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A REFEREED PAPER

SHORT-DURATION, HOT WATER TREATMENT FOR THE CONTROL OF CHILLING INJURY AND POSTHARVEST DECAY IN CITRUS

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Abstract. **Hot water treatments have been studied and used as non-chemical methods to reduce postharvest decay and chilling injury (CI) in fresh citrus fruit. While many studies have been performed in Mediterranean climates, there exists relatively little work evaluating the effects of hot water on Florida grapefruit quality and quality retention during postharvest handling. In current studies, 'Ruby Red' grapefruit dipped in water at 56 or 59 °C for 30 s developed 18% or 32%, respectively, less CI after storage at 5 °C for 6 weeks plus 1 week at 16 °C, compared to fruit dipped at 25 °C. The fruit were not washed or coated with shellac and no fungicides were used. Hot water dip treatment (HWDT) had the greatest effect on reducing CI of less CI-sensitive inner-canopy fruit (32%) compared to more CI-sensitive outer-canopy fruit (10%). In a separate experiment, washing and coating the fruit with shellac (no fungicide) immediately after the 30 s HWDT significantly reduced scalding (i.e., hot water injury) by 45% or 37% in fruit treated at 59 or 62 °C, respectively, compared to unwashed and uncoated fruit. Fruit treated at 56 or 59 °C developed less total decay after 12 weeks of storage at 10 °C than did 25, 53 or 62 °C-treated fruit. None of the treatments resulted in consistent differences in total soluble solids or titratable acidity in grapefruit. Higher electrolyte leakage and lower peroxidase activity were observed in heat-treated 'Valencia' oranges, but there was no correlation with visible heat injury. HWDT did not affect total phenolics or total protein content of 'Valencia' oranges.**

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The increasing demand for fresh fruits and vegetables with reduced residues of synthetic fungicide has led to the development and increased use of non-chemical methods to control postharvest diseases. Short-duration (as brief as 20 s) hot water treatment (HWT) is one physical method that can effectively reduce postharvest decay on fresh fruits and vegetables (Ben-Yehoshua et al., 2000; Lanza et al., 2000). For example, Lanza et al. (2000) reported that hot water dip at 52 °C for 180 s was as effective as non-heated imazalil in controlling postharvest decay of lemon. In addition, brushing grapefruit for 20 s with 56, 59, or 62 °C water reduced decay by 20%, 5% or 1%, respectively, compared to the control (Porat et al., 2000). Short-duration, hot water brushing is currently used in Israel for cleaning and disinfecting fresh fruits and vegetables (Ben-Yehoshua et al., 1998; Fallik et al., 1999; Prusky et al., 1999). Hot water drench at 62.8 °C for 30 s reduced green mold incidence to 14.5% and 9.4% on California lemons and oranges, respectively, compared to 97.9% and 98.0% on untreated lemons and oranges, respectively (Smilanick et al., 2003).

In addition to reducing postharvest decay, HWT also reduces the incidence of chilling injury (CI) (Rodov et al., 1995; Schirra et al., 1997). For example, grapefruit dipped in 53 °C water for 3 min had 40% less CI than the control, and developed only 2.5% decay compared to 60% in the control (Rodov et al., 1995). CI is a physiological disorder that is most often characterized by areas of the peel that collapse and darken to form pits. CI symptoms generally require at least 3 to 6 weeks to develop at low (e.g. 4.4 °C) shipping and storage temperatures.

The present study was conducted on Florida citrus to 1) determine the optimum temperature for a short-duration, hot water dip treatment (HWDT), and 2) study the physiological responses of citrus to HWDT.

