HEAT TREATMENTS FOR CONTROLLING POSTHARVEST DISEASES AND CHILLING INJURY IN FLORIDA CITRUS

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2004

This document is dedicated to my parents Dr. J. John Karuppiah and Mrs. J. Geetha.

ACKNOWLEDGMENTS

I would like to express my sincere thanks and gratitude to Dr. Mark A. Ritenour, my committee chair, for giving me the opportunity to work on postharvest physiology and for his valuable advice and guidance throughout my master's program. I thank my committee member Dr. Jeffrey K. Brecht who initially taught me to organize and conduct experiments. I would like to extend my thanks and gratitude to Dr. T. Gregory McCollum, my committee member, for teaching me numerous laboratory techniques and for helping me to statistically analyze my results. I would like to thank Kim Cordasco and Michael S. Burton for their valuable help in conducting my experiments.

I thank my parents Dr. J. John Karuppiah and Mrs. J. Geetha, my sister R. Denniz Arthi and her family for their love and encouragement during my whole life. I thank my friends S.K. Ashok Kumar, A. Nithya Devi, M. Janakiraman, R. Rajesh and other friends in India and in USA for their friendship and support.

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August 2004

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For years, heat treatment has been tested and used on various fresh horticultural commodities as a non-chemical method of reducing postharvest diseases, insects, and physiological disorders such as chilling injury (CI). In citrus, the increasing demand for fruit with less or no synthetic fungicide residues has led to the development and increased use of hot water (HW) treatments, especially in Mediterranean climates such as those found in Israel and California. However, the efficacy of these treatments on citrus under subtropical Florida conditions is unclear.

In evaluation of physiological effects of HW dip on Florida citrus, rind discs of 'Valencia' oranges dipped in 66, 68 or 70 °C water for 60 s had higher electrolyte leakage immediately after HW dip than non-treated fruit. In another experiment, HW-dipped fruit (66 °C for 60 s) had higher electrolyte leakage and lower peroxidase activity than non-treated fruit. In all these treatments, 100% of fruit developed scalding. Dipping 'Valencia' oranges in 60 °C water for 60 s caused about 20% scalding, but did not

increase electrolyte leakage. Higher electrolyte leakage and lower peroxidase activity were only observed when peel injury was severe. Hence, these parameters cannot be used as early indicators of heat injury.

Treating grapefruit with ethylene (2-3 ppm for 3 d) before or after HW dip at 62 °C for 60 s did not affect the response of grapefruit to HW dip. Washing and coating grapefruit with shellac immediately after HW dip at 56 or 59 °C reduced peel scalding by 45% or 37%, respectively, compared with fruit that were not washed and coated. Dipping grapefruit at 56 or 59 °C for 30 s followed by washing and shellac coating reduced incidence of CI to 2% or 1%, respectively, compared with 50% CI for fruit dipped at 25 °C followed by no post-dip treatment. In another experiment, HW dip 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 with fruit dipped at 25 °C. Hot water dip was more effective in reducing CI of inner canopy fruit (32%) compared with outer canopy fruit (10%).

After 12 weeks of storage, November-harvested grapefruit dipped in water at 56 or 59 °C for 30 s developed 25% or 18% decay, respectively, compared with 40% decay in fruit dipped at 25 °C water. In an experiment using February-harvested fruit, dipping in heated solutions of 3% or 6% sodium carbonate increased the incidence of stem-end rot by 50% compared with fruit dipped in water alone. Dipping fruit in heated solutions of 125 or 250 ppm imazalil did not have any effect in reducing decay after 12 weeks of storage. This could have been due to low incidence of decay in February-harvested fruit.

Dipping grapefruit in 59 °C water for 30 s induced significant peel scalding in all the experiments whereas treatment at 56 °C for 30 s did not induce peel scalding. Hot water dip treatment at 56 °C for 30 s followed by washing and shellac coating was effective in reducing postharvest decay and CI without causing damage to the peel.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

The origin of almost all citrus species was probably the southern slopes of the Himalayas in northeastern India and adjacent Burma. The origin of trifoliate orange and kumquat is eastern China. In Florida, sweet oranges could have been introduced in 1565 when the colony at St. Augustine was established. The important species of citrus are *Citrus sinensis* (sweet orange), *C. paradisi* (grapefruit), *C. reticulata* (mandarin), *C. aurantifolia* (sour lime) and *C. grandis* (pummelo). Citrus production in the United States is second only to Brazil. However, the U.S. ranks first worldwide in grapefruit production. Commercial citrus production in the U.S. is limited to the states of Florida, California, Texas and Arizona. Florida accounts for 74% of the total U.S. citrus production, California produces 23% and Texas and Arizona contribute the remaining 3% (NASS, 2003).

Though Florida is the number one grapefruit producer in the world, less than 50% of the grapefruit produced in Florida is suitable for the fresh market primarily due to cosmetic defects (NASS, 2003). In 2002-03, 65% of fresh grapefruit was exported from the U.S., of which 36% was exported to Japan (Fla. Dept. Citrus, 2004).

Because of the increased attention on the importance of fresh fruits and vegetables as part of good human nutrition, consumption of fresh fruits and vegetables continues to increase. Furthermore, worldwide marketing and shipment of produce has increased the requirement for maintaining fruit and vegetable quality throughout extended shipping and storage durations. For a 50% packout of fresh Florida grapefruit, meaning that 50% of the

fruit are suitable for fresh market, harvesting and postharvest handling accounts for 63% of the total cost of production and postharvest handling (University of Florida, 2003; University of Florida, 2004). Fresh grapefruit shipped from Florida to Japan must maintain its quality throughout the almost 30-d transit, plus an additional 1 to 3 months in storage at destination warehouses. During this time, as with most commodities, decay represents one of the greatest sources of product and economic loss. Grapefruit exported from the U.S. must meet U.S. grade standards of U.S. Fancy, U.S. #1 Bright, or U.S. #1. The tolerance level for decay for these grades is only 3% (USDA, 1997). So, reducing postharvest decay of citrus is of critical importance for maintaining Florida's competitiveness, especially in international markets.

The susceptibility of fresh horticultural commodities to postharvest diseases increases during prolonged storage as a result of physiological changes in the fruits and vegetables that enable pathogens to develop (Eckert and Ogawa, 1988). Postharvest chemical treatments are very effective in controlling decay and are widely used on citrus. Recently there has been an increased demand for fresh horticultural commodities with less or no chemical residues. A number of fungicides are no longer registered for use on fresh citrus, including those that were used to effectively control postharvest diseases. There are only three fungicides (imazalil, thiabendazole and sodium o-phenylphenate) currently registered for postharvest use on citrus and there are problems like development of resistant pathogenic strains and environmental concerns in disposing the chemicals. To minimize pre- or postharvest treatments of fresh citrus with synthetic fungicides, research efforts are currently focused on enhancement of host resistance to pathogens through

physical, chemical, or biological agents (Ben-Yehoshua et al., 1988, 1997; Wilson et al., 1994).

Of the physical treatments, prestorage heat treatment appears to be a promising method of decay control for fresh horticultural commodities in general (Couey, 1989; Klein and Lurie, 1991; Schirra and Ben-Yehoshua, 1999). Many fresh horticultural products can tolerate temperatures of 50 °C to 60 °C for up to 10 min, but shorter exposure at this temperature can control many postharvest diseases (Barkai-Golan and Phillips, 1991). Heat treatments may be applied to fruits and vegetables via: 1) long-duration (e.g., 3-7 d) curing at warm temperatures and high relative humidity (RH) (Kim et al., 1991), 2) long- or short-duration hot water (HW) dips (Schirra and Ben-Yehoshua, 1999), 3) vapor heat (Hallman et al., 1990a; Paull, 1994), 4) hot dry air (Lurie, 1998a, b; Schirra and Ben-Yehoshua, 1999) or 5) short-duration (< 1 min) HW sprays (Fallik et al., 1996).

Curing

Curing involves heat treatment for relatively long periods (i.e., 3-7 d). The first curing experiments on citrus fruit were conducted by Fawcett in 1922 to reduce *Phytophthora citrophthora*. In this case, fruit were held for 1-3 d at 30-36 °C in a water-saturated atmosphere. Ben-Yehoshua et al. (1987 a, b) showed that curing of seal-packaged citrus fruit at 36 °C and saturated humidity for 3 d effectively reduced decay without damage during subsequent storage at 17 °C for 35 d. Curing citrus fruits increases their resistance to green mold development (Brown and Barmore, 1983; Kim et al., 1991). Postharvest curing at 34-36 °C for 48-72 h effectively controls citrus decay

and reduces chilling injury (CI) symptoms (Ben-Yehoshua et al., 1987b; Del Rio et al., 1992).

Curing lemons for 3 d at 36 °C reduced the decline of anti-fungal compounds (that inhibit germ tube elongation) in the flavedo tissue, reduced the loss of citral, and thus suppressed decay development (Ben-Yehoshua et al., 1995).

In spite of its beneficial effects on reducing decay and CI in different citrus fruits, curing is not widely utilized on a commercial scale for citrus. Practical implementation of curing is difficult because of the long treatment durations, which result in fruit damage, and the high cost of heating large volumes of fruit for up to 3 d (Schirra et al., 2000).

Vapor Heat

At temperatures higher than what is normally used for curing, the major obstacle to widespread use of heat to reduce postharvest disease or insect infestation is the sensitivity of many fruits to the high temperatures required for effective treatment (Couey, 1989). For example, navel oranges, lemons, and avocados grown in California were easily damaged by the vapor heat treatment (Sinclair and Lindgren, 1955). Visible heat damage was reported for grapefruit exposed to forced vapor at 46 °C for 3.75 h (Hallman et al., 1990b). Flavor and appearance of grapefruit air-heated at 46 °C for 3 h with controlled atmosphere were inferior to those of non-heated fruit (Shellie et al., 1997). Vapor heat treatment at 59 °C for 180 s resulted in severe peel scalding of grapefruit (over 77% of the fruit surface) (Ritenour et al., 2003).

Hot Water Dipping

Hot water dip treatments are applied for only a few seconds to minutes at temperatures higher than those used for vapor heat or hot air. Hot water dips have been

used for many years as non-chemical methods to control postharvest decay in various fruits and vegetables (Barkai-Golan and Phillips, 1991; Couey, 1989; Lurie, 1998b). Fawcett (1922) conducted the first studies using HW treatments and first reported control of decay in oranges. For citrus, HW dips (2-3 min at 50-53 °C) were shown to be as effective as curing for 72 h at 36 °C in controlling postharvest decay and CI in various citrus fruits and are much less expensive, mainly because of shorter treatment duration (Rodov et al., 1993, 1995a).

Dipping grapefruit in water at 53 °C for 3 min resulted in about 50% reduction in decay (Rodov et al., 1995a). Ben-Yehoshua et al. (2000) reported that the effective temperature range for 2 min grapefruit dip treatments is between 51 and 54 °C; temperatures above 54 °C caused brown discoloration of the peel and temperatures below 51 °C were not effective in reducing decay.

Hot water dips at 52 °C for 3 min have been shown to reduce green mold in organic lemon inoculated with the spores of *Penicillium digitatum* (Lanza et al., 2000). In 'Bianchetto' and 'Verdello' lemons, there was only 1% and 0% decay, respectively, in the HW dip-treated fruit, which was as effective as non-heated imazalil treatment (1g a.i./L), which had no decay. The untreated 'Bianchetto' and 'Verdello' fruit developed 86% and 75% decay, respectively.

Most work on HW treatments of citrus has evaluated their effectiveness in reducing decay from *Penicillium* molds. While these represent the most important cause of citrus postharvest decay worldwide (Eckert and Brown, 1986), stem-end rot (SER; primarily from *Lasiodiplodia theobromae*) commonly is the primary cause of postharvest decay in Florida. Ritenour et al. (2003) reported that grapefruit dipped in water at 56 °C for 120 s

developed SER in only 8% of the fruit compared with 33% of the fruit with SER in fruit dipped in ambient water. However, they found very few instances where water temperatures and dipping times that reduced SER did not also injure the fruit.

Fungicides in heated solutions (50-60 °C) are more effective at controlling decay than non-heated solutions. Heated solutions leave higher fungicide residues on the fruit than non-heated solutions and are therefore effective even at much lower concentrations (Cabras et al., 1999). Heated solutions of thiabendazole (TBZ) and imazalil are more effective at controlling postharvest decay in citrus than non-heated solutions (McDonald et al., 1991; Schirra and Mulas, 1995a, 1995b; Wild, 1993).

Heated solutions of compounds generally recognized as safe (GRAS) like sulfur dioxide, ethanol, and sodium carbonate have been found to be efficient in controlling green mold in citrus (Smilanick et al., 1995, 1997). For example, navel and 'Valencia' oranges inoculated with *Penicillium digitatum* spores, and after 24 h immersed in 2%, 4% or 6% sodium carbonate solutions for 1 or 2 min at 35.0, 40.6, 43.3, or 46.1 °C, had 40%-70% less green mold than in fruit dipped in water alone (Smilanick et al., 1997). Treatments of 4% or 6% sodium carbonate at 40.6 or 43.3 °C provided better decay control than did 2% sodium carbonate at 35.0 or 46.1 °C.

In contrast to the report of Smilanick et al. (1997), Palou et al. (2001) found that temperature of the sodium carbonate solution had more effect than concentration of the sodium carbonate. Treating oranges in 3% or 4% sodium carbonate solution for 150 s at 45 °C reduced the incidence of green mold and blue mold to 1% and 14%, respectively, whereas the hot water without sodium carbonate reduced the incidence of green mold and blue mold to 12% and 27%, respectively. The untreated fruit had 100% decay incidence.

Palou et al. (2002) evaluated the effects of dipping 'Clementine' mandarins in 0%, 2% or 3% sodium carbonate solutions at 20, 45 or 50 °C for 60 or 150 s. Fruit were artificially inoculated with *Penicillium digitatum* 2 h prior to treatment. Treatment with 45 or 50 °C water alone did not effectively control the decay. Adding sodium carbonate to the solution enhanced decay control compared with water alone at all temperatures and immersion periods. Dipping the fruit in 3% sodium carbonate solution at 50 °C for 150 s completely controlled decay development. At lower temperatures, sodium carbonate solution was more effective than water alone and reduced the risk of peel damage by heat.

Hot Water Brushing

Recently, interest has been focused on short-duration HW rinsing and brushing of fresh fruits and vegetables (Fallik et al., 1996). In this method, HW is sprayed over the produce as it moves along a set of brush rollers, thus, simultaneously cleaning and disinfecting the produce (Porat et al., 2000a). Hot water brushing (10-30 s at 55-64 °C; Israeli patent 116965) has been commercially used in Israel with bell peppers (Fallik et al., 1999), mangoes (Prusky et al., 1999), kumquat (Ben-Yehoshua et al., 1998), citrus (Porat et al., 2000a), and several other crops to reduce postharvest decay. Hot water brushing of grapefruit for 20 s at 56, 59 or 62 °C, reduced decay by 80%, 95%, and 99%, respectively, compared with brushing in ambient water (Porat et al., 2000a).

In separate experiments, 'Oroblanco' citrus fruit (a pummelo-grapefruit hybrid) were either dipped in water for 2 min at 52 °C or HW brushed (10 s at 52, 56 or 60 °C) (Rodov et al., 2000). After air-drying and waxing, dipped fruit developed significantly less decay than did the non-treated fruit. Decay development was less after HW brushing

for 10 s at 56 or 60 °C than after HW brushing at 52 °C, but was higher than HW-dipped fruit.

For grapefruit, HW brushing at 62 °C for 20 s significantly reduced green mold even when the fruit were inoculated with *Penicillium digitatum*; only 22% or 32% of the inoculated wounds were infected with green mold if inoculated 1 or 3 d after HW treatment, respectively, compared with 50% or 59% infection if inoculated on the HW treatment day or 7 d later, respectively (Pavoncello, 2001). For grapefruit brushed and rinsed for 20 s with 20, 53, 56, 59, or 62 °C water and then inoculated with *Penicillium digitatum* 24 h later, treatments at 53 °C did not significantly reduce decay, whereas treatments at 56, 59, or 62 °C reduced postharvest decay by 20%, 52%, or 69%, respectively, compared with the untreated fruit (Porat et al., 2000b). Since inoculation occurred after HW brushing in these experiments, they demonstrate that HW treatment reduce decay by enhancing grapefruit resistance to the decay organisms.

Commodity Responses to Heat Treatments

When fruit are exposed to high temperatures, there is potential risk of injury. Symptoms of heat injury can be external (i.e., peel scalding, pitting, etc.) or internal (i.e., softening, discoloration, tissue disintegration, off-flavors, etc.) (Lurie, 1998a). For example, Miller et al. (1988) dipped grapefruit in 43.5 °C water for 4 h and observed resulting peel discoloration after 3 weeks.

Scald

Peel scalding is a brown discoloration of the flavedo that can be caused by heat treatment. Schirra and D'hallewin (1997) dipped 'Fortune' mandarins in 50, 54, 56, or 58 °C water for 3 min and then stored at 6 °C for 30 d followed by 3 d at 20 °C. After

storage, 10%, 70% or 100% of the fruit developed peel scalding after HW dip in 54, 56, or 58 °C water, respectively.

Dipping ‘Tarocco’ oranges in 53 °C water for 3 min caused little peel scald in fruit harvested in February and none in fruit harvested in March (Schirra et al., 1997). Fruit harvest before February developed 20-30% peel scald, whereas 70% of fruit harvested in April developed peel scald.

In preliminary experiments, HW brushing at 60 °C damaged ‘Shamouti’ oranges. In contrast, HW brushing treatment at 56 °C for 20 s is non-damaging in all tested cultivars of citrus (Porat et al., 2000a).

Ritenour et al. (2003) showed a time × temperature interaction for peel scalding in grapefruit. Fruit dipped in water at 56 °C for 120 s, 59 °C for 20-120 s or 62 °C for 10-120 s developed significant peel scalding after 33 d in storage at 10 °C. Hot water dips at 62 °C for 60 or 120 s resulted in 100% peel scalding.

Electrolyte Leakage

‘Fortune’ mandarins dipped in 50, 52, 54, 56 or 58 °C water for 3 min were stored at 6 °C for 30 d followed by 3 d at 20 °C (Schirra and D’hallewin, 1997). Immediately after treatment, there was no significant difference in electrolyte leakage, while at the end of storage period, fruit dipped in water at 56 or 58 °C had greater electrolyte leakage than did fruit dipped in water at 50, 52 or 54 °C. However, Schirra et al. (1997) reported no change in electrolyte leakage due to HW dips of ‘Tarocco’ oranges at 53 °C for 3 min.

