INFLUENCE OF HARVEST MATURITY AND PRE-STORAGE CONDITIONING ON QUALITY OF MELTING AND NON-MELTING FLESH PEACHES

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2011

To my family

ACKNOWLEDGMENTS

I am very grateful to both of my advisors, Dr. Jeffrey K. Brecht and Dr. Jeffrey G. Williamson, for their motivation, guidance, and support. I would like to thank Dr. Donald J. Huber for his assistance in solving problems associated with laboratory assays. I also would like to thank Dr. Jose Chaparro for establishing the peach journal club, which helped me to keep up with the literature. I am very appreciative of Dr. Elizabeth A. Baldwin for her willingness to participate in my supervisory committee. I would like to thank Kim Cordasco, Paul Miller, James Lee and Adrian Berry for their assistance in setting up my experiments both in the laboratory and peach orchard. Lastly, I would like to thank Bee Ling Poh, Angelos Deltsidis, Francisco Loayza, and Pei-Wen Huang, for their kind help during my treatment days.

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August 2011

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Major: Horticultural Sciences

Two melting flesh (MF) peach cultivars, ‘Flordaprince’ and ‘TropicBeauty’, and two non-melting flesh (NMF) peach cultivars, ‘UFSun’ and ‘Gulfking’, were sorted into different maturity groups (MG) at harvest based on peel ground color a* value (GCa*). The NMF cultivars harvested at different developmental stages generally produced higher climacteric ethylene at harvest and during ripening than the MF cultivars. The MF peaches were preclimacteric or at onset of the climacteric rise at harvest. The NMF cultivars can be harvested at more advanced developmental stages (MG 15-20) than the MF cultivars (MG 5-10) for immediate fresh market consumption due to the absence of rapid softening at late ripeness stage. The MF cultivars and NMF ‘Gulfking’ fruit intended for low temperature storage should be harvested at MG 0-10 for best quality when ripened, but NMF ‘UFSun’ needs to be harvested at a more advanced stage (MG 10-15) to avoid development of abnormal softening.

A second study focused on the effect of different pre-storage conditioning treatments on maintenance of fruit quality during ripening at ambient temperature (20°C) or after low temperature storage condition (0°C). NMF ‘UFSun’ and NMF ‘Delta’

peaches at commercial harvest maturity were immersed for 30 min in water at 25°C (Control) or 46°C (HW), or in 25°C water containing 100 µg/L 1-methylcyclopropene (1-MCP), or in 46°C water containing 100 µg/L 1-MCP (HW x 1-MCP). It was found that 100 µg/L 1-MCP was insufficient to inhibit fruit softening for both of those NMF cultivars. The experiment was repeated with higher 1-MCP concentrations based on the climacteric ethylene production rate of the fruit measured before the treatment. The results indicate the HW treatment alone was most potent in delaying fruit softening of the NMF peaches during ambient temperature storage. Although low temperature storage prolonged the inhibitory effect of 1-MCP, both HW and HW x 1-MCP treatments were more effective than 1-MCP application for firmness retention of the NMF peaches during ripening after low temperature storage.

CHAPTER 1
INTRODUCTION

Peaches are one of the most popular fruits in the world because of their dessert-like flavor and a long history of cultivation (Robertson et al., 1991; Li 1984). They can also be an important source of antioxidants for human nutrition (Tomas-Barberan et al., 2001). Peaches ranked 6th among 18 fruits in U.S. per capita consumption in 1997, higher than grapes (7th), cherries (16th), blueberries (17th), and cranberries (18th) that typically contain high polyphenol concentrations, but are underused in the average American diet (Vinson et al., 2001). Although peaches are flavorful and nutritious, the annual per person consumption of peaches (both fresh and canned) dropped from 13 lbs in the early 1970s to about 8.8 lbs by 2008 (Brunke et al., 2011). Fresh consumption was around 4.6 lbs in 2006 but increased to 5.5 lbs in 2008. Canned consumption decreased from 7 lbs per person in the 1970s to 3.0 lbs per person in 2008. This suggests that consumers would like to incorporate fresh peach fruit in their diet more than the canned fruit.

Lack of flavor and hard texture are two main complaints from consumers about fresh peaches (Bruhn et al., 1991). Traditional peach cultivars (melting-flesh types; MF) ripen quickly at ambient temperature and are extremely vulnerable to mechanical injuries after harvest due to extensive softening toward the end of ripening process. Thus, peaches grown for fresh consumption are harvested at “firm-mature” stage (Cascales et al., 2005; Williamson and Sargent, 1999). These fruit are often immature and cannot ripen to have the same qualities as tree-ripened fruit.

To extend the postharvest life of peaches, immediate refrigeration at low temperature is necessary. The recommended storage temperature for most peaches is

0°C for up to 2 or 3 weeks depending on the cultivar (Crisosto et al., 1999). Prolonged low temperature storage enhances the development of chilling injury (CI), which can result in peaches with poor flavor and dry and mealy texture (Lurie and Crisosto, 2005). Immature and unripe peaches are more susceptible to chilling injury than ripe fruit.

Another way to preserve the quality of peaches after harvest is to minimize ethylene production either by blocking ethylene synthesis or action. Pre-storage conditioning methods such as heat treatment (ethylene biosynthesis inhibitor) and 1-MCP application (ethylene action inhibitor) have the potential to be incorporated into commercial handling practices for peaches (Clemente Vitti et al., 2007; Kluge and Jacomino, 2002; Murray et al., 2007).

A trend in peach breeding is to combine the ideal fresh market quality of MF peaches with the firmer texture of non-melting flesh (NMF) peaches (Sherman et al., 1990). NMF peaches are generally used for canning since they are able to maintain their integrity throughout the high-temperature retort treatment (Robertson et al., 1992b). They retain initial texture longer after harvest and soften gradually during ripening, thus decreasing the chance of physical damages from handling. A NMF fruit with MF flavor may attain maximum sensory qualities on the tree and would be more resistant to the mechanical damage and decay associated with excessive softening.

Peach varieties that are suitable for growing in subtropical and tropical environments are continuously being released. Developing these low-chill subtropical cultivars is advantageous because they provide a fresh supply of fruit for local markets and the potential to ship fruit for higher values to more distant markets where peach production is not occurring (Rouse and Sherman, 2002). Although this deciduous fruit is

becoming more popular in the subtropical and tropical regions, information on optimum harvest maturity, maturity indicators, and postharvest handling practices are limited, especially for the new NMF varieties.

Using four, low-chill, subtropical cultivars, MF ‘Flordaprince’, MF ‘TropicBeauty’, NMF ‘UFSun’ and NMF ‘Gulfking’, the objectives of this study were 1) to determine optimum harvest maturity based on ambient and low temperature storage conditions and to develop indices that can best predict fruit maturity at harvest; and 2) to compare respiration rates and ethylene production for fruit harvested at different stages during ripening. The results indicate that the NMF cultivars can be harvested at more advanced stages than the MF cultivars for immediate fresh market consumption. The MF and NMF cultivars generally can be stored at 0°C for 2 weeks if harvested at lower maturity than those destined for immediate fresh market consumption. Harvest maturity for both peach types can be best predicted with ground color a* value and titratable acidity. The NMF peach cultivars harvested at different stages generally produced higher climacteric ethylene than the MF peaches at harvest and during ripening.

A second study was carried out using NMF ‘UFSun’ and NMF ‘Delta’ peaches to evaluate if some pre-storage conditioning methods, including hot water treatment, liquid 1-MCP application, a combination of the two, are effective to maintain quality, especially texture, of the fruit during ripening in ambient temperature storage (20°C) and after low temperature storage (0°C). The results suggest that HW treatment is more effective than 1-MCP treatment as a pre-storage conditioning method for quality maintenance of NMF peaches at 20°C. HW and HW x 1-MCP treatments have the potential to be used

to control ripening of NMF peaches that are held in low temperature storage. 1-MCP treatment can be considered for cultivars that develop surface injury under heat stress.

CHAPTER 2
LITERATURE REVIEW

Origin and Cultivation

Peach is native to China and its cultivation has been dated as early as 3,000 B.C (Li, 1984). The botanical name of peach, *Prunus persica* (L.) Batch, can be traced to Persia (Iran) (Bassi and Monet, 2008). It is believed that peaches were carried from China to Persia and quickly spread from there to Europe. The Spaniards first introduced peaches to America around 1565. The earliest cultivation was at St. Augustine, Florida and along the Savannah River in Georgia. Around the same time, peaches brought by the Spaniards disseminated quickly among the Aztecs in Mexico. From Mexico, peaches spread to New Mexico, Arizona, and California. Eventually, commercial peach production in California started around the mid 19th century (Faust and Timon, 1995).

According to the Food and Agriculture Organization (FAO) of the United Nations (FAO, 2009), China accounts for 50% of the world production of peaches and nectarines (a peach mutation lacking trichomes on the fruit) followed by Italy (10%), Spain (7%) and the USA (6%). In the USA, peaches are commercially produced in 23 states and the top four states are California, South Carolina, New Jersey and Georgia. California is a major producer of both fresh and processed peaches, while South Carolina, New Jersey, and Georgia mainly produce fresh peaches. In 2009, the USA produced a total of 1,103,770 tons of peaches, 46% of which were destined for fresh consumption and 54% were for processing (NASS, 2010). Major processed peach products include canned and frozen fruit. Other products include peach concentrate, baby food, dry fruit, jam, and jelly (Siddiq, 2006).

Peaches are generally pleasant to consumers due to dessert-like characteristics developed during ripening (Robertson et al., 1991). They can also be an important source of antioxidants (Tomas-Barberan et al., 2001). Vinson et al. (2001) presented data on per capita consumption of several fruits in 1997. Among 18 fruits, peach ranked 6th in per capita consumption, higher than grapes (7th), cherries (16th), blueberries (17th), and cranberries (18th) that typically have high phenolic concentrations but are underused in the average American diet.

Melting (MF) and non-melting flesh (NMF) are two types of peach fruit that are most commonly produced. Most fresh market peaches belong to the MF group. They soften relatively fast in the late stages of fruit ripening ('melting phase') compared to the NMF types that remain relatively firm. NMF peaches are generally used for canning since they are able to maintain their integrity throughout the high-temperature retort treatment (Robertson et al., 1992b). MF cultivars carry the dominant allele of the (M) locus that controls flesh firmness while NMF cultivars possess a homozygous recessive (mm) allele (Peace et al., 2005).

Various peach training systems are utilized in commercial orchards. Although the open vase system is the most popular, other training systems such as palmette, Y-trellis, and slender spindle (fusetto), offer higher density planting, which can be more beneficial in certain orchards (Corelli-Grappadelli and Marini, 2008). One of the major goals for any training and pruning system is to increase light interception without increasing vegetative growth potential (Grossman and DeJong, 1998). Light environment surrounding the fruit is able to positively influence fruit quality such as size,

ground color (GC), firmness (softening), and sugar content (Lewallen and Marini, 2003; Marini et al., 1991).

In the USA, the bulk of peach production areas are temperate, high-chill locations with fruit available beginning in late May; hence, developing low-chill subtropical cultivars is economically important because they provide a fresh supply of fruit for local markets and the potential to transport fruit for higher values to more distant markets with no peach production at certain times of the year. The difference between high-chill and low-chill cultivars depends on the amount of “chilling” or accumulation of cool temperature required during the winter dormancy before the resumption of growth in spring. The amount of chilling can be measured as chill units (CU) or chill hours, defined as 1 hour of exposure to an optimum air temperature range (Richardson et al., 1974). Temperatures between 32 and 45 °F (0 and 7.2°C) are believed to be the optimum chilling temperatures for temperate zone peaches (Weinberger, 1950). In the Southeastern USA, low-chill cultivars generally have less than a 500 chill hour requirement (Okie, 2004). North Carolina accumulates the largest number of chilling hours among the Southeastern states. The recommended varieties for North Carolina usually have a chilling requirement of 750 hours or greater (Parker and Werner, 1993). Varieties that require chilling between 500-750 hours are considered medium-chill in the Southeastern USA. Low-chill cultivars have been reported to set adequate crops after exposure to temperatures at or below 55 °F (12.8°C) (Rouse and Sherman, 2003). Low-chill cultivars usually have a relatively short fruit development period (days from bloom to mature fruit), resulting in earlier harvests than the medium- and high-chill cultivars grown in temperate regions (Andersen et al., 2001). Low-chill cultivars grown in

sub-tropical regions like Florida are also bred to withstand different climactic, disease, and pest pressures than higher chill cultivars grown in temperate regions.

Fruit Growth and Development

Peach fruit growth has been described as a double sigmoidal curve since the work of Connors (1919). The double sigmoidal curve is separated into three stages. Stage I includes the first exponential growth phase, which is characterized by rapid cell division and development of the endosperm. Endocarp sclerification or pit hardening occurs and rapid embryo development in Stage II, but little pericarp growth occurs. Stage II is followed by a second exponential growth phase characterized by rapid enlargement of mesocarp cells (Stage III), commonly known as the “final swell “. Stage III has been the primary focus of peach postharvest physiology research since fruit maturation, ripening, and senescence all occur during this stage. Cell division and cell expansion are both energy consuming process. Therefore, respiration rates are high during Stage I of fruit development, decreases through Stage II (pit hardening) and part of Stage III (second exponential growth phase), and rises gradually at the end of Stage III (Ramina et al., 2008).

The primary cell wall grows in the expansion phase as fruit increases in size. The growing wall behaves like a network of inextensible cellulose microfibrils laterally linked together via a complex matrix of flexible polysaccharides (glycans and pectins) that may bind to cellulose and to each other (Cosgrove, 1999). According to Brummell et al. (2004), pectins that are loosely attached to the wall through ionic calcium bonds increase in amount as peach fruit reaches full size. Pectins that are covalently attached to the wall are approximately half as much as those ionic bond pectins and the amount is fairly constant until early-ripe. It is expected that the latter will decline with an

increased of the former type of pectin in later stages. The amount of matrix glycans in the wall were relatively constant during the early developmental stages, with approximately 1/3 loosely bound to the wall and 2/3 tightly attached to the wall. The tightly bound matrix glycans decrease or are “solubilized” as loosely bound ones increase during ripening.

Fruit maturation is a stage of development leading to the attainment of physiological or horticultural maturity (Watada et al., 1984). Physiological maturity is defined as the stage of development at which a plant or plant part will continue ontogeny even if detached from the plant. A horticulturally mature commodity is defined as having reached a stage of development such that, after harvesting and postharvest handling, its quality will be at least the minimum acceptable to the ultimate consumer (Reid, 1992). For peaches, the horticultural maturity generally means when the fruit has reached or even passed the physiological maturity but has not undergone ripening (Delwiche and Baumgardner, 1983). In terms of postharvest physiology, a mature peach is generally referred to its horticultural maturity instead of physiological maturity. Ripening involves changes that transform the mature fruit into one that is ready to eat (Crisosto, 1994). These transformations can begin as early as the middle of the second exponential growth phase (Chalmers and Ende, 1975) to as late as when the exponential phase has plateaued (Kader and Mitchell, 1989). Tonutti et al. (1991) has further separated the ripening stage (Stage IV) from Stage III. Ethylene evolution is low and constant during development and generally begins to increase at Stage IV, reaching a peak that coincides with the last stage of peach fruit ripening.

Levels of different sugars vary greatly during fruit growth in peaches. The sucrose concentration remains low in the early stages of development. A large amount of sucrose accumulates during Stage III but these molecules do not come from starch to sugar conversion as in fruits like apple and banana (Brady, 1993). Although young peach fruit tend to store some carbon as starch, this starch is used before the fruit enters Stage III. Glucose and fructose are present at similar concentrations throughout fruit development, and are the predominant soluble carbohydrates of mesocarp tissue during the early stages of growth. Thereafter, their contents decrease concurrent with the rise in sucrose concentration.

Malate and citrate are the two major organic anions acids in peaches, but the ratio of the two from maturation to ripening is not constant, which reflects differential regulation of respiration at each developmental stage. Chapman et al. (1991) observed that during early development, the percentage of malic and citric acids in 'Majestic' peaches were similar. Malic acid increased and citric acid decreased in Stage III. At harvest maturity, malic acid composed about 50-60% of the total organic acid, citric (20-25%) and quinic (20-25%) being present in lesser quantities (Byrne et al., 1991).

As an immature fruit transforms into a mature one, there is a slight increase in total phenolic compounds and total carotenoids (Kader et al., 1982). In peaches, the peel has higher concentration of phenolics than the flesh (Wang et al., 2010). These phenolic compounds have a role in the visual appearance (pigmentation and browning), taste (astringency), and health-promoting properties (free-radical scavengers) (Tomas-Barberan et al., 2001). Peaches are rich in carotenoids such as β-carotene and β-cryptoxanthin (Caprioli et al., 2009). Chlorophyll content decreases significantly during

maturation parallel with an increase in carotenoid content (Cascales et al., 2005). Consuming carotenoids is also beneficial to heath since β-carotene and β-cryptoxanthin have been reported to be the primary provitamin A carotenoids (Curl, 1959). Vitamin C, a natural antioxidant, is higher in concentration in mature than in immature peach fruit (Kader et al., 1982). The increase in total ascorbic acid along with increased maturity is contributed by a higher level of dehydroascorbic acid (oxide form) (Camejo et al., 2010), indicating that more antioxidants are being used to offset oxidative stress brought by fruit ripening and senescence.

Peach Postharvest Physiology – Climacteric Ripening

Ethylene

Ethylene is a gaseous plant hormone with numerous important roles in plant growth, development, and senescence. Its production increases during leaf abscission and flower senescence as well as during fruit ripening. Furthermore, plants respond to environmental stimuli, such as touching, wounding, pathogen attack, and flooding with induction of ethylene production (Abeles et al., 1992). The biosynthetic pathway of ethylene from methionine consists of three steps: (1) S-adenosyl-L-methionine (SAM) synthetase catalyzes the conversion of methionine to SAM; (2) formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from SAM via ACC synthase (ACS) activity; (3) conversion of ACC to ethylene by ACC oxidase (ACO) (Yang and Hoffman, 1984). Two systems are hypothesized to regulate ethylene production in higher plants. System 1 is responsible for producing the basal levels of ethylene detectable in all tissues, in which ethylene inhibits its own biosynthesis during the preclimacteric stage of fruit development. When development shifts from preclimacteric to climacteric, System 2 is responsible for the upsurge in ethylene production in which ethylene biosynthesis

becomes autocatalytic (McMurchie et al., 1972). The initiation of autocatalytic ethylene production is accompanied by increases in ACO and ACS gene expression (Giovannoni, 2001).

Ethylene perception begins when ethylene binds to its receptor sites in the tissue and activates the downstream signal transduction. Treatment with ethylene hastens the ripening of climacteric fruit as a result of the complete saturation of all the available receptor sites (Payasi and Sanwal, 2010). In Arabidopsis, ethylene is perceived by a family of five receptors: ETR1, ETR2, ERS1, ERS2 and EIN4 (Guo and Ecker, 2004). In peaches, PpETR2 is induced at the very early steps of climacteric and displays a ripening related expression pattern (Trainotti et al., 2006). PpETR1 and PpERS1 expression does not appear to be controlled by ethylene (Ziliotto et al., 2008). Ethylene binding to the ETRs further inactivates CTR1, a key negative regulator of the ethylene receptor complex (Kieber et al., 1993). EIN2 is the first positive regulator in the ethylene signaling cascade acting downstream from CTR1. Transcript accumulation of PpEIN2 has been associated with ripening peaches (Begheldo et al., 2008; Trainotti et al., 2006). The nuclear protein EIN3 is downstream of EIN2 and acts as a transcription factor that regulates the expression of its immediate target genes such as ethylene response factor (ERF) (Solano and Ecker, 1998). ERFs are proteins that specifically bind to GCC box in promoters of ethylene-responsive genes and lead to the regulation of ethylene-controlled gene expression (Guo and Ecker, 2004). PpERF2 has been associated with peach fruit ripening and to the presence of ethylene (Trainotti et al., 2007).

The ripening process of peaches, like other climacteric fruits such as tomato, avocado, banana and apple, is regulated by System II ethylene for initiation and control of ripening (Lelievre et al., 1998). Ethylene production by peaches varies greatly from mature but unripe fruit (0.10 µl kg^{-1} h^{-1}) to ripe fruit (up to 160 µl kg^{-1} h^{-1}) (Crisosto and Kader, 2004). In peach fruit, a gradient of ethylene concentration is established between epicarp and mesocarp during ripening. High rates of ACO activity in the epicarp disk was proposed to be responsible for the higher values of ethylene evolution (Tonutti et al., 1991). Haji et al. (2004) reported that varietal difference determines whether ethylene biosynthesis is initiated before or after softening begins. For example, in early softening MF 'Kushigatahakuto', ethylene production and softening precede completion of fruit enlargement and changes in GC, as well as the rapid rise in sugar content. Supported by Brovelli et al. (1998b), who reported that for both NMF 'Oro A' and 'FL-28C', although maturity was reached (based on GC and firmness) fruit did not stop increasing in diameter and mass. In the late softening MF 'Nagasawahakuho', ethylene production starts after the fruit attain full size, complete sugar accumulation, and change GC from green to yellow. Tonutti et al. (1996) reported similar results with other MF cultivars.

Another flesh type in peach is called "Stony Hard" (SH). SH peaches do not produce any ethylene autocatalytically due to reduced expression of PpACS1 (Tatsuki et al., 2006). The stony hard trait is controlled by a single recessive gene (*hd*) which is inherited independently of the (M) locus (Haji et al., 2005). Consequently, this type of peach does not soften after harvest or even after the indicators for ripening such as GC, sugars and acids, have changed unless an external ethylene source is applied (Haji et

al., 2003). Stony hard (SH) peaches are useful for studying the regulation of ethylene on fruit ripening, especially on cell wall modification (softening).

Ethylene can be both beneficial and deleterious in postharvest physiology. It is essential for the ripening of climacteric fruits because without ethylene ripening does not proceed and the result is unpalatable fruit. However, once ripening is triggered by ethylene, the irreversible process can soon turn the beneficial aspects of ethylene for generating a high-quality product into over-ripening and decay (Barry and Giovannoni, 2007). Thus, controlling the biosynthesis and action of ethylene during ripening in climacteric fruits is a key point, but it may not be enough for delaying postharvest deterioration.

Both ethylene and developmental controls have been suggested to regulate climacteric ripening. Transgenic and mutant tomato lines with inhibited ethylene biosynthesis or perception demonstrate that ACS expression is initially induced during ripening by an unknown developmental signaling system (Barry et al., 2000). Hence, controlling both ethylene dependent and independent pathways involved in ripening can potentially lengthen the postharvest life of many fruits and vegetables.

Respiration

Aerobic respiration, which involves the oxidative breakdown of certain organic substances stored in the tissues, supplies the energy required by horticultural products to support a myriad of metabolic processes. In peaches, the burst in respiration associated with climacteric ripening occurs simultaneously with the increased levels of System 2 ethylene production (Ferrer et al., 2005; Madrid et al., 2000). The average respiration rate of peach fruit during ripening is classified as moderate when compared to other species (Wills et al., 2007). The respiration rate (CO_2 production) at the

climacteric peak has been reported to vary from 64 to 110 mg kg^{-1} h^{-1} at 20 ºC depending on the genotype (Crisosto and Kader, 2004). Marked differences in respiration rates have been observed in relation to the duration of the fruit developmental cycle. Ventura et al. (1998) reported that cultivars with shorter developmental cycles (earlier harvest dates) had higher and more pronounced respiration rates at the climacteric peak.

Compositional Changes

Sugars, acids, salts, bitter compounds such as alkaloids or flavonoids, and aroma volatiles contribute greatly to the flavor in fruits and vegetable (Baldwin et al., 2007). However, these compounds may only be at the minimum acceptable level at harvest maturity of a commodity. Compositional changes during ripening increase the desirable flavor. In peaches, the sugar to acid ratio in ripe fruit plays a major role in consumer acceptance (Crisosto et al., 2006). The pigment changes during peach fruit ripening provide visual attraction and boost quality (Francis, 1995).