Materials and Methods

Effect of HWDT on CI of 'Ruby Red' Grapefruit. 'Ruby Red' grapefruit were harvested on 3 Nov. 2003 at the Indian River Research and Education Center (IRREC) research grove in Fort Pierce, Fla. Fruit were harvested from 1-1.5 m above ground level on the tree, from the inner and outer canopy, and stored at room temperature overnight before receiving their respective HWDT. Fruit were dipped in water at 25, 53, 56, or 59 °C for 30 s. Dips were conducted using stainless steel tanks (Hogan Bros. Welding, Ft. Pierce, Fla.) holding ~95 L of rapidly stirred water. Heating was accomplished using a gas burner with the temperature varying by ~±1 °C during each treatment. Fruit were treated by placing them in perforated plastic crates that allowed water to circulate around the fruit. Each treatment had four replications and there were 30 fruit in each replicate. The fruit were not washed or coated with shellac and no fungicides were used. Inner- and outer-canopy fruit were kept separate. After the HWDT, half the fruit in each treatment and canopy position were stored at 5 °C (90%

RH) and the other half stored at 16 °C (90% RH). Five fruit from each replicate were randomly selected before storage and initial weights measured to follow weight loss during storage. Total soluble solids (TSS), titratable acidity (TA), peel puncture resistance (PPR) and percent juice were measured on four sets of five fruit each taken randomly from the initial harvested fruit population.

Juice TSS (°Brix) was measured using a refractometer (Abbe-3L, Spectronic Instruments Inc., Rochester, N.Y.) and the juice TA (% citric acid) was measured by titrating juice samples to pH 8.3 with NaOH using an automatic titrimeter (DL 12, Mettler-Toledo Inc., Columbus, Ohio). Peel puncture resistance was measured at two equidistant spots along the equator of each fruit using a texture analyzer (Model TAXT2i, Stable Micro Systems, Godalming, England) with a 2 mm diameter, flat-tipped, cylindrical probe. The analyzer was set such that the probe traveled at a speed of 2 mm s^{-1} and the maximum force exerted to puncture the peel was recorded. Peel puncture resistance is expressed in Newton. Percent juice was calculated from the total weight of fruit and total weight of juice.

Fruit were evaluated for peel scalding 1, 3, and 7 weeks after treatment. Weight loss was measured on designated fruit after 4 and 7 weeks in storage. After 4 weeks of storage, TSS, TA, PPR, and percent juice were evaluated from five fruit per replicate. Six weeks after the HWDT, fruit stored at 5 °C (90% RH) were transferred to 16 °C (90% RH) and evaluated for CI and decay after an additional 7 d. CI was rated from 0 to 3 (0-none, 1-slight, 2-moderate, and 3-severe). The number of fruit in each rating was multiplied by its corresponding rating number and the sum of these products was divided by the total number of fruit in the replicate to give an average CI for that replicate.

Effects of Washing and Coating on the Response of 'Ruby Red' Grapefruit to HWDT. 'Ruby Red' grapefruit were harvested on 12 Nov. 2003 at the IRREC research grove. Fruit were harvested from 1-1.5 m above ground level on the tree, stored at room temperature overnight, and HWDT administered the following day. Fruit were dipped in 25, 53, 56, 59, or 62 °C water for 30 s. There were three post dip treatments:

1. Hot water dip only without any post dip treatment

3. Hot water dip, followed immediately by a 1 min dip in water at ambient (~25 °C) temperature

4. Hot water dip, followed immediately by washing and coating (simulated commercial packinghouse treatment)

Hot water dip treatment was conducted as described in the previous experiment. Each treatment had four replications and there were 40 fruit per replicate. Fruit were washed over a brush bed, then coated with shellac (Sta-Fresh 590 HS, FMC Corporation, Lakeland, Fla.), and dried using a small, heated, forced-air dryer to simulate commercial handling. Fungicides were not used. Following HWDT and post dip treatment, the fruit were stored at 10 °C (90% RH). Ten fruit from each replicate were randomly selected, marked, and initial weights measured to follow weight loss during storage. Total soluble solids, TA, PPR, and percent juice were also measured on four sets of 10 fruit from the initial sample population. Fruit were evaluated for peel scalding 1 and 4 weeks after treatment. After 4 weeks of storage, marked fruit were evaluated for weight loss, TSS, TA, PPR, and percent juice. Decay was evaluated after 4, 8, and 12 weeks in storage.