Respiration and Juice Quality

Respiration is the process of breakdown of organic materials to simple end products, providing energy required for various metabolic processes and results in the loss of food reserves (Kader, 2002). Increased respiration allows enhanced metabolic

rates to occur in the commodity and thus supports more rapid senescence. Temperature affects the respiration rate of fruits and vegetables by increasing the demand for energy to drive metabolic reactions. The respiration rate thus increases with increase in temperature of the product.

After 33 d in storage, the respiration rate was higher in 'Fortune' mandarins previously dipped in 56 or 58 °C water (Schirra and D'hallewin, 1997). The rate of ethylene production was also higher in fruit treated at 58 °C. Total soluble solids (TSS) and titratable acidity (TA), however, did not show much difference between the untreated and the heat-treated (50, 54, 56 and 58 °C for 3 min) fruit.

Dipping 'Tarocco' oranges in 53 °C water for 3 min did not influence the respiration rate, ethylene production, TSS and TA (Schirra et al., 1997). Hot water brushing at 56 °C for 20 s did not affect juice TSS and TA in 'Minneola' tangerines, 'Shamouti' oranges and 'Star Ruby' red grapefruit (Porat et al., 2000a).

In general, respiration rate was higher immediately after heat treatment, but later decreased to levels like that of the non-treated fruit. Juice TSS and TA were not affected by heat treatments.

Peel Color

No significant difference could be found in rind color between untreated 'Fortune' mandarin fruit and those treated in water at 50 or 54 °C for 3 min (Schirra and D'hallewin, 1997). L* values were lower for mandarins treated at 58 °C, a* values were lower for fruit treated at 54, 56 and 58 °C, and b* values were lower for fruit treated at 56 and 58 °C. Hot water brushing at 60 °C for 10 s followed by waxing slowed the yellowing process in 'Oroblanco' (Rodov et al., 2000). There was a delay of about 2 weeks in the change of rind color as compared with the non-treated fruit.

Flesh and Peel Firmness

Firmness of citrus fruit depends mainly on turgidity and weight loss (Rodov et al., 1997). Heat treatment causes the redistribution of natural epicuticular wax on the fruit surface and closes many microscopic cuticular cracks (Rodov et al., 1996). This could be the reason for better maintenance of firmness in heat-treated citrus fruit. However, in further experiments, maintenance of citrus fruit firmness by heat treatment was not accompanied by reduction in weight loss (Rodov et al., 2000). Heat treatment could have inhibited enzymatic action involved in softening or enhanced cell wall strengthening processes like lignification. Hot water dips for 2 min at 52 °C maintained firmness by inhibiting fruit softening 'Oroblanco'. Hot water brushing at 60 °C for 10 s also maintained fruit firmness but brushing at 52 or 56 °C did not.

Structural Changes of Epicuticular Wax

A number of deep surface cracks that form an interconnected network on peel surface are observed on the epicuticular wax of non-heated apples (Roy et al., 1999). Changes in the structure of the epicuticular wax are quite similar following different types of heat treatment (Schirra et al., 2000). After heat treatment at 38 °C for 4 d, the cuticular cracks on apples disappeared, probably due to the melting of wax platelets (Roy et al., 1994). Changes in epicuticular wax structure have been noticed in several types of produce when heat-treated, such as after a 2-min water dip at 52 °C of 'Oroblanco' (Rodov et al., 1996), a 2-min water dip at 50-54 °C of 'Fortune' mandarins (Schirra and D'hallewin, 1996, 1997), and a 20 s HW brushing at 56-62 °C of grapefruit (Porat et al., 2000a).

Heat treated ‘Marsh’ grapefruit showed that during long term storage the cuticular cracks became wider and deeper (D’hallewin and Schirra, 2000). Also during long term storage, severe alterations occurred in the outer stomatal chambers, thus becoming important invasion sites for wound pathogens (Eckert and Eaks, 1988).

Host-Pathogen Interaction

Inoculum levels are directly related to decay potential of a particular commodity (Trapero-Casas and Kaiser, 1992; Yao and Tuite, 1989). The effect of heat on decay-causing organisms can be influenced by factors like moisture content of spores, age of the inoculum, and inoculum concentration (Barkai-Golan and Phillips, 1991), as well as factors inherent within the host (i.e., physiological maturity and stress) (Klein and Lurie, 1991). Schirra et al. (2000) reported that heat treatments have a direct effect on fungal pathogens by slowing germ tube elongation or by inactivating or killing the germinating spores.

Heat treatments may also have an indirect effect on decay development by inducing anti-fungal substances within the commodity that inhibit fungal development or by promoting the healing of wounds on the commodity (Schirra et al., 2000). Oil glands of citrus flavedo contain compounds, such as citral in lemons that have anti-fungal activity (Ben-Yehoshua et al., 1992; Kim et al., 1991; Rodov et al., 1995b). Heat treatments enhance wound healing by promoting the synthesis of lignin-like compounds and these compounds act as physical barriers to the penetration of pathogens (Schirra et al., 2000). High concentration of scoparone was found in heat-treated citrus and this could have anti-fungal properties (Kim et al., 1991).

Heat treatments may also influence decay susceptibility by altering surface features of the commodity. For example, HW brushing of ‘Minneola’ tangerines at 56 °C for 20 s

smoothed the fruit epicuticular waxes, which covered and sealed the stomata and microscopic cracks on the fruit (Porat et al., 2000a). This could reduce the entry of pathogens and thus reduce decay development.

Heat treatments can also damage tissues and thus make them more susceptible to invasion by pathogens. For example, HW and vapor heat treatments used to control Caribbean fruit fly resulted in increased decay compared with the non-treated fruit (Hallman et al., 1990a; Miller et al., 1988). The long duration of treatment caused damage to the tissue and this led to increased decay (Jacobi and Wong, 1992).

Water dips for 4.5 h at 43.5 °C increased decay of 'Marsh' grapefruit compared with fruit dipped in ambient water and the non-treated fruit (Miller et al., 1988). Heat-treated fruit developed 45% decay in 2 weeks whereas fruit dipped in ambient water and untreated fruit developed only 6% and 1% decay, respectively. Grapefruit dipped in 62 °C water for 30 s developed only 5% SER after 82 d in storage, whereas increasing the treatment duration to 120 s caused significant peel scalding (100%) and increased incidence of SER (23%) (Ritenour et al., 2003).

Stem-End Rot

Stem-end rot of citrus fruits is caused by either *Lasiodiplodia theobromae* (previously known as *Diplodia natalensis* and commonly called *Diplodia* stem-end rot) or *Phomopsis citri*. The pathogen infects the calyx of immature citrus fruit and remains quiescent (Eckert and Brown, 1986). After harvest, decay develops when the fungus grows from the calyx into the fruit. The fungus does not spread from fruit to fruit in packed containers. Stem-end rot may also begin in wounds in the rind and at the stylar end of the fruit.

Diplodia SER is the most serious postharvest disease in Florida citrus. The warm and humid conditions in Florida favor the development of SER. Development of *Diplodia* SER is hastened during degreening with ethylene and it is more serious in early season degreened fruits (Brooks, 1944; McCornack, 1972). *Phomopsis* SER is more prevalent later in the season when degreening is not necessary.

Stem-end rots appear as a leathery, pliable area encircling the button or stem-end of citrus fruit. The affected area is buff colored to brown. *Diplodia* proceeds more rapidly through the core than the rind and the decay often appears at both ends of the fruit. It usually develops unevenly in the rind and forms finger-like projections. *Phomopsis* SER spreads through the core and in a nearly even rind pattern from the stem-end without finger-like projections.

Anthracnose

Anthracnose of citrus fruits is caused by *Colletotrichum gloeosporioides*. This fungus also infects immature fruit before harvest and remains quiescent (Eckert and Brown, 1986). Degreening with ethylene enhances anthracnose development and so it is more severe in early season fruit (Brown, 1975, 1978). The lesions initially appear silvery gray and leathery. The rind becomes brown to grayish black and softens as the rot progresses. In humid conditions, pink masses of spores may form on the lesions. Infections do not spread to adjacent healthy fruit.

Green Mold

Green mold of citrus fruits is caused by *Penicillium digitatum*. The fungus enters the fruit only through injuries in the skin (Eckert and Brown, 1986). Initially the decay appears as soft, water-soaked spots. Later the white fungal mycelium appears on the surface and produces a mass of powdery olive green spores. As the disease advances, the

colored sporulating area is surrounded by a white margin. The fungus produces enzymes that break down the cell walls and macerate the tissues as the mycelium grows. Finally the decayed fruit becomes soft, shrunken, and shriveled and is entirely covered with green spores. Green mold is the major decay causing organism in citrus fruits worldwide. But in Florida this disease is less severe than SER and anthracnose.

Minor Postharvest Diseases

Some of the other citrus postharvest diseases that are of less importance are black rot (*Alternaria citri*), blue mold (*Penicillium italicum*) and sour rot (*Galactomyces citri-aurantii*).

Physiological Disorders

Physiological disorders are caused by nutritional imbalances, improper harvesting and handling practices or storage at undesirable temperatures (Grierson, 1986). Some of the common physiological disorders in citrus fruits are CI, stem-end rind breakdown, postharvest pitting, blossom-end clearing, and oleocellosis.

Chilling injury occurs mainly in tropical and subtropical commodities when held below a critical threshold temperature, but above their freezing point (Kader, 2002). Grapefruit develops CI when stored at temperatures below 10-12 °C (Chace et al., 1966). Symptoms of CI become more noticeable when the fruit are transferred to non-chilling temperatures. Grapefruit harvested early and late in the season are more susceptible to CI than are fruit harvested in mid-season (Grierson and Hatton, 1977). Fruit from the outer-canopy are more susceptible to CI than are fruit from the inner-canopy (Purvis, 1980). Common symptoms of CI are surface pitting, discoloration of the skin or water-soaked area of the rind (Grierson, 1986).

Chilling injury in many horticultural commodities has been successfully reduced by high temperature prestorage conditioning or by curing treatments (Wang, 1990). Changes in respiratory rate, soluble carbohydrates, and starch content in 'Fortune' mandarin due to conditioning (3 d at 37 °C) were evaluated by Holland et al. (2002). They found that changes in carbohydrate concentration were mainly due to the consumption of carbohydrates for respiration, which was highest in the heat-treated fruit. There was no relation between CI and changes in carbohydrates. The sucrose levels in untreated fruit were 57-79% lower than the heat-treated fruit. But there were losses in glucose, fructose, and starch in heat-treated fruit. So sucrose could be involved in heat-induced chilling tolerance of citrus fruit.

Rodov et al. (1995a) studied the effect of curing and HW dip treatments on CI development of grapefruit. Curing (36 °C for 72 h), HW dip (53 °C for 3 min) or hot imazalil dip (53 °C for 3 min) each reduced CI by about 40%. When grapefruit were sealed in plastic film (D-950 film, Cryovac) after HW dip treatment, CI was reduced by about 60%. But curing and sealing the fruit did not provide better decay control than curing alone.

'Tarocco' oranges, harvested monthly from November to April and then dipped in 53 °C water for 3 min, were stored at 3 °C for 10 weeks followed by 1 week at 20 °C (Schirra et al., 1997). The fruit harvested between November and January were more susceptible to CI than fruit harvested in February, March or April. Heat treatment reduced the development of CI, especially in fruit that were more susceptible to CI.

'Tarocco' oranges harvested monthly from December to April were dipped in water or TBZ solution (200 ppm) at 50 °C for 3 min (Schirra et al., 1998). Dipping fruit in HW

alone reduced CI only in fruit that were harvested during January or April. But, dipping the fruit in heated TBZ solution significantly reduced CI throughout the season.

'Valencia' oranges dipped in water, benomyl (500 mg/L) or TBZ (1000 mg/L) for 2 min at ambient temperature or 53 °C developed less CI after 15 weeks of storage at 1 °C when dipped at 53 °C (Wild and Hood, 1989). In another experiment, grapefruit dipped in water, TBZ (1000mg/L) or benomyl (500 mg/L) for 2 min at 14 °C or 50 °C developed significantly less CI after 8 weeks storage at 1 °C when dipped at the higher temperature (Wild, 1993).

Objectives

- Determine the effects of ethylene treatment of grapefruit on response to hot water dip treatment
- Determine the physiological responses of oranges to hot water dip treatment
- Determine if postharvest diseases in Florida grapefruit can be reduced by hot water dip treatment
- Determine if hot water dip treatment can reduce chilling injury in Florida grapefruit

CHAPTER 2
EFFECTS OF ETHYLENE TREATMENT ON 'RUBY RED' GRAPEFRUIT QUALITY AFTER SHORT-DURATION HOT WATER DIP TREATMENTS

Introduction

Degreening with ethylene is a common practice with early season grapefruit that enhances rind color without affecting the fruit's internal quality. But ethylene treatment can have adverse effects like making the fruit susceptible to *Diplodia* stem-end rot (Brooks, 1944; McCornack, 1972). The objective of this experiment was to determine if ethylene treatment affects the response of grapefruit to hot water (HW) dip treatments.

Materials and Methods

Fruit

Commercially mature (TSS:TA ≥ 7:1) and healthy 'Ruby Red' grapefruit were harvested randomly from 1-1.5 m above ground level on healthy trees, evenly spaced around the tree and distributed through the inner and outer canopy on 22 Nov. 2002 at the Indian River Research and Education Center research grove in Fort Pierce, Fla. The trees received standard commercial care. The fruit were transported to Gainesville, Fla. on the day of harvest and stored at 10 °C overnight before receiving their respective treatments the following day.

Heat Treatment and Degreening

The fruit were exposed to one of the following treatments.

1. Control (no HW dip or degreening)
2. Degreening only
3. Hot water dip only

4. Hot water dip followed by degreening
5. Degreening followed by HW dip

Each treatment had three replicates of 20 fruit each. Hot water dips were done at 62 °C for 60 s. Hot water dip was administered using a laboratory scale fruit heating system (Model HWH–2, Gaffney Engineering, Gainesville, Fla.) capable of maintaining water temperatures up to 65 °C, to a stability within $\pm$2.0 °C of the initial water temperature for the first 4–5 min after submerging up to 32 kg of fruit with an initial fruit temperature of 20 °C. Following treatment, the fruit were air dried and stored at 20 °C. Degreening was accomplished by treating fruit with 2-3 ppm ethylene for 3 d at 29 °C and 95% relative humidity (RH). Fruit that were not degreened were held at 20 °C and 95% RH while fruit from the other treatments were being degreened. Initial total soluble solids (TSS) and titratable acidity (TA) were measured from three replicates of 10 fruit each. Following treatments, fruit were stored at 20 °C, with 95% RH. Rates of respiration and ethylene production were measured for 6 d. On the 7th d of storage, half the fruit were evaluated for peel scalding, TSS and TA, and on the 10th d, the remaining 10 fruit per replication were evaluated.

Respiration Rate and Ethylene Production

Rates of respiration and ethylene production were measured by the static system. Three fruit per replicate were weighed and sealed together in a 3 L container for 2 h. Gas samples (0.5 mL) were withdrawn through a rubber septum using a syringe and the percentage of carbon dioxide determined using a Gow-Mac gas chromatograph (Series 580, Bridgewater, N.J.) equipped with a thermal conductivity detector. The respiration rate was calculated using the following formula:

$$\text{Respiration rate (mL } CO_2 \cdot kg^{-1} \cdot h^{-1}) = \frac{\%\ CO_2 \cdot \text{void volume (mL)}}{\text{Sample weight (kg)} \cdot \text{sealed time (h)} \cdot 100}$$

Ethylene production was measured by injecting a 1 mL gas sample into a HP 5890 gas chromatograph (Hewlett Packard, Avondale, Pa.) equipped with a flame ionization detector. The rate of ethylene production was calculated using the following formula:

$$\mu L\ C_2H_4 \cdot kg^{-1} \cdot h^{-1} = \frac{\text{ppm } C_2H_4 \cdot \text{void volume (mL)}}{\text{Sample weight (kg)} \cdot \text{sealed time (h)} \cdot 1000}$$

Peel Scalding

Peel scalding was evaluated on each fruit and the percentage of fruit showing any peel scalding was calculated.

Total Soluble Solids and Titratable Acidity

A 13 mm thick cross sectional slice was removed from the equatorial region of each fruit and the peel removed. The samples were macerated in a blender and then centrifuged at 4,000 g_n for 20 min. The supernatant solution was used for measuring TSS and TA. Total soluble solids were measured using a Mark II refractometer (Model 10480, Reichert-Jung, Depew, N.Y.) and the values expressed as °Brix. Titratable acidity was measured with an automatic titrimeter (Fisher Titrimeter II, No. 9-313-10, Pittsburg, Pa.) using 6.00 g of juice that was diluted with 50 mL of distilled water and titrated with 0.1 N NaOH to an endpoint of pH 8.2. Titratable acidity was expressed as percentage citric acid. The TA was calculated as follows:

$$\text{TA (\% citric acid)} = \frac{\text{mL NaOH} \cdot \text{Normality of NaOH} \cdot 0.064 \cdot 100}{\text{6 g of juice}}$$

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

Dipping grapefruit in 62 °C water for 60 s before or after degreening did not result in consistent differences in the respiration rate (Figure 2-1). Respiration increased dramatically in fruit from all treatments on the 6th d of storage, but there were no significant differences between treatments. There were no decay of fruit and hence the sudden increase can be due to mistake in calibration of the gas chromatograph. Ethylene production was low (<0.1 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) and not significantly different among treatments throughout the entire 6-d monitoring period (data not shown). Schirra and D'hallewin (1997) have shown that ethylene production from 'Fortune' mandarins was stimulated by treatment at 58 °C for 3 min. But, Schirra et al. (1997) reported that a heat treatment at 53 °C for 3 min did not affect the respiration rate and ethylene production of 'Tarocco' oranges.