Sugars

The main sugars in a peach fruit at harvest maturity are sucrose, fructose, and glucose in a proportion of about 3:2:1 (Byrne et al., 1991) or 3:1:1 (Génard et al., 2003). A rapid accumulation of sucrose starts at the beginning of Stage III and peaks when ripening is initiated (Chapman et al., 1991; Vizzotto et al., 1996). The glucose:fructose ratio is about 1 during early Stage III and falls to 0.8 during ripening, showing that glucose is preferentially used for respiration during ripening (Souty et al., 1998). Peaches with high eating quality are considered to have relatively large amounts of fructose and low quantities of glucose and sorbitol (Brooks et al., 1993) because fructose is 3.0, 2.3, and 1.7 times sweeter than sorbitol, glucose, and sucrose,

respectively (Kulp et al., 1991). Although sugars are commonly thought to be synonymous with soluble solids content (SSC), salts, proteins, acids are also included in the measurement since SSC measures all the soluble solids dissolved in water. The sugar content of peach juice can be conveniently estimated using a refractometer. There is high consumer acceptance for peaches with high SSC (Crisosto et al., 2006). Overall, the SSC in mature nectarines and peaches should be in excess of 10% for acceptable quality (Beckman and Krewer, 1999; Kader and Mitchell, 1989).

Acids

Peach fruit undergoes a continuous accumulation of organic acids, mainly malic and citric acid, during development. These organic acids are used as respiratory substrates (Etienne et al., 2002). A common method for measuring the level of the acids is by titration with sodium hydroxide (Jones and Scott, 1984). Titratable acidity (TA) of peaches declines from Stage III to the end of ripening process (Bakshi and Masoodi, 2009; Kwon et al., 2007; Moing et al., 1999). Malic acid generally remains constant throughout ripening while citric acid either significantly increases (Borsani et al., 2009) or decreases (Robertson et al., 1991) depending on the peach genotype. The TA of the fruits in the former case did not change significantly during the post-harvest ripening process.

Different acids can affect perception of sourness differently depending on their chemical structure. An increase in molecular weight can increase sourness perception while increasing carboxyl groups can decrease sourness (Hartwig and Mcdaniel, 1995). The concentration of acids correlates highly with sourness in ripe peaches (Crisosto et al., 2006), Although titratable acidity (TA) has been reported to play an important role in consumer acceptance for grapes (Crisosto and Labavitch, 2002), cherries (Crisosto et

al., 2003), and kiwifruit (Marsh et al., 2004), SSC/TA ratio or pH can sometimes relate better to sourness perception than TA itself (Malundo et al., 2001).

Peach cultivars can be further categorized by the level of TA into high-acid and low-acid (sub-acid) cultivars. The high-acid 'Elegant Lady' has an average of 0.75% TA whereas the low-acid 'Ivory Princess' has an average of 0.22% TA within the populations tested (Crisosto et al., 2003). Moing et al. (1998) found 10 fold differences in TA between 'Fantasia' (high-acid) and 'Jalousia' (low-acid) peaches. Consumer acceptance for low-acid was always greater than for high-acid cultivars regardless of fruit maturity (Iglesias and Echeverria, 2009).

Pigments

Color changes associated with ripening strongly influence visual and eating quality of fruits and vegetables (McGuire, 1992). Changes in color in many fruits involve loss of chlorophyll, synthesis of new pigments such as carotenoids and/or anthocyanins, and unmasking of other pigments that are formed previously during development (Ferrer et al., 2005). Martinez- Madrid et al. (2000) reported that changes in peel GC and flesh color (FC) initiated around the same time during onset of peach fruit ripening but the changes in GC were more dramatic than in FC.

Color can be measured objectively in the CIE (L*, a*, b*) color sphere using a reflectance colorimeter. CIE refers to the Commission Internationale de l'Eclairage (International Commission on Illumination). For all peach genotypes the chromaticity a* value (green-red) of the epidermal GC (GCa* value) increases the most with maturation and ripening, whereas the L* value (lightness, from black to white) and b* (yellow-blue) value change only slightly (Delwiche and Baumgardner, 1985). Thus, the a* value has been suggested as the primary coordinate of change near harvest and has been

described as a reliable color index of maturity for both MF and NMF peaches (Byrne et al., 1991; Delwiche and Baumgardner, 1983; Robertson et al., 1993). The FCa* value has been selected as a good maturity index for processing peaches (Fuleki and Cook, 1976; Kader et al., 1982). Lessertois and Moneger (1978) showed that pigments of the mesocarp are the same as foliar types, i.e. chlorophylls, α- and β-carotene, lutein, epoxylutein, violaxanthin and neoxanthin. The increase of FCa* value from negative to positive as maturity advances also denotes the loss of green color related to the disappearance of chlorophyll in the fruit flesh (Ferrer et al., 2005). Other color measurements can be obtained from calculating hue angle (h°; arctangent of b*/a*) and chroma [C*; $(a^{*2} + b^{*2})^{½}$], an index analogous to color saturation or intensity (McGuire, 1992). In peaches, a sharper decline in h° was found in peel than flesh and the C* of both remained constant throughout ripening (Madrid et al., 2000).

Evidence has been found to support ethylene regulation of color changes during the onset of peach fruit ripening (Amoros et al., 1989; Robertson et al., 1992a). For example, anoxia or 1-MCP treatments, which inhibit ethylene biosynthesis and ethylene perception, respectively, when applied at the preclimacteric stage, reduced the level of pigments in peaches (Mencarelli et al., 1998). Although Luchsinger and Walsh (1998) showed a good correlation between ethylene production and changes in GCa* values in ‘Redhaven’ and ‘Elegant Lady’ peaches, exceptions were reported by Haji et al. (2004). MF ‘Nagasawahakuho’ underwent GC change before the onset of ethylene evolution and the GC-a* value of the stony hard (SH) cultivar ‘Yumyeong’ increased as maturity advanced without any application of ethylene.

The results from transcriptome profiling of nectarines treated with 1-MCP indicated that β-carotene hydroxylase was the earliest gene induced at the onset of ripening and appeared to be the only one among the selected targets that was significantly affected by 1-MCP (Ziliotto et al., 2008). Therefore, the accumulation pattern and abundance of specific carotenoid compounds (i.e., lycopene, β-carotene, and xanthophylls) induced by ethylene depended largely on the genetic background rather than the presence or not of the ethylene climacteric at ripening. These results reinforce the hypothesis that both development and ethylene synergistically control peach fruit color development during ripening.

Textural Changes

One of the most important determinants of peach fruit quality and consumer acceptability is the transformation of texture from hard to soft during fruit ripening. MF peach cultivars lose firmness quickly once ripening is initiated, but NMF cultivars maintain their textural integrity for a longer period of time; thus, NMF peaches can be harvested at a more advanced stage of ripeness and have a longer potential shelf life than the traditional MF cultivars (Byrne, 2002; Sherman et al., 1990). The texture differences of MF and NMF cultivars are controlled by a single gene with the NMF characteristic being recessive (Bailey and French, 1949). The limited softening of NMF cultivars during ripening coincides with their reduced capacity to degrade cell walls (Lester et al., 1994; Lester et al., 1996; Pressey and Avants, 1978). Furthermore, the reduced capacity to degrade cell wall is not due to ethylene production since NMF fruit can produce higher levels of ethylene than MF fruit (Brovelli et al., 1999a).

NMF cultivars are believed to have high potential for the fresh market, primarily because their improved firmness retention allows the harvest of NMF fruit to be delayed

until ripening has begun. However, some NMF fruit have inherently superior flavor and aroma. In one study, the flavor of a NMF cultivar was rated higher than that of MF peaches when fruit were ripened at 20 ºC for 3 days following low temperature storage, and the firmer texture of the NMF cultivar did not appear to negatively impact consumer acceptance (Williamson and Sargent, 1999). While differences in pH, titratable acidity (TA), and soluble solids content (SSC) were detected among MF and NMF genotypes, no consistent grouping could be concluded based on the MF/NMF nature of the fruit (Brovelli et al., 1999b). Recently released NMF cultivars ‘Gulfking’, ‘Gulfcrimson’ and ‘Gulfprince’ showed superior post-storage firmness, quality, and flavor compared to the current commercial MF cultivars grown in the same region and having comparable harvest seasons (Beckman et al., 2008). These results indicate that NMF cultivars are a viable alternative to conventional MF cultivars and can be very beneficial to the early season shipping industry.

Cell Wall Modifications associated with Fruit Softening

Fruit softening is an intricate process that involves dissolution of the middle lamella, the primary determinant of intercellular adhesion, and disruption of the primary cell wall, which is composed of rigid cellulose held together by a network of hemicellulose and pectin (Brummell and Harpster, 2001; Toivonen and Brummell, 2008) 2008). Brovelli et al. (1998a) observed that a similar anatomical structure of the mesocarp cells between unripe MF and NMF peaches. The middle lamella was well bonded between cells and the intercellular spaces were sharply defined. During fruit ripening, cells underwent separation and intercellular spaces adopted the form of crevices among cells in MF fruit. Cells in NMF fruit retained good contact and showed less expansion of the intercellular spaces than that of the MF fruit.

The sequence of cell wall modifications in peaches from the end of maturation to the final melting phase of ripening has been described in detail by Brummell (2006). In the beginning of the softening process, pectin starts to lose galactose and arabinose side chains while depolymerization of hemicelluloses occurs; both processes continue until the end of ripening. As ripening progresses, pectin de-methyl-esterification precedes pectin solubilization. The final melting phase is achieved by pectin depolymerization. It is not clear why pectin depolymerization begins relatively early in tomato and avocado but late in the ripening of MF peaches (Brummell et al., 2004; Huber and Odonoghue, 1993). In NMF peaches, the final melting phase is absent and the pectin undergoes little solubilization. Furthermore, the NMF peaches possess higher content of water-insoluble pectin and higher capacity for calcium binding to this fraction compared to MF peaches, which generally have higher content of water-soluble pectin as the fruit ripen (Karakurt et al., 2000b; Manganaris et al., 2006b).

Progressive disassembly of the cell wall structural network is achieved by the concerted and synergistic action of several different enzymes, in which the action of one family of cell wall-modifying enzymes may mediate the activity of another, resulting in synchronized cell wall modification during fruit softening (Trainotti et al., 2003). Pectin methyl esterase (PME) and polygalacturonase (PG) are believed to have important roles in peach fruit softening. For example, a common physiological disorder of peaches related to cold storage is abnormal softening. This is related to an imbalance between PME and PG (Ben Arie and Lavee, 1971). The PME activity can increase, decrease, or remain unchanged during cold storage while PG activity is commonly inhibited (Lurie and Crisosto, 2005).

Pectin methyl esterase (PME)

In plants, de novo synthesized pectin in Golgi is secreted into the cell wall in a highly esterified form (Mohnen, 2008). The degree of pectin esterification directly influences cell wall rigidity, gel-forming ability, overall porosity, pH, and charge distribution (Carpita and Gibeaut, 1993; Grignon and Sentenac, 1991). Among cell wall changes during ripening fruit, pectins generally undergo the earliest modification (Li et al., 2010). PME (EC 3.1.1.11) is responsible for hydrolyzing the ester bond in the carboxymethyl groups of galacturonic residues of pectin, resulting in the release of methyl groups and exposure of carboxyl groups (Tijskens et al., 1999). De-methylesterification by the action of PME is a prelude to PG-mediated pectin disassembly since the resulting pectin contains mainly homogalacturonan, the preferred substrate for PG (Wakabayashi, 2000). Thus, de-methylesterification is an indispensable step for cell wall degradation. Muramatsu et al. (2004) observed that the activity of endo-PG had little effect on solubilization of peach pectin derived from immature green fruit even though the enzyme was active. The rate of pectin solubilization accelerated when active PME was added to the crude cell wall fraction with active endo-PG.

In MF peaches, PME activity increases sharply at an early stage of ripening and remains constant or decreases throughout the cell wall depolymerization phase (Brummell et al., 2004; Glover and Brady, 1995). PME mRNA expression also appeared to be highest at harvest and decrease during storage at 20°C (Zhou et al., 2000a). Two PME genes have been found in peaches and designated as PpPME1 and PpPME2 ((Murayama et al., 2009). PpPME2 might be critical for fruit softening since ethylene treatment was able to induce a higher level of PpPME2 expression in SH ‘Mihara

Hakuto' (Murayama et al., 2009). However, ethylene application or 1-MCP treatment did not influence PME activity in other peaches stored at 20 ºC, which may reflect post-translational regulation that is developmentally controlled (Girardi et al., 2005). Hayama et al. (2006a) also demonstrated that there was no significant difference in PME activity between ethylene treated SH 'Manami' and ethylene-free 'Manimi'. PpPME1 expression was found to be fruit specific but the transcription was probably not ethylene mediated since up-regulation of expression was observed during ripening of both MF and SH peaches stored in ethylene-free air (Hayama et al., 2003). PME activity of NMF 'Andross' peaches was found to follow a trend that was similar to MF 'Caldesi 2000' during ripening, but 'Andross' PME activity was significantly lower than that of 'Caldesi 2000' (Manganaris et al., 2006b).

Polygalacturonase (PG)

The role of PG in cell wall degradation during ripening has received broad attention. A rapid rise in PG activity that parallels increased solubilization of pectic substances and progressive loss of flesh firmness is observed in many fruits during ripening (Li et al., 2010). Both endo-PG (EC 3.2.1.15) and exo-PG (EC 3.2.1.67) are found in ripening peaches. Ripening-related exo-PG activity is found in both MF and NMF peaches but an increase of endo-PG activity is observed only in MF peaches. The reduced or undetectable endo-PG mRNA accumulation and activity in ripening NMF peaches may be due to a partial or complete deletion of genes encoding endo-PG (Callahan et al., 2004; Pressey and Avants, 1978). Thus, endo-PG is regarded as the key enzyme related to the textural difference between MF and NMF peaches. More detailed examinations of endo-PG revealed that the endo-PG activity of the MF cultivars increases gradually as the fruit soften but the rate of increase in activity accelerates only

when the fruit are already very soft (<20 N), suggesting that the initiation of softening is not associated with endo-PG activity (Orr and Brady, 1993).

There are, to date, six PG genes that have been found in peaches and nectarines. The mRNA of PpPG2/PRF5 was found to substantially accumulate in late softening of ‘Flavorcrest’(MF) peaches (Lester et al., 1994), while eight NMF cultivars each had a deletion in at least one of their PpPG2 sequences (Callahan et al., 2004). The importance of PpPG2/PRF5 during ripening was confirmed by Murayama et al. (2009) who reported that PpPG2 was expressed throughout 7 days of storage at 20°C in MF ‘Kawanakajima Kakuto’ peaches and only expressed in SH ‘Mihara Hakuto’ during ethylene treatment. Hence, it is likely that the transcription of PpPG2 corresponds to the ripening related endo-PG (Lester et al., 1994). The levels of PpPG1 and PpPG3 are apparently regulated independently of ethylene since their expressions were barely detectable in MF and ethylene treated SH peaches during ripening.

Although PG has been proposed as a major contributor to fruit softening, studies using antisense and overexpression technologies in several fruit species have revealed that PG is not the sole determinant of fruit softening (Toivonen and Brummell 2008). Polyuronide depolymerization was inhibited in antisense tomato expressing 1% of wild-type PG activity and with reduced PG mRNA, but the fruit sustained almost normal pectin solubilization (Smith et al., 1990). In this case, the reduction of PG mRNA and activity did not prevent fruit softening or the fruit remained just slightly firmer than the controls. Moreover, no significant effect on fruit softening was observed in mutant rin (ripening-inhibitor) tomatoes that overexpressed PG to 60% of normal activity (Dellapenna et al., 1990; Giovannoni et al., 1989). In avocados, polyuronide

solubilization occurs before the increase of PG activity, suggesting a PG-independent mechanism of pectin depolymerization. As a result, while PG is still proposed as the primary determinant for cell wall depolymerization, its activity alone is not sufficient to promote softening (Huber and Odonoghue, 1993).

Chilling Injury

Chilling injury (CI), which is commonly called internal breakdown (IB) for peaches, is a physiological disorder due to stress from low temperature storage. Internal breakdown is a primary factor that negatively influences consumer acceptance of peaches (Stockwin, 1996). The consumer can easily recognize CI in peach fruit since the symptoms develop quickly upon transfer from chilling temperature to non-chilling temperature (Lill et al., 1989). The development of CI can be affected by a combination of storage temperature and duration, fruit maturity, and genotype (Brovelli et al., 1998c; Ju and Duan, 2000). Although the recommended storage temperature for most peaches is 0°C, peaches can develop CI when they are exposed to 0°C for 3 or more weeks. However, peaches develop CI symptoms faster and more intensely when stored between 2.2°C to7.6°C (‘killing zone’ temperature) (Crisosto et al., 1999). Chilling injury can be directly related to the harvest maturity of peaches (Crisosto and Labavitch, 2002). Early harvest fruit are more susceptible to CI since they are presumably more likely to be immature than later harvest fruit (Ju et al., 2000). Less mature fruit that have been exposed to chilling temperature are more likely to not ripen normally as a consequence of impaired ethylene synthesis (Obenland et al., 2008; Zhou et al., 2001). Chilling injury development has been suggested to affect the quality of MF cultivars more than NMF cultivars. MF peaches were described as more “mealy”, less “sweet”, and less “peachy” than NMF peaches following chilling exposure (Brovelli et al., 1998c).

NMF peaches with CI were reported to have rubbery texture and off-flavors such as astringency, bitterness, and fermentative taste after ripening from cold storage (Karakurt et al., 2000a). These analyses demonstrated that NMF peaches have more genotypic advantages over MF peaches due to their slow softening rate and reduced CI susceptibility.

Symptoms of CI in peach vary among genotypes and typically include flesh discoloration such as internal bleeding and flesh browning, abnormal softening, low aroma, and dry texture (i.e., mealiness). Flesh discoloration and mealiness are the most frequently reported symptoms of CI (Murray et al., 2007). It is believed that flesh discoloration is related to tissue deterioration or senescence, which leads to changes in membrane permeability. Consequently, phenolic substrates and polyphenol oxidase (PPO) are able to interact because they are no longer compartmentalized separately in the cell (Lurie and Crisosto, 2005). Decreased activities of both PG and PME accompanied with decreased levels of cell wall binding cations (primarily Ca^{2+}) were observed in the brown fleshed tissues (Manganaris et al., 2006a). Mealiness is hypothesized to be caused by the interaction of extracellular water with highly polymerized insoluble pectic substances that have low degrees of esterification and depolymerization. These insoluble pectic substances sequester water through the aid of cell wall Ca^{2+} resulting in a gel-like consistency in the middle lamella (Lurie et al., 2003; Zhou et al., 2000b). Reduced endo-PG activity and enhanced or stable PME activity in low temperature storage have been observed in mealy tissues of peaches (Brummell et al., 2004; Zhou et al., 2000b). Furthermore, a dramatic decline in the arabinose (Ara) content of cell wall polysaccharides was detected in chilled peaches exhibiting mealy

texture (Brummell et al., 2004). The relevance of PG and PME in the development of the CI in peach fruit was confirmed in a proteomic analysis (Nilo et al., 2010).

Harvest Maturity

The stage of development at which a peach fruit is harvested strongly affects its flavor components, susceptibility to mechanical injuries, resistance to moisture loss, resistance to pathogen invasion, ability to ripen, and shelf life (Crisosto, 1994; Shewfelt et al., 1987). The physiological maturity and the horticultural maturity of a commodity can be distinct or overlapping with each other. According to Watada et al. (1984), physiological maturity is defined as the stage of development at which a plant or plant part will continue ontogeny even if detached from the parent plant. For a climacteric fruit like peach, that means that it will be able to ripen normally. Horticultural maturity is defined as the stage of development when a plant or plant part possesses the minimum acceptable quality for utilization by consumers (Watada et al., 1984). For peaches, minimum horticultural maturity coincides with physiological maturity because the harvested fruit must be able to complete ripening in order to be acceptable to consumers. However, a minimally mature peach fruit that is harvested prior to ripening initiation is likely to develop flavor, aroma and texture that are much inferior to that of a peach that was allowed to initiate ripening prior to harvesting.

To ensure that a commodity will always be harvested at an optimum maturity, indices are developed and often can be used as standards for trade regulation. Crisosto (1994) suggested that a maturity index must ensure an acceptable eating quality and provide for adequate storage life for the commodity. Ideally, a maturity index should be easy to measure, objective, applicable to all growing conditions, and if possible, nondestructive (Crisosto, 1994). The common nondestructive maturity indices for

peaches include size, fresh weight, and GC change; the destructive indices include flesh firmness, SSC, TA, pH and FC change.

Determination of optimum harvest maturity for peaches, especially for MF cultivars, is crucial. Due to the fast softening characteristic of MF peaches, fruit are often harvested when the GC changes from green to yellow (firm-mature or semi-ripe stage) in order to have higher pack-out, less spoilage, and to allow for long distance shipment (Sherman et al., 1990). Growers tend to harvest fruit before it reaches the recommended maturity stage in order to meet the demand. Shewfelt et al. (1987) showed that out of 5 packing houses sampled, one packing house had 70% of fruit below color reference # 3, the minimum maturity standard adopted by both California Tree Fruit Agreement and South Carolina Peach Board (Delwiche et al., 1987). Therefore, these fruit are often not mature enough to develop good flavor like tree-ripe fruit and frequently score low in consumer acceptance (Meredith et al., 1989). Crisosto (2002) indicated that hard fruit, mealiness, lack of taste, and failure to ripen are the main reasons consumers do not eat more stone fruit. Ripe fruit have a short postharvest life, primarily because of rapid softening and because they are already approaching a senescent stage at harvest. The soft texture of ripe peaches renders them highly susceptible to mechanical injury and they are also more susceptible to fungal infection (Casals et al., 2010a). By the time such fruit reach the consumer they may have become overripe, with poor eating quality including off-flavors and irregular or mushy texture (Meredith et al., 1989).

Many attempts have been made to determine the best maturity index for different peach cultivars. Strong correlation between flesh firmness and ground color were found

for ‘Halehaven’ peaches (Sims and Comin, 1963). For ‘Redhaven’ peaches, flesh firmness and the SSC/TA were reported to be the best indicators of maturity (Salunkhe et al., 1968). Minimum quality criteria have been proposed for MF cultivars both at commercial harvest stage and ready to eat stage. Fruit at picking generally need to have fresh mass greater than 90 g, diameter greater or equal to 57mm (2.25”), and approximately 40 to 48 N (9-11 lbf) in flesh firmness for normal softening to occur. It is generally accepted that when peach fruit reach 8.8–13.2 N (2-3 lbf) in flesh firmness, 0.5-0.8% TA, greater than or equal to 10% SSC or a SSC/TA ratio of 15, the fruit are “ready to eat” (Beckman and Krewer, 1999; Kader and Mitchell, 1989; Malakou and Nanos, 2005). The balance between TA and SSC are important to consumer acceptance. High acidity of peach fruit is not a negative quality attribute if balanced with the adequate amount of SSC at ripeness (Crisosto and Crisosto, 2005).

Few attempts have been made to identify harvest indices for NMF peaches because differences in harvest maturity, peel ground color, and flesh firmness between MF and NMF cultivars complicate the use of existing peach maturity indices when applied to the NMF cultivars. Robertson et al. (1993) found that size, weight, and CIE Lab a* values of the epidermis increased significantly during maturation for the three NMF cultivars measured. Despite the fact that GCa* value appears to be a universal maturity index for both MF and NMF genotypes, a different range of GCa* values or a different GCa* value threshold should be used for determining harvest timing of NMF cultivars since they can be harvested at more advanced ripeness (Robertson et al., 1991). Furthermore, newer cultivars are more highly red colored with less visible GC than older cultivars, making assessment of GC more difficult. Flesh color can be used

as a maturity index for NMF peaches when GC is not visible but destructive analysis is not practical for determining which fruit to harvest (Fuleki and Cook, 1976; Josan and Chohan, 1982; Kader et al., 1982). Brovelli et al. (1998b) identified cheek and blossom end firmness as potential indices common for both of the NMF flesh genotypes tested, but expert training was required for this method to be used. Clearly more work is needed to determine simple and reliable maturity indices for harvesting NMF peaches, especially when more new cultivars are released for commercial operations.