Physiological Responses of 'Valencia' Orange to HWDT. 'Valencia' oranges were harvested on 28 July 2003 from the inner canopy of trees at the IRREC research grove. The fruit were harvested in the morning and on the same day dipped in water at 60 or 66 °C for 60 s. Hot water dip treatment was administered in a temperature-controlled water bath (Optima series immersion circulators, Boekel Scientific, Feasterville, Pa.). Control fruit were not dipped in water. Each treatment had three replicates of five fruit each. Four sets of each treatment were done; one set was evaluated immediately after HWDT and the other sets were stored at 10 °C (90% RH) and evaluated after 2, 4, or 7 d. At each evaluation, electrolyte leakage, total phenolics, protein content, and peroxidase activity were measured in the flavedo.

Electrolyte leakage was determined following the method described by McCollum and McDonald (1991). Protein was determined using the Lowry assay (Lowry et al., 1951) and peroxidase activity was determined following the method described by Worthington (1972). For phenolics estimation, the method described by Swain and Hillis (1959) was followed.

Statistical Analysis. Percentage data were transformed to arcsine values and analyzed by ANOVA using SAS (PROC GLM) for PC (SAS Institute Inc., Cary, N.C.). When differences were significant ($P < 0.05$), individual treatment means were separated using Duncan's Multiple Range Test ($P = 0.05$). Means presented are untransformed values.

Results and Discussion

Effect of HWDT on CI of 'Ruby Red' Grapefruit. Compared to fruit dipped at 25 °C, dipping fruit in 53, 56, or 59 °C water reduced CI by 3%, 6% or 10%, respectively, in outer canopy fruit stored at 5 °C, and reduced CI by 11%, 18% or 32%, respectively, in inner canopy fruit stored at 5 °C (Fig. 1). Hence, HWDT had a greater effect on reducing CI of inner canopy fruit than of outer canopy fruit. Purvis (1980) previously reported that outer canopy fruit are more susceptible to CI than inner canopy fruit. However, our results indicated little effect of canopy position on non-heated fruit. Thus, heat treatment by itself had a major role in reducing CI in inner canopy fruit. None of the fruit stored at 16 °C developed CI.

Fruit dipped in 59 °C water developed significantly less decay than did fruit from other treatments after 6 weeks of

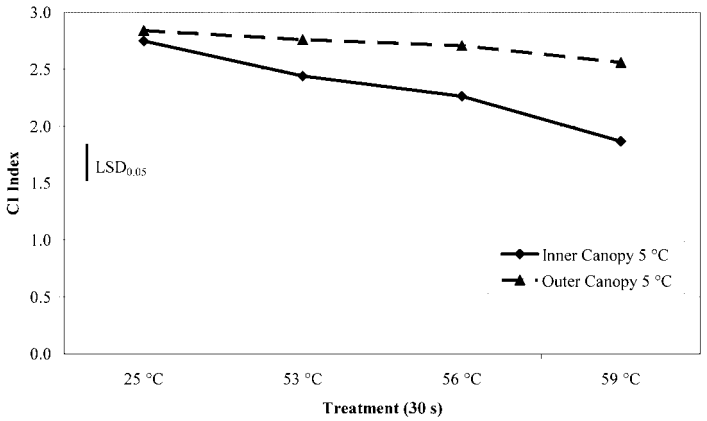

Fig. 1. Chilling injury (CI) of 'Ruby Red' grapefruit after 30-s hot water dip treatments and storage for 6 weeks at 5 °C (90% RH) plus 1 week at 16 °C (90% RH). CI was rated from 0 (none) to 3 (severe). Vertical bar represents the 5% LSD value.

Table 1. Peel puncture resistance (PPR), decay, and weight loss of 'Ruby Red' grapefruit after 30-s dip treatments in water at the designated temperatures. Fruit were stored for 6 weeks at 5 or 16 °C (90% RH), plus 1 week at 16 °C (90% RH).

