	C (53 °C/1 h)	2.17 e	43.18 c	44.83 c	1.24 e	11.68 a	9.41 b	3.8 ab
	C (53 °C/6 h)	2.11 e	44.73 ab	45.96 bc	1.20 f	11.96 a	9.97 b	3.0 bc
60	Control	4.18 a	38.91 g	38.48 e	1.15 g	10.42 c	9.06 c	2.5 c
	HWD (48 °C/12 min)	3.88 b	41.34 ef	43.68 cd	1.16 g	10.80 b	9.31 b	3.3 b
	HWD (53 °C/3 min)	3.24 d	37.68 h	42.67 de	1.12 gh	11.00 b	9.82 b	3.6 ab
	HWD (53 °C/6 min)	3.41 cd	42.45 d	43.56 cd	1.15 g	11.00 b	9.56 b	3.7 ab
	C (48 °C/6 h)	3.98 ab	41.73 e	41.76 d	1.09 h	11.35 a	10.41 a	2.4 c
	C (53 °C/1 h)	3.56 c	42.38 d	42.23 d	1.04 i	11.28 a	10.84 a	2.9 bc
	C (53 °C+6 h)	4.12 a	43.52 c	43.75 cd	1.00 i	11.39 a	11.46 a	2.3 c
	Significance[bc]							
	ST	***	**	***	***	**	**	***
	HT	**	**	**	**	**	**	**
	ST x HT	*	**	**	***	**	***	**

[y]Hot water; [z]Curing; [b]ST: storage time; HT: heat treatments;
[c]*significant at 5 % level, **significant at 1 % level, ***significant at 0.1 % level .
[d]Overall quality : 4 = excellent, 3 = good, 2 = fair, 1 = poor, 0 = very poor
[a]Mean separation by Duncan's multiple range test. Values followed by different letters within a column are significantly different at the 5 % level.

There were also statistically significant differences among storage periods.

Total soluble solids increased in cured fruit during the first 40-day of storage period, then decreased during the rest of storage and significant differences were found among different hot water and curing treatments. However, total soluble solids decreased continuously or remained at the same level in hot water dipped and control fruit with increasing storage duration (Table 2).

TSS/acid ratio was higher in cured fruit compared to hot water and control fruit and the longer the storage duration was, the higher was the TSS/acid ratio was (Table 2).

The external appearance and eating quality of control and treated fruit either, with hot water or curing after 20 days was judged to be excellent. After 40-days of storage, external appearance, and eating quality of hot water dipped mandarins declined slightly or remained at the same level as compared to control and cured fruit, but still were judged to be excellent. The overall quality of the hot water dipped mandarins in both external appearance and eating quality still remained acceptable after 60 days of storage, but overall quality was lower in cured fruit than control and hot water dipped fruit (Table 2). SHELLIE and MANGAN (1994) stated that exposure to moist forced air of 'Valencia' oranges at 46 °C did not significantly affect fruit flavour and oranges can toler-

ate exposure to moist forced air at 46 °C for up to 230 min without compromising market quality.

There were no statistical differences between control, hot water dipped or cured mandarins in terms of peel colour (data not shown).

The objective of this research was to test if the heat treatments could reduce decay and chilling injury, and maintain eating quality of 'Clementine' mandarins after 2 months at 1 °C. The present study showed that hot water and curing treatments were a valid method to reduce the development of chilling injury and decay in 'Clementine' mandarins. The mode of action of heat treatment in reducing decay and chilling injury of fresh produce is not clearly understood. However, evidence has accumulated that high temperatures could increase the transcript levels of heat shock proteins (HSPs) (SABEHAT et al.1996; WOOLF et al. 1999; DING et al. 2001; WANG et al. 2001). These HSPs are thought to serve as molecular chaperons that could control correct folding, oligomeric assembly, and transport across membranes or disposal by degradation of other conformer unstable proteins by binding and releasing these proteins. In addition, the molecular chaperones could prevent incorrect interaction within and between non-native polypeptides, which results in their irreversible aggregation. Therefore, these HSPs act to protect other proteins from breaking down and thus, maintain integrity of cells and prevent the tissues from developing chilling injury. Heat treatment could also enhance activity of anti-fungal substances (BEN-YEHOSHUA et al. 1995) rendering the heat treated tissues more resistant to decay. According to RODOV et al. (1995) an increase may occur in the putrescine level that may help to reduce chilling injury and decay in citrus fruits. MCDONALD et al. (1993) stated that hot water treatment increases the natural defence system of the fruit against chilling injury.

We conclude that heat treatments (curing and hot water) are an effective and environmentally safe treatment, which reduces decay and chilling injury in mandarins. In our study, all heat treatments were found to be effective in reducing decay and chilling injury and maintaining the quality of 'Clementine' mandarins, with the treatment of 53 °C for 6 h more effective than the others. However, it should be noted that curing treatments at high temperatures may cause heat damage to sensitive cultivars (PORAT et al. 2000b). In our study we did not observe any heat damage in 'Clementine' mandarins.

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Addresses of authors: Mustafa Erkan (corresponding author), Mustafa Pekmezci, Işılay Karaşahin, Hüseyin Uslu, Department of Horticulture, Faculty of Agriculture, Akdeniz University, 07059 Antalya, Turkey, e-mail: erkan@akdeniz.edu.tr.