Storage

Temperature Management

Peaches are usually stored at low temperatures immediately after harvest because they ripen and deteriorate quickly at ambient temperature. As mentioned in the chilling injury section, storage at 0°C is better than 5°C for peaches in general because CI symptoms develop faster and more intensely at 5°C (Crisosto et al., 1999). Higher degree of unsaturation in the plasma membrane and higher membrane fluidity were proposed as the main reasons for the enhanced tolerance of peach fruit stored at 0 ºC relative to those stored at 5°C (Zhang and Tian, 2010). Therefore, temperatures between -1 to 0°C (30.5 to 32 °F) are currently recommended to store peaches (Crisosto and Kader, 2004).

It was reported that NMF peaches stored for 8 weeks (wk) at 0°C and subsequently ripened at 20°C showed no significant changes in physical characteristics for both immature and threshold mature (at physiological maturity) fruit except for a higher firmness compared to those ripened directly at 20 ºC (Robertson et al., 1992b). Hue angle slightly decreased with storage time, which was mainly attributed to the increase of GCa*. Weight of peaches of all maturity grades decreased significantly

during storage probably due to dehydration. Brown rot development became an issue after 8 wk of low temperature storage. Similar to the NMF peaches reported by Robertson et al. (1992b), SSC of ‘Maycrest’ peaches were constant during 5 wk of storage at 1°C. Sensory analysis of ‘Cresthaven’ peaches stored for 1 wk at 0°C and then ripened at 20 ºC were very similar to those stored at 2 wk (Lyon et al., 1993). Peach aroma and taste continuously decrease after peaches stored in 0°C for more than 4 wk.

Atmosphere Modification

Low O_2 and high CO_2 atmospheres were first shown to be beneficial to long term storage of apples by inhibiting the climacteric respiration (Kidd and C. West, 1934). The regulation may be directed towards the pathways involved in respiration and the fermentative metabolism, presumably through its influence on the synthesis, degradation, inactivation and/or activation of the respective enzymes (Mathooko, 1996). Furthermore, high CO_2 has been shown to suppress ethylene action in apples by effectively inhibiting both ACS and ACO activity (Gorny and Kader, 1996). Thus, reduction of both climacteric respiration and autocatalytic ethylene production can be achieved with high levels of CO_2 coupled with low levels of O_2. Consequently, modified (MA) or controlled atmosphere (CA) has been commonly used to improve the shelf-life of fruit and vegetables (Kader et al., 1989). 3–5% CO_2 + 1–2% O_2 at 0°C were the original recommendation of CA conditions for peaches and nectarines (Kader, 1986). However, higher levels of CO_2 were found to delay appearance of CI symptoms better than the recommended CA conditions. It was reported that ‘Rich Lady’ fruit stored under 3% O_2 + 10% CO_2 at 2°C for 15 days had improved juiciness, sweetness, perception of

peach flavor, emission of aroma volatile compounds and sensory acceptance in comparison with fruit stored in cold air (Ortiz et al., 2009).

Ethylene Control - Inhibitors of Ethylene Biosynthesis

ACC formation is critical for ethylene biosynthesis. Although ACS is generally considered to be the rate-limiting enzyme in the ethylene biosynthetic pathway, suppressing ACO activity by inhibitors are also effective in reducing ethylene production. Examples of ACS inhibitors include aminoethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA). Co^{2+}, α-aminoisobutyric acid (AIB), ethanol, and acetaldehyde vapors, are compounds that can depress ACO activity (Martinez-Romero et al., 2007). In addition, heat treatments applied to apples and tomatoes prior to storage have been shown to inhibit ethylene synthesis, acting on both ACO and ACS activities, although ACS is less heat-sensitive than ACO (Lurie 1998).

Heat Treatment

Heat is a type of abiotic stresses (Wang et al., 2003). Interestingly, the adaptive responses developed against this type of abiotic stress are known to protect plants from other biotic or abiotic stresses that can potentially lead to serious crop loss (Margosan et al., 1997; Serrano et al., 2004). Conditioning peach fruit using heat treatment before storage has the potential to reduce some of the major problems associated with low consumer acceptance. Heat treatment applied to peaches alone or combined with other treatments has been reported to reduce CI (Cao et al., 2010; Murray et al., 2007), control postharvest decay (Casals et al., 2010b; Karabulut et al., 2010; Malakou and Nanos, 2005; Obenland et al., 2005), and delay softening (Budde et al., 2006; Steiner et al., 2006).

Heat treatment is a pre-storage conditioning method that utilizes a reversible temperature stress to stimulate a defense reaction in plant tissues that is capable of protecting them from other stresses (Murray et al., 2007). The term ‘heat treatment’ has been used to describe exposure to temperatures higher than 33°C (Li and Han, 1998). It is an ideal way to minimize chemical usage to control insect infestation and prolong postharvest life of peaches for food safety and environmental reasons (Serrano et al., 2004).

Heat treatment is capable of causing the biological system to synthesize heat shock proteins (HSP), which have been suggested to induce plant resistance to CI by physical interaction with proteins or regulation of gene expression (Lurie, 1998; Zhou et al., 2002). For example, one HSP (VIS1) regulated by both high temperature and fruit ripening in tomatoes is proposed to act as a chaperone that binds reversibly to cell wall modifying enzymes and protect them from thermal denaturation (Ramakrishna et al., 2003). The altered gene expression patterns caused by the transient heat stress, including down-regulation of ACS and ACO, are proposed to result in a reduction in cell wall catabolism, delaying normal fruit softening (Martinez and Civello, 2008). In heat-treated MF peaches, PME was activated, resulting in production of more pectin carboxyl groups, while PG was inactivated (Koukounaras et al., 2008). Although the activities of PME and PG in heat-treated fruit were similar to that of the chilling-injured fruit mentioned above, it was proposed that the retention of firmness was caused by excess carboxyl groups of heat-treated fruit bonding with endogenous calcium to form Ca-pectates, resulting in increased rigidity of the cell wall and middle lamella (Steiner et al., 2006). Bakshi and Masoodi (2010) reported that the decline in pectin (% of Ca-pectate)

was less in ripe, heat-treated fruit, supporting the idea that the formation of Ca-pectates in the cell wall and middle lamella restrict access and activities of cell wall degradation enzymes such as PG.

The most common postharvest heat treatments applied to fruits are hot water, hot water vapor, and hot air (Zhang et al., 2007). Heating by using radio-frequency to control brown rot in peaches and nectarines was also reported (Casals et al., 2010c). The internal temperature of peaches treated with hot water increases more rapidly than in fruit treated with hot moist air, due to the higher convective heat transfer coefficient for water compared with air (Zhou et al., 2002). The most common temperatures applied to peaches to delay ripening are between 40 to 50°C and the length of application time varies from 10 min to 2 days (hot air). Peaches have been shown to tolerate hot water at 43°C for 24 min and 46°C for 25 min without showing any external measurable injury (skin browning or pitting) (Malakou and Nanos, 2005; Wells, 1971). Peaches heated at 50°C for 10 min, 4 h before fresh-cut processing, had a significantly prolonged shelf life (Koukounara et al., 2008). These peach slices exhibited minimal changes in hue (h^o) and L* values in modified atmosphere packages (MAP). One possibility could be due to reduction in PPO activity (Bakshi and Masoodi, 2010). Firmness retention observed in peaches treated with hot air could be due to temporarily repressed ethylene synthesis and was suggested to be ripening related since heat treatments had no influence on fruit firmness when ethylene production was already triggered (Budde et al., 2006). Fruit ripened after heat treatment were found to have positive qualities such as higher fructose content, lower total TA, and increased red pigments in the flesh and peel (Budde et al., 2006; Lara et al., 2009) or the changes

were negligible (Obenland et al., 2005). On the contrary, when the stress applied is too extreme and thus irreversible, heat treatment can lead to damages that can significantly affect the appearance and nutrition of the fruit such as causing substantial surface browning, increased total carotenoid loss, and lower chroma values of the flesh (Kerbel et al., 1985; Koukounaras et al., 2008; Steiner et al., 2006). Therefore, an optimum combination of temperature and exposure time must be determined specifically for each cultivar in order to avoid heat injuries that can possibly result in negative consumer acceptance.

Ethylene Control - Inhibitors of Ethylene Action

Before the discovery of 1-methylcyclopropene, silver ions (applied as silver thiosulfate, STS) (Beyer, 1976), 2,5-norbornadiene (2,5-NBD) (Blankenship and Sisler, 1989), and diazocyclopentadiene (DACP) (Sisler and Blankenship, 1993) have been shown to successfully inhibit ethylene action. There are disadvantageous of these compounds. Silver ion cannot be used on foods since it is a heavy metal and a potential pollutant (Martinez-Romero et al., 2007). Continuous application of 2,5-NBD in high concentration is required to inhibit ethylene perception. Furthermore, the gas itself has a foul odor (Huber, 2008; Robbins et al., 1985). DACP is highly explosive and toxic (Sisler and Blankenship, 1993). 1-MCP is currently the most potent ethylene antagonist that can be applied on food crops. The capacity of uniform delivery of 1-MCP to intact organs generates a major benefit over STS that required vascular access for uniform delivery, restricting its use to cut flowers (Huber, 2008). Other advantages of 1-MCP include low phytotoxicity, short exposure period, and prolonged ethylene action inhibition after a single exposure at relatively low concentrations (Sisler, 2006). Since 1-MCP has been used successfully to extend the postharvest life of various climacteric

fruits including apple, avocado, banana, pear and tomato by inhibiting the onset and progression of ripening (Watkins, 2006), it has tremendous potential on extending postharvest life of peaches. Therefore, 1-MCP is discussed more in detail herein.

1-Methylcyclopropene (1-MCP)

1-MCP is an ethylene antagonist that competes with ethylene for its receptor and interacts with ethylene receptors by irreversible binding, thereby blocking ethylene-dependent ripening responses (Sisler and Serek, 1997). Liguori et al. (2004) reported delayed flesh softening and extended shelf life with no incidence of flesh browning or breakdown in MF peaches treated with 5µL/L 1-MCP at 20°C or 0 ºC for 20 h. Kluge and Jacomino (2002) reported delayed flesh softening, less ground color loss and reduced incidence of fruit rot from *Monilinia* for preclimacteric peaches treated with 100 nL/L 1-MCP at 20°C for 24 h. TA loss in peaches is generally inhibited by 1-MCP, but the influence of 1-MCP on respiration and SSC appears to be cultivar dependent (Liguori et al., 2004). Transcriptome analysis indicates that 1-MCP is capable of inhibiting genes associated with ripening in peach. Compared with samples at harvest, only nine genes appeared to be differentially expressed when peach fruit were sampled immediately after treatment with 1 µL/L 1-MCP at 20°C for 24 h, while a total of 90 targets were up- or down-regulated in untreated fruit (Ziliotto et al., 2008). Reported effective gaseous 1-MCP concentrations for peaches vary greatly: from as low as 0.4 µL/L (Liu et al., 2005) to 5 µL/L (Liguori et al., 2004). However, 5 µL/L is higher than the maximum concentration registered for use (Watkins 2006).

Despite the fact that 1-MCP has been shown to be able to delay ripening in many climacteric fruits, the inhibitory effect of 1-MCP on peaches is often transitory. For example, preclimacteric MF peaches (ethylene production rate of 0.4 nL/g FW/h and

flesh firmness about 80 N) treated with 1 µL/L 1-MCP gas for 12 h at 25°C began to soften only 1-2 days after the treatment ended (Rasori et al., 2002). Hayama et al. (2008) reported that MF peaches harvested at the “commercial stage” and treated with 1 µL/L 1-MCP for 16 h at 25°C softened to a similar degree of flesh firmness as that of untreated fruit after 4 days of ripening at 20°C. Although repeated 1-MCP applications on peaches were shown to be more effective in terms of softening inhibition than a single application (Liu et al., 2005), it is difficult to apply repeated applications in a commercial operation.

Application of 1µL/L 1-MCP for 24 h at 20°C had a relatively small effect on peaches at “commercial ripeness” in comparison with apples at “commercial ripeness” with regard to gene expression and activities of the enzymes (PpACS1), regulators (PpCTR1), and receptors (PpETR1, PpERS1) involved in the ethylene biosynthetic and signal transduction pathways (Dal Cin et al., 2006). It was proposed that the ethylene receptors were regenerated within a short period of time after a single 1-MCP treatment (Mathooko et al., 2001). Another possibility is that the system 2 ethylene production in peaches is regulated differently than in tomatoes and apples. PpACS1 might represent one crucial factor in the modulation of responses to 1-MCP application (Mathooko et al., 2001; Ziliotto et al., 2008). Expression of PpACS1 may be negatively regulated by ethylene, unlike the positively regulated LeACS2 and MdACS1 (Tatsuki, 2010). It is also possible that another hormone such as auxin (NAA) influences the PpACS1 expression more strongly than ethylene during ripening (Trainotti et al., 2007).

The effectiveness of gaseous 1-MCP application appears to be limited by storage temperature, which can be a potential problem since low temperature storage is

commonly required for peaches. 1-MCP-treated nectarine fruit stored at 25°C had a longer shelf life than those stored at 4°C (Bregoli et al., 2005). The inhibition of softening was greater when peach fruit were 1-MCP-treated and held at 20°C than when they were treated and held at 0°C before ripening at 20°C (Liguori et al., 2004). In some cases, use of 1-MCP on peaches has been associated with increased IB depending on the storage condition. It is possible that ethylene synthesis and action can be blocked by 1-MCP even after cold storage, subsequently leading to abnormal ripening and emergence of severe CI disorders such as IB (Dong et al., 2001; Fan et al., 2002; Girardi et al., 2005). Adding ethylene during cold storage was able to delay the development of mealiness (Zhou et al., 2001; Dong et al., 2001). These studies indicated that using gaseous 1-MCP to extend the shelf life of peaches after low temperature storage is currently limited.

Liquid or spray formulations of 1-MCP have been developed recently, but this has not yet become commercially available for postharvest application. Aqueous 1-MCP [1-$MCP_{(aq)}$] application may have better potential than the gaseous application for postharvest purposes because it appears to be more efficient in inhibiting ripening and does not require tightly sealed rooms. Choi and Huber (2008) demonstrated that 1-MCP(aq) strongly delays ripening in both tomato and avocado fruit. Fruit immersed in 625 µg/L 1-$MCP_{(aq)}$ for 1 min had all the examined ripening parameters strongly suppressed, including ethylene biosynthesis, respiration, softening, surface color changes, lycopene and PG accumulation. Manganaris et al. (2007) demonstrated the beneficial effects of dipping ‘Harrow Sun’ plums in 1-$MCP_{(aq)}$ to delay ripening and control CI. They reported that plums immersed in 100 µg/L of -$MCP_{(aq)}$ for 5 min had

reduced respiration and ethylene production, and better firmness retention. Furthermore, reduction in the activity of cell wall modifying enzymes such as PG, endo-β-1,4-glucanase (EGase), and β-galactosidase (β-Gal) were observed. The treated fruit that were ripened after being stored at 5°C for 10 days showed less flesh reddening, a CI symptom. In a more recent report, Manganaris et al. (2008) determined that 1 µg/L of 1-$MCP_{(aq)}$ was the most effective rate to control ripening changes and extend shelf life of 'Joanna Red' plums harvested at early ripeness stage. Therefore, 1-$MCP_{(aq)}$ can be a potential pre-storage conditioning method for both MF and NMF peaches since it is more efficient compared to the traditional gaseous application and can be applied to stone fruit harvested after ripening has initiated.

CHAPTER 3
OPTIMUM HARVEST AND POSTHARVEST HANDLING PRACTICES FOR LOW-CHILL, MELTING AND NON-MELTING FLESH PEACH VARIETIES IN TROPICAL AND SUBTROPICAL CLIMATES

Overview

The bulk of peach production in the USA occurs in temperate, high-chill locations with fruit available from late May through October. Developing low-chill subtropical cultivars is economically important because they provide a fresh supply of fruit for local markets and the potential to transport fruit for higher values to more distant markets that have no peach production at the time (Rouse and Sherman, 2002). The traditional melting flesh (MF) cultivars are primarily grown for fresh market whereas non-melting flesh (NMF) cultivars are commonly grown for canning because the fruit maintain their integrity during the high-temperature retort treatment (Robertson et al., 1992b). The main distinction between NMF and MF peaches is that the former lack the rapid loss of firmness or “melting of the fruit” toward the end of ripening, which coincides with their reduced capacity to degrade cell walls (Lester et al., 1994; 1996; Pressey and Avants, 1978).

Early season peaches have a poor reputation among consumers. The major complaints are poor and inconsistent fruit textural quality and flavor (Beckman and Krewer 1999). A common problem for traditional MF peaches is that the fruit are harvested at the “firm-mature” stage to minimize mechanical injuries, but consequently have considerably lower eating quality because these fruit have not completed or just reached physiological maturity at harvest (Cascales et al., 2005; Williamson and Sargent, 1999). A major goal of the University of Florida *Prunus* breeding program is to develop low-chill NMF peaches for fresh consumption with color, aroma, and flavor

typical of MF cultivars while still possessing enough flesh firmness to prevent mechanical damage during postharvest handling (Sherman et al., 1990).

Little is known about the best harvesting procedures for NMF peaches and other low-chill subtropical MF varieties that would result in fruit of satisfactory condition after handling and shipping. For peaches, horticultural maturity coincides with physiological maturity. Physiological maturity is defined as the stage of development at which a plant or plant part will continue ontogeny even if detached from the parent plant (Watada et al., 1984). Thus, peaches at horticultural maturity can continue ripening to develop high flavor characteristics; at the same time, the fruit is still firm enough to prevent bruising and premature softening during shipping and storage (Wells et al., 1989). Since NMF peaches can retain firm texture longer than MF peaches, standard harvest maturity of MF cultivars may not be suitable for the NMF cultivars.

Many of the physical and chemical characteristics of maturing peaches, typically of the MF cultivars, have been studied in order to obtain suitable indices of harvest maturity. Ideally, a maturity index should be easy to measure, objective, applicable to all growing conditions, and if possible, nondestructive (Crisosto, 1994). Flesh firmness (Rood, 1957; Salunkhe et al., 1968) and peel ground color (Baumgardner and Delwiche, 1983; Delwiche and Baumgardner, 1985) have been suggested as reliable maturity indices for MF cultivars, while flesh color was suggested for NMF cultivars (Fuleki and Cook, 1976; Josan and Chohan, 1982; Kader et al., 1982). Information on the maturity indices of fresh market NMF cultivars is currently limited because they are not traditionally used for fresh consumption. Furthermore, the newer cultivars are more

highly red colored on the skin making the assessment of the correct harvest maturity via peel ground color more difficult.

Refrigeration is the most common practice to retard ripening and lengthen storage life of fruits and vegetables; however, low temperature can affect fruit qualities in a negative way commonly known as chilling injury (CI). Although the recommended storage temperature for most peaches is 0°C, fruit can develop CI when they are exposed to 0°C for 3 or more weeks. Symptoms are even more pronounced when the chilling injured fruit are transferred from 1 or 2 weeks of storage at 2.2°C to 7.6°C (the 'killing zone' temperature) to room temperature (Crisosto et al., 1999). Flesh discoloration such as internal bleeding and flesh browning, abnormal softening, low aroma, and dry texture (i.e., mealiness) are typical symptoms of CI (Lurie and Crisosto, 2005). The development of CI is usually related to a combination of storage temperature and duration, fruit maturity, and genotype (Brovelli et al., 1998a; Ju et al., 2000; Ju et al., 2000). It has been reported that symptoms were more severe in unripe than in ripe fruit (Ben Arie and Lavee, 1971). Chilling injury development has been suggested to affect the quality of MF cultivars more than NMF cultivars (Brovelli et al., 1998c).

The purpose of this study was to investigate the changes of physical and chemical characteristics of the newer, low-chill, MF and NMF varieties at harvest to aid in the development of indices for predicting the maturity of peaches. The second objective was to determine optimum harvest maturity for each peach cultivar based on the effect of ripening and low temperature storage on fruit qualities.

Materials and Methods

Plant Materials

During the spring seasons of 2007 and 2008, samples from two MF cultivars, 'Flordaprince' and 'TropicBeauty', and two NMF cultivars 'UFSun' and 'Gulfking', were harvested three times from the UF-IFAS Plant Science Research & Education Unit (PSREU) at Citra, FL. The harvest time was based on the development of 100 tagged fruit for each cultivar after fruit thinning and natural fruit drop. These fruit were randomly selected and considered as the representative population for each cultivar. Samples from the four cultivars were harvested when 50%, 70%, and 90% of the tagged fruit reached commercial harvest stage (ground color change from green to yellow). For each harvest, a 50-fruit sample from four trees was sorted according to ground color (subjective) and fruit diameter. Ten fruit were used for destructive analyses immediately after harvest. The remaining fruit were divided into two groups of 20 for two storage treatments. The first group was stored (ripened) at 20°C for 7 days in 2007 and 5 days in 2008. The second group was stored at 0°C for 14 days, and then ripened at 20°C for 7 days in 2007 and 5 days in 2008. After storage and ripening, non-destructive analyses (fresh weight, size, peel blush and ground color), and destructive analyses (flesh color, firmness, soluble solids content, total sugars, pH and titratable acidity) were performed. Relative humidity (RH) of both storage conditions ranged from 83 to 97%.

Size, Fresh Weight, and Peel Blush Determination

Fruit size was determined by measuring the diameter midway between the stem and blossom end with a vernier caliper. Peel blush (PB) was subjectively measured by estimating the total percentage of each fruit that was red. Fresh weight (FW) of individual fruit was recorded using a weighing balance. Measurements of size, FW, and

PB were taken at harvest (initial) and after ripening from each storage condition (final). The measurements of weight loss (WL), size loss (SL), and change in peel blush (ΔPB) were determined in 2008 for samples stored directly at 20°C. WL was calculated by subtracting the final FW of the fruit from the initial FW. The difference obtained was divided by the initial FW and converted to percentage by multiplying by 100. The SL was calculated in the same fashion as WL. ΔPB was calculated by subtracting the final PB from the initial PB. In addition, weight loss was measured immediately after 14 days of low temperature storage in 2008 (WL-m).

Color Determination

Ground color (GCa*) and flesh color (FCa*) were objectively measured using a reflectance colorimeter (Minolta CR-400, Konica Minolta, Japan) and expressed as C.I.E. a* values (green-red) since a* value increases with increasing maturation and ripening both in the peel (Delwiche and Baumgardner, 1985) and in the flesh (Kader et al., 1982; Robertson et al., 1991). Ground color was measured on the greenest portion of the peel. Flesh color was measured after removing a small section of the epidermis on two sides of each fruit at the equator on the cheeks using a potato peeler. Changes in GC (ΔGCa*) were calculated using the a* values measured after ripening from 20°C or 0°C storage treatments minus the initial a* values.

Compositional Analysis

Fruit were sliced, pitted, and pureed in a Waring blender for 1 min. After the slurry was centrifuged (20 min; 15,000 x g_n; 4°C), the clear solution was used to determine soluble solids content (SSC) and titratable acidity (TA). SSC was measured with a temperature compensated digital refractometer (model ABBE Mark II, Cambridge Instruments Inc, U.S.A) and expressed as percent FW. TA was determined by titration

(model 719 S. Titrino, Metrohm, Switzerland). A 0.1N sodium hydroxide solution was used to titrate 6 g of peach juice until pH 8.2 was reached. The TA was expressed as percent malic acid. The juice pH was measured using the same equipment for TA determination.

Total soluble sugar (TS) determination was performed using the phenol-sulfuric assay (Dubois et al., 1956) modified as follows: 5 µL of extracted sample was diluted with 5 mL of 80% ethanol. Further dilution was performed if the concentration of the sample was out of the range of the standard curve. A 500-µL aliquot of the diluted sample was added to 500 µL of 5 % phenol solution (Fisher Scientific, New Jersey, USA; certified grade) then vortexed. Then 2.5 mL of concentrated sulfuric acid (Fisher Scientific; certified ACS grade) was added to the mixture and vortexed. The mixture was left for 10 min at room temperature for color development. The absorbance of the sample at 490 nm was read on a microplate with glucose (Fisher Scientific, New Jersey, USA; certified ACS grade) as the standard. Total sugar was expressed as a percentage of FW.