Peel scalding (Figire 2-2) did not develop on fruit that were not dipped in heated water, regardless if they were degreened or not (Figure 2-3). However, 50% of the fruit receiving the HW dip treatments developed peel scalding after 10 d in storage. Scalding was not significantly affected by the degreening treatments. There were no consistent differences in TSS (Figure 2-4) or TA (Figure 2-5). Porat et al. (1999) reported that ethylene degreening does not affect the juice TSS and TA of 'Shamouti' oranges. Neither did HW dips at 53 °C for 3 min have any effect on the TSS and TA of 'Tarocco' oranges (Schirra et al., 1997). Heat treating grapefruit before or after degreening did not have any effect on the incidence of peel scalding, rates of respiration and ethylene production, or

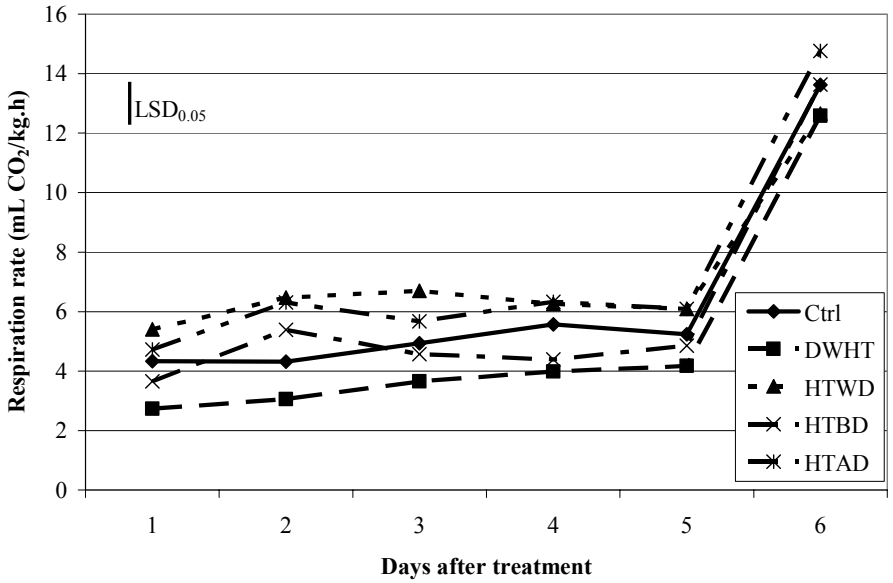

Figure 2-1. Rates of respiration of 'Ruby Red' grapefruit for the first six d after 62 °C hot water treatment for 60 s and stored at 20 °C. Vertical bar represents the 5% LSD value. Ctrl – Control (no HW dip or degreening), DWHT – Degreening without heat treatment, HTWD – Heat treatment without degreening, HTBD – Heat treatment before degreening and HTAD – Heat treatment after degreening.

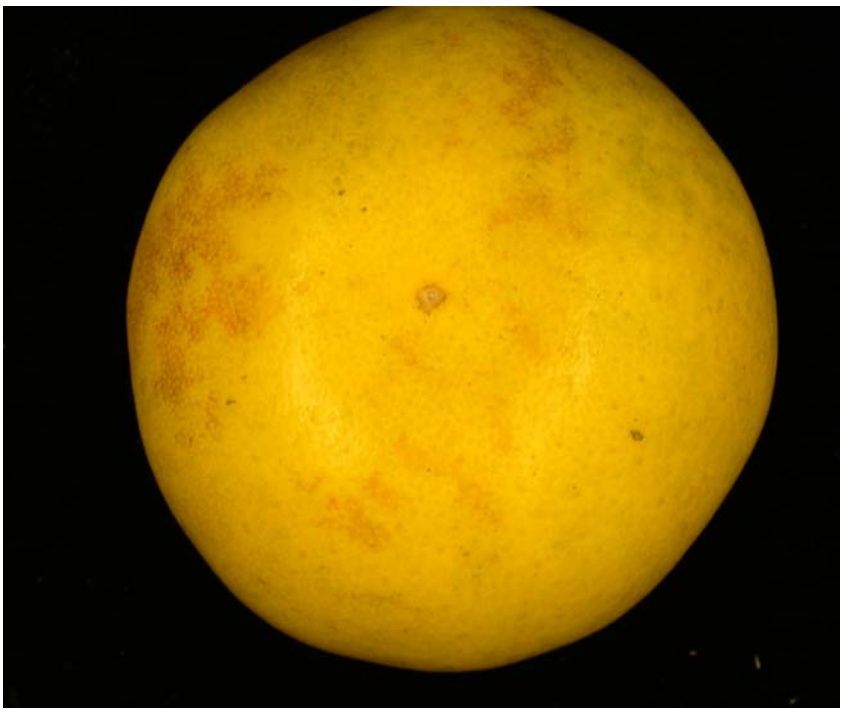

Figure 2-2. Peel scalding of 'Ruby Red' grapefruit after 10 d of storage at 20 °C. Fruit were dipped in 62 °C water for 60 s.

on juice quality (TSS and TA). From these results it may be concluded that degreening grapefruit with ethylene had no effect on the response to HW dip treatment.

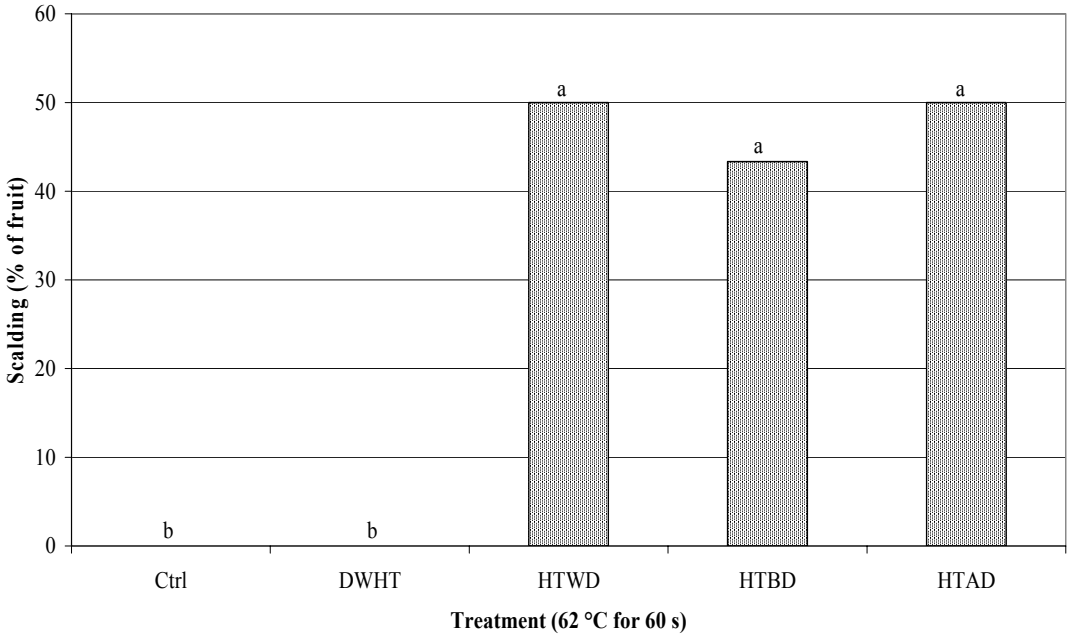

Figure 2-3. Peel scalding (% of fruit scalded) of 'Ruby Red' grapefruit due to 62 °C hot water dip for 60 s after 10 d of storage at 20 °C. Bars with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip or degreening), DWHT – Degreening without heat treatment, HTWD – Heat treatment without degreening, HTBD – Heat treatment before degreening and HTAD – Heat treatment after degreening.

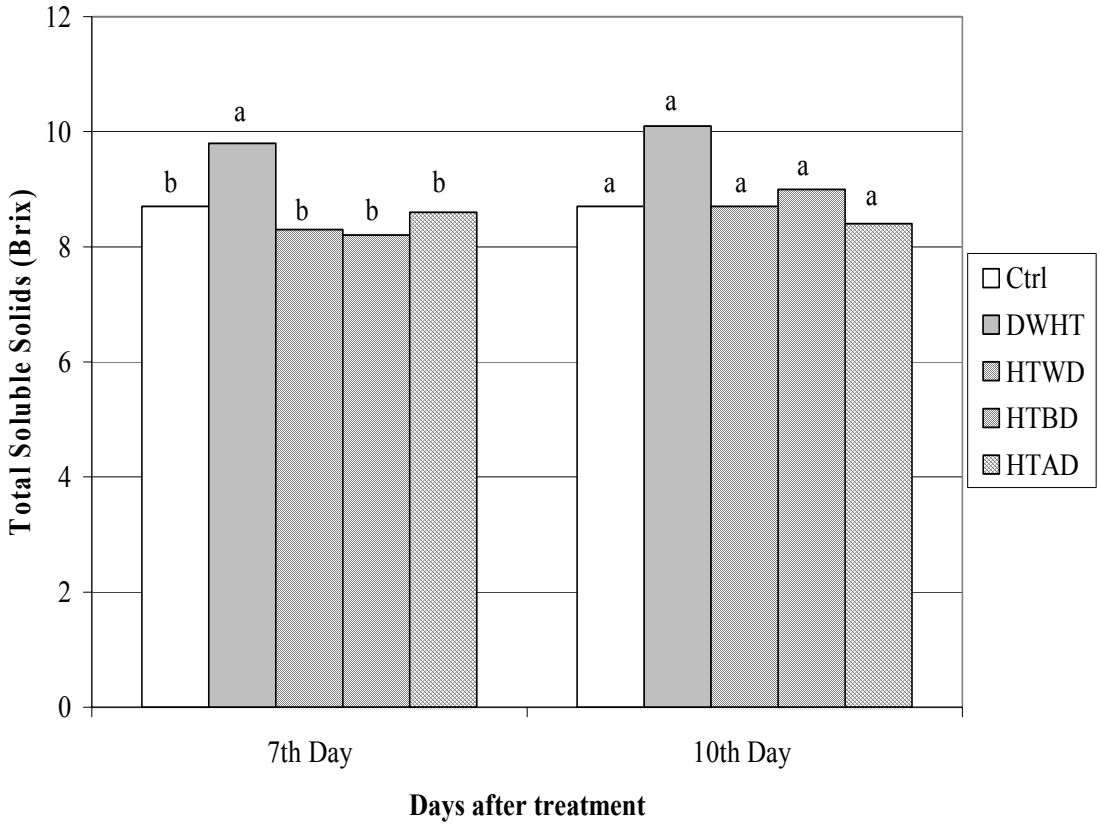

Figure 2-4. Total soluble solids content in 'Ruby Red' grapefruit on the 7th and 10th d after 62 °C hot water treatment for 60 s. Bars within each day with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip or degreening), DWHT – Degreening without heat treatment, HTWD – Heat treatment without degreening, HTBD – Heat treatment before degreening and HTAD – Heat treatment after degreening.

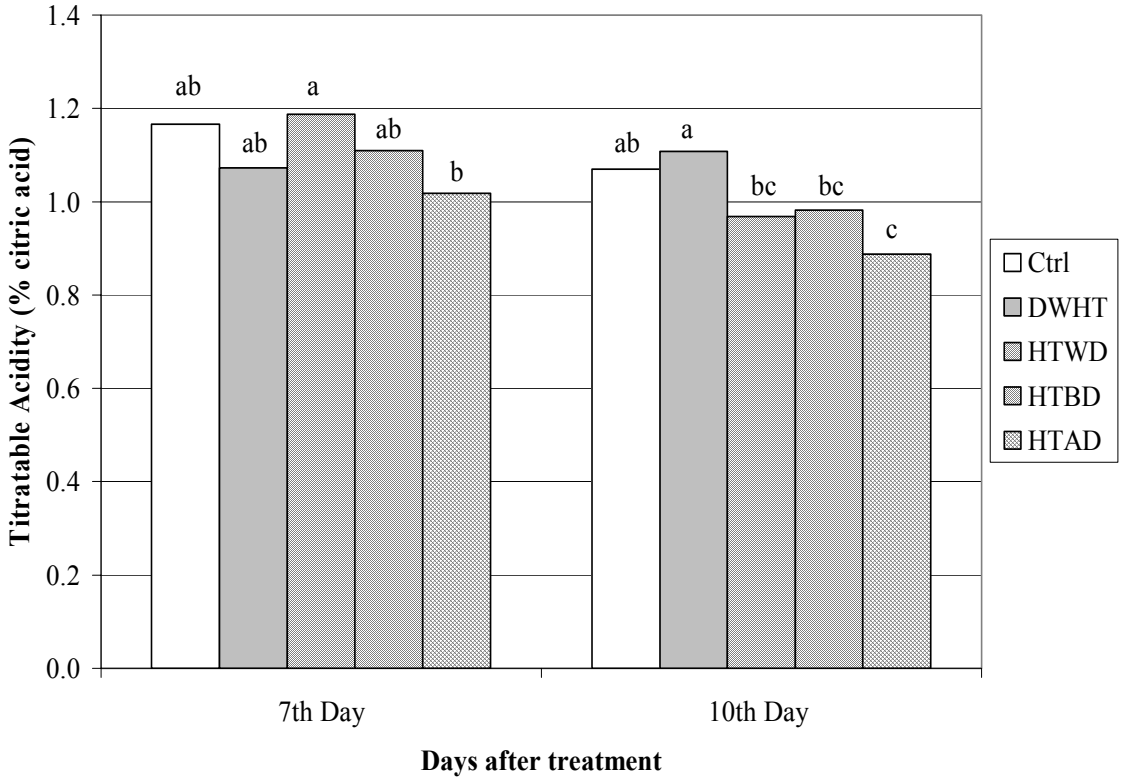

Figure 2-5. Titratable acidity in 'Ruby Red' grapefruit on the 7th and 10th d after 62 °C hot water treatment for 60 s. Bars within each day with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip or degreening), DWHT – Degreening without heat treatment, HTWD – Heat treatment without degreening, HTBD – Heat treatment before degreening and HTAD – Heat treatment after degreening.

CHAPTER 3
PHYSIOLOGICAL RESPONSES OF 'VALENCIA' ORANGES TO SHORT-DURATION HOT WATER DIP TREATMENT

Introduction

When fruit are dipped in hot water (HW), there is a potential risk of damaging the tissues. Tissue damage may be associated with changes in peel electrolyte leakage, peroxidase activity, total phenolics or total protein content. If so, such changes could even serve as early, quantitative measures of heat injury. The following experiments were conducted to evaluate the physiological responses of 'Valencia' oranges to HW dips.

Materials and Methods

Fruit

'Valencia' oranges were harvested at the Indian River Research and Education Center research grove in Fort Pierce, Fla. between June and August 2003. Inner-canopy fruit harvested from 1-1.5 m above ground level were used in all experiments. The fruit were harvested in the morning and were treated later the same day.

Experiment 1

Harvested fruit were dipped in water at 56 °C for 10, 20, 30, 60 or 120 s. Hot water dips were conducted in a temperature controlled water bath (Optima series immersion circulators, Boekel Scientific, Feasterville, Pa.). Fruit were dipped such that half the surface of each fruit along the meridian was immersed in the water. Control fruit were not dipped. Each treatment had three replicates of three fruit each. Electrolyte leakage from flavedo tissue was measured using the procedure described below on the treated and

untreated sides of each fruit immediately after treatment. After taking discs of peel for electrolyte leakage measurement, the fruit were stored at 10 °C (90% RH). This resulted in decay development on the 12th d after treatment at which time scald was evaluated and the experiment terminated.

Experiment 2

Because treatments in the previous experiment did not result in measurable differences, fruit were dipped in water at 50, 60, 70, 80 or 90 °C for 60 s. Dipping was conducted as described in experiment 1. Each treatment had three replicates of three fruit each and these were duplicated so that one set of three replicates was used to measure electrolyte leakage immediately after treatment, and the other set of three replicates was stored at 10 °C (90% RH). After 14 d of storage, fruit were evaluated for scald, peel color, and electrolyte leakage.

Experiment 3

Based on results from the second experiment and to focus on the temperature range where changes in electrolyte leakage occurred, harvested fruit were dipped in water at 60, 62, 64, 66, 68 or 70 °C for 60 s. Hot water dip was administered as described in experiment 1. Each treatment had three replicates of five fruit each and these were duplicated so that one set of three replicates was used to measure electrolyte leakage immediately after treatment, and the other set of three replicates was stored at 10 °C (90% RH). After 8 d of storage, fruit were evaluated for scald, peel color, and electrolyte leakage.

Experiment 4

Because peel electrolyte leakage and scalding changed significantly between 60 and 66 °C, harvested fruit were dipped in water at 60 or 66 °C for 60 s and peel color,

total phenolics, protein content, and peroxidase activity were evaluated in addition to electrolyte leakage and peel scalding over time. Dipping was conducted as described in experiment 1 except that the whole fruit were completely submerged. Each treatment had three replicates of five fruit each and these were duplicated four times so that one set was evaluated at each of four sampling times: immediately after treatment, and after storage at 10 °C (90% RH) for 2, 4, or 7 d.

Peel Scalding

Peel scalding was evaluated on each fruit and expressed on a 0 to 10 scale with 0 representing no scalding and 10 representing scald on 100% of the fruit surface. The percentage of fruit with any peel scalding (rated as a 1 or above) was also calculated.

Peel Color

Peel color was measured using a Minolta Chroma Meter (CR-300 series, Minolta Co. Ltd., Japan) at three equidistant locations on each fruit along the equator of the fruit and expressed as L*, a* and b* values. The hue and chroma values were calculated from a* and b* values using the following formula:

$$\text{Hue} = \text{arc tangent } (b^* \cdot a^{*-1})$$

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

Electrolyte Leakage

Electrolyte leakage of flavedo tissue was determined following the procedure of McCollum and McDonald (1991). Discs of peel tissue were taken from the equator of each fruit using a 13-mm cork borer. Two discs were taken per fruit during the first three experiments, and only one during the fourth experiment. The albedo was removed using a razor blade and the flavedo discs were incubated in 25 mL of 0.4 M mannitol solution for 4 h at room temperature with constant shaking. After 4 h, the initial conductivity was

measured using a conductivity meter (D-24, Horiba Ltd., Japan). The samples were then frozen overnight. Following thawing and warming to room temperature total conductivity was measured. Electrolyte leakage was calculated using the following formula:

$$\text{Electrolyte leakage (\%)} = \frac{\text{Initial conductivity } (S \cdot m^{-1}) \cdot 100}{\text{Total conductivity } (S \cdot m^{-1})}$$

Preparation of Flavedo Samples for Protein, Phenolic and Peroxidase Assays

Flavedo was peeled from one meridian half of each fruit using a fruit peeler, frozen in liquid nitrogen, crushed into small pieces with a mortar and pestle, and then freeze dried. After drying, the samples were ground into a fine powder and stored at -20 °C.