	4 weeks[z]		7 weeks			
	PPR (N)		Total decay (%)		Weight loss (%)	
Treatment	5 °C[y]	16 °C	5 °C[y]	16 °C	5 °C[y]	16 °C
25 °C	17.86 b[x]	14.04 b	56.50 b	1.00	4.38 a	3.80 a
53 °C	18.15 b	14.45 b	42.50 b	2.50	3.92 b	3.58 b
56 °C	18.31 b	14.14 b	44.63 b	3.02	3.84 b	3.21 b
59 °C	19.27 a	15.04 a	31.50 a	0.50	4.07 b	3.49 b
Significance	*	*	*	ns	*	*

[z]Weeks after storage at 5 °C or at 16 °C.
[y]Values for each treatment temperature are averages of inner and outer canopy fruit.
[x]Values within each column followed by different letters are significantly different by Duncan's multiple range test at $P < 0.05$.
*Significant at $P \leq 0.05$.
ns = Not significant at $P \leq 0.05$.

storage at 5 °C plus 1 week at 16 °C (Table 1). Decay did not differ significantly among fruit dipped in 25, 53 or 56 °C water. Interactions between treatment temperature, canopy position, and storage temperature on decay were not significant. Decay was low and not significant among heat-treated fruit continually stored at 16 °C. Most of the decay was due to anthracnose (*Colletotrichum gloeosporioides*; data not shown) with a high incidence of anthracnose observed on fruit that developed CI. As a result, fruit stored at the chilling temperature (5 °C) developed more decay (44%) than did fruit stored at the non-chilling (16 °C) temperature (2% decay).

After 3 weeks of storage, 2% of fruit treated at 59 °C developed visible peel scalding (data not shown). No scalding was observed on fruit dipped in 25, 53 or 56 °C water. Hot water dip treatment did not affect the percent juice, TSS, or TA of the fruit (data not shown). Peel puncture resistance was significantly greater in fruit treated at 59 °C than in all other treatments. At the end of the experiment, weight loss from fruit treated at 25 °C was significantly greater than from the other three treatment temperatures (Table 1). The weight loss was higher in the fruit stored at 5 °C than the fruit stored at 16 °C. Higher weight loss at 5 °C was likely due to accelerated weight loss in fruit that developed CI. Purvis (1984) has correlated higher weight loss during storage with CI development in citrus fruit. Cohen et al. (1994) have used weight loss as an early indicator of CI.

Effects of Washing and Coating on the Response of 'Ruby Red' Grapefruit to HWDT. After 4 weeks of storage at 10 °C, 25% and 62% of the fruit dipped in water at 59 or 62 °C, respectively, for 30 s developed peel scalding (Table 2). None of the fruit dipped in water ≤56 °C developed scalding. In addition, grapefruit dipped in 56 or 59 °C water developed significantly less decay after 12 weeks of storage at 10 °C than did fruit exposed to higher or lower water temperatures (Table 2). Fruit dipped in 62 °C water were injured by the treatment which likely negated any beneficial effects of the HWDT. Hot water (Miller et al., 1988) and vapor heat (Hallman et al., 1990) treatments of grapefruit that caused scalding were reported to also result in increased decay, which was suggested to be a result of the damaged tissue being more susceptible to pathogen invasion. Heat treatment did not affect the sugar/acid ratio or the amount of juice (Table 2). There were no consistent differences in weight loss or peel puncture resistance.

Washing and coating the fruit immediately after HWDT significantly reduced the development of peel scalding (Table 3). Only 13% of the fruit developed scald when washed and coated, whereas 21% of the fruit developed scald when they were not washed and coated after heat treatment. Peel scalding was not significantly different when fruit were dipped in ambient water immediately after HWDT than fruit that received only HWDT.

Table 2. Percent of fruit scalded, weight loss, peel puncture resistance (PPR), juice content, and total soluble solids: titratable acidity ratio (TSS/TA) of 'Ruby Red' grapefruit after 30-s dip treatments in water at the designated temperatures and storage at 10 °C (90% RH) for the indicated durations.