Flesh Firmness Determination

Flesh firmness was measured with an Instron Universal Testing Instrument (Model 4411-C8009, Canton, MA, USA) that applied a compressive force from a 50 kg load cell. A convex tip probe (Magness-Taylor type), 7.9 mm in diameter, was attached to the load cell moving at a speed of 12 cm/min. Flesh firmness was measured on two sides of each fruit at the equator on the cheeks without peel and expressed as the maximum bioyield force (N).

Statistical Analysis

Since differences in the physical and chemical characteristics for all the cultivars were not noticeable among the three harvests, the data for all harvests were pooled together for statistical analysis. Samples were divided into 7 maturity groups (MG) in 2007, ranging from the least mature to most mature based on GCa* measurements. More than 7 MG were assigned to 'Flordaprince' and 'UFSun' in 2008 because those varieties had broader ranges of GCa* than in 2007. Data were analyzed by the General Linear Model (GLM) program of the Statistical Analysis System (SAS) (SAS Institute, Cary, NC). One way – Analysis of Variance (ANOVA) was used to detect significant differences at the 5% level among the MG for each cultivar. The least significant difference (LSD) test was used for mean separation. Correlation coefficients (r) were obtained both at 1% and 5% level of significance.

Results and Discussion

Effect of Maturity at Harvest on Physical and Chemical Characteristics

Ground color (GCa*) and flesh color (FCa*) increased significantly as maturity increased for all the cultivars (Table 3-1, 3-4, 3-7, 3-10, 3-13, 3-16, 3-19, 3-22). A change of negative a* value to positive a* value represents the increase of orange and red coloration and decrease of green. Therefore, peaches accumulate orange and red pigments while losing chlorophyll as maturity advances. The percentage of the fruit surface covered by red blush or peel blush (PB) is an important quality indicator to the consumer. The current U.S. Standard for Grades of Peaches includes minimum PB as a requirement for the U.S. Fancy and U.S. No. 1 Extra grades (AMS, 2004). The PB of 'UFSun' was significantly affected by maturity (Table 3-7, 3-19) while PB of 'Gulfking' was not (Table 3-10, 3-22). The significance of PB development related to maturity in

both MF cultivars varied between the 2 years. The consistent pattern of PB development within the NMF cultivars in both years suggests that this trait is genetically regulated in the NMF cultivars and may be influenced more by the environment in the MF cultivars. A minimum of 60% red blush is considered to be sufficient for new varieties in the UF breeding program and would score as 7 on a 10-point scale (Beckman et al., 2008). 'Flordaprince', 'TropicBeauty' and 'UFSun' picked at commercial harvest stage in Gainesville, FL were reported to have 70, 80, and 80% red blush, respectively (Rouse et al., 2004). The reported % red blush was only observed in 2008. 'Gulfking' peaches had the highest PB and thus were highly covered with red blush in 2008 (Table 3-22).

Regardless of maturity, all the cultivars achieved 90 g FW and 57 mm size in 2007 (Table 3-1, 3-4, 3-7, 3-10), the most common size sold in the early season market (Beckman et al. 2008). Smaller fruit size was observed in 2008 for all the cultivars although 'TropicBeauty' still managed to achieve the standard size (Table 3-16). Since all the cultivars were affected, climate, crop load, and time of thinning were possible reasons that may have contributed to smaller fruit size in 2008 (Drogoudi et al., 2009). FW and size were highly correlated for both MF and NMF cultivars in both years (Table 3-26, 3-27). Therefore, as the FW increases the fruit also expands in size.

The softening pattern was different between MF and NMF cultivars as the fruit started to ripen. Fruit of the MF cultivars were very firm until they reached MG 10 to15 (Table 3-1, 3-4) in 2007 and MG 20 to 25 in 2008 (Table 3-13, 3-16). Flesh firmness dropped tremendously as the 'softening' process began. Flesh firmness of the NMF cultivars decreased relatively slowly as maturity increased (Table 3-7, 3-10, 3-19, 3-22).

Fruit softening in ‘Flordaprince’ and ‘Gulfking’ peaches started prior to attainment of full size in 2007, (Table 3-1, 3-10) similar to the result reported by (Brovelli et al., 1998b). This indicates that cell wall synthesis and cell wall degradation may overlap each other when the fruit switches from the preclimacteric to the climacteric stage of development (Rose and Bennett, 1999). In peaches, different isozymes of expansins are suggested to be responsible for loosening the cell wall during this overlapping phase (Hayama et al., 2003; Hayama et al., 2006a; Hayama et al., 2006b). Up-regulation of cellulose synthase catalytic subunit expression during this period demonstrates that newly synthesized cellulose can be integrated into the cell wall while fruit softening progresses (Trainotti et al., 2003).

Soluble solids content (SSC) was generally not affected by maturity for all the cultivars. It was possible that most of these fruit had already reached physiological maturity at harvest. Secondly, there might be large variations of SSC within the MG since fruit at different positions within the canopy showed significant differences in SSC (Mitchell et al., 1990). ‘Flordaprince’ had approximately 10-11% SSC at harvest (Table 3-1, 3- 13) and ‘TropicBeauty’ had around 12-13% SSC (Table 3-4, 3-16), which was similar and higher than the values reported by Karakurt et al. (2000b). Both ‘UFSun’ and ‘Gulfking’ had approximately 10-12% SSC at harvest (Table 3-10, 3-19, 3-22). Total soluble sugar (TS) was usually no different among the MG for both MF and NMF peaches. This implies that the quantity of sugars in peaches does not vary significantly throughout the latter stages of development although the types may differ. The main sugars in peach fruit at harvest maturity are sucrose, fructose, and glucose, with sucrose being dominant (Byrne et al., 1991; Génard et al., 2003). It is possible that

while sucrose decreases, glucose and fructose increase during ripening, thus maintaining a constant TS level (Borsani et al., 2009). SSC/TA generally increased as maturity increased for all of the cultivars. Since SSC was not significantly different among the MG, the increase in SSC/TA was due to a gradual decrease of TA along with advanced maturity.

The pH of 'TropicBeauty', 'UFSun', and 'Gulfking' increased from low MG to high MG in 2007; however, the pH of 'Flordaprince' did not change in either year (Table 3-1, 3-13). Similar results were observed previously for 'TropicBeauty' (Brovelli et al., 1998c) and 'Fantasia' peaches (Moing et al., 1998). The major amino acid in peach flesh, asparagine, may contribute to the constant cytoplasmic pH of 'Flordaprince' and 'TropicBeauty' as maturity increases (Moing et al., 1998).

Effect of Maturity and Ripening on Physical and Chemical Characteristics

Color changes associated with ripening strongly influence visual and eating quality of fruits and vegetables (McGuire 1992). Significant increases in ground color change (ΔGCa*) were observed in all the cultivars, especially the least mature fruit, after ripening for 7 d at 20°C (Table 3-2, 3-5, 3-8, 3-11, 3-14, 3-17, 3-20, 3-23). This result agrees with Robertson et al. (1993) who reported that immature fruit (Maturity 1) had the most increase in a* on the skin color and the mature fruit (Maturity 3) had the least increase after ripening. Ripening induced higher a* in flesh color (FCa*) in all the cultivars thus fruit flesh became more red and orange. Maturity did not significantly affect FCa* of ripened 'UFSun' peaches in either year (Table 3-8, 3-20), demonstrating that synthesis and accumulation of pigments in the flesh of this cultivar cease earlier than in the other cultivars. The FCa* was relatively lower in 'Flordaprince' after ripening in both years, which suggests that 'Flordaprince' fruit maintained more green color in

the flesh than did the other cultivars. Change of peel blush (ΔPB) after direct ripening at 20°C was only measured in 2008 (Table 3-14, 3-17, 3-20, 3-23). The ΔPB was not significantly affected by maturity in any of the cultivars. The changes were often close to zero, suggesting that the red pigment is not synthesized postharvest. The changes were also random, indicating that this subjective method is not a reliable indicator of fruit maturity.

The WL and SL after storage for 7 d at 20°C were only determined in 2008 (Table 3-14, 3-17, 3-20, 3-23). The WL and SL were similar among the MG. The WL for 'Flordaprince', 'UFSun', and 'Gulfking' were around 11-12%, similar to the value reported by (Robertson et al., 1990b). 'TropicBeauty' lost around 8-9% WL. Shrinkage in size was generally around 5% or less.

The NMF cultivars maintained texture better than the MF cultivars after ripening. The MF fruit were very soft (≤ 5N) after ripening regardless of maturity (Table 3-2, 3-5, 3-14, 3-17). The NMF cultivars were approximately 3-5 times firmer than the MF types (Table 3-8, 3-11, 3-20, 3-23). Levels of SSC and TS were generally not affected by maturity and ripening, similar to the results reported by Byrne et al. (1991). 'TropicBeauty' had the highest ripe SSC among the cultivars, which might lead to higher consumer acceptance (Crisosto et al., 2006). In general, SSC/TA and pH increased while TA decreased after ripening compared to the values measured at harvest. Maturity had a significant effect on these chemical characteristics. Fruit in more advanced MG generally had higher SSC/TA, indicating that fruit harvested at more advanced stages had a sweeter taste than those in the lower MG after ripening. Robertson et al. (1993) reported that threshold mature (maturity 2) fruit ripened for 7 d

at 20°C had significantly higher attribute scores for fruity, peachy, sweet, and juicy and had greater overall acceptability than the less mature peaches.

Effect of Maturity and Storage on Physical and Chemical Characteristics

Slight increases in GCa* were observed in all the cultivars after ripening following low temperature storage relative to ripening directly at 20°C (Table 3-3, 3-6, 3-9, 3-12). Robertson et al. (1992b) did not find any changes in GCa* in fruit stored for 8 weeks at 0°C and subsequently ripened. Low temperature had a major impact on FCa* of both NMF cultivars (Table 3-12, 3-21, 3-24). The FCa* increased markedly compared to fruit ripened directly at 20°C (Table 3-11, 3-20, 3-23). It has been reported that total carotenes and xanthophylls increased in three NMF cultivars and decreased in MF cultivars ‘Flordaprince’ and ‘Tropic Beauty’ during 2 to 5 d of storage at 8°C (Karakurt et al., 2000b). Since no browning was observed, this development was not considered to be related to CI.

The WL for all the cultivars was more severe after cold temperature storage compared to ambient temperature storage and was independent of the degree of maturity. Longer period of storage was the main reason that fruit lost more weight after ripening following cold temperature storage, presumably due to dehydration (Lyon et al., 1993).

The WL of ‘Flordaprince’, ‘UFSun’, and ‘Gulfking’ peaches were around 20% and fruit shrank about 5-10% in size after ripened following 0°C storage in both years (Table 3-3, 3-12, 3-15, 3-21, 3-24). ‘TropicBeauty’ lost the least amount of weight (14% in 2007 and 10% in 2008) and shrank approximately 5% in size (Table 3-6, 3-18). Weight loss was also determined immediately after the fruit were transferred from 0°C to 20°C in

2008 (WL-m). For ‘Flordaprince’, ‘UFSun’, and ‘Gulfking’, the WL-m was similar to that of fruit ripened at ambient temperature, and was approximately half of the total WL. Therefore, fruit lost about 3-5% of their initial weight each week under the 0°C storage condition.

Low temperature affected the flesh firmness of ‘UFSun’ peaches the most. Low temperature-stored ‘Flordaprince’ and ‘TropicBeauty’’ fruit all ripened to have similar flesh firmness as those stored directly at 20°C. Low temperature-stored ‘UFSun’ fruit in MG ≤10 did not soften as much as those ripened at ambient temperature in 2007 (Table 3-8, 3-9). In 2008, ‘UFSun’ fruit in MG 0 to 5 were firmer after ripening following low temperature storage compared to those ripened directly at ambient temperature storage (Table 3-20, 3-21). This abnormal softening pattern indicated that less mature fruit had greater susceptibility to CI (Fernandez-Trujillo and Artes, 1997). Fruit with abnormal softening appeared to be similar in color and composition to other fruit in the nearby MG. Robertson et al. (1992b) reported similar observations. In their study, NMF peaches had no significant changes in physical characteristics after ripening except firmness, which increased during 8 weeks of storage at 0°C plus 6 d of ripening at 20°C regardless of maturity. Since only one NMF cultivar was affected by low temperature in this study, it is difficult to conclude that chilling affects the quality of MF more than NMF genotypes as suggested by (Brovelli et al., 1998c).

Fruit SSC and TS were not significantly affected by maturity and the values determined after low temperature storage were similar to those of fruit ripened directly at ambient temperature for all the cultivars. After ripening from the low temperature storage, most fruit in lower MG were able to attain SSC/TA of 15, the minimum

acceptable quality standard for ripe fruit (Kader and Mitchell, 1989; Robertson et al., 1990a). This indicates that more organic acids are being consumed during the prolonged storage period, resulting in even lower TA and higher pH compared to fruit ripened directly at 20°C. This was a beneficial aspect of low temperature storage if no CI developed. A wider maturity range can be used with low temperature storage because the fruit with lower maturity will have enough time to develop an acceptable flavor compared with those ripened directly at 20°C.

Optimum Harvest Maturity Determination

Peach fruit are considered “ready-to-eat” when the firmness reaches 8.8–13.2 N. Other minimum quality standards for ripe fruit include TA > 0.8%, and 10-11% SSC or 15% SSC/TA (Kader and Mitchell, 1989; Robertson et al., 1990a; Kader et al., 1989). Typically, consumer acceptance is controlled by the interaction between TA and SSC rather than SSC alone (Crisosto and Crisosto, 2005). After ripening directly at 20°C or following low temperature storage, ‘Flordaprince’ and TropicBeauty’ fruit of different MG all softened to 5 N or less. Thus, all were “ready-to-eat” (Table 3-2, 3-5, 3-14, 3-17). In 2007, ‘Flordaprince’ of MG 5 to10 was particularly suitable for fresh consumption because softening was not initiated (Table 3-1) and fruit attained SSC/TA of 17.02 after ripening at 20°C (Table 3-2). MG -5 to10 were suitable for low temperature storage (Table 3-3). MG< -5 contained high TA (Table 3-3) with SSC/TA less than 15 and thus was not recommended for low temperature storage. MG 10 to 20+ was not a suitable range for either storage condition because the fruit were already too soft at harvest (Table 3-1). In 2008, fruit from different MG were separated into two groups based on flesh firmness (Table 3-13). Fruit harvested between MG <0 to 20 had enough firmness (approximately 45 N) to allow softening to occur during storage. These fruit possessed

desirable flavor after ripening at 20°C (i.e. 11% SSC, <0.8% TA, and >15 SSC/TA) (Table 3-14). MG < 0 was not recommended for low temperature storage due to persistence of high TA after ripening (Table 3-15).

In 2007, MG 0 to10 was the ideal range for harvesting 'TropicBeauty' destined for both storage conditions. Fruit of MG < 0 might be too immature, still having TA > 0.80% after ripening directly at 20°C or following storage at 0°C (Table 3-5, 3-6). Similar to 'Flordaprince', two groups of 'TropicBeauty' fruit could be separated based on their flesh firmness at harvest in 2008 (Table 3-16). MG 5 to 20 was selected as the ideal range for fresh market use mainly because of the acceptable TA (Table 3-17). A wider range of maturity, MG < 0 to 20, was selected for low temperature storage for 'TropicBeauty' (Table 3-18).

Since NMF peaches soften relatively slowly compared to the MF types, peaches with flesh firmness less than 45 N were considered suitable for both fresh and distant markets. A critical bruising threshold for NMF peaches was proposed by (Metheney et al., 2002). Out of the sample population, only 1 percentile of bruises greater than 100 mm^2 occurred when flesh firmness was greater than 27 N. 'UFSun' peaches were reported to have flesh firmness of 14 N at harvest maturity (Rouse et al., 2004). Therefore, flesh firmness around 14 N was considered as the standard firmness for fresh market NMF peaches. Fruit with flesh firmness around 27 N were generally considered best for distant markets in this study. In 2007, MG 5 to 20+ of 'UFSun' was ideal for fresh consumption since they ripened to have high SSC/TA (Table 3-8). MG ≤10 of 'UFSun' were highly susceptible to CI (Table 3-25) because fruit within this maturity range were unable to soften to the same extent as those ripened directly at 20

°C (Table 3-8). Thus, only fruit at more advanced stages, i.e., MG 10-20+, were suitable for low temperature storage. Abnormal softening also occurred in low temperature-stored 'UFSun' peaches in MG 0 to 5 in 2008 (Table 3-21). MG 5 to15 was a better range for low temperature storage for 'UFSun' in 2008 since the flesh firmness at harvest was greater than the critical bruising threshold (Table 3-19) and those fruit were able to attain SSC/TA of 20 after ripening (Table 3-21). 'UFSun' fruit of MG 15 to 30+ were recommended for fresh consumption due to their high quality developed after ripening (Table 3-20).

The ideal harvest maturity for 'Gulfking' peaches destined for fresh consumption was MG 15+ in 2007 (Table 3-11) and MG 10-25+ in 2008 (Table 3-20) because these fruit had flesh firmness around 14 N at harvest and had the preferred quality suggested by (Leonard et al., 1961). Leonard et al., (1961) found that SSC/TA above 25 and acidity below 0.5% in fresh clingstone peaches resulted in canned fruit with good flavor. MG 0 to15 in 2007 (Table 3-12) and MG <0 to 10 (Table 3-24) appeared to be the optimum harvest maturity stage for low temperatures storage due to their suitable firmness at harvest (Table 3-10, 3-22).

Based on the results from this 2-year study, the optimum harvest maturities associated with direct ripening or for ripening after low temperature storage for both MF and NMF cultivars are summarized in Table 3-25. The common MG shows the overlapping optimum maturity stages between 2007 and 2008. NMF peaches destined for fresh consumption can be picked at more advanced stages than MF peaches. MF peaches must be picked at an earlier maturity to avoid handling soft fruit that are prone to mechanical injuries. For both MF varieties and NMF 'Gulfking' peaches, fruit

destined for low temperature storage can be picked at less mature stages than those destined for fresh market. Since ‘UFSun’ peaches in lower MG are more susceptible to CI, they must be harvested at more advanced stages to avoid abnormal softening.

Potential Maturity Indices Determination

High correlations of GCa* with maturity (GC-MG) were found in all the cultivars over the 2 years (r = 0.98 to 0.99) (Table 3-26, 3-27, 3-28), confirming that GCa* is a good indicator of ground color changes for both MF and NMF types (Delwiche and Baumgardner, 1985). FCa* was highly correlated with MG in both MF cultivars and in ‘UFSun’ peaches in 2007 and 2008 (Table 3-26, 3-27). This result was unanticipated since FC has been suggested as a maturity index for NMF peaches when GC is not visible; however, it was correlated with both MF cultivars but only one NMF cultivar in this study (Fuleki and Cook, 1976; Kader et al., 1982). Consistent correlation of size and PB was only observed on ‘Gulfking’ peaches (Table 3-26, 3-27). This positive correlation of size and PB in ‘Gulfking’ may lead to better consumer acceptance since consumer perception depends primarily on external qualities such as size and appearance (Iglesias and Echeverria, 2009).

High correlations were found between MG and firmness (MG-Firm) and GCa* and firmness (GC-Firm) for both MF and NMF cultivars in Year 2007 (Table 3-26). The results agree with (Brovelli et al., 1998b) who demonstrated the importance of firmness as a potential maturity index, even in NMF genotypes. However, the correlations of MG-Firm and GC-Firm were only observed in MF in 2008 (Table 3-27, 3-28), confirming that the firmness is the most consistent maturity indicator for MF types (Byrne et al., 1991; Sims and Comin, 1963).

The TA was inversely correlated with MG in both 2007 and 2008 (Table 3-26, 3-27), indicating that TA can be used as a reliable maturity index for both MF and NMF peaches (Table 3-28). The pH was highly correlated with TA in both NMF peaches and 'Flordaprince' (Table 3-26, 3-27). Fruit pH may be a better maturity index for NMF peaches than TA since it can be easily measured in the field.

Chapter Conclusion

This study demonstrates that NMF peaches do not need to be harvested at the same maturity as MF peaches. NMF peaches for fresh consumption can be picked at more advanced stages to attain the desirable 'tree-ripe' flavor. The low temperature storage study shows that 2 weeks of 0°C storage are not enough to induce severe CI in peaches but can induce minor symptom like abnormal softening. Low temperature storage can actually be beneficial for fruit harvested at less mature stages because it provides time for the fruit to develop proper flavor. Varieties that are susceptible to CI should be harvested at more advanced stages. Finally, the results of this study suggest that GCa* and TA are reliable maturity indicators of low-chill subtropical MF and NMF cultivars. GCa* is preferred over TA since it is easy to measure and non-destructive. TA may be used as a supplementary indicator if fruit is covered with blush. FCa* may be used as a secondary indicator for the MF cultivars while pH may be used specifically for the NMF cultivars.