Acetone-washed flavedo powder was prepared by shaking 1 g of freeze dried flavedo in 25 mL of acetone for 1 h. The samples were filtered through Whatman No.1 filter paper under vacuum and washed with 25 mL acetone before being collected in beakers and dried overnight.

Protein was extracted by adding 25 mL borate buffer (0.1 M, pH 8.8) to 0.3 g of the acetone-washed powder and shaking at 5 °C for 1 h. Extracts were then filtered through 4-6 layers of cheesecloth and the volume brought up to 25 mL with the borate buffer before centrifugation at 25,000 g_n for 10 min. Supernatants were decanted, brought to 70% saturation with solid ammonium sulfate and shaken overnight at 5 °C. Samples were centrifuged at 25,000 g_n for 10 min the following day and the supernatant discarded. The pellets were dissolved in 4 mL borate buffer. Desalting was done by dialysis using dialysis tubes with molecular weight cut-off = 6,000-8,000.

Lowry Assay for Protein

For protein measurement, standards for the Lowry assay (Lowry et al., 1951) were prepared from 0, 4, 8, 12, 16, and 20 µg of bovine serum albumin. Samples were prepared by adding 190 µL of water to 10 µL of protein extract. Working solution of copper-tartrate-carbonate (CTC) was prepared by mixing 5 mL of CTC, 5 mL of NaOH (0.8 M), and 10 mL of water. The CTC working solution (200 µL) was added to the protein solution, and the mixture was vortexed and allowed to stand for 10 min. Working solution of Folin-Ciocalteu was prepared by mixing one volume of Folin-Ciocalteu phenol reagent with five volumes of water. Then 100 µL of Folin-Ciocalteu working solution was added to the protein and CTC solution, and the mixture was vortexed and allowed to stand for 30 min. Absorbance was read at 750 nm using a microplate reader (Ultramark, BioRad, Hercules, Calif.). The results were expressed as mg of protein per g dry weight of the tissue.

Estimation of Total Phenolics

For phenolics estimation (Swain and Hillis, 1959), methanol (10 mL) was added to 200 mg of freeze dried flavedo acetone powder and kept on a shaker overnight. The extracts were filtered through Whatman No.1 filter paper under vacuum. To 0.2 mL of filtered extract, Folin-Ciocalteu reagent (0.4 mL) and 0.5 M ethanolamine (0.9 mL) were added and the mixture vortexed. After 20 min, 200 µL aliquot aliquots were applied to a microplate reader (Ultramark, Bio-Rad Laboratories, Hercules, Calif.) and absorbance was measured at 630 nm. Gallic acid solutions in methanol at 0, 50, 100, 150, and 200 ppm were used as standards. The results were expressed as mg of total phenolics per g dry weight of the tissue.

Peroxidase Assay

For peroxidase assay (Worthington, 1972), protein extract (100 µL) was added to 1.4 mL of 0.0025 M 4-aminoantipyrine and 1.5 mL of 0.0017 M H_2O_2 and mixed well by shaking. The absorbance was read in a UV-visible recording spectrophotometer (UV160U, Shimadzu, Japan) at 510 nm over a period of time. The spectrophotometer was set under kinetic program to have a lag time of 30 s, rate time of 180 s and interval time of 30 s. The results were expressed as peroxidase activity per minute per g dry weight of the tissue.

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

Experiment 1

Electrolyte leakage on the heat-treated sides of fruit from each treatment averaged between 33% and 42%, and was not significantly different from each other (Figure 3-1). Schirra and D'hallewin (1997) reported that HW dip at 50, 52, 54, 56 or 58 °C for 3 min of 'Fortune' mandarin did not affect the electrolyte leakage immediately after treatment. However, they observed higher electrolyte leakage after 30 d of storage in fruit dipped at 56 or 58 °C. After 12 d of storage, only fruit dipped in 56 °C water for 120 s developed peel scalding (Table 3-1). Of these fruit, about 67% were scalded and an average of 89% of the fruit surface was scalded, but this did not result in a significant change in

electrolyte leakage. Therefore, HW dip at 56 °C for 10-120 s did not result in significant changes in electrolyte leakage.

Table 3-1. Peel scalding of 'Valencia' oranges on the heat-treated side of the fruit after 12 d at 10 °C.

Treatment (56 °C)	Scalding (% of fruit surface)	Scalding (% of fruit)
0 s	0.00 b[z]	0.00 b
10 s	0.00 b	0.00 b
20 s	0.00 b	0.00 b
30 s	0.00 b	0.00 b
60 s	0.00 b	0.00 b
120 s	89.00 a	66.67 a
Significance	*	*

[z] Values within each column followed by different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$.
* Significant at $P \leq 0.05$.

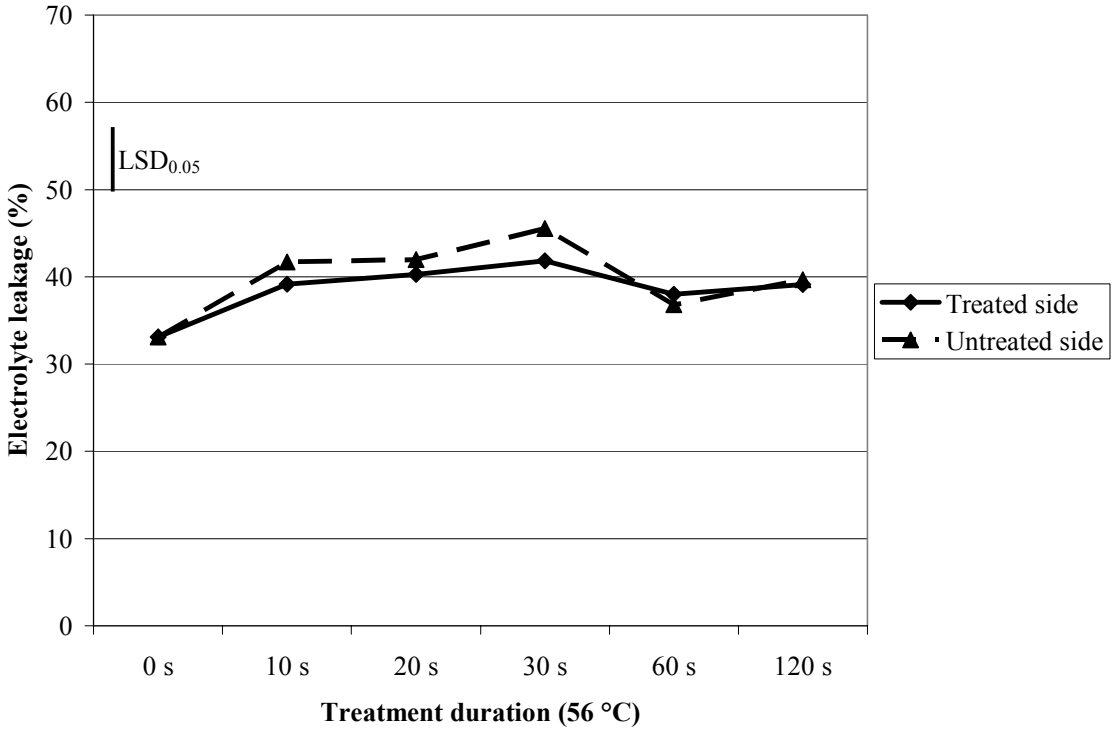

Figure 3-1. Electrolyte leakage from flavedo of 'Valencia' oranges immediately after 56 °C hot water treatment. Vertical bar represents the 5% LSD value.

Experiment 2

To further evaluate the effect of HW dip on electrolyte leakage, the range of treatment temperatures was increased to between 50 and 90 °C, all for 60 s. Hot water dip at 60, 70, 80 or 90 °C for 60 s resulted in all fruit developing peel scalding (Figure 3-2). An average of 64% of the fruit surface was scalded in fruit dipped at 60 °C, which was significantly less than the 100% of the fruit surface scalded in fruit dipped at 70, 80 or 90 °C (Figure 3-3). Scalding incidence was still high (56%) on fruit dipped at 50 °C for 60 s, but was significantly lower than on fruit dipped at higher temperatures (Figure 3-2); 20% of the fruit surface was scalded when dipped at 50 °C (Figure 3-3). The untreated half of the fruit did not develop peel scalding in any of the treatment temperatures (data not shown).

Electrolyte leakage from fruit dipped at 50 or 60 °C was not significantly different from the non-treated fruit when evaluated immediately after the treatments, or after 14 d of storage (Figure 3-4). Fruit dipped in 70, 80 or 90 °C water had higher electrolyte leakage (79-97%) compared with the non-treated fruit (35%), 50 °C (35%), or 60 °C (39%) treated fruit. Electrolyte leakage from 50 and 60 °C-dipped fruit was not significantly different from the non-treated fruit, although the 60 °C HW dip resulted in significant peel scalding (Figures 3-2 and Figure 3-3). Schirra and D'hallewin (1997) reported that HW dip at 54, 56 or 58 °C for 3 min did not result in significant difference in electrolyte leakage immediately after treatment though the scalding incidence was 10%, 70%, or 100%, respectively, after 33 d in storage. Thus, flavedo electrolyte leakage is not an early indicator of peel scalding. Electrolyte leakage of flavedo from the untreated sides of fruit from all treatments did not result in significant differences from the non-treated fruit throughout the 14 d experiment (data not shown).

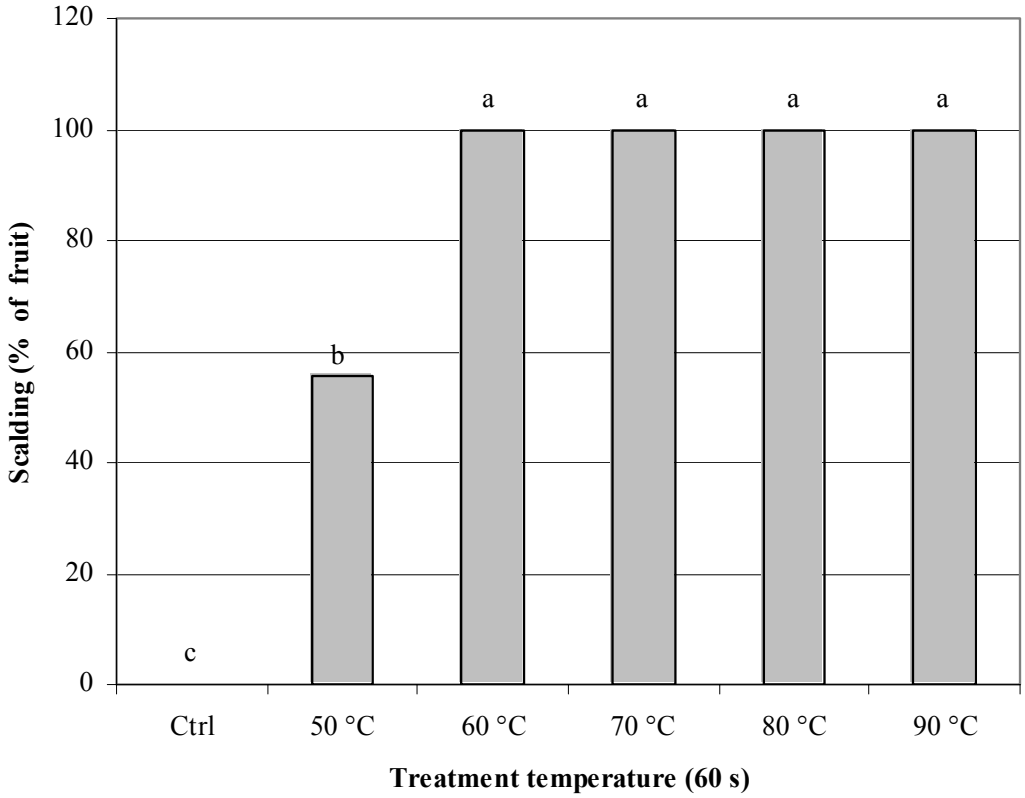

Figure 3-2. Peel scalding (percent of fruit scalded) of 'Valencia' oranges on the heat-treated side of the fruit after 14 d of storage at 10 °C. Bars with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip).

Peel hue and chroma values did not show consistent treatment differences (Table 3-2). The L* values from fruit treated at 70, 80 or 90 °C, which had scalding on 100% of the peel surface, were significantly lower than the non-treated fruit. Though not significantly lower, L* values also tended to decline at dipping temperatures above 50 or 60 °C treatment. Schirra and D'hallewin (1997) reported that 'Fortune' mandarins dipped at 58 °C for 3 min had lower L* values after 33 d in storage. Differences in treatment duration and orange cultivar studied likely explain the slight differences in results.

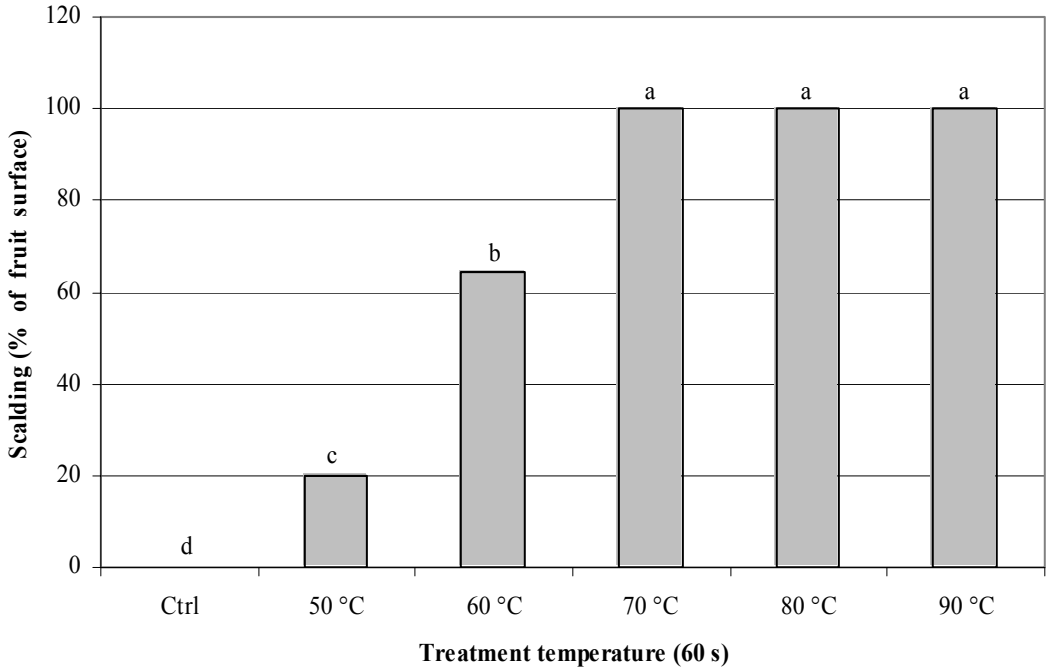

Figure 3-3. Peel scalding (percent of fruit surface scalded) of 'Valencia' oranges on the heat-treated side of the fruit after 14 d of storage at 10 °C. Bars with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip).

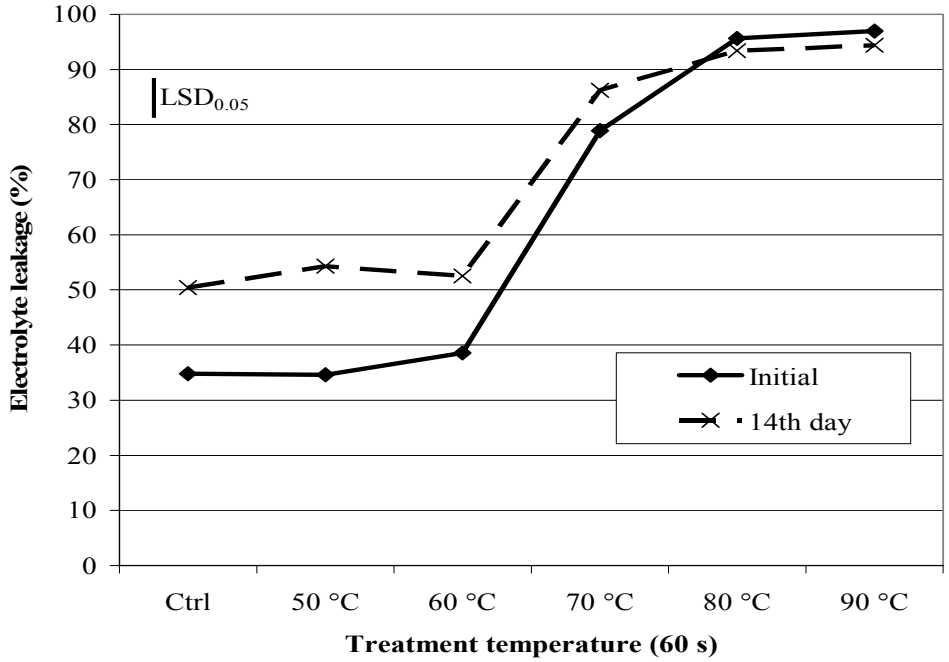

Figure 3-4. Electrolyte leakage from flavedo of the heat-treated sides of 'Valencia' oranges immediately after treatment and after 14 d of storage at 10 °C. Vertical bar represents the 5% LSD value. Ctrl – Control (no HW dip).

Table 3-2. Peel hue, chroma and L* values of 'Valencia' oranges after 14 d of storage at 10 °C.

	Hue		Chroma		L*	
Treatment (60 s)	Treated side	Untreated side	Treated side	Untreated side	Treated side	Untreated side
Ctrl	75.109 b c[z]	75.109	55.607	55.607	56.090 a	56.090
50 °C	77.630 a b	77.141	53.671	54.707	55.240 a b	56.097
60 °C	79.236 a	77.600	51.146	54.563	54.09 a b c	55.783
70 °C	73.128 c d	77.230	49.837	57.930	51.047 c d	56.644
80 °C	70.28 d	71.949	49.054	57.498	50.197 d	54.791
90 °C	73.619 c d	75.297	51.438	58.128	52.373 b c d	57.596
Significance	*	ns	ns	ns	*	ns

[z] 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 $P \leq 0.05$.
Ctrl – Control (no HW dip).