	4 weeks[z]					12 Weeks
Treatment	Scald (%)[y]	Weight loss (%)	PPR (N)	Juice (%)	TSS/TA	Decay (%)
25 °C	0.00 c[x]	3.12 ab	16.76 a	57.90	8.79	62.26 a
53 °C	0.00 c	2.70 c	15.47 b	58.65	8.61	69.85 a
56 °C	0.00 c	2.74 bc	15.73 b	58.65	8.67	40.98 b
59 °C	25.42 b	2.95 bc	15.83 b	58.95	9.16	40.66 b
62 °C	61.88 a	3.43 a	17.49 a	58.91	9.73	65.94 a
Significance	*	*	*	ns	ns	*

[z]Weeks after storage at 10 °C with 90% relative humidity.
[y]Values for each treatment temperature are averages of all the three coating treatments (none, water dip and shellac).
[x]Values within each column followed by different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$.
*Significant at $P \leq 0.05$.
ns = Not significant at $P \leq 0.05$.

Table 3. Percent of fruit scalded, weight loss, peel puncture resistance (PPR), juice content, and total soluble solids: titratable acidity ratio (TSS/TA) of 'Ruby Red' grapefruit after 30-s hot-water dip treatments immediately followed by: 1) no post-dip treatment, 2) an ambient water dip, or 3) a shellac coating. Fruit were then stored at 10 °C (90% RH) for the indicated durations.

	4 weeks[z]					12 Weeks
Treatment	Scald (%)[y]	Weight loss (%)	PPR (N)	Juice (%)	TSS/TA	Decay (%)
None	20.50 a[x]	2.89	16.48 a	58.40	9.24	60.72 b
Water dip	19.38 a	3.10	16.51 a	58.41	8.51	71.87 a
Shellac	12.50 b	2.97	15.77 b	59.05	9.22	35.23 c
Significance	*	ns	*	ns	ns	*

[z]Weeks after storage at 10 °C with 90% relative humidity.
[y]Values for each treatment are the averages of all three temperatures.
[x]Values within each column followed by different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$.
*Significant at $P \leq 0.05$.
ns = Not significant at $P \leq 0.05$.

Though others have reported the development of CI of Florida grapefruit stored at 10 °C (Grierson and Hatton, 1977), it is fairly uncommon commercially because of the almost universal use of wax coatings that restrict gas exchange to varying degrees and reduce chilling sensitivity. Early season fruit (September-November) are more susceptible to CI than fruit harvested during the middle of the season (December-February) (Grierson and Hatton, 1977; Schirra et al., 2000). The fruit utilized for the current studies were still very chilling sensitive and developed CI during storage at 10 °C. Hot water dip at 56 or 59 °C reduced the development of CI to 19% or 13%, respectively, whereas 42% of fruit dipped at 25 °C developed CI (data not shown).

Physiological Responses of 'Valencia' Orange to HWDT. All 'Valencia' oranges dipped in 66 °C water for 60 s developed peel scalding within 7 d of HWDT, whereas 20% of the fruit dipped in 60 °C water developed scalding (Fig. 2). Significantly higher electrolyte leakage was also observed in the flavedo of 66 °C dipped fruit immediately after HWDT and throughout the 7 d storage period (Fig. 3). Electrolyte leakage from the flavedo of fruit treated at 60 °C was not significantly different from the control.

Peroxidase activity in the flavedo of fruit treated at 66 °C was lower than the control and 60 °C-treated fruit (data not shown). Higher electrolyte leakage and lower peroxidase activity were observed only from fruit that were dipped at 66 °C, which also resulted in significant scalding. Heat treatment did not significantly affect total phenolics or total protein content in the peel (data not shown). Peel browning is generally caused by the oxidation of phenols mainly by the enzymes polyphenol oxidase (PPO) and peroxidase (Lattanzio et al., 1994). The total phenolics did not change with heat treatment and there was lower peroxidase activity in heat-treated compared to control fruit. So browning could be due to oxidation by PPO. Martínez-Tellez and Lafuente (1993) have reported that chilling induced browning had no correlation with PPO and peroxidase activities. There are also non-enzymatic browning reactions in which colored complexes are formed by the interactions between phenolics and heavy metals (Lattanzio et al., 1994). These could have also contributed to the peel browning due to HWDT.