YEAR 2007

Table 3-1. 'Flordaprince' - physical and chemical characteristics of the least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	-8.81 g	87.23 c	50.76 b	23.00 c	-7.85 b	68.45 a	10.00 a	1.07 a	9.40	3.60	4.58
-5 to 0	-3.20 f	92.30 bc	52.71 b	40.00 bc	-3.97 b	60.14 a	9.64 ab	1.04 ab	9.33	3.72	5.17
0 to 5	2.26 e	106.26 abc	55.60 ab	70.00 a	-2.35 b	59.07 a	10.77 a	1.01 ab	10.83	3.80	5.61
5 to 10	7.22 d	101.12 abc	53.83 b	67.50 a	-2.02 b	65.57 a	10.23 a	0.92 bc	11.19	3.72	5.47
10 to 15	13.00 c	124.62 ab	61.10 a	87.50 a	4.62 a	31.56 b	10.65 a	0.74d	11.92	4.02	5.91
15 to 20	17.95 b	111.78 abc	57.13 ab	66.67 ab	4.96 a	4.91 c	7.83 c	0.74d	10.59	3.88	4.26
20+	24.81 a	129.69 a	61.27 a	80.00 a	9.12 a	4.18 c	8.40 bc	0.78 cd	10.76	3.82	5.60
Significance	*	*	*	*	*	*	*	*	NS	NS	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = Non-significant
Values with different letters were significantly different at p ≤ 0.05

Table 3-2. 'Flordaprince' - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	14.05 a	6.36 c	4.39 a	11.27	1.07 a	10.97 e	3.82 d	5.02
-5 to 0	15.40 ab	8.96 abc	3.37 bc	11.25	0.80 bc	14.82 de	4.10 bc	6.01
0 to 5	13.06 ab	7.83 bc	3.49 b	11.17	0.88 b	12.93 de	3.96 cd	5.48
5 to 10	13.43 ab	11.29 a	2.90 cd	10.80	0.68 cd	17.02 cd	4.25 ab	5.91
10 to 15	11.88 ab	9.91 ab	2.64 de	10.50	0.50 e	21.45 ab	4.42 a	5.44
15 to 20	10.35 b	10.11 ab	2.49 de	10.58	0.57 de	19.26 bc	4.37 a	5.25
20+	5.43 c	11.08 a	2.18 e	12.14	0.52 de	24.31 a	4.37 a	7.01
Significance	*	*	*	NS	*	*	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05

Table 3-3. ‘Flordaprince’ - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	19.60 a	23.28	9.65	5.25 a	9.80	4.38 a	12.94	0.98 a	13.58 e	3.95 c	7.19
-5 to 0	18.04 a	23.63	9.77	-2.50 ab	11.45	3.33 cd	11.90	0.79 b	16.54 de	4.21 b	7.65
0 to 5	17.85 a	19.65	7.53	0.00 ab	10.85	3.41ab	11.48	0.65 bc	17.77 cd	4.29 b	9.05
5 to 10	15.58 a	23.15	10.55	-3.33 ab	11.04	2.43 bcd	12.08	0.60 c	20.79 bc	4.30 b	7.48
10 to 15	8.53 b	21.67	9.60	-4.17 ab	11.07	2.94 bcd	12.01	0.56 cd	22.26 b	4.40 ab	8.98
15 to 20	8.81 b	22.46	8.81	-6.43 b	11.97	2.34 cd	10.93	0.53 cd	20.42 bc	4.40 ab	6.30
20+	3.37 c	21.86	9.30	-10.89 b	10.22	2.36 d	11.94	0.42 d	28.35 a	4.57 a	7.74
Significance	*	NS	NS	*	NS	*	NS	*	*	*	NS

* = Significant differences detected among the MG at $p \leq 0.05$

NS = non-significant

Values with different letters were significantly different at $p \leq 0.05$

Table 3-4. 'TropicBeauty' - physical and chemical characteristics of least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	-6.37 e	105.51 c	56.23 b	31.67 d	-3.70 d	80.18 a	12.51	1.13 a	11.13 b	4.07 bc	7.32
-5 to 0	-2.16 d	120.83 bc	58.13 b	69.00 bc	1.26 bc	62.23 b	12.02	1.12 a	11.09 b	4.02 ab	8.02
0 to 5	0.86 d	132.08 b	60.65 ab	50.00 cd	4.83 b	57.29 b	12.65	0.99 b	12.82 b	3.71 c	10.03
5 to 10	8.05 c	142.93 ab	63.20 ab	30.00 d	3.39 b	58.01 b	12.80	1.04 ab	12.35 b	4.07 ab	9.07
10 to 15	12.53 b	137.30 b	59.60 b	65.00 bc	14.50 a	3.89 d	11.20	0.56 c	12.49 b	3.94 bc	7.63
15 to 20	15.73 b	120.19 bc	61.15 ab	75.00 ab	5.40 b	47.81 c	12.40	1.02 ab	12.12 b	3.91 bc	10.03
20+	28.35a	166.52 a	67.88 a	93.00 a	13.48 a	3.88 d	13.02	0.51 c	25.45 a	4.24 a	9.76
Significance	*	*	*	*	*	*	NS	*	*	*	NS

* = Significant differences detected among the MG at $p \leq 0.05$
NS = non-significant
Values with different letters were significantly different at $p \leq 0.05$

Table 3-5. 'TropicBeauty' - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	12.56 a	9.56 c	4.04 a	13.39	1.19 a	11.47 e	4.08	7.42 b
-5 to 0	13.61 a	12.03 b	3.44 ab	13.44	1.00 a	13.42 de	4.15	7.09 b
0 to 5	11.09 a	12.86 ab	3.54 ab	13.21	0.76 cd	16.86 cde	4.15	7.67 b
5 to 10	12.97 a	11.74 bc	3.00 bc	13.22	0.79 c	17.74 cd	4.25	8.19 b
10 to 15	7.36 b	14.80 a	2.97 bc	13.75	0.58 e	20.70 bc	4.36	12.10 a
15 to 20	7.99 bc	13.37 ab	2.76 c	14.23	0.62 de	27.20 a	4.31	7.02 b
20+	3.51 c	11.86 bc	2.90 bc	12.35	0.51 e	25.13 ab	4.21	7.58 b
Significance	*	*	*	NS	*	*	NS	*

* = Significant differences detected among the MG at $p \leq 0.05$
NS = non-significant
Values with different letters were significantly different at $p \leq 0.05$

Table 3-6. 'TropicBeauty' - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL %	SL%	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	16.77 a	14.68 ab	6.20	0.83	13.47	3.74	14.45 a	1.05 a	14.15 d	4.32 bc	6.91
-5 to 0	15.99 a	14.33 abc	5.09	3.33	12.99	3.20	13.85 ab	0.83 b	16.86 cd	4.28 c	7.72
0 to 5	13.70 ab	14.99 a	5.50	2.86	12.27	3.58	14.10 a	0.76 b	17.81 cd	4.40 bc	8.37
5 to 10	11.58 b	12.55 c	4.12	-4.50	13.33	3.16	12.55 bc	0.55 c	22.63 bc	4.40 bc	7.35
10 to 15	7.89 c	13.44 abc	4.67	-4.29	13.81	3.25	13.63 ab	0.52 cd	27.54 ab	4.29 c	9.33
15 to 20	4.89 c	12.35 c	3.43	-2.50	11.71	3.57	13.35 abc	0.46 de	31.63 a	4.63 a	10.91
20+	0.46 d	12.67 bc	4.50	7.78	12.36	3.36	12.11 c	0.40 e	30.08 a	4.54 ab	7.79
Significance	*	*	NS	NS	NS	NS	*	*	*	*	NS

* = Significant differences detected among the MG at $p \leq 0.05$

NS = non-significant

Values with different letters were significantly different at $p \leq 0.05$

Table 3-7. ‘UFSun’ - physical and chemical characteristics of least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a-value)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	-7.53 g	98.32	55.05	35.5 c	-0.82 d	53.67 a	9.93	1.03 a	9.64 c	3.83 bc	4.69
-5 to 0	-3.25 f	124.60	60.80	39.5 bc	3.59 cd	49.00 a	10.30	0.98 ab	10.55 bc	3.79 c	5.70
0 to 5	1.96 e	127.99	60.90	60.0 abc	3.76 cd	42.65 a	10.25	0.94 ab	10.92 bc	3.82 bc	5.15
5 to 10	5.76 d	120.67	60.00	50.0 bc	14.26 ab	22.89 b	8.20	0.74 ab	11.12 abc	4.22 a	6.08
10 to 15	13.76 c	152.46	65.03	50.0 bc	7.00 bcd	17.60 b	11.55	0.78 ab	16.01 a	4.04 ab	6.49
15 to 20	17.44 b	142.67	63.34	74.0 ab	9.52 abc	25.46 b	9.63	0.73 ab	13.13 abc	3.99 abc	5.20
20+	29.07 a	137.61	63.55	93.5 a	17.65 a	18.74 b	10.85	0.71 b	15.29 ab	4.02 abc	6.00
Significance	*	NS	NS	*	*	*	NS	*	*	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05

Table 3-8. ‘UFSun’ - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	20.10 a	10.53	20.76 a	10.65	0.83 a	13.46 c	4.21 b	7.81
-5 to 0	16.99 ab	12.31	18.25 ab	11.34	0.64 b	17.44 bc	4.30 b	7.55
0 to 5	14.02 bc	11.18	16.05 ab	9.54	0.58 bc	17.04 c	4.41 b	5.33
5 to 10	14.50 bc	11.65	13.98 bc	9.88	0.46 cd	22.89 ab	4.64 a	7.91
10 to 15	9.59 c	12.96	9.18 c	10.13	0.40 d	26.43 a	4.67 a	6.74
15 to 20	4.10 d	14.34	10.12 c	9.88	0.39d	26.36 a	4.71 a	5.04
20+	-2.22 e	13.49	10.28 c	9.30	0.40 d	23.43 a	4.68 a	5.65
Significance	*	NS	*	NS	*	*	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05

Table 3-9. ‘UFSun’ - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	22.39 a	20.76	8.77 a	12.06 a	12.07 d	26.26 a	11.72 ab	0.76 a	15.75 c	4.31 d	7.24
-5 to 0	20.57 a	22.43	8.85 a	11.25 a	18.51 bc	23.31 a	12.50 a	0.62 ab	21.20 b	4.46 bcd	6.95
0 to 5	15.41 b	20.16	9.13 a	2.67 a	15.35 cd	19.96 a	11.24 ab	0.65 ab	18.22 bc	4.44 cd	7.13
5 to 10	12.74 b	16.28	6.88 ab	10.00 ab	17.79 bc	20.94 a	10.78 bc	0.52 bc	21.17 b	4.51 bc	7.09
10 to 15	6.04 c	16.02	4.82 ab	-6.00 bc	23.26 ab	11.97 b	7.80 d	0.37 cd	21.07 b	4.80 a	4.51
15 to 20	3.71 c	12.90	2.44 b	-1.67 abc	25.07 a	12.31 b	11.35 ab	0.46 cd	25.50 a	4.64 ab	8.18
20+	-2.60 d	15.75	4.07 ab	-11.30 c	23.43 ab	9.11 b	9.53 c	0.36 d	27.36 a	4.82 a	5.66
Significance	*	NS	*	*	*	*	*	*	*	*	NS

* = Significant differences detected among the MG at p ≤ 0.05

NS = non-significant

Values with different letters were significantly different at p ≤ 0.05

Table 3-10. ‘Gulfking’ - physical and chemical characteristics of least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	-7.78 f	95.72 b	54.40 b	40.83	-0.07 c	47.02 a	11.13	0.87ab	12.89	3.86 de	6.49
-5 to 0	-2.55 ef	102.16 b	55.93 b	69.00	3.88 bc	42.37 ab	11.75	0.99 a	11.99	3.82 e	8.68
0 to 5	2.84 de	91.68 b	53.35 b	54.00	8.45 b	23.43 bc	10.30	0.58 cd	17.77	4.19 b	6.00
5 to 10	9.02 cd	116.92 ab	58.77 ab	67.33	6.92 b	21.54 bc	NA	NA	NA	NA	NA
10 to 15	13.17 bc	123.42 ab	58.30 ab	85.00	24.89 a	32.74 abc	10.10	0.70 bc	14.48	3.99 cd	6.08
15 to 20	18.65 b	159.18 a	65.10 a	75.00	7.80 b	17.95 c	9.00	0.59 cd	15.63	4.08 bc	5.46
20+	30.27 a	156.25 a	65.90 a	90.00	9.77 b	13.77c	9.10	0.35 d	25.95	4.41 a	11.89
Significance	*	*	*	NS	*	*	NS	*	NS	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05

Table 3-11. ‘Gulfking’ - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	17.45a	9.60	18.01 a	11.83	0.67 a	18.24 c	4.29b	10.84 a
-5 to 0	13.74ab	12.23	20.51 a	10.46	0.50 b	20.94 c	4.45b	7.43 abc
0 to 5	12.92b	11.94	19.83 a	10.37	0.52 b	19.97 c	4.46b	6.91 abc
5 to 10	15.61ab	11.70	16.49 ab	9.95	0.35 c	28.54 ab	4.71a	4.89 abc
10 to 15	5.64c	17.71	12.98 ab	8.05	0.37 c	22.36 bc	4.79a	4.89 c
15 to 20	6.89c	12.91	14.09 ab	10.84	0.39 c	28.95 ab	4.77a	6.48 bc
20+	0.85d	13.03	8.70 b	11.54	0.35 c	33.74 a	4.74a	9.31 ab
Significance	*	NS	*	NS	*	*	*	*

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05

Table 3-12. ‘Gulfking’ - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
-5 >	18.71 a	19.37 ab	7.21	10.25 ab	13.99 b	18.71	12.18	0.71 a	18.04 c	4.28 c	7.70
-5 to 0	18.28 a	21.33 a	7.91	22.00 a	20.87 ab	23.83	11.88	0.57 b	21.54 bc	4.50 bc	6.56
0 to 5	18.67 a	20.22 ab	7.28	2.17 b	23.51 a	18.76	11.37	0.49 bc	23.72 abc	4.54 b	9.25
5 to 10	8.49 b	16.40 bc	3.94	6.25 ab	20.60 ab	17.61	9.75	0.48 bc	19.97 c	4.56 ab	6.51
10 to 15	8.26 b	14.78 c	4.71	0.00 b	26.54 a	12.74	9.68	0.36 c	27.32 ab	4.77 a	6.86
15 to 20	9.43 b	19.89 ab	7.65	1.17 b	22.99 a	17.70	11.70	0.40 c	28.94 a	4.59 ab	8.53
20+	0.88 c	16.34 bc	4.89	7.00 ab	26.83 a	12.31	11.47	0.41 c	28.40 a	4.55 ab	8.25
Significance	*	*	NS	*	*	NS	NS	*	*	*	NS

* = Significant differences detected among the MG at $p \leq 0.05$

NS = non-significant

Values with different letters were significantly different at $p \leq 0.05$

YEAR 2008

Table 3-13. 'Flordaprince' - physical and chemical characteristics of least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	-2.48 g	73.10 b	52.50 b	72.50	1.77 abc	45.48 a	10.30	0.89 a	11.57 d	3.83	6.21
0 to 5	3.18 f	73.50 b	53.67 b	55.00	-1.25 c	50.42 a	10.03	0.79 ab	12.72 cd	3.88	4.93
5 to 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10 to 15	14.08 e	70.49 b	51.33 b	63.33	0.62 bc	43.53 a	10.73	0.71 bc	15.42 bc	3.95	5.41
15 to 20	17.47 d	81.12 b	55.55 ab	65.00	3.66 ab	42.49 a	11.05	0.64 bc	17.79 ab	3.96	8.14
20-25	23.42 c	86.42 ab	56.63 ab	66.67	3.27 ab	19.27 b	9.77	0.60 cd	14.69 bcd	3.95	5.10
25-30	27.19 b	116.28 a	61.39 a	75.00	5.99 a	14.03 b	10.44	0.66 bc	16.10 cd	3.89	5.56
30+	32.37 a	81.48 b	54.52 ab	85.00	6.09 a	7.03 b	10.50	0.47 d	21.36 a	4.12	5.77
Significance	*	*	*	NS	*	*	NS	*	*	NS	*

* = Significant differences detected among the MG at $p \leq 0.05$
NS = non-significant
Values with different letters were significantly different at $p \leq 0.05$

Table 3-14. ‘Flordaprince’ - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GC a*)	ΔGC (a*)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	10.56 a	10.54	3.64	17.50	6.89 b	5.37 a	11.75 ab	0.77 a	15.33	3.80 c	7.14
0 to 5	5.55 ab	12.30	5.65	2.50	5.96 b	3.43 bc	12.40 a	0.67 ab	18.72	3.90 bc	8.27
5 to 10	7.87 ab	10.65	4.09	17.86	6.95 b	3.60 b	11.24 ab	0.58 bc	19.21	3.89 bc	7.20
10 to 15	8.46 ab	10.41	3.88	10.63	6.72 b	3.74 b	10.91 b	0.54 c	19.89	4.22 ab	7.00
15 to 20	5.69 ab	9.79	3.65	12.00	6.79 b	2.96 bc	10.94 b	0.52 c	21.64	4.21 ab	6.22
20-25	4.46 abc	10.03	3.34	4.55	8.78 ab	2.92 bc	11.33 ab	0.52 c	22.67	4.25 ab	6.76
25-30	3.86 bc	10.91	4.11	10.63	9.33 ab	2.85 bc	11.10 ab	0.47 c	24.21	4.28 a	5.64
30+	-0.69 c	10.91	3.90	8.25	11.52 a	2.39 c	11.81 ab	0.50 c	23.48	4.31 a	7.34
Significance	*	NS	NS	NS	*	*	*	*	NS	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-15. 'Flordaprince' - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL-m (%)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	13.03 a	13.41	21.08	10.78	5.00	8.76 ab	3.11 b	14.30	0.97 a	14.80 c	4.00 c	8.48
0 to 5	14.09 ab	10.70	17.78	7.89	0.00	7.32 b	5.58 a	12.40	0.63 bc	19.80 bc	4.28 ab	6.96
5 to 10	9.14 abc	10.24	17.79	6.18	-3.13	9.51 ab	4.87 a	13.38	0.70 b	19.27 bc	4.18 bc	7.89
10 to 15	10.31 abc	9.75	17.08	7.96	0.00	11.07 ab	2.72 b	12.20	0.58 bcd	21.34 bc	4.30 ab	5.85
15 to 20	8.49 bcd	10.10	17.46	7.02	0.00	10.37 ab	2.87 b	12.46	0.55 bcd	23.29 ab	4.26 ab	6.57
20-25	5.82 cd	8.46	15.74	6.17	-10.0	10.12 ab	2.58 b	11.91	0.48 cd	25.23 ab	4.38 ab	6.44
25-30	4.11 de	9.21	16.11	6.13	5.00	11.69 ab	2.54 b	11.82	0.50 cd	24.03 ab	4.34 ab	6.41
30+	-0.76 e	9.65	17.91	6.20	2.41	13.28 a	2.14 b	12.89	0.44 d	30.69 a	4.49 a	8.48
Significance	*	NS	NS	NS	*	*	*	NS	*	*	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-16. 'TropicBeauty' - physical and chemical characteristics of least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	-4.61 g	79.10	54.95	58.75	-3.00 c	63.02 a	12.53	0.89 a	14.25	3.90	4.52
0 to 5	2.00 f	100.41	58.81	68.13	1.74 bc	45.88 a	12.69	0.84 a	15.70	3.98	5.67
5 to 10	7.11 e	97.36	58.10	73.75	2.63 bc	53.56 a	12.88	0.78 a	15.68	3.93	5.93
10 to 15	12.93 d	92.20	57.80	83.75	4.33 ab	49.66 a	12.90	0.78 a	15.42	3.79	6.14
15 to 20	17.82 c	110.62	61.77	78.33	6.14 ab	38.63 a	12.62	0.71 a	18.51	4.03	5.80
20-25	24.95 b	128.02	64.40	80.00	11.33 a	9.23 b	13.35	0.70 ab	19.38	3.78	6.73
25+	26.70 a	103.32	57.10	70.00	4.56 ab	4.27 b	12.3	0.57 b	25.38	4.27	6.58
Significance	*	NS	NS	NS	*	*	NS	*	NS	NS	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-17. ‘TropicBeauty’ - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	8.56 ab	8.08	4.29	0.33	6.70 c	4.80 a	13.37	0.94 a	14.54 e	3.93 c	6.66
0 to 5	9.81a	6.76	2.90	1.00	7.92 bc	3.53 b	12.48	0.80 ab	15.62 de	4.04 bc	6.60
5 to 10	8.50 abc	9.54	4.15	-3.64	8.79 abc	3.61 b	13.31	0.75 bc	18.82 cde	4.12 abc	6.51
10 to 15	5.90 c	8.81	5.17	-5.71	9.54 ab	3.07 bc	13.61	0.62 cd	22.59 bc	4.25 ab	8.52
15 to 20	6.24 bc	9.40	3.66	-10.00	9.50 ab	3.47 bc	12.92	0.63 cd	21.17 cd	4.24 ab	6.82
20-25	1.65 d	9.77	5.23	-4.29	9.56 ab	3.08 bc	13.63	0.51 d	29.16 a	4.34 a	7.13
25+	0.45 d	7.80	3.27	-8.57	10.95 a	2.46 c	12.77	0.49 d	27.93 ab	4.34 a	8.05
Significance	*	NS	NS	NS	*	*	NS	*	*	*	NS

* = Significant differences detected among the MG at $p \leq 0.05$
NS = non-significant
Values with different letters were significantly different at $p \leq 0.05$.

Table 3-18. ‘TropicBeauty’ - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL-m (%)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	13.10 a	6.06	9.17 bc	3.17 b	1.18	9.80	3.60 a	13.14	0.69 a	20.43 b	4.26 c	5.96
0 to 5	11.03 a	6.87	9.77 bc	2.63 bc	-1.11	11.07	3.21 ab	13.76	0.65 ab	22.81 b	4.25 c	7.22
5 to 10	10.48 a	5.97	8.72 bc	1.81 bc	-0.56	10.65	3.34 a	13.27	0.53 abc	26.14 b	4.33 bc	5.98
10 to 15	9.07 a	8.52	12.94 a	5.43 a	0.00	11.72	3.57 a	13.13	0.48 bcd	27.45 b	4.47 b	7.24
15 to 20	4.23 b	6.56	10.41 ab	2.67 bc	-0.91	11.75	3.20 ab	12.64	0.47 bcd	28.56 b	4.50 b	5.99
20-25	4.16 b	6.24	7.55 c	0.66 c	1.67	10.20	2.67 ab	11.80	0.46 cd	27.25 b	4.52 ab	5.99
25+	-0.97 c	6.38	9.51 bc	2.93 b	-2.86	13.62	2.34 b	12.91	0.33 d	45.11 a	4.72 a	6.34
Significance	*	NS	*	*	NS	NS	*	NS	*	*	*	NS

* = Significant differences detected among the MG at $p \leq 0.05$
NS = non-significant
Values with different letters were significantly different at $p \leq 0.05$.

Table 3-19. ‘UFSun’ - physical and chemical characteristics of least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	-2.82 i	80.88	54.97	58.33 bc	0.21 c	38.15 a	11.53	0.95a	12.12	3.82	5.81
0 to 5	1.89 h	83.73	57.00	85.00 ab	7.15 bc	17.78 c	13.00	0.76abc	17.15	4.11	7.07
5 to 10	6.37 g	95.46	57.93	55.00 c	5.46 bc	35.94 ab	10.07	0.77ab	13.44	4.02	4.90
10 to 15	12.07 f	74.47	52.65	75.00a bc	4.88 bc	35.92 ab	12.45	0.90a	13.91	3.98	5.58
15 to 20	17.48 e	87.83	56.53	60.00 bc	8.69 abc	22.14 c	11.60	0.59bc	17.60	4.07	5.19
20-25	22.02 d	94.42	57.13	67.50 abc	10.48 ab	18.52 c	12.02	0.55bc	18.56	4.13	6.00
25-30	28.46 c	90.38	56.40	83.00 abc	17.34 a	18.37 c	10.52	0.48c	19.41	4.30	5.81
30-35	31.48 b	91.58	56.10	85.00 ab	10.41 ab	26.83 bc	11.60	0.58bc	16.86	4.04	3.81
35 +	36.02 a	95.63	57.40	89.00 a	9.70 ab	22.84 c	11.20	0.59bc	19.47	4.12	4.85
Significance	*	NS	NS	*	*	*	NS	*	NS	NS	NS

* = Significant differences detected among the MG at $p \leq 0.05$
NS = non-significant
Values with different letters were significantly different at $p \leq 0.05$.

Table 3-20. ‘UFSun’ - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	9.71 ab	13.04	5.32	-5.00	8.65	16.67	9.70 c	0.75 a	12.91 c	4.25 a	5.07
0 to 5	13.98 a	12.18	5.05	2.50	10.85	20.75	11.15 bc	0.73 a	15.92 bc	3.70 c	6.43
5 to 10	12.96 a	12.42	5.12	-2.50	10.11	10.80	11.20 bc	0.77 a	14.70 bc	4.22 b	4.29
10 to 15	5.33 bc	14.38	3.76	0.71	11.70	16.71	13.60 a	0.61 ab	23.61 abc	4.36 ab	8.57
15 to 20	5.06 bc	12.36	5.04	0.38	11.84	16.45	11.36 bc	0.46 bc	24.18 abc	4.55 ab	5.38
20-25	3.10 b	13.24	5.56	-0.63	12.00	13.98	12.33 abc	0.49 bc	25.68 ab	4.55 ab	6.37
25-30	1.59 b	11.94	4.63	5.00	14.21	18.05	12.32 ab	0.50 bc	20.20 abc	4.46 ab	6.59
30-35	-0.06 cd	11.64	3.86	-1.80	14.36	17.45	10.87 bc	0.46 bc	26.34 ab	4.45 ab	6.45
35 +	-4.56 d	13.00	4.58	2.30	12.31	18.08	12.00 ab	0.39 c	31.87 a	4.67 a	6.93
Significance	*	NS	NS	NS	NS	NS	*	*	*	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-21. ‘UFSun - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a*)	WL-m (%)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	17.23 a	10.77	18.04	7.46	1.25	10.91 c	13.07 c	11.73 bc	0.81 a	15.55 d	4.25 d	5.04 bc
0 to 5	16.89 a	11.24	21.57	9.44	5.00	14.87 bcd	26.26 a	10.33 c	0.58 bc	17.91 d	4.31bcd	4.11 c
5 to 10	14.84 a	14.76	24.81	6.83	1.25	16.82 abc	21.10 b	11.95 bc	0.60 b	20.00 cd	4.37 cd	5.13 bc
10 to 15	9.67 b	9.68	18.63	6.71	6.43	14.00 cd	17.47 bc	12.66 abc	0.63 b	20.71 bcd	4.41 bcd	6.01 bc
15 to 20	5.56 bc	9.50	19.54	5.98	1.50	19.40 ab	14.86 c	13.66 ab	0.51 bc	26.67 abc	4.58 abc	7.15 ab
20-25	7.46 b	9.60	19.01	6.39	7.22	21.20 a	16.60 bc	11.12 bc	0.43 c	27.74 a	4.74 a	5.68 bc
25-30	1.95 cd	9.69	18.99	5.84	10.00	18.45 abc	16.90 bc	11.99 bc	0.44 c	27.86 a	4.70 ab	6.51 bc
30-35	-1.29 d	9.40	19.50	8.46	11.29	17.73 abc	16.11 bc	15.40 a	0.56 bc	27.43 ab	4.45 abcd	9.66 a
35+	-6.46 e	8.78	18.86	6.74	0.17	21.62 a	14.97 c	13.05 abc	0.48 bc	27.64 a	4.49 abcd	6.97 ab
Significance	*	NS	NS	NS	NS	*	*	*	*	*	*	*

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-22. 'Gulfking' - physical and chemical characteristics of least mature to most advanced fruit at harvest based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	GC (a*)	FW (g)	Size (mm)	PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
5 to 10	8.16 e	83.71 b	53.36 bc	90.80	12.04	29.38 a	12.54 a	0.70 a	17.99	4.00 c	6.98
10 to 15	13.40 d	105.00 a	58.42 a	95.00	13.40	20.85 b	11.88 a	0.60 b	19.93	4.11 bc	6.40
15 to 20	16.79 c	89.65 b	55.65 ab	92.70	14.90	19.80 bc	11.59 a	0.54 c	21.68	4.19 b	6.26
20-25	22.82 b	85.08 b	53.43 bc	86.17	18.48	13.75 c	9.62 b	0.42 d	22.68	4.49 a	5.81
25+	26.98 a	57.28 c	50.20 c	85.75	16.03	18.17 bc	7.30 c	0.35 e	21.36	4.58 a	3.79
Significance	*	*	*	NS	NS	*	*	*	NS	*	NS

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-23. 'Gulfking' - physical and chemical characteristics of least mature to most advanced fruit after 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage.