Experiment 3

Because flavedo electrolyte leakage increased markedly following dip treatments between 60 and 70 °C, experiments were conducted further evaluating this range of temperatures. There was a distinct increase in peel scalding at treatment temperatures of 64 °C and above (Figure 3-5, Figure 3-6); fruit dipped at 60 or 62 °C developed no scalding, whereas all fruit dipped at 66, 68 or 70 °C developed scalding after 8 d of storage. Parallel increases in the percentage of fruit surface scalded at higher dip temperatures were also observed (Figure 3-7).

Electrolyte leakage immediately after the treatments also increased significantly at treatment temperatures of 64 °C and above (Figure 3-8); values for fruit dipped in 66, 68, or 70 °C water were 45%, 53%, and 78%, respectively, higher than non-treated fruit. After 8 d of storage, electrolyte leakage was significantly higher only in fruit dipped at 68 or 70 °C, which were 23% and 36% higher, respectively, than the non-treated fruit. Electrolyte leakage on the untreated side of fruit did not vary significantly immediately after treatments or after 8 d of storage (data not shown). While scalding developed in fruit

dipped at 64 °C or higher, increased electrolyte leakage was only detected in tissue exposed to 66 °C or higher immediately after treatment, and at 68 °C or higher after 8 d storage. Thus, increased electrolyte leakage was only observed if severe scalding developed, and is not suitable as an early indicator of peel scalding.

After 8 d of storage, there was no significant change in hue angle between the treated and untreated tissue (Table 3-3). However, chroma and L* values for fruit dipped at 66, 68 or 70 °C were significantly lower than for fruit not dipped. These results are similar to the previous experiment where HW dips at higher temperatures resulted in lower L* values. But the chroma values in the previous experiment were not affected by HW dips. The untreated sides of the fruit did not have any significant changes in the chroma and L* values.

Figure 3-5. Peel scalding of ‘Valencia’ oranges after 8 d of storage at 10 °C. Fruit were dipped in 66, 68, or 70 °C water for 60 s.

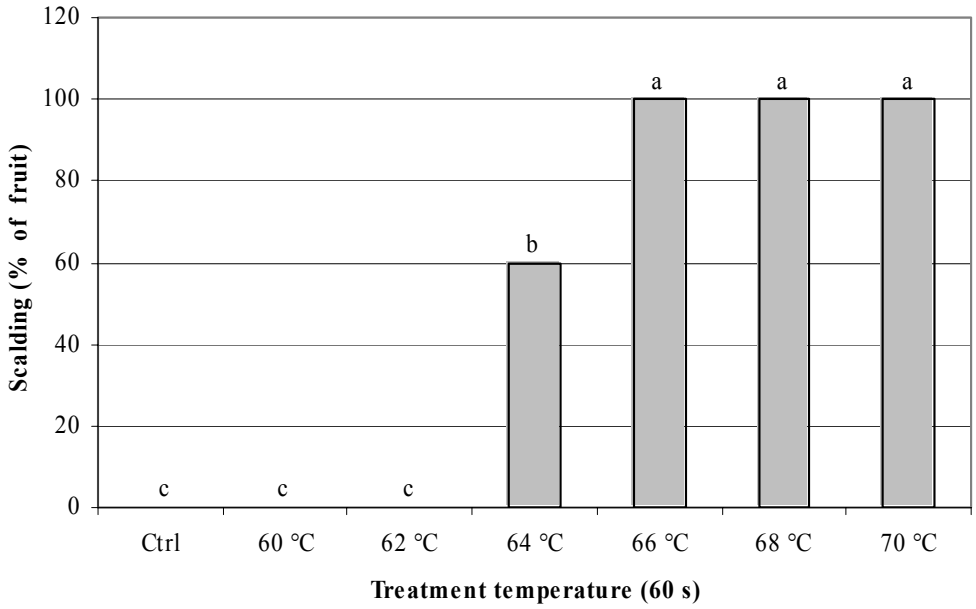

Figure 3-6. Peel scalding (percent of fruit scalded) of 'Valencia' oranges on the heat treated side of the fruit after 8 d of storage at 10 °C. Fruit were dipped in 66, 68, or 70 °C water for 60 s. Bars with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip).

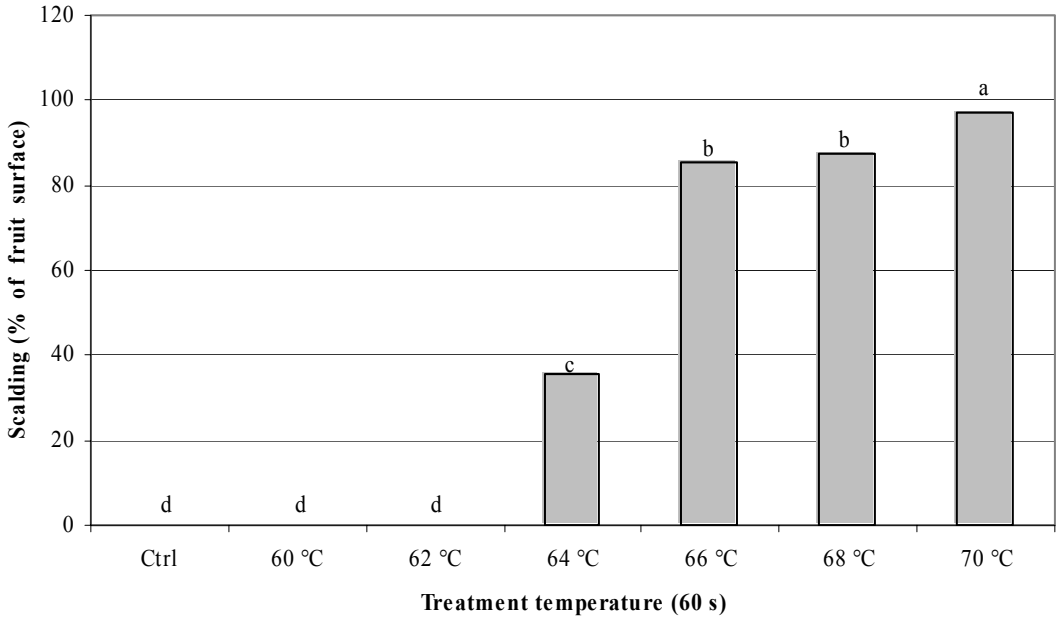

Figure 3-7. Peel scalding (percent of fruit surface scalded) of 'Valencia' oranges on the heat-treated side of the fruit after 8 d of storage at 10 °C. Bars with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip).

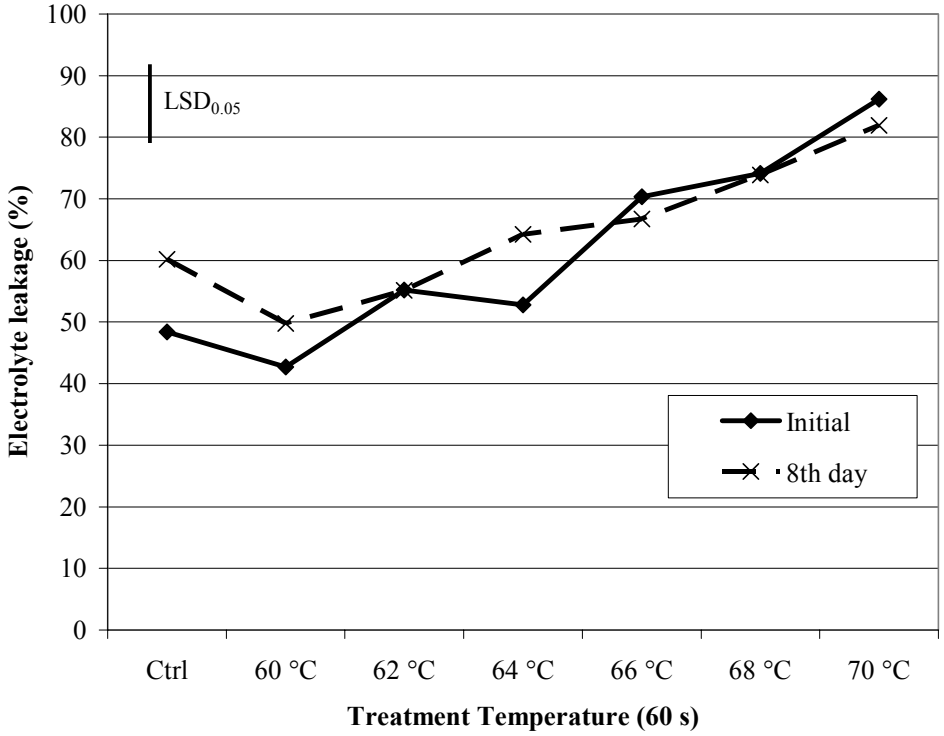

Figure 3-8. Electrolyte leakage from flavedo of the heat-treated sides of 'Valencia' oranges immediately after treatment and after 8 d of storage at 10 °C. Vertical bar represents the 5% LSD value. Ctrl – Control (no HW dip).

Table 3-3. Peel hue, chroma and L* values of the heat-treated and untreated sides of 'Valencia' oranges after 8 d of storage at 10 °C. Ctrl – Control (no HW dip).

	Hue		Chroma		L*	
Treatment (60 s)	Treated side	Untreated side	Treated side	Untreated side	Treated side	Untreated side
Ctrl	75.877	75.877	70.252 a b[z]	70.252	63.913 a	63.913
60 °C	66.157	65.508	71.247 a	71.917	63.880 a	64.809
62 °C	77.358	77.746	70.859 a b	69.186	63.895 a	63.826
64 °C	77.576	77.407	66.957 b c	64.884	62.011 a	61.531
66 °C	75.559	79.141	64.957 c	69.835	59.047 b	63.44
68 °C	77.465	78.958	63.320 c	68.322	59.529 b	62.933
70 °C	74.348	75.686	63.844 c	71.066	58.529 b	63.317
Sinificance	ns	ns	*	ns	*	ns

[z] 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 $P \leq 0.05$.

Experiment 4

In testing for other potential physiological changes in heat-treated 'Valencia' oranges, all fruit dipped in 66 °C water for 60 s developed peel scalding within 7 d, whereas only 20% of the fruit developed scalding when dipped in 60 °C water (Figure 3-9). In experiment 2, all the fruit dipped at 60 °C for 60 s were scalded after 14 d of storage and in experiment 3, none of the fruit dipped at 60 °C for 60 s were scalded after 8 d of storage. The differences in sensitivity to heat injury could be due to the differences in the time of harvest which was between 1st week of June and 1st week of August. Again, only severely scalded fruit from the 66 °C water treatment developed significantly higher electrolyte leakage throughout the 7 d storage period (Figure 3-10). Electrolyte leakage averaged 19% higher in 66 °C-treated fruit than in untreated fruit. These results are consistent with the previous experiments.

Peroxidase activity of fruit treated at 66 °C was lower than the non-treated fruit and 60 °C treatment (Figure 3-11). Higher electrolyte leakage and lower peroxidase activity were observed only on fruit that were dipped at temperatures of 66 °C or greater, which resulted in significant peel scalding. The HW dip treatments did not significantly affect total phenolics or total protein contents in the peel (Table 3-4 and Table 3-5).

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. So the flavedo browning was likely 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

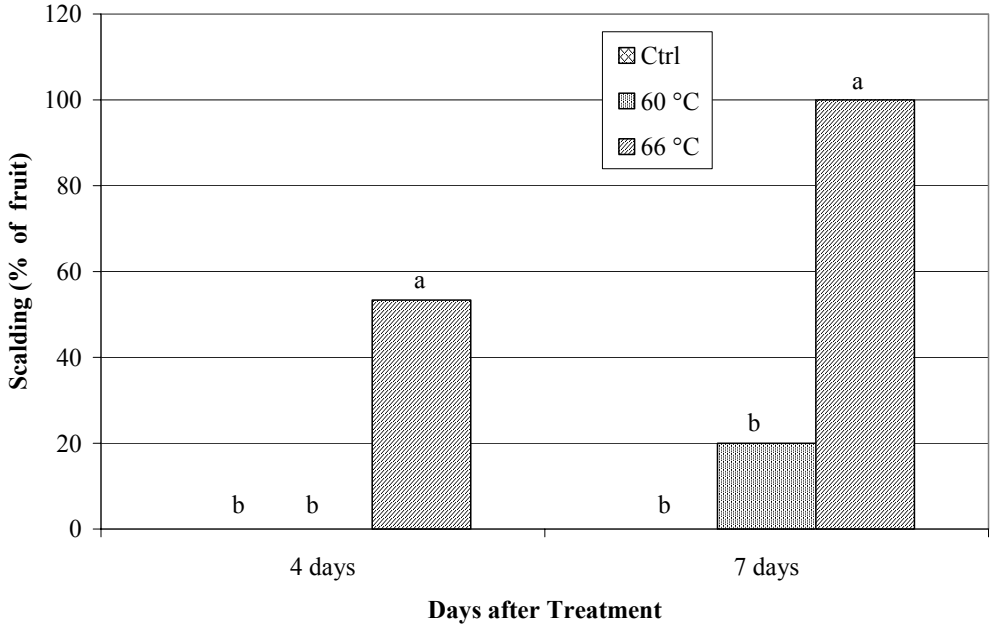

Figure 3-9. Peel scalding (percent of fruit scalded) of 'Valencia' oranges after heat treatment at 60 and 66 °C for 60 s. Scald was evaluated after 4 and 7 d of storage at 10 °C. Bars within each day with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip).

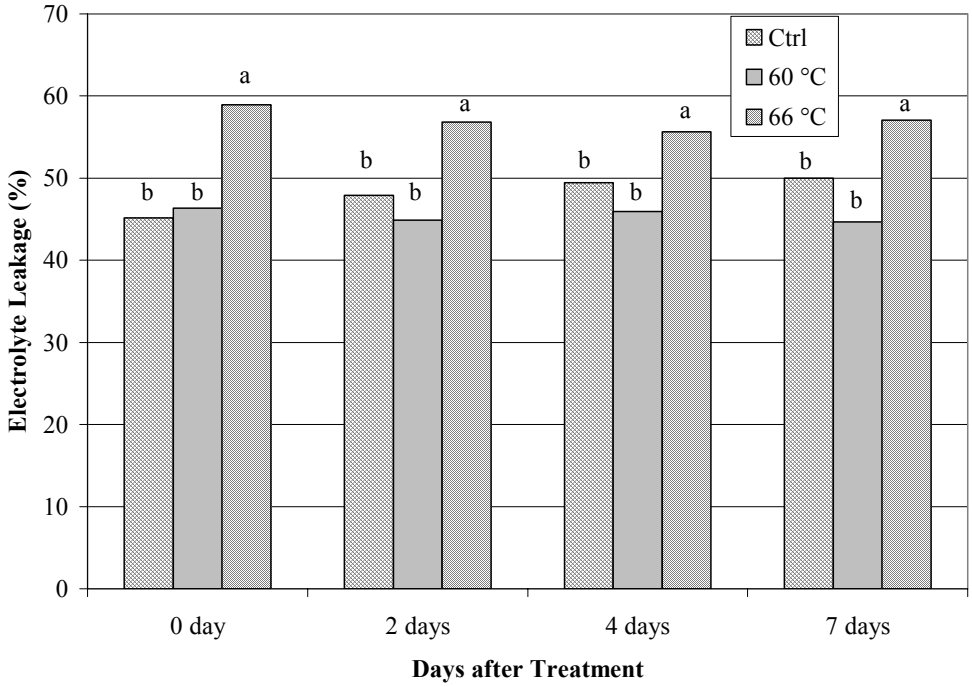

Figure 3-10. Electrolyte leakage from flavedo of 'Valencia' oranges immediately after hot water dip for 60 s and after 2, 4, and 7 d of storage at 10 °C. Bars within each day with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. Ctrl – Control (no HW dip).

et al., 1994). These could have also contributed to the peel browning caused by HW treatment.

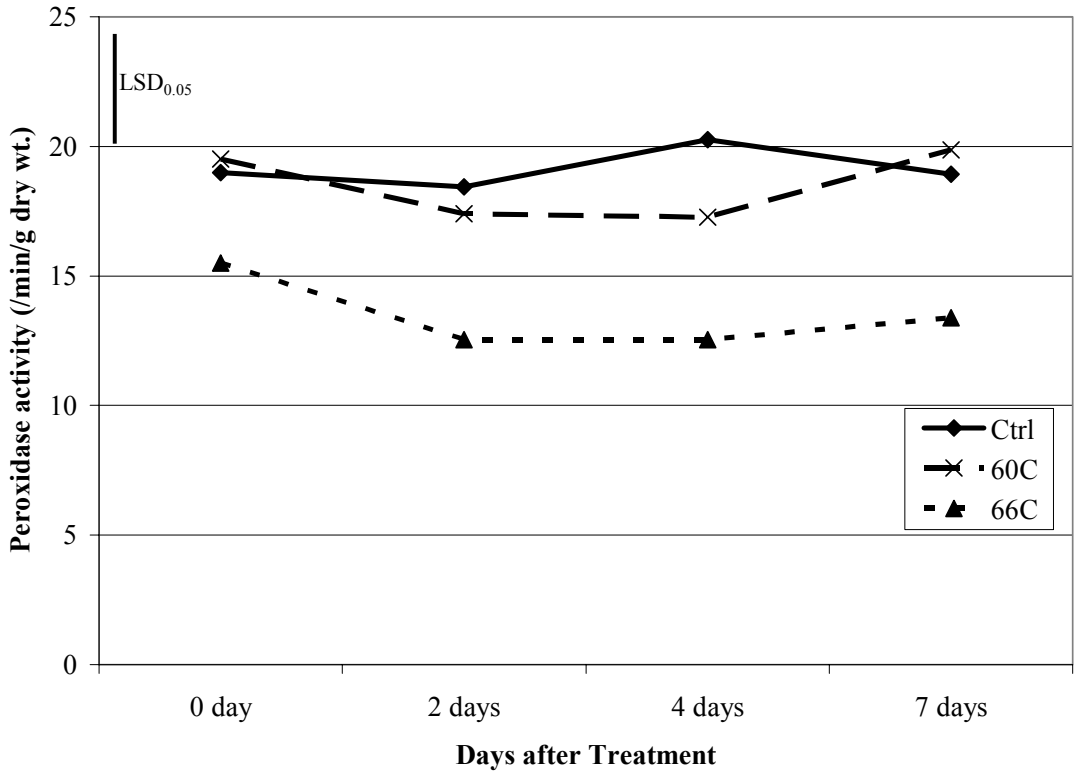

Figure 3-11. Peroxidase activity from flavedo of 'Valencia' oranges immediately after hot water dip for 60 s and after 2, 4, and 7 d of storage at 10 °C. Vertical bar represents the 5% LSD value. Ctrl – Control (no HW dip).