Conclusions

Hot water dips at 56 or 59 °C for 30 s significantly reduced CI and decay development in Florida grapefruit. However, treatment at 59 °C for 30 s resulted in some peel scalding. Our results indicate that hot water dip at 56 °C for 30 s was the safest treatment for grapefruit. Although this treatment reduced

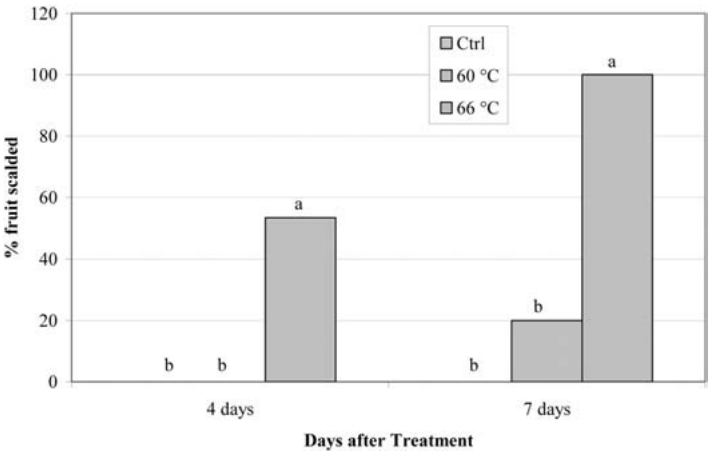

Fig. 2. Percent of fruit scalded in 'Valencia' oranges after 30-s hot water dip treatments and storage at 10 °C (90% RH) for 4 and 7 d. Bars within each day with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$.

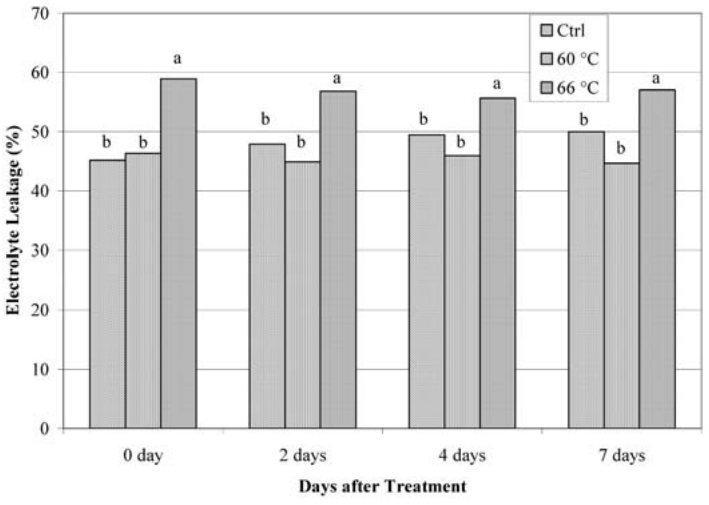

Fig. 3. Electrolyte leakage in 'Valencia' orange flavedo tissue immediately after 30-s hot water dip treatment and after storage at 10 °C (90% RH) for 2, 4, or 7 d after treatments. Bars within each day with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$.

decay development, it did not completely control the decay. Further research should be conducted using heated solutions of compounds generally recognized as safe like sodium carbonate, sulfur dioxide or ethanol that have shown some success in reducing postharvest decay in citrus (Smilanick et al., 1997). In addition, further work adding fungicides to the short-duration hot water solutions is warranted because heated fungicide solutions have been reported to be more effective than non-heated solutions and therefore fungicides could be used at lower concentrations (Schirra and Mulas, 1995).

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