Maturity group (GCa*)	ΔGC (a*)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	19.83 a	7.49	3.54	-2.50	7.29 b	11.32	11.00 a	0.73 a	15.14 b	4.18 c	5.45 ab
0 to 5	12.68 b	8.63	2.32	0.25	10.94 ab	15.24	11.88 a	0.52 b	23.06 ab	4.39 bc	6.92 a
5 to 10	10.22 c	12.80	2.21	-2.86	12.03 ab	14.46	11.01 a	0.44 c	24.93 ab	4.46 bc	6.41 a
10 to 15	8.41 d	13.71	3.48	-2.00	19.19 a	12.75	12.20 a	0.39 d	31.23 a	4.48 bc	7.35 a
15 to 20	6.22 e	11.26	4.01	-3.00	17.51 a	12.74	10.35 a	0.33 e	31.79 a	4.65 ab	5.78 ab
20-25	4.29 f	11.65	4.25	0.22	17.62 a	13.50	8.81 ab	0.27 f	32.95 a	4.90 a	4.46 ab
25+	3.09 g	11.22	-8.99	30.00	19.03 a	8.938	6.20 b	0.23 g	26.50 a	5.04 a	2.90 b
Significance	*	NS	NS	NS	*	NS	*	*	*	*	*

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-24. 'Gulfking' - physical and chemical characteristics of least mature to most advanced fruit after 14 days at 0°C and 7 days at 20°C based on GCa*. Fruit were obtained when 50%, 70%, or 90% of tagged fruit reached commercial harvest stage

Maturity group (GCa*)	ΔGC (a-value)	WL-m (%)	WL (%)	SL (%)	Δ PB (%)	FC (a*)	Firmness (N)	SSC (%)	TA (%)	SSC/TA	pH	TS (%)
0 >	18.67 a	6.29	13.08	2.74 bc	3.75	16.23 b	19.73	12.18 ab	0.65 a	18.94 d	4.24 e	5.71 bc
0 to 5	14.84 b	5.80	13.47	1.55 c	5.36	19.69 b	16.46	12.49 ab	0.56 b	22.32 cd	4.29 de	6.62 abc
5 to 10	12.63 c	6.90	15.99	5.17 abc	-1.29	16.84 b	16.08	12.89 a	0.51 c	25.36 bcd	4.42 d	6.73 ab
10 to 15	9.66 d	8.66	18.42	3.32 bc	1.27	27.38 a	15.63	12.85 a	0.44 d	29.38 bc	4.58 c	7.02 a
15 to 20	7.74 e	6.14	16.22	3.36 bc	1.14	26.21 a	14.73	12.43 ab	0.39 e	31.69 ab	4.61 c	7.05 a
20-25	5.21 f	7.84	18.94	6.43 ab	3.29	28.61 a	13.60	10.16 b	0.33 f	30.57 b	4.85 b	4.96 bc
25+	2.50 g	9.05	20.73	8.49 a	0.83	26.88 a	13.15	10.18 b	0.27 g	39.45 a	5.08 a	4.75 c
Significance	*	NS	NS	*	NS	*	NS	*	*	*	*	*

* = Significant differences detected among the MG at p ≤ 0.05
NS = non-significant
Values with different letters were significantly different at p ≤ 0.05.

Table 3-25. Summary of optimum harvest maturities and the common maturity range between the two years for all cultivars

	Storage at 20°C for 7D			Storage at 0°C for 14D Plus 20°C for 7D		
Cultivar	Year 2007	Year 2008	Common Maturity Range	Year 2007	Year 2008	Common Maturity Range
'Flordaprince'	MG 5-10	MG < 0 to 20	MG 5-10	MG -5 to 10	MG 0-20	MG 0-10
'TropicBeauty'	MG 0 to 10	MG 5-20	MG 5-10	MG 0 to 10	MG < 0 to20	MG 0-10
'UFSun'	MG 5-20+	MG 10 to 35+	MG 10-20	MG 10-20+	MG 5-15	MG10-15
'Gulfking'	MG 15-20+	MG 10-25+	MG 15-25	MG 0 to 15	MG <0 to 10	MG 0-10

Table 3-26. Correlation coefficient (r) between maturity groups and fruit qualities, and among fruit qualities at harvest for both MF and NMF peaches in 2007

Year 2007	MF		NMF	
	'Flordaprince'	'Tropic Beauty'	'UFSun'	'Gulfking'
Significant at α≤ 0.01	MG-GC(0.99) MG-FW(0.90) MG-Size(0.87) MG-FC(0.98) MG-Firm(-0.90) MG-TA(-0.92) GC-FW(0.91) GC-Size(0.88) GC-FC(0.98) GC-Firm(-0.90) GC-TA(-0.92) FW-Size(0.99) FW-PB(0.90) FW-FC(0.95) Size-PB(0.89) Size-FC(0.94) PB-SSC/TA(0.92) FC-Firm(-0.93) FC-TA(-0.92) Firm-TA(0.87)	MG-GC(0.98) GC-FC(0.92) FW-Size(0.93) Size-FC(0.94) FC-Firm(-0.97) Firm-TA(0.98)	MG-GC(0.98) MG-PB(0.88) MG-Firm(-0.90) MG-TA(-0.93) GC-Peel(0.91) GC-TA(-0.88) FW-Size(0.99) FC-TA(-0.90) Firm-TA(0.96)	MG-GC (0.99) MG-FW(0.91) MG-Size (0.9) GC-Mass(0.99) GC-Size (0.9) FW-Size (0.99) Firm-TA(0.93) TA-pH(0.97) SSC/TA-pH (0.97)
Significant at α≤ 0.05	MG-PB(0.82) GC-PB(0.82) FW-Firm(-0.81) FW-TA(-0.87) FW-pH(0.82) Size-Firm(-0.81) Size-TA(-0.87) Size-pH(0.86) PB-FC(0.82) PB-TA(-0.73) PB-pH(0.85) FC-pH(0.76) Firm-SSC(0.77) TA-pH(-0.83) SSC/TA-pH(0.78)	MG-Size(0.82) MG-FC(0.90) MG-Firm(-0.91) MG-TA(-0.90) GC-Mass(0.80) GC-Size(0.87) GC-Firm(-0.88) GC-TA(-0.89) GC-SS/TA(0.79) FW-FC(0.89) FW-SSC/TA(0.83) Size-SSC/TA(0.86) FC-TA(-0.94) FC-SSC/TA(0.86)	MG-FW(0.79) MG-Size(0.82) MG-FC(0.84) MG-SSC/TA(0.85) GC-Size(0.78) GC-FC(0.83) GC-Firm(-0.86) GC-SSC/TA(0.87) FW-Firm(-0.76) FW-SSC/TA(0.86) Size-Firm(-0.78) Size-SSC/TA(0.86) FC-Firm(-0.84) FC-pH (0.78) Firm-SSC/TA(-0.85) Firm-pH(-0.83) TA-pH(-0.84)	MG-PB(0.86) MG-Firm (-0.85) MG-SSC(-0.91) MG-TA(-0.83) GC-Peel (0.9) GC-Firm(-0.85) GC-SSC(-0.9) GC-TA(-0.86) FW-PB (0.76) FW-SSC(-0.85) Size-PB (0.76) Size-SSC(-0.82) Firm-SSC(0.91) Firm-pH (-0.90) TA-SSC/TA(-0.91)

Table 3-27. Correlation coefficient (r) between maturity groups and fruit qualities, and among fruit qualities at harvest for both MF and NMF peaches in 2008

Year 2008	MF		NMF	
	'Flordaprince'	'Tropic Beauty'	'UFSun'	'Gulfking'
Significant at α≤ 0.01	MG-GC(0.98) MG-Firm(-0.93) MG-TA(-0.93) MG-SSC/TA(0.83) GC-FC(0.82) GC-Firm(-0.89) GC-TA(-0.94) FW-Size(0.97) TA-SSC/TA(-0.91) TA-pH(-0.91) SSC/TA-pH(0.92)	MG-GC(0.99) MG-Firm(-0.91) MG-TA(-0.96) MG-SSC/TA(0.88) GC-Firm(-0.90) GC-TA(-0.94) GC-TS(0.89) FW-Size(0.94) FW-FC(0.94) Size-FC(0.90) Firm-TA(0.89) Firm-SSC/TA(-0.91) TA-SSC/TA(-0.95)	MG-GC(0.99) MG-TA(-0.80) GC-TA(-0.82) FW-Size(0.88) FC-TA(-0.92) FC-SSC/TA(0.86) FC-pH(0.94) Firm-TA(0.80) Firm-SSC/TA(-0.92) Firm-pH(-0.83) TA-SSC/TA(-0.90) TA-pH(-0.84) SSC/TA-pH(0.87)	MG-GC(0.99) MG-TA(-0.99) MG-pH(0.98) GC-TA(-0.99) GC-pH(0.99) FW-Size(0.97) Firm-SSC/TA(-0.96) SSC-pH(-0.96) SSC-TS(0.97) TA-pH(-0.99)
Significant at α≤ 0.05	MG-FC(0.86) GC-FC(0.822) GC-SSC/TA(0.84) GC-pH(0.75) GC-SSCTA(0.79) PB-FC(0.85) PB-Firm(-0.78) FC-Firm(-0.87) SSC-TS(0.76)	MG-FC(0.80) MG-TS(0.87) GC-FW(0.77) GC-FC(0.85) GC-SSC/TA(0.84) FW-Firm(-0.76) FW-TS(0.77) PB-FC(0.79) FC-TS(-0.81) Firm-TS(-0.81) SSC-pH(-0.82) TA-TS(-0.80)	MG-FC(0.74) MG-SSC/TA(0.76) GC-FC(0.77) GC-SSC/TA(0.77) FW-TA(-0.72) FC-Firm(-0.76)	MG-SSC(-0.94) MG-TS(-0.90) Size-PB(0.90) FC-Firm(-0.93) FC-SSC/TA(0.93) SSC-TA(0.95) pH-TS(-0.88)

Table 3-28. Two year summary of potential maturity indices for all cultivars, MF specific, or NMF specific based on the correlation coefficient (r) between maturity groups and fruit qualities, and among the fruit qualities at harvest

Year	All Cultivar	MF Specific	NMF Specific
2007	MG-GC MG-Firm MG-TA MG-Size Firm-TA GC-TA GC-Firm GC-Size FW-Size	MG-FC GC-FC GC-FW FC-Firm FC-Size FC-FW FC-TA	MG-PB Firm-pH TA-pH
2008	MG-GC MG-TA GC-TA FW-Size	MG-Firm MG-SSC/TA MG-FC GC-Firm GC-SSC/TA TA-SSC/TA	Firm-SSC/TA TA-pH FC-Firm FC-SSC/TA

CHAPTER 4
RIPENING AND QUALITY DEVELOPMENT OF LOW-CHILL SUBTROPICAL MELTING AND NON-MELTING FLESH PEACH VARIETIES HARVESTED AT DIFFERENT MATURITIES

Overview

Growing high quality peaches (*Prunus persica* (L.) Batch) i.e., with good flavor and fruit size, is appealing to homeowners, landscapers, and commercial fruit growers located in the tropical and sub-tropical regions of the U.S. (Rouse et al., 2006). Peaches adapted to tropical and sub-tropical climates have a low-chilling requirement for fruit set (less than 250 chill units) and, in Florida, ripen in a market window when peaches from other areas of the U.S. are not available. Supplied of fruit from Chile usually are depleted before early market Florida peaches mature (about early April) and the earliest higher-chill peach cultivars from Georgia, Carolina and California appear after they ripen (Rouse and Sherman, 2002).Therefore, growing low-chill peach cultivars in Florida may be economically advantageous.

Early season peaches suffer from relatively low soluble solids content (SSC). Thus, fruit growers generally rely on cultural practices to improve the fruit quality (Mercier et al., 2009). Moreover, the traditional melting flesh (MF) peaches become extremely soft (i.e., “melting”) quickly after ripening is triggered. Thus, they need to be harvested at ‘firm-mature’ stage to minimize mechanical injuries, but consequently have considerably lower eating quality than tree-ripened fruit (Cascales et al., 2005; Delwiche and Baumgardner, 1983; Williamson and Sargent, 1999). Developing non-melting flesh (NMF) peach cultivars that are traditionally used for canning with the flavor and external red color of MF peaches is desirable because the relatively slow softening characteristics of NMF peaches will allow growers to pick the fruit at a riper stage, thus

providing eating quality for consumers without sacrificing shipping ability (Beckman et al., 2008).

Ripening initiation of peaches is regulated by System II ethylene. In peaches, the burst in respiration associated with climacteric ripening coincides with the increased levels of System II ethylene production (Ferrer et al., 2005; Madrid et al., 2000). Peaches have a moderate respiration rate during ripening relative to other horticultural commodities (Wills et al., 2007). It was reported that peach cultivars with shorter developmental cycles (earlier harvest dates) had higher and more pronounced respiration rates at the climacteric peak (DeJong et al., 1987).

There are no clear distinctions between the sensory aspects of MF and NMF peaches after normal ripening except texture (Brovelli et al., 1999b). The dominant (M) allele controls the flesh firmness of MF cultivars, while the allele of NMF cultivars is homozygous recessive (mm) (Peace et al., 2005). This genetic difference in texture translates into a reduced capacity for cell wall degradation in the NMF fruit, which could be attributed to either a partial or complete deletion (Callahan et al., 2004; Pressey and Avants, 1978), or mutation (Morgutti et al., 2006) of the endo-polygalacturonase (endo-PG) gene. Endo-PG (E.C. 3.2.1.15) is a cell wall modification enzyme that randomly cleaves specific pectin molecules (homogalacturonans) and effectively reduces their molecular size (Pressey 1978). Since endo-PG mRNA is highly expressed after the ethylene climacteric rise and the increased enzyme activity is accompanied by increases in water-soluble pectin during the melting phase (Orr and Brady, 1993; Pressey and Avants, 1978), endo-PG is regarded as the primary enzyme responsible for peach softening (Lester et al., 1994; 1996)

In contrast to endo-PG, exo-PG (PG, E.C. 3.2.1.67) removes monomer units from the non-reducing ends of the pectin chains and has a minimal effect on the size of the macromolecule (Pressey and Avants 1978). Exo-PG activity in NMF peaches can be similar or higher than in MF peaches during ripening (Manganaris et al., 2006a; 2006b; Pressey and Avants, 1973; 1978b). Two forms of exo-PG in the mesocarp tissue of ripe MF peaches were distinguished, and increased enzyme activity occurred only when the mesocarp tissue was very soft (Downs and Brady, 1990). Therefore, exo-PG may not have an important role in the initiation or promotion of fruit softening during ripening, but it may act together with endo-PG to produce the MF texture (Orr and Brady 1993).

Based on previous research, a direct relationship between endo-PG and peach softening may be assumed; however, antisense RNA work in transgenic tomato did not support a direct relationship between endo-PG and softening (Carrington et al., 1993). Increased endo-PG activity in tomato fruit was found to occur mostly during the final (postclimacteric) stage of ripening, which could be termed the “over-ripening” phase. Furthermore, ripening inhibition of avocados using 1-methylcyclopropene (1-MCP) also showed that endo-PG is not required for the extensive softening that occurs in ripening avocado fruit (Jeong et al., 2002).

Another enzyme that may affect cell wall degradation during ripening is pectin methylesterase (PME; E.C 3.1.1.11). PME activity is required to initiate pectin degradation (Li et al., 2010) by hydrolyzing the ester bond in the carboxymethyl groups of galacturonic residues of pectin, resulting in the release of methyl groups and exposure of carboxyl groups (Tijskens et al., 1999). PG has no substrates with which to

react until demethylesterification occurs (Fischer and Bennett, 1991). PME activity has been shown to increase sharply at an early stage of peach ripening and remains constant or decreases throughout the cell wall depolymerization phase in MF cultivars (Brummell et al., 2004; Glover and Brady, 1995). PME activity of NMF ‘Andross’ peaches was reported to be significantly lower than that of MF ‘Caldesi 2000’ (Manganaris et al., 2006b).

Compositional changes during ripening increase the desirable flavor of peaches. Assessment of fruit quality after ripening is important because consumers ultimately will taste the ripened fruit and make decisions regarding repeat purchases base on this experience. Currently, there is little information available on the qualities of the low-chill peaches after ripening, especially for the NMF cultivars that have been recently released. Thus, the objective of the this study was to characterize the ripening of low-chill, subtropical MF and NMF peach cultivars during storage at 20 ºC based on their respiration rates and ethylene production rates when harvested at various developmental stages; 2) to quantify the qualities of the fruit objectively after postharvest ripening at 20 ºC for 5 days; and, 3) to investigate the relationship between cell wall modifying enzymes and softening of MF and NMF cultivars via measurement of PME and PG activities during ripening.

Materials and Methods

Plant Materials

In 2007, 2008, and 2009, two MF cultivars, ‘Flordaprince’ and ‘TropicBeauty’, and two NMF cultivars, ‘Gulfking’ and ‘UFSun’, were selected from the peach collection at the UF Plant Science Research & Education Center at Citra, Florida and a group of 100 marker fruit from four trees for each cultivar were tagged after fruit thinning and natural

fruit drop. These fruit were randomly selected and considered to be representative of the population of each cultivar. During later stages of fruit development, as the fruit approached full size, the marker fruit were monitored visually for changes in peel ground color (GC). When the peel GC of 50% of the marker fruit changed from green to yellow, the peel GC (C.I.E. L*, a*, and b* values) of all 100 marker fruit were objectively measured using a reflectance colorimeter (Minolta CR-400, Konica Minolta, Japan). Ground color was measured on the greenest portion of the peel. For all peach genotypes, the chromaticity "a*" (green-red) of the epidermal GC (GCa*) increases the most with increasing maturation and ripening, whereas L* (lightness) and b* (yellow-blue) values change only slightly with maturation and ripening (Delwiche and Baumgardner, 1985). Three fruit per tree (12 fruit per cultivar) with GCa* that was within one standard deviation of the 100 marker fruit were harvested. The harvested fruit were subjectively separated into different maturity groups (MG) according to their GC and ripened at 20 ºC for 5 days.

Since PG activity is expected to be higher at more advanced ripeness stages, at least 9 fruit from each of the four cultivars with initial GC a*-values ≥ 15 were pooled together from three harvests in 2007. In 2010, fruit collected from one harvest of 'Flordaprince' and 'Gulfking' were compared separately from 'TropicBeauty' and 'UFSun' since the former pair of cultivars had initial GC a*-values ≥ 15 and the latter pair had a*- values < 15. All of the fruit were ripened at 20°C for 5 days before firmness measurements and tissue collection. Fruit tissue was diced and stored at -30°C until enzyme analyses were conducted.

Ethylene Production and Respiration Rate Determination

In 2008, respiration rate (CO_2 production) and ethylene production were monitored in a static system consisting of 550-ml glass containers with air-tight lids containing individual fruit that were sealed for 0.5 h before 1-ml headspace gas samples were withdrawn for gas chromatograph (GC) injection. The CO_2 was determined by GC using a thermal conductivity detector with a molecular sieve column. Ethylene was measured by GC with a photoionization detector and activated alumina column. Certified gas standards were used to determine the concentration of CO_2 and ethylene. Measured concentrations of CO_2 and ethylene were converted into rates of production by calculation based on the mass of fruit in a jar, the void volume, and the duration of sealing. Respiration rate and ethylene production were monitored every day for 5 days at 20°C. In 2009, an ethylene and CO_2 gas analyzer ETH-1010 (Fluid Analytics, Inc., West Linn, Oregon) was used to measure respiration rate and ethylene production. One to two fruit were sealed in a 2.735L Plexiglas container connected to the device. Five replicate measurements per sample were made.

Quality Analysis

Ground and flesh color determination

Ground color (GCa*) and flesh color (FCa*) were objectively measured using a reflectance colorimeter (Minolta CR-400, Konica Minolta, Japan) and expressed as C.I.E. a* values (green-red) since a* value increases the most with increasing maturation and ripening of peaches, both in the peel (Delwiche and Baumgardner, 1985) and the flesh (Fuleki and Cook, 1976; Kader et al., 1982; Robertson et al., 1991). Ground color was measured on the greenest portion of the peel. Flesh color was measured after removing a small (circa 2 cm diameter) patch of peel.

Flesh firmness determination

Flesh firmness was measured with an Instron Universal Testing Instrument (Model 4411-C8009, Canton, MA, USA) that applied a compressive force from a 50 kg load cell. A convex tip probe (Magness-Taylor type), 7.9 mm in diameter, was attached to the load cell moving at a speed of 12 cm/min. Flesh firmness was measured at the fruit equator on two sides, on the cheeks, without peel, and expressed as the bioyield force (N). Following color and firmness measurements, fruit samples were placed in quart size (17.7cm x 20.3cm), zipper locking plastic freezer bags and stored at -30 ºC for later compositional analyses.

Soluble solids content, titratable acidity, and total sugar determinations

Frozen fruit tissues were pureed in a Waring blender for 1 min. The resulting slurry was centrifuged (20 min; 15,000 × g_n; 4°C) and the clear supernatant was used to determine soluble solids content (SSC) and titratable acidity (TA). The SSC was measured with a temperature compensated digital refractometer (model ABBE Mark II, Cambridge Instruments Inc, U.S.A) and expressed as per cent FW. The TA was determined by titration (model 719 S. Titrino, Metrohm, Switzerland) of 6.0 g of juice plus 50 mL of water with 0.1N sodium hydroxide solution until pH 8.2 was reached and the TA was expressed as percent malic acid. The pH of the diluted juice was determined automatically by the Titrino equipped with a pH electrode.

Total soluble sugar (TS) measurement was performed using the phenol-sulfuric assay (Dubois et al., 1956) modified as follows: 5 µL of extracted juice was diluted with 5 mL of 80% ethanol. Further dilution was performed if the concentration of the sample was out of the range of the standard curve. A 500 µL aliquot of the diluted sample was added to 500 µL of 5 % phenol solution (Fisher Scientific, New Jersey, USA; certified

grade) and vortexed. Next, 2.5 mL of concentrated sulfuric acid (Fisher Scientific; certified ACS grade) was slowly added to the mixture and vortexed. The mixture was left for 10 min at room temperature for color development. The absorbance of the sample at 490 nm was read on a microplate with glucose (Fisher Scientific, New Jersey, USA; certified ACS grade) as the standard. Total sugar in the juice was expressed as a percentage.