Table 3-4. Total phenolics from flavedo of 'Valencia' oranges immediately after hot water dip and after 2, 4, and 7 d of storage at 10 °C. Ctrl – Control (no HW dip).

	Total phenolics (mg/g dry wt.)			
Treatment (60 s)	0 day	2 days	4 days	7 days
Ctrl	3.101	3.499	3.510	3.746
60 °C	3.336	4.071	3.900	3.333
66 °C	3.808	3.117	3.320	3.695
Significance	ns	ns	ns	ns

ns Not significant at $P \leq 0.05$.

Table 3-5. Total protein content from flavedo of 'Valencia' oranges immediately after hot water dip and after 2, 4, and 7 d of storage at 10 °C. Ctrl – Control (no HW dip).

	Total protein (mg/g dry wt.)			
Treatment (60 s)	0 day	2 days	4 days	7 days
Ctrl	18.969	21.241	25.569	23.732
60 °C	20.468	19.096	23.543	23.897
66 °C	18.906	14.812	16.459	19.501
Significance	ns	ns	ns	ns

ns Not significant at $P \leq 0.05$.

CHAPTER 4
CONTROL OF POSTHARVEST DISEASES IN 'RUBY RED' GRAPEFRUIT BY HOT WATER DIP TREATMENT

Introduction

Short duration, hot water (HW) dip treatments appear to be a promising method to control postharvest decay in citrus (Rodov et al., 1995a). Heated solutions of sodium carbonate have been more effective in controlling decay than non-heated solutions (Palou et al., 2002). The following experiments were conducted to evaluate the effectiveness of HW dip, hot sodium carbonate dip, and hot imazalil dip for decay control in Florida grapefruit. The effect of washing or coating the fruit immediately after HW dip was also studied.

Materials and Methods

Fruit

Three experiments were conducted between November 2003 and May 2004. For the first experiment, fruit were harvested during November 2003 and for the last two experiments, fruit were harvested during February 2004 at the Indian River Research and Education Center research grove in Fort Pierce, Fla. Commercially mature (TSS:TA ≥ 7:1) and healthy 'Ruby Red' grapefruit were harvested randomly from 1-1.5 m above ground level on healthy trees, evenly spaced around the tree and distributed through the inner and outer canopy. Harvested fruit were stored at room temperature overnight and heat treatment was done on the next day.

Experiment 1

Harvested 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
2. Hot water dip, followed immediately by a 1 min dip in water at ambient (~25 °C) temperature
3. Hot water dip, followed immediately by washing and coating (simulated commercial packinghouse treatment)

The HW dip treatments were conducted using stainless steel tanks holding ~ 95 L of rapidly stirred water. Heating was accomplished using a large gas burner with the temperature varying by ~$\pm$1 °C during each treatment. Fruit were treated by placing them into perforated plastic crates that allowed water circulation past the fruit. Each treatment had four replicates of 40 fruit each. Fruit were washed over a brush bed and then coated with shellac (FMC Foodtech., Lakeland, Fla.) to simulate commercial handling. Fungicides were not used. Following treatment, the fruit were stored at 10 °C (90% RH). Ten fruit from each replicate were randomly selected, marked and weighed to follow weight loss during storage. Initial analyses of peel color, total soluble solids (TSS), titratable acidity (TA), peel puncture resistance (PPR), and percent juice were done using four replications of 10 fruit each. Fruit were evaluated for peel scalding 1 and 4 weeks after treatment. After 4 weeks of storage, marked fruit were weighed, and peel color, TSS, TA, PPR, and percent juice were determined. Decay was evaluated after 4, 8, and 12 weeks in storage.

Experiment 2

Harvested fruit were dipped in water at 25, 56 or 59 °C for 30 s. There were three post-dip treatments:

1. Hot water dip only
2. Hot water dip, followed immediately by washing (~25 °C water) through a commercial packing line
3. Hot water dip, followed immediately by washing (~25 °C water) and coating (simulated commercial packinghouse treatment)

The HW dips were administered as described in experiment 1. Each treatment had four replicates of 40 fruit each. Fungicides were not used. Following treatment, the fruit were stored at 16 °C (90% RH). Ten fruit from each replicate were randomly selected, marked, and weighed to follow weight loss measurement during storage. Fruit were evaluated for peel scalding 1, 2, and 4 weeks after treatment. After 4 weeks of storage, marked fruit were weighed. Decay was evaluated after 4, 8, and 12 weeks in storage.

Experiment 3

There were two dip temperatures and six chemical treatments:

1. Water alone
2. 3% sodium carbonate solution
3. 6% sodium carbonate solution
4. 125 ppm imazalil solution
5. 250 ppm imazalil solution
6. 3% sodium carbonate + 125 ppm imazalil solution

Harvested fruit were dipped at 25 or 56 °C for 30 s for each of the above treatments. The HW dips were administered as described in experiment 1. Each treatment had four replicates of 40 fruit each. There were five additional fruit in each replication for the imazalil treatments, which were taken for residue analysis after coating the fruit with shellac. Fruit were washed and shellac coated immediately after HW dip treatment. Following treatment, fruit were stored at 16 °C (90% RH). Ten fruit from each replicate

were randomly selected, marked, and weighed to follow weight loss during storage. Initial analyses of peel color, TSS, TA, PPR and percent juice were done using four replications of 10 fruit each. Fruit were evaluated for peel scalding 1 and 4 weeks after treatment. After 4 weeks of storage, marked fruit were weighed and peel color, TSS, TA, PPR, and percent juice were determined. Decay was evaluated after 4, 8, and 12 weeks in storage.

Peel Scalding

Peel scalding was evaluated as described in chapter 3.

Residue Analysis

Residue analysis was done by Decco, Ceraxagri, Inc., Fort Pierce, Fla. Fruit were cut into quarters and one section from each fruit was used. The samples were weighed and then blended. To the blended samples, DI water (100 mL), 5N NaOH (10 mL) and NaCl (50 g) were added and the homogenate was weighed. After homogenizing for 3 min, 50 g of the sample was taken in centrifuge tubes and 10 mL of iso-octane was added. After centrifuging at 1800 g_n for 10 min, 5 mL of supernatant solution was decanted into a 25 mL beaker. The samples were dried with anhydrous sodium sulfate. A 2 µL sample of the extract was injected into the gas chromatograph with electron capture (EC) or nitrogen phosphorus (NP) detector. The residue level was calculated from the following formula:

$$\text{Imazalil (ppm)} = \frac{\text{Chromatogram reading} \cdot 10 \cdot \text{Total weight}}{\text{Weight of blended fruit taken} \cdot \text{Initial weight}}$$

Weight Loss

Fruit were weighed on the 1st d after treatment and then again on the 4th week after treatment. Weight loss was calculated as follows:

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

Peel Color

Peel color was measured as described in chapter 3.

Total Soluble Solids and Titratable Acidity

The fruit were cut into halves along the equator and juice was extracted using a test juice extractor (Model 2700, Brown Citrus Systems Inc., Winter Haven, Fla.). 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 40 mL of juice samples to pH 8.3 with 0.3125 N NaOH using an automatic titrimeter (DL 12, Mettler-Toledo Inc., Columbus, Ohio).

Percent Juice

Percent juice was calculated from the total weight of fruit and total weight of juice.

$$\text{Percent juice} = \frac{\text{Juice weight (g)}}{\text{Fruit weight (g)}} \cdot 100$$

Peel Puncture Resistance

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 $mm \cdot s^{-1}$ and the maximum force exerted to puncture the peel was recorded. Peel puncture resistance was expressed in Newtons.

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

Experiment 1

Dipping fruit in 25, 53, or 56 °C water for 30 s did not cause any peel scalding after 4 weeks of storage at 10 °C (Table 4-1). Fruit dipped in 59 °C water for 30 s developed 17%-31% peel scalding and fruit dipped in 62 °C water for 30 s developed 46%-72% peel scalding depending on the post-dip treatment (Table 4-1). Schirra and D'hallewin (1997) reported peel scalding in 'Fortune' mandarins after HW dips at 56 or 58 °C for 3 min. Washing and shellac coating of fruit immediately after HW dip reduced the development of peel scalding by 45% or 37% in fruit dipped at 59 or 62 °C, respectively, compared with fruit that were not washed and coated. Dipping fruit in ambient water immediately after HW dip reduced the development of scalding by only 6% or 5% in fruit dipped at 59 or 62 °C, respectively, compared with fruit that were not dipped in ambient water after HW dips. So, washing and coating fruit immediately after HW dip can significantly reduce the heat damage.

Grapefruit dipped in 56 or 59 °C water followed by washing and shellac coating showed the best result in reducing decay to 25% or 18%, respectively, after 12 weeks of storage at 10 °C (Figure 4-1). Fruit dipped in 25 °C water followed by no post-dip treatment developed 75% decay. Decay development in fruit dipped in ambient water after HW dip at 62 °C did not differ from fruit that received no post-dip treatment after HW dip at 62 °C. But fruit treated at other temperatures followed by dipping in ambient water developed higher decay than fruit that received no post-dip treatment. Fruit dipped in 62 °C water were injured by the treatment, which negated the beneficial effect of

reducing decay. 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.

Though others have reported the development of chilling injury (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. Dipping fruit in 56 or 59 °C water followed by washing and

Table 4-1. Peel scalding (percent of fruit scalded) of 'Ruby Red' grapefruit after 30 s HW dip treatments followed by post-dip treatments. Fruit were evaluated after 4 weeks of storage at 10 °C. Dip – Fruit were dipped in ambient water for 1 min and Shellac – Fruit were washed over a brush bed and coated with shellac.

	Post-dip treatment		
Tmnt. temp.	None	Dip	Shellac
25 °C	0.00	0.00	0.00
53 °C	0.00	0.00	0.00
56 °C	0.00	0.00	0.00
59 °C	30.63	28.75	16.88
62 °C	71.88	68.13	45.63

$LSD_{0.05} = 14.37$

shellac coating reduced CI to 2% or 1%, respectively, after 12 weeks of storage at 10 °C (Figure 4-2). Fruit dipped in 25 °C water followed by no post-dip treatment developed 50% CI. Hot water dip did not affect the TSS, TA or the amount of juice (data not

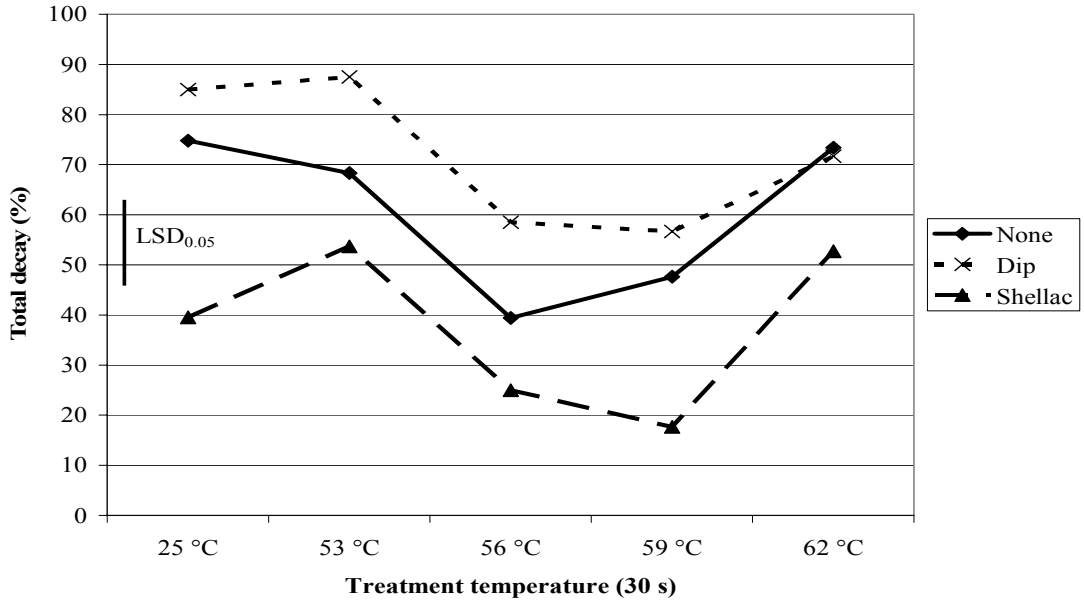

Figure 4-1. Total decay of 'Ruby Red' grapefruit after 30 s HW dip treatments followed by post-dip treatments. Fruit were evaluated after 12 weeks of storage at 10 °C. Vertical bar represents the 5% LSD value. Dip – Fruit were dipped in ambient water for 1 min and Shellac – Fruit were washed over a brush bed and coated with shellac.

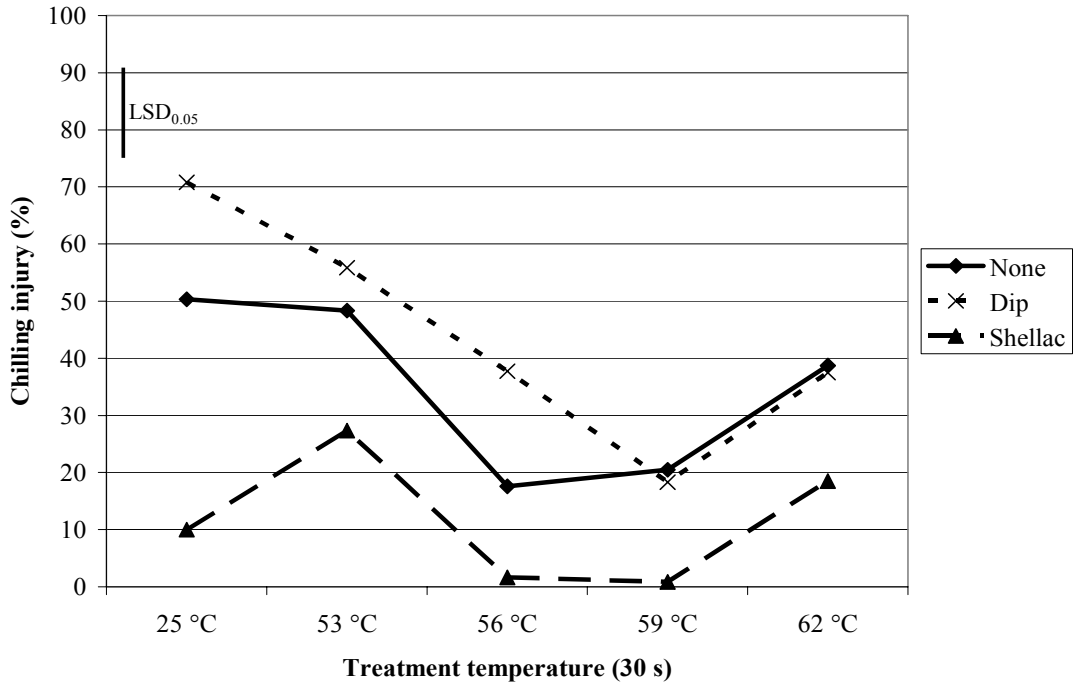

Figure 4-2. Chilling injury of 'Ruby Red' grapefruit after 30 s HW dip treatments followed by post-dip treatments. Fruit were evaluated after 12 weeks of storage at 10 °C. Vertical bar represents the 5% LSD value. Dip – Fruit were dipped in ambient water for 1 min and Shellac – Fruit were washed over a brush bed and coated with shellac.

shown). There were no consistent differences in weight loss or peel puncture resistance (data not shown).

Experiment 2

Fruit dipped in 59 °C water for 30 s had significantly more scalding than the fruit that were dipped in 25 °C water after 1, 2, and 4 weeks of storage at 16 °C. After 4 weeks in storage, the incidence of peel scalding for fruit dipped in 59 °C water for 30 s was 3%-5% depending on the post-dip treatment (Table 4-2) and peel scalding severity ranged from 8%-14% of the fruit surface (Table 4-3). In the previous experiment using November-harvested fruit, HW dips at 59 °C resulted in 17%-31% peel scalding incidence after 4 weeks of storage. So, the late season (February-harvested) fruit used in the second experiment may have been more resistant to heat damage. Hot water dips at 56 °C water for 30 s followed by no post-dip treatment resulted in only 2% incidence of peel scalding whereas fruit that were shellac coated after HW dipping at 56 °C did not develop any peel scalding. Washing alone or washing and coating the fruit immediately after HW dip did not have a significant effect on the development of peel scalding. Since the level of scalding was very low compared with the previous experiment, the effect of coating the fruit on heat damage could not be seen.

None of the HW or post-dip treatments were effective in controlling decay (Figure 4-3). Heat treatment and washing alone or washing and coating the fruit did not result in significant reduction in postharvest decay. The incidence of total decay was very low in this season compared with the previous experiment. This could be a reason why significant effect of treatments could not be seen.

Table 4-2. Peel scalding incidence (percent of fruit scalded) of 'Ruby Red' grapefruit after 30 s HW dip treatments followed by post-dip treatments. Fruit were evaluated after 4 weeks of storage at 16 °C. Wash – Fruit were washed over a brush bed and Shellac – Fruit were washed over a brush bed and coated with shellac.