Enzyme Assays

Preparation of cell-free protein extract

Enzyme extracts were prepared similarly to the method of Jeong et al. (2002). Partially thawed mesocarp tissue (15 g) was homogenized with 25 mL of ice-cold 95% ethanol for 1 min in an Omnimixer (Model GLH-01, New-town, CT, USA) and centrifuged at 15,000 × g_n for 10 min at 4°C. The supernatant was discarded and the pellets were resuspended in 25 mL of ice-cold 80% ethanol for 1 min and centrifuged again at 15, 000 × g_n for 10 min at 4°C. The pellets were transferred to 10 mL of 50 mM Na-acetate buffer, pH 5, containing 0.5 M NaCl, for 30 min in an ice-cold water bath followed by centrifugation 15, 000 × g_n for 10 min at 4°C. The resulting supernatant was analyzed for enzyme activities. Total soluble protein in the supernatant was measured using the bicinchoninic acid method with bovine serum albumin as the standard (Smith et al., 1985).

Pectinmethylesterase activity determination

Pectinmethylesterase (PME, E.C. 3.1.1.11) was measured using modifications of the method of (Jeong et al., 2002). A 1% (w/v) solution of 93% esterified citrus pectin (Sigma Chemical Co., St. Louis, MO, USA) was prepared in 0.1 M NaCl and adjusted to pH 7.5 with dilute NaOH. A 0.01% solution of bromothymol blue was prepared in 0.003

M potassium phosphate buffer, pH 7.5. A 166 µL volume of 1% citrus pectin was mixed with 12 µL of 0.01% bromothymol blue and 70 µL of water, and the pH adjusted to 7.5 with dilute NaOH. The reaction was initiated by adding 6 µL of the cell-free protein extract that had been adjusted to pH 7.5 with dilute NaOH. The decrease in A_{620} over a 10 min reaction time was recorded and PME activity was expressed as ΔA_{620} mg^{-1} protein min^{-1}.

Polygalacturonase activity determination

Endo-PG (E.C. 3.2.1.15) activity was assayed by mixing 250 µL of enzyme extract with 250 µL of 0.5% polygalacturonic acid (from orange peel, Sigma Chemical Co., St. Louis, MO, USA) in 50 mM Na-acetate buffer (pH 4.4) and incubated at 30°C for 16 h (Pressey and Avants, 1973). For measurement of exo-PG (E.C. 3.2.1.67) activity, 250 µL of enzyme extract was mixed with 250 µL of 0.5% polygalacturonic acid in 50 mM Na acetate buffer (pH 5.5) containing 2 mM $CaCl_2$, and incubated at 30°C for 16 h. Uronic acid (UA) reducing groups released were measured using the method of Milner and Avigad (1967) with mono-D-galacturonic acid as the standard. One unit of activity was defined as 1 µg galacturonic acid produced mg^{-1} protein h^{-1}.

Statistical Analysis

The General Linear Model program of the Statistical Analysis System (SAS) (SAS Institute, Cary, NC) was used. One way – Analysis of Variance (ANOVA) was used to detect significant differences at the 5% level among the cultivars and between years. Since there were, in general, no significance differences after ripening among the MGs for the qualities listed in Table 4-1, all of the data were combined for analysis. The least significant difference (LSD) test was used for mean separation.

Results and Discussion

Respiration and Ethylene Production

In 2008 and 2009, both MF ‘Flordaprince’ and MF ‘TropicBeauty’ were on the climacteric rise at the time of harvest as shown by increased respiration and ethylene production measured after harvest (Fig. 4-1A to 4-1D; Fig. 4-2A to 4-2D). The ethylene production measured at harvest indicates that the MF cultivars only required low levels of ethylene to initiate ripening, since the fruit were apparently producing minimal amounts of ethylene on the tree. Fruit from different MGs were generally not significantly different in respiration rate and ethylene production for both MF cultivars. The peak levels of ethylene and CO_2 production in peaches were reported to occur around the same time (Amoros et al., 1989) . This was only observed on Day 4 of storage for ‘TropicBeauty’ of MG 5 to 10 in 2008 (Fig. 4-1C, 4-1D) and Day 3 for ‘Flordaprince’ in 2009 regardless of MG (Fig. 4-2A, 4-2B). The ethylene production rates at harvest in 2008 for ‘TropicBeauty’ from the most mature to the least mature fruit were similar to values reported by (Brovelli et al., 1999a), but higher than the rates in 2009. The ethylene production measured during ripening at 20°C in 2008 was in accordance with (Brovelli et al., 1999b). Although respiration rates during ripening for ‘TropicBeauty’ in both 2008 and 2009 were higher than previously reported values, the trends were comparable (Brovelli et al., 1998b).

For NMF ‘UFSun’, peaches with GCa* values of 10 to 15 and 20 to 25 were postclimacteric at the time of harvest in 2008, indicating that fruit from the more advanced MGs were producing climacteric ethylene while ripening on the tree (Fig. 4-1E, 4-1F). In 2009, postclimacteric ethylene production was not observed on any ‘UFSun’ fruit (Fig. 4-2E, 4-2F), which suggests a possible difference in harvest maturity

between the two years. The least mature fruit (GCa* 0 to 5) had peak ethylene production on Day 3 while other MG had the peaks on Day 2. Climacteric respiration peaked approximately 1 to 2 days after maximum ethylene production was reached (Fig. 4-2E). For NMF 'Gulfking' peaches, fruit in the lower MGs (GCa* < 15) had higher respiration rates than the most mature fruit (GCa* 20 to 25) in 2008 (Fig. 4-1G). The respiration climacteric of 'Gulfking' fruit with GCa* of 20 to 25 was 1 day in advance (Day 2) of the less mature fruit, which had their climacteric peaks delayed until Day 3. Fruit with GCa* of 20 to 25 had significantly lower ethylene production than fruit from the other MGs during storage (Table 4-1H). Therefore, 'Gulfking' fruit from this maturity range reached the postclimacteric stage during storage. In 2009, 'Gulfking' fruit with GCa* > 20 had their respiration climacteric on Day 1, 2 days earlier than the fruit from lower MG (Fig. 4-2G), indicating that the fruit were quickly approaching the postclimacteric stage. The ethylene climacteric peak for 'Gulfking' fruit generally occurred between Day 2 and Day 3 after harvest, similar to results in 2008 (Table 4-2H). 'Gulfking' fruit with GCa* of 5 to 10 reached their maximum respiration rate and ethylene production on the tree prior to harvest since they were already at the postclimacteric stage during storage (Table 4-2G, 4-2H).

In summary, the respiration rates were similar for MF and NMF peaches, but the ethylene production was usually higher in the NMF cultivars during ripening. Similar results have been reported, confirming that the NMF trait in peaches is not related to low ethylene production (Brovelli et al., 1999a; Haji et al., 2001; Manganaris et al., 2006b). The MF cultivars were mostly preclimacteric or at onset of ripening regardless

of the different MG at harvest. The more advanced NMF peaches had already started the ripening process on the tree and became postclimacteric during storage.

The results from a previous experiment (Chapter 3) indicate that peaches destined for fresh market should be harvested when the GCa* reaches 5 to 10 for MF cultivars, 15 to 20 for NMF 'UFSun' and 15 to 25 for NMF 'Gulfking'. In this study, MF fruit harvested within a GCa* range of 5 to 10 were at the beginning of the climacteric rise with very low ethylene production, while both NMF peach cultivars were further along on the climacteric rise. This is expected, because MF cultivars have been selected by breeders so that the optimum harvest maturity occurs when softening has not yet been initiated. Since NMF peaches soften relatively slowly, the fruit can be harvested when ripening has already been initiated. Based on the results of this study, the optimum harvest maturity for 'Gulfking' peaches should be amended from GCa* 15 to 25 to GCa* 15 to 20 to avoid fruit that are approaching the postclimacteric phase. At the postclimacteric developmental stage, fruit are over-ripe and more susceptible to decay caused by pathogens (Gradziel, 1994).

Quality Analysis

Ground color (GCa*) increased tremendously after 5 days of storage (Table 4-1). In peaches, the increase in a* denotes an increase in red carotenoid pigments and a loss of green color that is related to the disappearance of chlorophyll in the skin or flesh of the fruit (Ferrer et al., 2005; Madrid et al., 2000). The initial GCa* and final GCa* of 'TropicBeauty' peaches were the lowest among all the cultivars in 2008 and 2009, indicating that this MF cultivar generally has more green color and less red pigment accumulation in the peel than the other cultivars that were included in this study. MF 'Flordaprince' had the lowest FCa* compared to the other cultivars, indicating that

‘Flordaprince’ retained the highest amount of chlorophyll in the mesocarp and the flesh appeared to be less red.

Flesh firmness and flavor composition were compared to the minimum eating qualities of MF peaches stated in the literature. MF peaches at eating ripe stages should be close to 13 N flesh firmness, 0.5-0.8% TA, and at least 10% SSC or SSC/TA ≥ 15 (Beckman and Krewer, 1999; Malakou and Nanos, 2005). Currently, there are no minimum eating qualities defined for NMF peaches although it is known that a ripe NMF fruit may soften to around 16 N in firmness (Lurie and Crisosto, 2005). As was expected, the MF cultivars in this study became extremely soft during storage (flesh firmness < 4N) and the NMF cultivars retained greater flesh firmness longer than the MF cultivars. Both NMF cultivars maintained flesh firmness of 12 to 17 N after ripening for 5 days at 20°C. ‘TropicBeauty’ had highest SSC throughout the 3 years of this study (11.50 -12.50%), followed by ‘Flordaprince’ (10-11%), ‘UFSun’ (9-11%), and ‘Gulfking’ (9-10%). Interestingly, the higher TA of the MF cultivars was balanced by higher SSC, while the lower TA of the NMF cultivars was balanced by lower SSC. As a result, an SSC/TA of more than 15 was consistently observed in the NMF cultivars but was only achieved in the MF cultivars in two out of three years (2008 and 2009), indicating that the NMF cultivars may have higher consumer acceptance than MF peaches after ripening (Williamson and Sargent 1999). Consumers may perceive ‘Gulfking' most favorably among the cultivars studied because it had the highest SSC/TA due to it having the lowest TA. Consumers reportedly prefer low-acid over the high-acid cultivars regardless of fruit maturity (Iglesias and Echeverria, 2009).

The pH was significantly different between the MF and NMF peaches. The MF cultivars consistently had lower pH than the NMF cultivars after ripening. It has been shown that pH can sometimes relate better to sourness perception than TA in mangoes (Malundo et al., 2001). Hence, the MF cultivars would be expected to be perceived as more sour than the NMF cultivars.

The TS concentrations were similar among the four cultivars in 2008 and 2009, but all were significantly less than the SSC. Sucrose, fructose, and glucose are the main sugars in peaches, with sucrose being the dominant sugar at harvest (Byrne et al., 1991; Génard et al., 2003). The TS is generally lower than the SSC because SSC can also include solutes that are not sugars such as pigments, salts, proteins, and acids. Furthermore, it is possible that alcohol-soluble substances present in ripe peaches, including pigments, lipids and proteins, react with the concentrated sulfuric acid in the TS assay and therefore may significantly interfere with the absorbance reading (Ashwell, 1957).

Enzyme Assays

In 2007, MF ‘TropicBeauty’ differed from the other MF cultivar in having a higher level of endo-PG activity than the two NMF cultivars after being stored at 20°C for 5 days (Table 4-2). In 2010, endo-PG activity was significantly higher in MF ‘TropicBeauty’ than NMF ‘UFSun’ (Table 4-3). The NMF cultivar ‘Gulfking’ had endo-PG activity that was similar to the MF cultivar ‘Flordaprince’ in 2007, but ’Gulfking’ was approximately five times firmer than ‘Flordaprince’. A similar pattern was observed in 2010. There were no differences in endo-PG activity between MF ‘Flordaprince’ and NMF ‘Gulfking’ although ‘Gulfking’ was 2.5 times firmer than ‘Flordaprince’. Since MF and NMF cultivars can have similar endo-PG activities, lack of endo-PG mRNA

accumulation as suggested by Callahan et al. (2004) may not fully explain the delayed softening characteristic of NMF peaches. This confirms the report of Morgutti et al. (2006), who observed that peach fruit with essentially the same flesh firmness (46 N) showed barely detectable accumulation of endo-PG polypeptide in NMF 'OroA' and much higher accumulation in MF 'Bolero'. It may also be possible that the standard firmness measurement procedure using a Magness-Taylor type probe, which was developed for MF peaches, does not accurately measure the different texture of NMF peaches.

Exo-PG activities of the two NMF cultivars and MF 'Flordaprince' were not significantly different after storage in 2007 (Table 4-2). In 2010, NMF 'Gulfking' had significantly higher exo-PG activity than MF 'Flordaprince', whereas exo-PG activities were not significantly different between NMF 'UFSun' and MF 'TropicBeauty' (Table 4-3). These results demonstrate that the exo-PG activity of NMF fruit can be similar or higher than that of MF fruit after ripening (Pressey and Avants 1978; Manganaris et al., 2006). When comparing the endo-PG and exo-PG activities for each cultivar, MF peaches appeared to have higher endo-PG activity than exo-PG activity, except for 'Flordaprince' in 2007. NMF 'UFSun' differed between the two seasons in that it appeared to have higher exo-PG than endo-PG activity in 2007 (Table 4-2) and the reverse in 2010 (Table 4-3). Therefore, it may be possible for ripe NMF peaches to have lower, similar or higher exo-PG activity than endo-PG activity.

The PME activity after ripening was significantly higher in both MF cultivars than in the two NMF cultivars in 2007 (Table 4-2), which was similar to the results reported by Manganaris et al. (2006). However, this relationship was only observed between MF

‘Flordaprince’ and NMF ‘Gulfking’ in 2010 (Table 4-3) but not in MF ‘TropicBeauty’ and NMF ‘UFSun’, demonstrating that the softening variation between MF and NMF cultivars after ripening cannot be explained by PME activity, which differed significantly. It is possible that the PME determination in this study was not performed at the period of development when the enzyme is most active. PME is most active during the early stage of ripening and remains constant or decreases throughout the cell wall depolymerization phase (Glover and Brady 1995; Brummell et al., 2004). Porter et al. (2000) reported that there was no apparent relationship between PME activity and major changes in firmness for low *vs*. high-chill, or MF *vs*. NMF peaches from the mature green developmental stage to the early stages of fruit ripening.

Ortiz et al. (2010) reported that PG and PME activity detected in MF ‘Snow Queen’ nectarines had a similar trend during ripening but there was no apparent coincidence for either enzyme with the melting phase of fruit softening. PG and PME activities were highest at the mature-unripe stage and declined noticeably during the melting phase (late climacteric), but slowly regained activities as fruit became over-ripe. This may be indicative of a situation such that once ripening is initiated in MF fruit, the onset of events is irreversible, leading to the extensive, melting softening. It may also be possible that the presence of different PG and/or PME isozymes that contribute to the total activity of each enzyme measured masks the activity of individual softening related isozymes. The result would be that the changes in overall PG or PME enzyme activities would not correlate with the changes in flesh firmness. This was observed in tomatoes that contained a high level of β-galactosidase (β-Gal) activity, which was due to three forms of the enzyme. During tomato ripening, the sum of their activities remained

relatively constant, but the levels of the individual forms of β-Gal changed markedly (Pressey, 1983). Only one of the β-Gal forms was correlated with softening.

Different rates of softening in different fruit are contributed to inherent differences in composition and in the nature of the cell wall polysaccharides and other cell wall structural components (Li et al., 2010). Manganaris et al. (2006) reported that MF peach fruit underwent greater loss of neutral sugars, especially arabinose (Ara) and galactose (Gal), than NMF peaches during ripening. This is supported by Yoshioka et al. (2010) who observed that pectin solubilization and loss of neutral sugars, Ara and Gal, occurred to a limited extend in NMF peaches compared to that of MF peaches.

Galactosyl- and arabinosyl-containing side-chains on pectin backbones are thought to control pore size in the cell wall, thus limiting the accessibility of the pectin to pectolytic hydrolases and thereby protecting cell wall polysaccharides from extensive depolymerization (Brummell and Harpster, 2001; Brummell, 2006). The loss of Ara might be attributed to the sharp increase in α-arabinofuranosidase (ARF) activity from the preclimacteric to climacteric stage in MF peaches (Brummell et al., 2004; Carolina Di Santo et al., 2009; Santo et al., 2009). It has been shown in MF ‘Snow Queen’ nectarines that the highest β-Gal activity occurs in mature but unripe fruit and a loss of Gal from cell wall material is followed immediately afterwards, together with a significant decline in fruit firmness (Ortiz et al., 2010). Hence, the activities of ARF and β-Gal may be related to the difference in texture between MF and NMF peaches more directly than PME and PG activities.

Chapter Conclusion

The low-chill subtropical NMF cultivars ‘Gulfking’ and ‘UFSun’ had higher ethylene production at harvest and throughout ripening than the MF cultivars ‘TropicBeauty’ and

‘Flordaprince’. MF fruit harvested at different maturity stages were mostly preclimacteric or at onset of ripening. NMF fruit harvested at different maturities generally had started ripening on the tree and the fruit were at more advanced ripeness stages and some became postclimacteric after harvest. The NMF cultivars, especially ‘Gulfking’, have potential to be perceived as having higher sensory quality by consumers due to higher SSC/TA than the MF cultivars. PME and PG activities determined after ripening appeared to have no direct relationship with softening in either MF and NMF peaches.

Year 2008

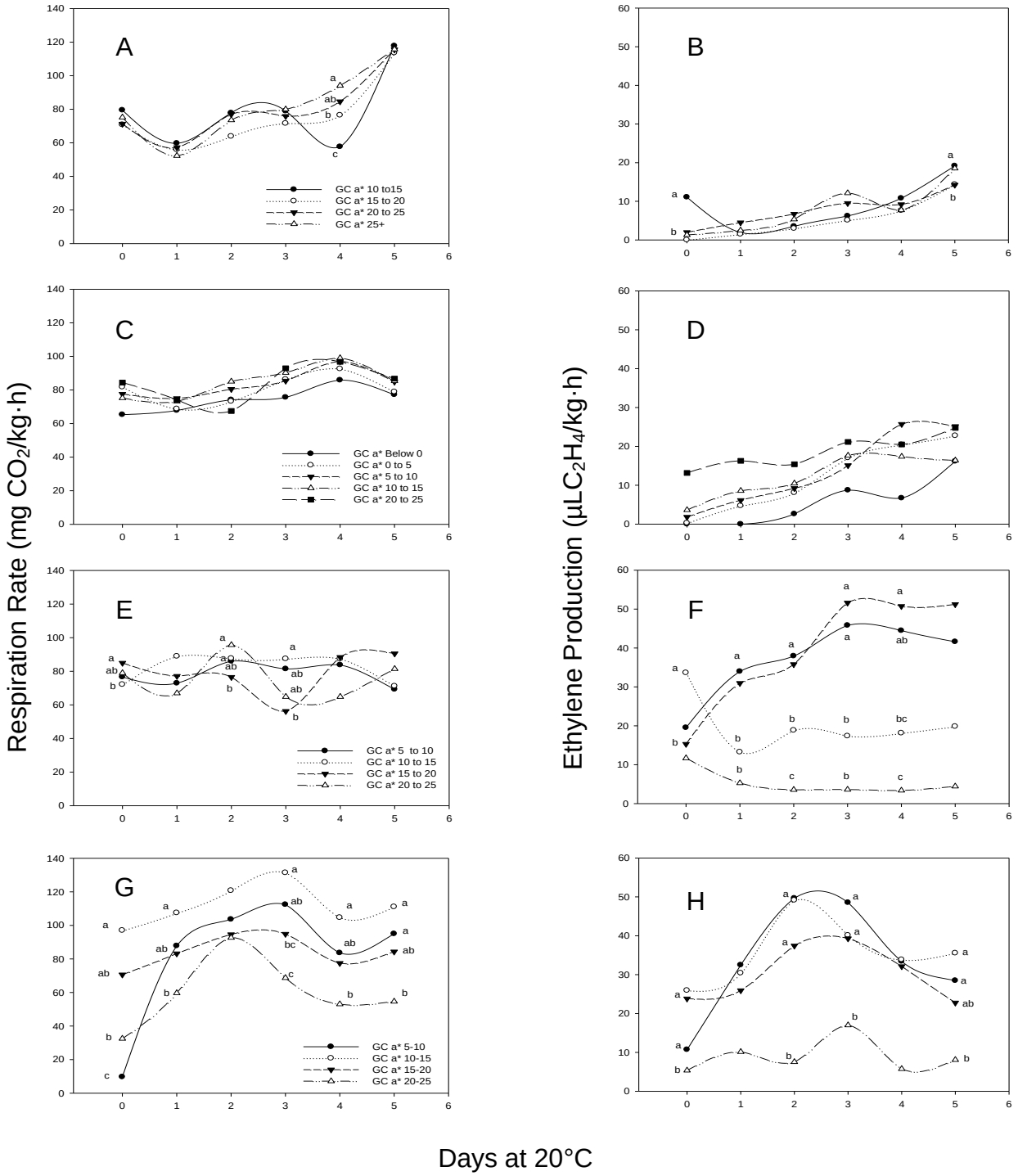

Figure 4-1.Respiration rate and ethylene production at 20°C storage of different maturity groups of MF 'Flordaprince' (A, B), MF 'TropicBeauty' (C, D), NMF 'UFSun' (E, F), and NMF 'Gulfking' (G, H) peaches in 2008 based on GCa*.

Year 2009

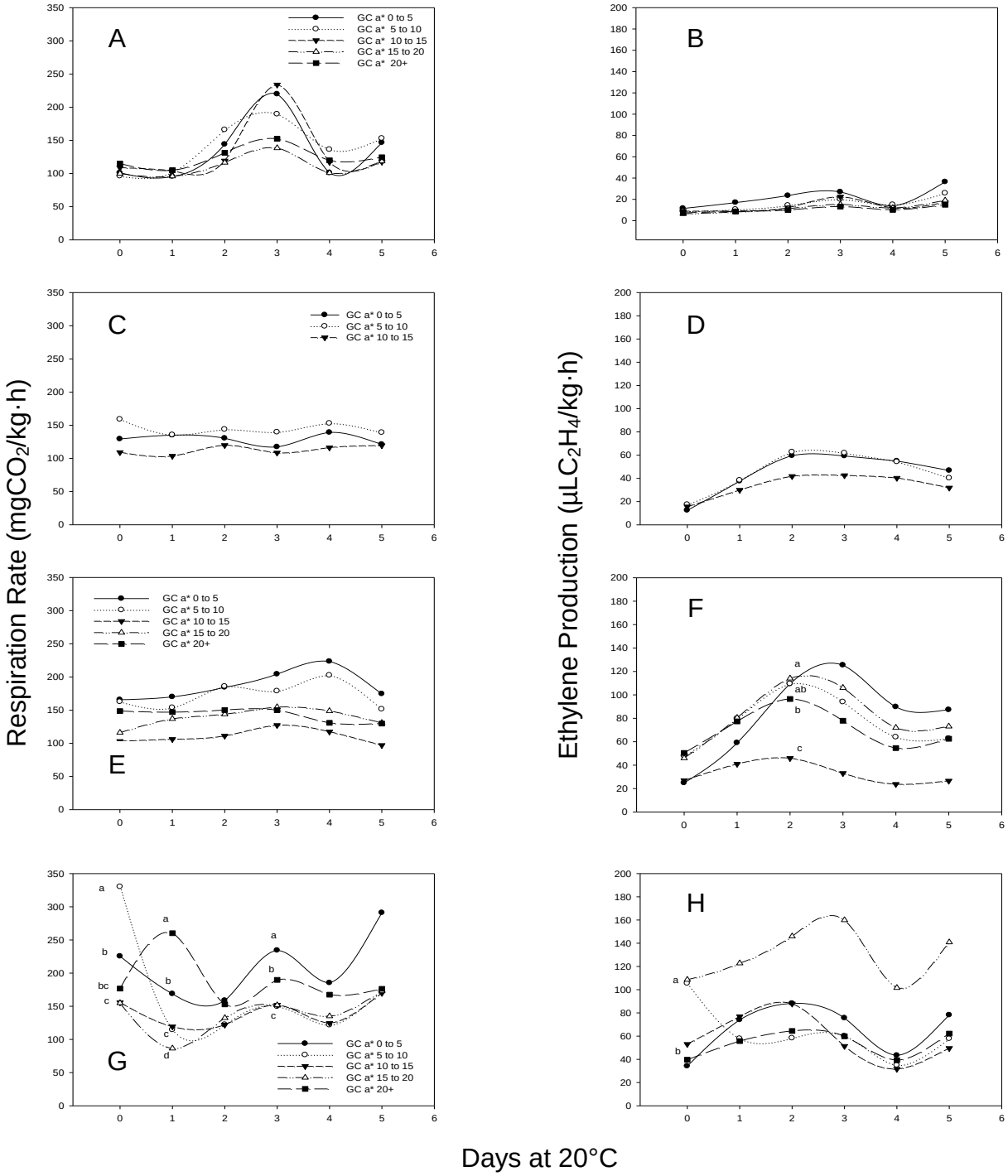

Figure 4-2. Respiration rate and ethylene production at 20°C storage of different maturity groups of MF ‘Flordaprince’ (A, B), MF ‘TropicBeauty’ (C, D), NMF ‘UFSun’ (E, F), and NMF ‘Gulfking’ (G, H) peaches in 2009 based on GCa*.