	Post-dip treatment		
Tmnt. temp.	None	Wash	Shellac
25 °C	0.00	0.00	0.00
56 °C	1.88	3.13	0.00
59 °C	2.50	3.75	5.00

$LSD_{0.05} = 2.05$

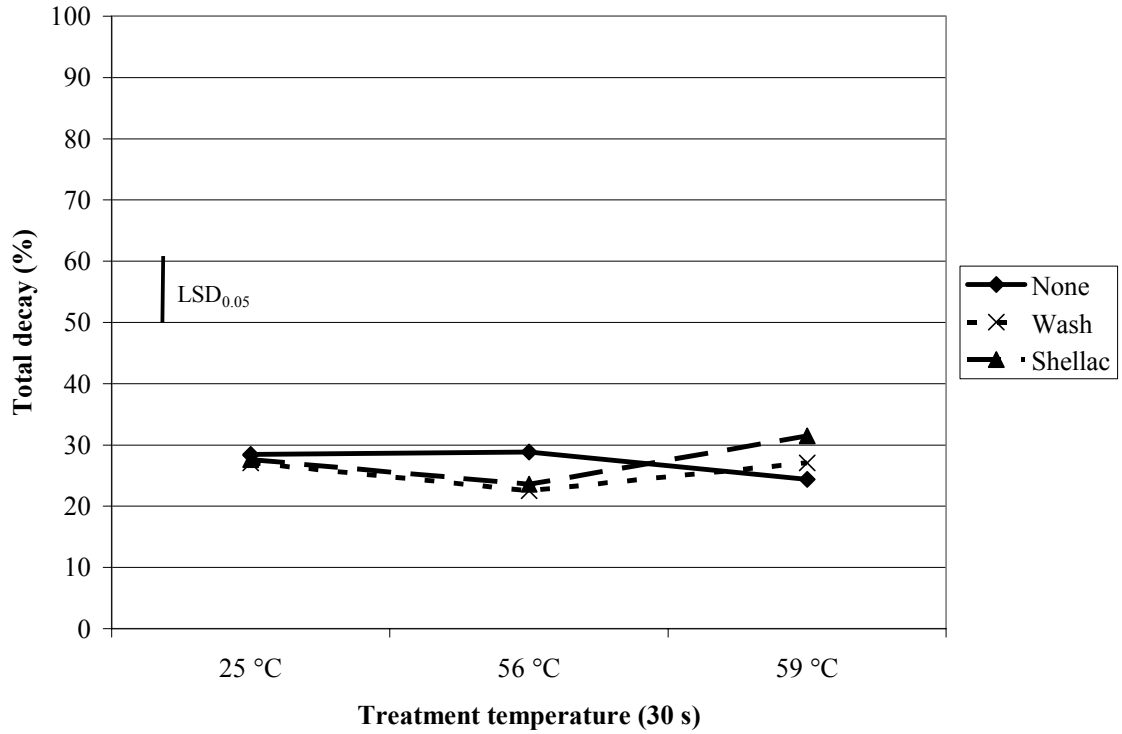

Figure 4-3. Total decay of 'Ruby Red' grapefruit after 30 s HW dip treatments followed by post-dip treatments. Fruit were evaluated after 12 weeks of storage at 16 °C. Vertical bar represents the 5% LSD value. Wash – Fruit were washed over a brush bed and Shellac – Fruit were washed over a brush bed and coated with shellac.

Table 4-3. Peel scalding severity (percent of fruit surface scalded) of 'Ruby Red' grapefruit after 30 s HW dip treatments followed by post-dip treatments. Fruit were evaluated after 4 weeks of storage at 16 °C. Wash – Fruit were washed over a brush bed and Shellac – Fruit were washed over a brush bed and coated with shellac.

	Post-dip treatment		
Tmnt. temp.	None	Wash	Shellac
25 °C	0.00	0.00	0.00
56 °C	6.67	11.25	0.00
59 °C	7.50	13.75	9.17

$LSD_{0.05} = 2.05$

Experiment 3

Fruit dipped in heated solutions of imazalil had greater residue levels immediately after dipping than fruit dipped in non-heated solutions of imazalil (Figure 4-4). Fruit dipped in heated solutions of imazalil had 0.6-1 ppm of residue whereas fruit dipped in ambient temperature solution had only 0.1 ppm of residue. 'Salustiana' oranges dipped in imazalil solution at 50 °C had 8-fold greater residue than when dipping was done at 20 °C (Cabras et al., 1999). Hence, lower concentrations of imazalil could be more effective when the application occurs at higher temperatures.

After 4 weeks of storage, no significant scalding was observed in fruit dipped at 56 °C for 30 s. Only 0.2% of the fruit were scalded. Most decay was due to stem-end rot (SER). After 12 weeks of storage at 16 °C, HW dipping at 56 °C for 30 s resulted in lower SER than the non-heated solutions for all the imazalil and sodium carbonate solutions except for the water dip treatment (Figure 4-5). Ritenour et al. (2003) reported that a HW dip at 56 °C for 30 s decreased SER to 20% after 12 weeks in storage compared with 33% SER in fruit dipped in ambient water. Treatments with 3% or 6% sodium carbonate solutions at 25 °C increased the incidence of SER to 27% or 23%,

respectively, compared with water dip treatment at 25 °C, which developed 6% SER. Treatments with 3% or 6% sodium carbonate solutions at 56 °C also increased the incidence of SER (to 14% or 19%, respectively) compared with the water dip treatment at 56 °C, which developed 11% SER. This is in contrast to the effect of hot sodium carbonate solutions on green and blue molds. Smilanick et al. (1997) and Palou et al. (2001, 2002) have shown that sodium carbonate solutions, especially heated solutions, significantly reduce the development of green and blue molds in citrus. The SER may be enhanced by the high pH (10-11) of the sodium carbonate solutions. Treating the fruit with imazalil solution did not result in significant reduction in SER though the fruit dipped in heated imazalil solutions had higher residue levels. Since the incidence of natural decay was low in fruit harvested during February, the effect of imazalil could not be observed.

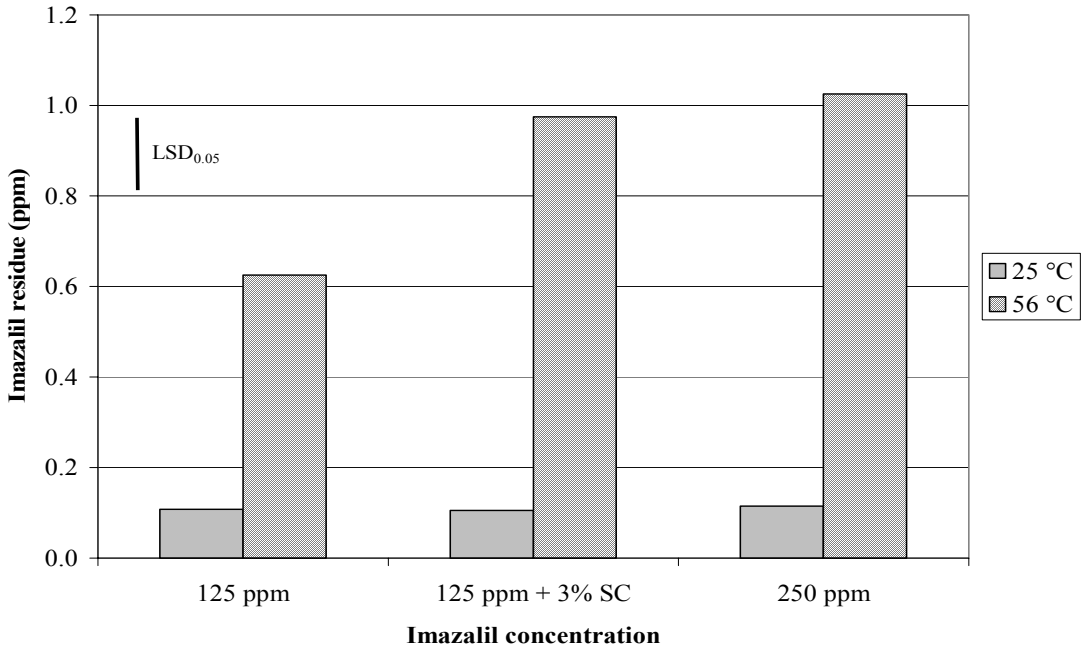

Figure 4-4. Imazalil residue in 'Ruby Red' grapefruit immediately after the 30 s dip treatments at 25 and 56 °C. Vertical bar represents the 5% LSD value. SC – Sodium carbonate.

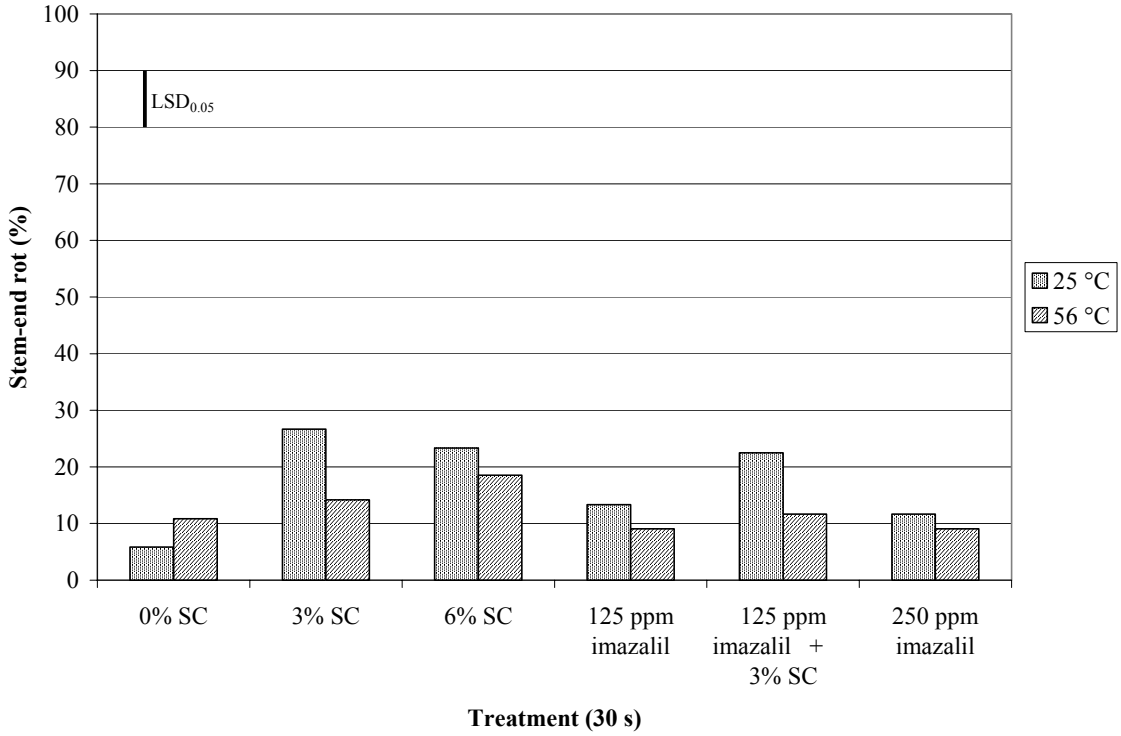

Figure 4-5. Stem-end rot for different treatment temperatures after 12 weeks of storage at 16 °C. Bars with different letters are significantly different by Duncan's multiple range test at $P \leq 0.05$. SC – Sodium carbonate.

CHAPTER 5
CONTROL OF CHILLING INJURY IN 'RUBY RED' GRAPEFRUIT BY HOT WATER DIP TREATMENT

Introduction

Grapefruit develops chilling injury (CI) when stored at temperatures below 10-12 °C (Chace et al., 1966). Grapefruit harvested early and late in the season are more susceptible to CI than are fruit harvested in mid-season (Grierson and Hatton, 1977). Fruit from the outer-canopy are more susceptible to CI than are fruit from the inner-canopy (Purvis, 1980). Heat treatments have been successful in reducing CI in many citrus varieties (Rodov et al., 1995a; Schirra et al., 1997). The following experiment was conducted to study the effect of short duration, hot water (HW) dip treatments in controlling CI in Florida grapefruit.

Materials and Methods

Fruit

Commercially mature (TSS:TA ≥ 7:1) and healthy 'Ruby Red' grapefruit were harvested randomly from 1-1.5 m above ground level on healthy trees, evenly spaced around the tree on 3 Nov. 2003 at the Indian River Research and Education Center research grove in Fort Pierce, Fla. Fruit were harvested separately from the inner and outer canopies of the tree. The harvested fruit were stored at room temperature overnight and heat treatment was done on the next day.

Heat Treatment

Fruit were dipped in water at 25, 53, 56 or 59 °C for 30 s. Hot water dips were administered as described in chapter 4. Each treatment had four replicates of 30 fruit each. After the HW dip, half of the fruit from 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, marked, and weighed to follow weight loss during storage. Initial analyses of peel color, total soluble solids (TSS), titratable acidity (TA), peel puncture resistance (PPR) and percent juice were done using four replicates of five fruit each. Fruit were evaluated for peel scalding 1, 3, and 7 weeks after treatment. After 4 and 7 weeks of storage, marked fruit were evaluated for weight loss. After 4 weeks of storage, peel color, TSS, TA, PPR, and percent juice were evaluated from another five fruit randomly selected from each replicate. After 6 weeks of storage, fruit stored at 5 °C (90% RH) were transferred to 16 °C (90%RH). After 7 weeks of storage, remaining fruit were evaluated for CI and decay.

Chilling Injury

Chilling injury severity 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 the average CI severity for that replicate.

Peel Scalding

Peel scalding was evaluated as described in chapter 3.

Weight Loss

Weight loss was calculated as described in chapter 4.

Peel Color

Peel color was measured as described in chapter 3.

Total Soluble Solids and Titratable Acidity

Total soluble solids and titratable acidity were measured as described in chapter 4.

Peel Puncture Resistance

Peel puncture resistance was measured as described in chapter 4.

Percent Juice

Percent juice was calculated as described in chapter 4.

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

Hot water dip had a greater effect in reducing CI severity of inner canopy fruit than of outer canopy fruit. Compared to fruit dipped at 25 °C, dipping fruit in 53, 56, or 59 °C water for 30 s reduced CI severity by 3%, 6% or 10%, respectively, in outer canopy fruit stored at 5 °C, but reduced CI severity by 11%, 18% or 32%, respectively, in inner canopy fruit (Figure 5-1). Purvis (1980) has reported that outer canopy fruit are more susceptible to CI than the inner canopy fruit, but our results indicated little effect of canopy position on non-heated fruit with both inner and outer canopy fruit severely affected by CI. So, heat treatment by itself had a major role in reducing the CI severity in inner canopy fruit. None of the fruit stored at 16 °C developed CI.

Fruit stored at chilling temperature (5 °C) developed more decay than fruit stored at non-chilling temperature (16 °C). After 7 weeks of storage, fruit stored at 16 °C developed only 0%-4% decay whereas fruit stored at 5 °C developed 27%-58% decay (Figure 5-2). Most decay was due to anthracnose (*Colletotrichum gloeosporioides*). High incidence of anthracnose was observed on fruit that developed CI. For fruit stored at 5 °C, HW dipping at 53, 56 or 59 °C for 30 s reduced decay by 25%, 21% or 44%, respectively, compared with dipping in 25 °C water for 30 s.

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. After 4 weeks of storage, TSS in fruit from outer canopy was significantly higher than in fruit from inner canopy and TA was significantly lower in outer canopy fruit than in inner canopy fruit. The percent juice was not affected by the canopy position. The percent juice and TA was significantly lower in fruit stored at 5 °C than in fruit stored at 16 °C. However, HW dipping did not affect the percent juice, TSS, and TA of the fruit (data not shown). After 4 weeks of storage, PPR was significantly greater in fruit treated at 59 °C than in all other treatments (Table 5-1). 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 5-2). 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 could be due to accelerated weight loss in fruit that developed CI. Purvis (1984) correlated higher weight loss during storage with CI development in citrus fruit. Cohen et al. (1994) used weight loss as an early indicator of CI.

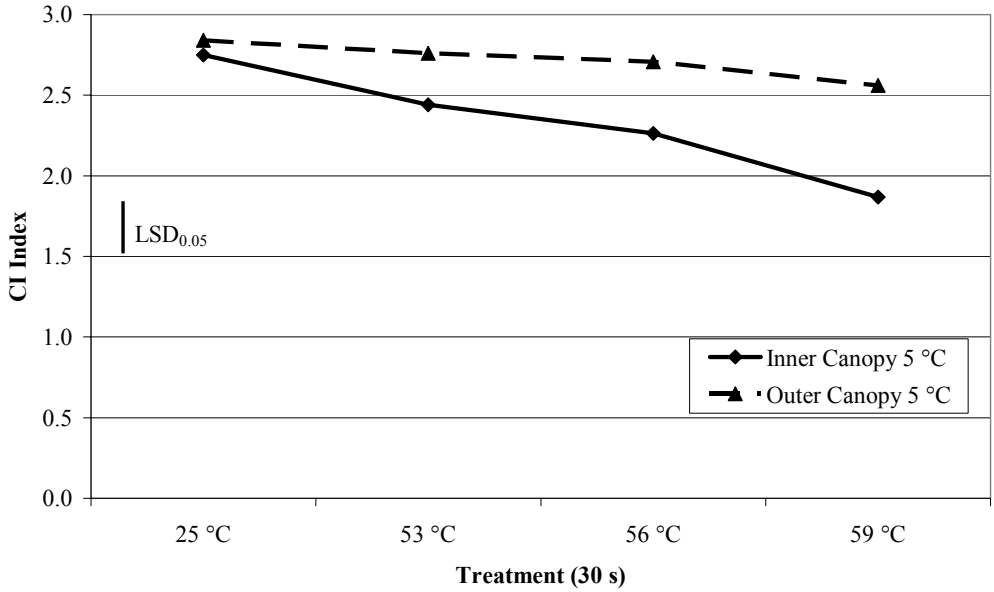

Figure 5-1. Chilling injury in 'Ruby Red' grapefruit after 30 s dip treatments of inner and outer canopy fruit. Fruit were evaluated after 6 weeks of storage at 5 °C plus 1 week at 16 °C. Chilling injury was rated from 0 (none) to 3 (severe). Vertical bar represents the 5% LSD value.

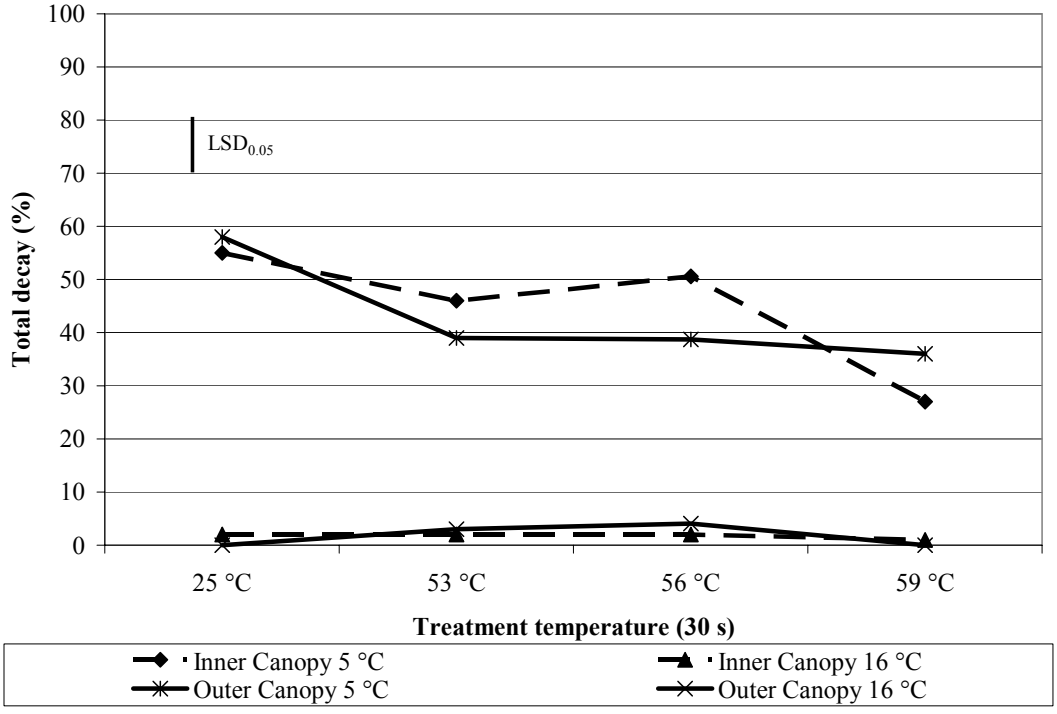

Figure 5-2. Total decay in 'Ruby Red' grapefruit after 30 s dip treatments of inner and outer canopy fruit. Fruit were evaluated after 7 weeks of storage at 5 or 16 °C. Vertical bar represents the 5% LSD value.

Table 5-1. Peel puncture resistance in Newtons of 'Ruby Red' grapefruit after 30 s dip treatments. Fruit were evaluated after 4 weeks of storage at 5 or 16 °C.

	Inner Canopy		Outer Canopy	
Tmnt. Temp.	5 °C	16 °C	5 °C	16 °C
25 °C	17.24	14.39	18.47	13.68
53 °C	18.19	14.53	18.12	14.37
56 °C	18.29	14.73	18.34	13.55
59 °C	18.25	14.91	20.28	15.17

$LSD_{0.05} = 1.66$

Table 5-2. Weight loss (%) from 'Ruby Red' grapefruit after 30 s dip treatments. Fruit were evaluated after 7 weeks of storage at 5 or 16 °C.

	Inner Canopy		Outer Canopy	
Tmnt. Temp.	5 °C	16 °C	5 °C	16 °C
25 °C	4.34	3.77	4.42	3.84
53 °C	4.15	3.61	3.68	3.55
56 °C	3.96	3.05	3.71	3.37
59 °C	4.23	3.61	3.92	3.36

$LSD_{0.05} = 0.61$

CHAPTER 6
CONCLUSIONS

The research reported in this thesis has shown the effects on ethylene treatment of grapefruit and physiological responses of oranges due to hot water (HW) dip treatments and also the effects of HW dip treatment in reducing postharvest decay and chilling injury (CI) in Florida grapefruit. As a result of these studies, it was found that HW dips administered before or after grapefruit degreening did not affect subsequent peel injury, respiration rate, ethylene production, total soluble solids (TSS) or titratable acidity (TA). Under commercial conditions, the various uses of ethylene treatment would not appear to have any affect on the use of HW treatments on grapefruit.

Significantly higher electrolyte leakage was found only in fruit dipped at temperatures greater than 66 °C for 60 s. In 'Valencia' oranges, a HW dip at 56 °C for up to 120 s or 60 °C for 60 s did not affect electrolyte leakage though it resulted in 67% or 100% incidence of peel scalding, respectively. None of these treatments affected electrolyte leakage than the non-treated fruit. Hence electrolyte leakage is not an early indicator of peel injury.

'Valencia' oranges dipped at temperatures above 66 °C for 60 s had lower L* values than non-treated fruit. Hot water dip at 70-90 °C for 60 s did not result in significant difference in chroma value though fruit developed visible peel discoloration. But, in a subsequent experiment, fruit dipped at 66-70 °C for 60 s had significantly lower chroma value. The hue value was not affected by HW dip. The peroxidase activity of heat-treated fruit (66 °C for 60 s) was lower than that of non-treated fruit or fruit treated

at 60 °C for 60 s. Hot water dips did not affect the total phenolics or the total protein content.

To study the effects of washing or shellac coating of fruit immediately after a HW dip, two experiments were conducted with ‘Ruby Red’ grapefruit. After 4 weeks of storage, washing and shellac coating of fruit immediately after HW dip reduced the development of peel scalding by 45% or 37% in fruit dipped at 59 or 62 °C, respectively, compared with fruit that were not washed and coated. Dipping the fruit in ambient water or washing through brush beds after HW treatment did not significantly reduce peel scalding.

Since the fruit used in the first coating experiment were harvested in November, they were highly susceptible to CI with 50% CI incidence in control fruit dipped in 25 °C water followed by no post-dip treatment and stored at 10 °C. After 12 weeks of storage, fruit dipped in 56 or 59 °C water followed by washing and shellac coating reduced CI to 2% or 1%, respectively. To study the effects of HW dip on CI, fruit were harvested separately from the inner and outer canopy and stored at 5 °C for 6 weeks followed by 1 week at 16 °C. Hot water dip at 53, 56 or 59 °C reduced CI by 3%, 6% or 10%, respectively, in outer canopy fruit, but reduced CI by 11%, 18% or 32%, respectively, in inner canopy fruit.

Scalding was not significant in fruit dipped at 56 °C water for 30 s. HW dip at 56 or 59 °C for 30 s significantly reduced decay in fruit harvested in November. The fruit harvested in February were less susceptible to decay and so the effects of HW dip could not be seen. In none of the experiments did HW dip affect TSS or TA of the fruit.

Although HW treatments reduced significantly the development of postharvest decay, there was still a substantial amount of decay. So, HW treatment alone did not effectively control the decay development. Heated solutions of sodium carbonate and imazalil were used at lower concentrations than used commercially at ambient temperature. Unfortunately, solutions of 3% or 6% sodium carbonate enhanced the development of stem-end rot. Imazalil at 125 and 250 ppm did not effectively control decay compared with fruit that were dipped in water only.

From the experiments conducted on ‘Ruby Red’ grapefruit, HW dip at 56 °C for 30 s followed by washing and shellac coating significantly reduced development of postharvest decay and CI without causing significant damage to the peel and without affecting the juice quality of the fruit. Hence, HW dip at 56 °C for 30 s followed by washing and shellac coating was the best treatment for ‘Ruby Red’ grapefruit.

Future experiments should be conducted to increase the efficacy of HW dip by using heated solutions of compounds generally regarded as safe or heated solutions of fungicides at lower concentrations. In the current study, heated solutions of sodium carbonate or imazalil did not effectively control postharvest decay in late season fruit. However, others have reported the efficacy of these compounds in heated solutions, which suggests they can be effective under modified conditions. Hence more work should be conducted on early season fruit when they are more susceptible to natural decay and economic losses from such decay is often more extensive.

APPENDIX A
ANALYSIS OF VARIANCE FOR CHAPTER 2

Table A-1. Analysis of variance for respiration rates of 'Ruby Red' grapefruit during the first six days in storage at 20 °C.

Sources of variation	d.f.	Mean squares of respiration rate					
		1 day	2 days	3 days	4 days	5 days	6 days
Treatment	4	3.12*	6.13**	3.95**	3.45*	2.05*	2.37
Error	10	0.65	0.81	0.12	0.72	0.18	4.34

* F values significant at 5%
** F values significant at 1%

Table A-2. Analysis of variance for ethylene production of 'Ruby Red' grapefruit during the first six days in storage at 20 °C.

Sources of variation	d.f.	Mean squares of ethylene production					
		1 day	2 days	3 days	4 days	5 days	6 days
Treatment	4	0.0012	0.0004	0.0007	0.0005	0.0027	$0.0080
Error	10	0.0012	0.0003	0.0004	0.0005	0.0017	0.0037

Table A-3. Analysis of variance for percent of fruit scalded, total soluble solids and titratable acidity of 'Ruby Red' grapefruit after 10 d of storage at 20 °C.

Sources of variation	d.f.	Mean squares		
		Scald	Total soluble solids	Titratable acidity
Treatment	4	0.5255**	1.2723	0.0228*
Error	10	0.0090	2.0180	0.0041

* F values significant at 5%
** F values significant at 1%

APPENDIX B
ANALYSIS OF VARIANCE FOR CHAPTER 3

Experiment 1

Table B-1. Analysis of variance for percent of fruit scalded after 12 d of storage at 10 °C, electrolyte leakage of treated and untreated sides of 'Valencia' oranges immediately after treatment.

		Mean squares		
Sources of variation	d.f.	Scald	Electrolyte leakage (treated side)	Electrolyte leakage (untreated side)
Treatment	5	0.4560**	26.789	57.141*
Error	12	0.0005	21.600	14.659

* F values significant at 5%
** F values significant at 1%

Experiment 2

Table B-2. Analysis of variance for peel scalding of 'Valencia' oranges after 14 d of storage at 10 °C.

Sources of variation	d.f.	Mean squares: Percent of fruit scalded	Mean squares: Percent of fruit surface scalded
Treatment	5	0.5096**	0.5952**
Error	12	0.0064	0.0007

** F values significant at 1%

Table B-3. Analysis of variance for electrolyte leakage of treated and untreated sides of 'Valencia' oranges immediately after treatment and after 14 d of storage at 10 °C.

		Mean squares			
		0 day		14 days	
Sources of variation	d.f.	Electrolyte leakage (treated side)	Electrolyte leakage (untreated side)	Electrolyte leakage (treated side)	Electrolyte leakage (untreated side)
Treatment	5	0.2803**	0.0025	0.1390**	0.0011
Error	12	0.0020	0.0027	0.0014	0.0009

** F values significant at 1%

Experiment 3

Table B-4. Analysis of variance for peel scalding of 'Valencia' oranges after 8 d of storage at 10 °C.

Sources of variation	d.f.	Mean squares: Percent of fruit scalded	Mean squares: Percent of fruit surface scalded
Treatment	6	1.8531**	1.2430**
Error	14	0.0064	0.0084

** F values significant at 1%

Table B-5. Analysis of variance for electrolyte leakage of treated and untreated sides of 'Valencia' oranges immediately after treatment and after 8 d of storage at 10 °C.

		Mean squares			
		0 day		8 days	
Sources of variation	d.f.	Electrolyte leakage (treated side)	Electrolyte leakage (untreated side)	Electrolyte leakage (treated side)	Electrolyte leakage (untreated side)
Treatment	6	0.0744**	0.0020	0.0360*	0.0013
Error	14	0.0046	0.0017	0.0051	0.0123

* F values significant at 5%

** F values significant at 1%

Experiment 4

Table B-6. Analysis of variance for peel scalding of 'Valencia' oranges after 4 and 7 d of storage at 10 °C.

Sources of variation	d.f.	Mean squares			
		4 days		7 days	
		Percent of fruit scalded	Percent of fruit surface scalded	Percent of fruit scalded	Percent of fruit surface scalded
Treatment	2	0.6691**	0.2513**	2.0104**	0.4761**
Error	6	0.0045	0.0015	0.0406	0.0154

** F values significant at 1%

Table B-7. Analysis of variance for electrolyte leakage from flavedo of 'Valencia' oranges immediately after treatment and after 2, 4 and 7 d of storage at 10 °C.

Sources of variation	d.f.	Mean squares of electrolyte leakage			
		0 day	2 days	4 days	7 days
Treatment	2	0.0176**	0.0116*	0.0073*	0.0117**
Error	6	0.0006	0.0012	0.0009	0.0008

* F values significant at 5%
** F values significant at 1%

Table B-8. Analysis of variance for peroxidase activity in flavedo of 'Valencia' oranges immediately after treatment and after 2, 4 and 7 d of storage at 10 °C.

Sources of variation	d.f.	Mean squares of peroxidase activity			
		0 day	2 days	4 days	7 days
Treatment	2	14.22*	29.78*	45.31	36.82*
Error	6	3.40	5.43	14.27	9.35

* F values significant at 5%

Table B-9. Analysis of variance for total phenolics in flavedo of 'Valencia' oranges immediately after treatment and after 2, 4 and 7 d of storage at 10 °C.

Sources of variation	d.f.	Mean squares of total phenolics			
		0 day	2 days	4 days	7 days
Treatment	2	0.3890	0.6916	0.2632	0.1522
Error	6	0.5317	0.6104	0.1957	0.3273

Table B-10. Analysis of variance for total protein in flavedo of 'Valencia' oranges immediately after treatment and after 2, 4 and 7 d of storage at 10 °C.

Sources of variation	d.f.	Mean squares of total protein content			
		0 day	2 days	4 days	7 days
Treatment	2	0.0939	1.2856	2.7458	0.7453
Error	6	0.4346	0.3432	1.6853	2.8706

APPENDIX C
ANALYSIS OF VARIANCE FOR CHAPTER 4

Experiment 1

Table C-1. Analysis of variance for peel scalding, total decay and chilling injury of 'Ruby Red' grapefruit. Peel scalding was evaluated after 4 weeks of storage at 10 °C. Total decay and chilling injury were evaluated after 12 weeks of storage at 10 °C.

		Mean squares			
Sources of variation	d.f.	Percent of fruit scalded	Percent of fruit surface scalded	Total decay	Chilling injury
Water temperature (WT)	4	2.0818**	0.8977**	0.3174**	0.4455**
Post dip treatment (PDT)	2	0.0453*	0.0045	0.7405**	1.0092**
WT x PDT	8	0.0195	0.0042*	0.0225	0.0494
Error	45	0.0123	0.0017	0.0279	0.0242

* F values significant at 5%
** F values significant at 1%

Table C-2. Analysis of variance for quality of 'Ruby Red' grapefruit after 4 weeks of storage at 10 °C.

		Mean squares				
Sources of variation	d.f.	Weight loss	Peel puncture resistance	Juice content	Total soluble solids	Titratable acidity
Water temperature (WT)	4	1.0734**	8.6801**	2.0819	0.2332	0.0136
Post dip treatment (PDT)	2	0.2176	3.7180*	2.8395	0.0922	0.0275
WT x PDT	8	0.2889	0.8983	2.9977	0.2822	0.0092
Error	45	0.1962	0.8120	2.0396	0.3028	0.0085

* F values significant at 5%
** F values significant at 1%

Experiment 2

Table C-3. Analysis of variance for peel scalding and total decay of 'Ruby Red' grapefruit. Peel scalding was evaluated after 4 weeks and total decay was evaluated after 12 weeks of storage at 16 °C.

		Mean squares		
Sources of variation	d.f.	Percent of fruit scalded	Percent of fruit surface scalded	Total decay
Water temperature (WT)	2	42.36*	311.67**	29.08
Post dip treatment (PDT)	2	2.26	87.34	13.97
WT x PDT	4	6.94	41.29	42.61
Error	27	7.99	42.14	109.37

* F values significant at 5%
** F values significant at 1%

Experiment 3

Table C-4. Analysis of variance for imazalil residue of 'Ruby Red' grapefruit immediately after treatment.

Sources of variation	d.f.	Mean squares of imazalil residue
Water temperature (WT)	1	3.5190**
Imazalil treatment (IT)	2	0.0968**
WT x IT	2	0.0933*
Error	18	0.0157

* F values significant at 5%
** F values significant at 1%

Table C-5. Analysis of variance for peel scalding and stem-end rot of 'Ruby Red' grapefruit. Peel scalding was evaluated after 4 weeks and stem-end rot was evaluated after 12 weeks of storage at 16 °C.

		Mean squares		
Sources of variation	d.f.	Percent of fruit scalded	Percent of fruit surface scalded	Stem-end rot
Water temperature (WT)	1	0.5208	18.75	300.25*
Chemical treatment (CT)	5	0.2083	8.75	238.14**
WT x CT	5	0.2083	8.75	78.62
Error	36	0.2604	10.42	49.26

* F values significant at 5%
** F values significant at 1%

APPENDIX D
ANALYSIS OF VARIANCE FOR CHAPTER 5

Table D-1. Analysis of variance for peel scalding, chilling injury index and total decay of 'Ruby Red' grapefruit. Peel scalding was evaluated at 4 weeks of storage. Chilling injury and total decay were evaluated after 7 weeks of storage.

		Mean squares			
Sources of variation	d.f.	Percent of fruit scalded	Percent of fruit surface scalded	Chilling injury index	Total decay
Water temperature (WT)	3	11.11*	25.00**	0.2359**	0.0718*
Canopy position (CP)	1	0.70	6.25	0.5968**	0.0016
Storage temperature (ST)	1	0.00	0.00	101.9090**	6.5993**
WT x CP	3	0.70	6.25	0.0630	0.0043
WT x ST	3	0.00	0.00	0.2359**	0.0504
CP x ST	1	6.26	6.25	0.5968**	0.0001
WT x CP x ST	3	6.26	6.25	0.0630	0.0320
Error	48	2.66	5.21	0.0451	0.0184

* F values significant at 5%
** F values significant at 1%

Table D-2. Analysis of variance for quality of 'Ruby Red' grapefruit after 4 weeks of storage.

		Mean squares			
Sources of variation	d.f.	Peel puncture resistance	Juice content	Total soluble solids	Titratable acidity
Water temperature (WT)	3	4.3335*	2.6888	0.1242	0.0026
Canopy position (CP)	1	0.5293	0.0923	1.0000**	0.0225**
Storage temperature (ST)	1	253.6853**	289.1275**	1.0000**	0.0286**
WT x CP	3	2.1114	2.0580	0.2083	0.0004
WT x ST	3	0.2691	0.0613	0.2283	0.0003
CP x ST	1	6.3252*	0.0083	0.0225	0.0084
WT x CP x ST	3	0.6842	7.2340	0.1975	0.0016
Error	48	1.3632	4.8838	0.0783	0.0023

* F values significant at 5%
** F values significant at 1%

Table D-3. Analysis of variance for weight loss in 'Ruby Red' grapefruit after 4 and 7 weeks of storage.

		Mean squares	
Sources of variation	d.f.	Weight loss (4th week)	Weight loss (7th week)
Water temperature (WT)	3	0.4211**	0.8772**
Canopy position (CP)	1	0.0054	0.1828
Storage temperature (ST)	1	0.0375	4.5263**
WT x CP	3	0.0293	0.1445
WT x ST	3	0.0245	0.0720
CP x ST	1	0.0147	0.2678
WT x CP x ST	3	0.0087	0.0749
Error	48	0.0672	0.1862

** F values significant at 1%

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BIOGRAPHICAL SKETCH

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