Table 4-1. Mean fruit quality of MF and NMF peaches in three seasons after 5 days of storage at 20 ºC except initial GCa*, which was determined at harvest

	Year	MF 'Flordaprince'	MF 'TropicBeauty'	NMF 'UFSun'	NMF 'Gulfking'
Initial GCa* [y]	2007	2.66ab	9.30a	-0.48b	6.65a
	2008	22.97a	8.68c	14.48b	17.49b
	2009	12.72a	6.38b	14.16a	12.72a
Final GCa* [y]	2007	17.13b	21.55a	15.54b	15.9b
	2008	28.57a	17.56c	21.35b	23.87b
	2009	24.25a	15.92b	23.27a	21.61a
FC (a* value) [y]	2007	7.32b	12.81a	12.43a	12.95a
	2008	8.62c	10.28bc	12.43b	15.92a
	2009	2.61c	9.11a	8.04ab	6.08b
Firmness (N) [y]	2007	3.78b	3.32b	17.03a	14.56a
	2008	3.37c	3.86c	12.66b	17.27a
	2009	2.41b	2.76b	13.99a	12.74a
SSC (ºBrix) [y]	2007	9.73b	11.49a	9.01bc	8.83c
	2008	10.82b	12.80a	9.99b	10.38b
	2009	11.16b	12.55a	11.08bc	10.04c
TA (%) [y]	2007	0.75a	0.83a	0.60b	0.42c
	2008	0.62a	0.64a	0.47b	0.33c
	2009	0.51b	0.70a	0.40c	0.39c
SSC/TA [y]	2007	13.04c	14.07bc	15.40b	21.54a
	2008	17.89b	20.97b	21.58b	31.55a
	2009	22.16bc	18.16c	29.86a	25.49ab
pH [y]	2007	NA	NA	NA	NA
	2008	4.23b	4.24b	4.55a	4.68a
	2009	4.11b	4.00b	4.64a	4.56a
TS (%) [y]	2007	6.97b	9.78a	9.37a	7.60b
	2008 [x]	4.42	5.59	5.06	5.18
	2009	6.70a	6.37a	5.96ab	5.29b

[x] = No significant differences of total sugar among the cultivars in 2008

[y] = Significant difference among three years at $p \leq 0.05$

Table 4-2. Flesh firmness and cell wall modification enzyme activities for MF and NMF peaches with initial GC a*-values ≥ 15 after 5 days of storage at 20 ºC in 2007

Cultivar	PME activity (units)[m]	Endo-PG activity (units)[g]	Exo-PG activity (units)[g]	Flesh firmness (N)
MF 'Flordaprince'	0.57a	0.67 bc	0.73 b	2.34 b
MF 'TropicBeauty'	0.42b	2.86 a	2.17 a	2.86 b
NMF 'UFSun'	0.11c	0.24 c	0.32 b	11.40 a
NMF 'Gulfking'	0.11c	1.17 b	0.63 b	10.77 a
Significance ($p \leq 0.05$)	*	*	*	*

[m] = 1 unit of PME activity = ΔA_{620} mg^{-1} protein min^{-1}
[g] = 1 unit of PG activity = 1 µg galacturonic acid mg^{-1} protein h^{-1}
* = Significant
NS = non-significant

Table 4-3. Flesh firmness and cell wall modification enzyme activities of MF and NMF peaches with initial GC a*-values < 15 and ≥ 15 after 5 days of storage at 20 ºC in 2010

Cultivar Initial GCa* < 15	PME activity (units)[m]	Endo-PG activity (units)[g]	Exo-PG activity (units)[g]	Flesh firmness (N)
MF 'TropicBeauty'	0.50	1.96	0.86	2.89
NMF 'UFSun'	0.43	0.98	0.74	8.62
Significance ($p \leq 0.05$)	NS	*	NS	*
Cultivar Initial GCa* ≥ 15	**PME activity (units)[m]**	**Endo-PG activity (units)[g]**	**Exo-PG activity (units)[g]**	**Flesh firmness (N)**
MF 'Flordaprince'	0.66	1.38	0.30	2.97
NMF 'Gulfking'	0.19	1.48	1.06	8.03
Significance ($p \leq 0.05$)	*	NS	*	*

[m] = 1 unit of PME activity = ΔA_{620} mg^{-1} protein min^{-1}
1 unit of PG activity = 1 µg galacturonic acid mg^{-1} protein h^{-1}
* = Significant
NS = non-significant

CHAPTER 5
EFFECT OF PRE-STORAGE HOT WATER TREATMENT ALONE OR COMBINED WITH AQUEOUS 1-METHYCLCOPROPENE ON RIPENING OF NON-MELTING FLESH PEACHES

Overview

Ripening is a part of development that transforms a low quality fruit into one with acceptable eating texture, attractive color, pleasant aroma and flavor, and various health-promoting phytochemicals. Among these quality attributes, fruit texture is pivotal in terms of its relationship to consumer preference, fruit storability, transportability, shelf-life, and pathogen resistance (Li et al., 2010). Traditional melting flesh (MF) peaches have dessert-like qualities after ripening but have to be harvested at “firm-mature” stage to avoid over-softening during distribution (Cascales et al., 2005; Williamson and Sargent, 1999). Consequently, the flavor is sacrificed because these fruit are too immature to be ripened with desirable qualities. Newer varieties of non-melting flesh (NMF) peaches, which have historically been use for canning, have become more popular fresh-market peaches due to their slower rate of softening as well as terminal firmness retention at the full ripe stage compared with the MF varieties. NMF peaches can be left on the tree longer to achieve maximum quality and still have sufficient firmness to be handled successfully during shipping and long-term storage due to this trait (Sherman et al., 1990).

Controlling the irreversible ripening process is crucial for reducing distribution loss and supplying high quality fruit to consumers. As a climacteric fruit, peach ripening is promoted by ethylene (Lelievre et al., 1998). Pre-storage hot water and 1-methylcylopropene (1-MCP) applications have been reported separately to maintain postharvest quality of MF peaches by lowering ethylene production (Kerbel et al., 1985;

Liguori et al., 2004), but no studies have been conducted with NMF varieties harvested at more advanced developmental stages than those typically utilized for MF varieties.

The term ‘heat treatment’ has been used to describe exposure to temperatures higher than 33 °C (Li and Han, 1998). Heat treatment is an attractive approach to minimize chemical usage in controlling insect infestation and prolonging the postharvest life of the fruit (Serrano et al., 2004). It extends storability and marketing of fruit by inhibiting ripening, inducing plant resistance to chilling injury (CI), and reducing external skin damage during storage (Lurie, 1998; Paull and Chen, 2000). Under certain conditions, heat-shock may be induced by heat treatment, resulting in delay of normal fruit softening, possibly due to disruption of mRNA synthesis and stability or protein synthesis and degradation of essential enzymes such as those responsible for ethylene biosynthesis (i.e., ACC oxidase and ACC synthase) and cell wall catabolism (i.e., polygalacturonase) (Lurie, 1998; Martinez and Civello, 2008). The most common application methods include hot water, hot water vapor, and hot air. Water is the preferred medium for most applications since it is more efficient than air in transferring heat (Zhou et al., 2002). Typical treatments usually consist of dipping fruit in 43–49 ºC water from several minutes to 2 hours (Fallik, 2004).

1-Methylcyclopropene (1-MCP), an ethylene action inhibitor, prevents ripening in climacteric fruits such as apple, banana, pear, plum, tomato, and avocado (Blankenship and Dole, 2003; Watkins, 2006). 1-MCP is traditionally applied in gaseous form, which requires incubating the fruit in a tightly sealed room on the order of 24 h at 20 ºC. Effective gaseous 1-MCP concentrations for delaying softening of MF peaches have been reported to range from 100ppb to 5ppm (Kluge and Jacomino 2002; Liguori et al.,

2004). Liquid formulated 1-MCP has been recently developed and appeared to be as efficient in inhibiting ripening as the gaseous method for avocado (Choi and Huber, 2008). Manganaris et al. (2007) demonstrated the beneficial effects of dipping 'Harrow Sun' plums in 1-MCP to delay ripening and control CI. They reported that plums immersed in 100 ppb of 1-MCP for 5 min had reduced respiration and ethylene production, and better firmness retention than untreated fruit, which could be related to the reduction in the activity of cell wall modifying enzymes. No information on ripening inhibition of peach fruit using liquid formulated 1-MCP can be found currently.

Endo- and exo-polygalacturonase (PG) are pectin hydrolyzing enzymes that have been shown to increase in transcription and activity at the same time when degradation of pectin molecules occurs during ripening (Downs and Brady, 1990; Lester et al., 1994). Endo-PG is regarded as the key enzyme related to the textural difference between MF and NMF varieties (Pressey and Avants, 1978). Ripened MF peaches have higher or similar levels of endo-PG activity compared to exo-PG activity, whereas ripened NMF peaches possess high exo-PG activity but very low endo-PG activity. Partial or complete deletion of gene encoding endo-PG may be responsible for the reduced or lack of endo-PG mRNA accumulation and enzyme activity in NMF peaches (Callahan et al., 2004; Lester et al., 1996).

De-methylesterification by the action of pectin methylesterase (PME) is a prelude to PG-mediated pectin disassembly since the resulting pectin after PME action contains mainly homogalacturonan, the preferred substrate for PG (Wakabayashi, 2000). For MF peaches, PME activity increases sharply at an early stage of ripening and remains constant or decreases throughout the cell wall depolymerization phase (Brummell et al.,

2004; Glover and Brady, 1995). PME activity of NMF ‘Andross’ peaches was found to follow a trend that was similar to MF ‘Caldesi 2000’ during ripening, but the magnitude of PME activity in ‘Andross’ was significantly lower than in ‘Caldesi 2000’ (Manganaris et al., 2006b).

The objective of this study was to evaluate the effectiveness of pre-storage hot water and liquid formulated 1-MCP treatment alone or combined in inhibiting ripening of NMF peaches. We hypothesized that hot water combined with 1-MCP may be more potent than either treatment alone in delaying softening by altering the activities of PME and PG, maintaining fruit qualities such as ground color (GC), flesh color (FC), soluble solids content (SSC), and titratable acidity (TA), and reducing weight loss (WL) and incidence of decay.

Materials and Methods

Preliminary Study 1- Determination of Optimum Hot Water Temperature, 1-MCP Concentration, and Exposure time

In 2008, a batch of 168 NMF ‘Last Tango’ peaches at commercial harvest maturity (peel GC changed from green to slightly yellow) was obtained from California and divided into two groups. One group was used to determine the optimum temperature and exposure time for hot water (HW) treatment and the other group was used to determine the optimum aqueous 1-MCP (AF x RD-038, AgroFresh, Inc.) concentration and exposure time. For both groups, four peaches were used to determine the initial flesh firmness. The rest were divided into four sub-groups of temperatures (25, 43, 46, and 50 ºC) for HW treatments or 1-MCP concentrations (0, 100, 500, and 1000 µg/L), which were all applied using 25 ºC water. Each sub-group was immersed for 1, 5, 10, 20, or 30 min. The treated fruit were air-dried before being placed on trays in a 20°C

storage room. The trays of fruit were inserted into 30 x 45 mil thickness unsealed plastic trash can liners to prevent moisture lost. All the fruit were ripened directly at 20°C for 3 days and flesh firmness was determined immediately after storage.

Preliminary Study 2 - Effect Of 1.5 Mg/L 1-MCP On Ripening of MF 'September Sun' Peaches Alone or Combined with HW Treatment

The firmness data indicated that the 1-MCP concentration (100 µg/L) applied to NMF 'UFSun' and NMF 'Delta' peaches during the first year of the 2-year study described below was not effective in inhibiting fruit softening; therefore, a higher 1-MCP concentration (1.5 mg/L) was tested before the start of the second Florida harvest season. Since there were no NMF peaches available at the time, MF 'September Sun' peaches from Chile were used in this study. A batch of 720 fruit at minimum maturity was divided into 4 treatment groups before storage at 20 ºC. The fruit were immersed for 30 min in water at 25°C (Control) or 46 ºC (HW), or immersed in 25 ºC water containing 1.5 mg/L aqueous 1-MCP, or 46 ºC water containing 1.5 mg/L aqueous 1-MCP (HW x 1-MCP). The fruit GC, FC, flesh firmness, WL ethylene production and respiration rate were measured on 3 replications of 10 fruit during 5 days of storage.

Two Year Study on Ripening of NMF Peaches Pre-conditioned with HW, 1-MCP, or HW x 1-MCP at 20 ºC

Plant material

In May of 2009 and 2010, NMF 'UFSun' peaches were commercially harvested from Punta Gorda, FL when fruit were considered as 'tree-ripe' (peel GC was yellow; diameter ≥ 57mm). In June of 2009 and 2010, NMF 'Delta' peaches were commercially harvested from Mershon, GA when the peel GC started to change from green to yellow and the diameter was ≥ 64mm. Peaches of both cultivars were transported to the University of Florida in Gainesville, FL by air-conditioned vehicle. In 2009, fruit were

immersed for 30 min in water at 25°C (Control) or 46 ºC (HW), or immersed in 25 ºC water containing 100 µg/L aqueous 1-MCP, or 46 ºC water containing 100 µg/L aqueous 1-MCP (HW x 1-MCP). Zipper-lock bags were used in all treatments to trap off-gas released by 1-MCP when it was introduced to water. Fruit were stored at 20 ºC for 5 days after the treatment.

In 2010, the 1-MCP concentration was increased to 1.5 mg/L or 1.5 ppm for 'UFSun' because the 1-MCP concentration used in the previous year (100 µg/L) did not inhibit fruit softening. All the fruit were stored at 20°C for 7 days. Respiration rate, ethylene production, and physical characteristics (GC, FC, flesh firmness, WL) were measured on all of the fruit in both years. Due to the failure of 1-MCP to produce softening inhibition during the first year of study, chemical characteristics (SSC, TA, pH) and PME and PG activities were measured only on the control and HW fruit in 2009 but on all the fruit in 2010. Incidence of decay was determined at the end of the ripening period for all the fruit in both years.

In a separate experiment during the second year of study, ethylene production and respiration rates of 'UFSun' peaches were measured as the fruit progressed from the preclimacteric to climacteric stages in order to better understand the ripeness stage of the fruit from the main experiment when 1-MCP was applied. The samples were obtained from the UF Plant Science Research and Education Unit at Citra, FL. A batch of 15 fruit was harvested with green GC and an average size of 82 g. The fruit were divided into 3 replications and stored at 20°C until Day 8. Ethylene production and respiration rate were measured every other day in order to construct curves for the

respiratory and ethylene climacterics to use for comparison with the ethylene productions and respiration rates of the fruit from the main experiment.

Ethylene production and respiration rate determination

In 2009, a gas analyzer ETH-1010 (Fluid Analytics, Inc., West Linn, Oregon) with an infrared detector for CO_2 measurement and a gold catalyst detector for ethylene measurement was used to measure respiration rate and ethylene production. 10 Fruit were equally divided into five replications and fruit of each replication were sealed in a 2.735L container that was connected to the device. In 2010, ethylene production and respiration rate for each treatment were monitored using a static system consisting of three, 18.9-L glass jars each containing 10 fruit. The jars were sealed for 15 min before 5 mL headspace gas samples were withdrawn. The concentrations of gases were determined using a Varian gas chromatograph (GC) (CP-3800, Middelburg, The Netherlands) equipped with a Valco valve system (Houston, Texas, USA). Ethylene was separated on a molecular sieve column (Ultimetal, 1.5 m*1/8”; 13x80-100 mesh) and a Pulse Discharge Helium Ionization Detector (PDHID) was used for detection. Measured concentrations of ethylene were converted into rates of production based on the mass of fruit in a jar, the void volume, and the duration of sealing.

Physical characteristics

Ground and flesh color determination

GC and FC were determined using a reflectance colorimeter that measured in C.I.E. L*, a*, b* values (Minolta CR-400, Konica Minolta, Japan). The shade of color, which is best described by hue angle (h°; arctangent of b*/a*), can often change after postharvest treatment (McGuire, 1992). Therefore, GCh°; and FCh°; were presented in

this study. GC was measured on the greenest portion of the peel. FC was measured after removing a small (circa 2 cm diameter) patch of peel.

Flesh firmness determination

Flesh firmness was measured with an Instron (Model 1132) that applied a compressive force from a 50 kg load cell. A convex tip probe (Magness-Taylor type), 7.9 mm in diameter, was attached to the load cell and the force applied with the probe moving at a speed of 12 cm/min. Flesh firmness was measured on the cheeks of the fruit at the fruit equator on both sides with peel removed and was expressed as the bioyield force (N). Following color and firmness measurements, fruit samples were placed in quart size (17.7 cm x 20.3 cm) zipper locking, plastic freezer bags and stored at -30 ºC for later compositional analyses.

Weight loss determination

Weight loss (WL) was calculated by subtracting the final (after storage) fresh weight of the fruit from the initial fresh weight and dividing the difference by the initial fresh weight. The resulting values were converted to percentage by multiplying by 100.

Chemical characteristics

Soluble solids content, titratable acidity, and pH determination

Frozen fruit tissues were pureed in a Waring blender for 1 min. The resulting slurry was centrifuged (20 min; 15,000 × g_n; 4°C) and the clear supernatant was used to determine SSC and TA. The SSC was measured with a temperature compensated digital refractometer (model ABBE Mark II, Cambridge Instruments Inc, U.S.A) and expressed as per cent FW. TA was determined by titration (model 719 S. Titrino, Metrohm, Switzerland) of 6.0 g of juice plus 50 mL of water with 0.1N sodium hydroxide solution until pH 8.2 was reached and the TA was expressed as percent malic acid. The

pH of the diluted juice was determined automatically by the Titrino equipped with a pH electrode.

Enzyme assays

Preparation of cell-free protein extract

Enzyme extracts were prepared similarly to the method of Jeong et al. (2002). Partially thawed mesocarp tissue (15 g) was homogenized with 25 mL of ice-cold 95% ethanol for 1 min in an Omnimixer (Model GLH-01, New-town, CT, USA) and centrifuged at 15,000 × g_n for 10 min at 4°C. The supernatant was discarded and the pellets were resuspended in 25 mL of ice-cold 80% ethanol for 1 min and centrifuged again at 15, 000 × g_n for 10 min at 4°C. The pellets were transferred to 10 mL of 50 mM Na-acetate buffer, pH 5, containing 0.5 M NaCl, for 30 min in an ice-cold water bath followed by centrifugation 15,000 × g_n for 10 min at 4°C. The resulting supernatant was analyzed for enzyme activities. Total soluble protein in the supernatant was measured using the bicinchoninic acid method with bovine serum albumin as the standard (Smith et al., 1985).

Pectinmethylesterase activity determination

Pectinmethylesterase (PME, E.C. 3.1.1.11) was measured using modifications of the method of (Jeong et al., 2002). A 1% (w/v) solution of 93% esterified citrus pectin (Sigma Chemical Co., St. Louis, MO, USA) was prepared in 0.1M NaCl and adjusted to pH 7.5 with dilute NaOH. A 0.01% solution of bromothymol blue was prepared in 0.003M potassium phosphate buffer, pH 7.5. A 166 µL volume of 1% citrus pectin was mixed with 12 µL of 0.01% bromothymol blue and 70 µL of water on a microplate, and the pH adjusted to 7.5 with dilute NaOH. The reaction was initiated by adding 2 µL of the cell-free protein extract adjusted to pH 7.5 with dilute NaOH. The decrease in A_{620}

over a 10 min reaction time was recorded and PME activity was expressed as Δ A_{620} mg^{-1} protein min^{-1}.

Polygalacturonase activity determination

In 2009, endo-PG (E.C. 3.2.1.15) was assayed by mixing 250 µL of enzyme extract with 250 µL of 0.5% polygalacturonic acid (from orange peel, Sigma Chemical Co., St. Louis, MO, USA) in 50 mM Na-acetate buffer (pH 4.4) and incubated at 30°C for 16 h (Pressey and Avants, 1973). Exo-PG (E.C. 3.2.1.67) activity was assayed by adding 250 µL of enzyme extract to 250 µL of 0.5% polygalacturonic acid in 50 mM Na acetate buffer (pH 5.5) containing 2 mM $CaCl_2$, and incubating at 30°C for 16 h. In 2010, the endo-PG assay was amended by using 100 µL of enzyme extract with 400 µL of 0.5% polygalacturonic acid. The purpose was to reduce concentration of NaCl to approximately 0.15M to maximize PG activity *in vitro* (Huber and Lee1989). Uronic acid (UA) reducing groups released were measured using the method of Milner and Avigad (1967) with mono-D-galacturonic acid as the standard. One unit of activity was defined as 1 µg galacturonic acid produced mg^{-1} protein h^{-1}.

Statistical Analysis

Randomized complete design was utilized in Preliminary Study 1 and one way – ANOVA was used to detect significant differences among the treatments. Preliminary Study 2 and studies associated with ripening of NMF peaches were conducted using a randomized complete design with a factorial arrangement of treatments. When a significant difference was detected in ethylene production, respiration rate, and flesh firmness, the data were analyzed by two-way ANOVA to examine the influence of HW (factor A), 1-MCP (factor B), and A x B interaction at both $p \leq 0.01$ and $p \leq 0.05$. Differences among the treatments were analyzed by LSD at $p \leq 0.05$..

Results and Discussion

Preliminary Study 1 - Determination of Optimum Hot Water Temperature, 1-MCP Concentration, and Exposure time for Pre-storage Conditioning Treatments

Flesh firmness of 'Last Tango' peaches after 3 days of storage at 20 ºC was significantly different among various temperatures only for fruit immersed in water for 5 min and 30 min (Table 5-1). However, the control fruit immersed for 5 min were firmer than the other treated fruit; therefore, 5 min was not considered to be an effective HW exposure time. HW, 1-MCP, and HW x 1-MCP fruit had better firmness retention than the control fruit when the exposure time was increased to 30 min. Abnormal texture was found in fruit immersed in 50 ºC water for 30 min because flesh firmness after storage (53.39 N) was actually greater than the initial value measured before the treatment (46.89 ± 4.13 N). Internal damage might have occurred for fruit treated with this combination of temperature and exposure time (Paull and Chen, 2000). Fruit immersed in 43 ºC or 46 ºC water for 30 min were firmer than those immersed at other temperatures without showing any external injuries. Therefore, the 46 ºC for 30 min treatment was selected as the maximum temp x time combination tolerated and was subsequently used in later experiments to delay softening of NMF peaches.

'Last Tango' fruit immersed in 1-MCP solutions of 100 to 1000 µg/L for 5 min and 30 min retained greater flesh firmness than the control fruit (Table 5-2). Fruit treated with different 1-MCP concentrations showed similar flesh firmness after storage indicating that the lowest 1-MCP concentration applied (100 µg/L) was sufficient to inhibit ripening. The 100 µg/L of 1-MCP for 30 min treatment was chosen to be the optimum combination in this study because the exposure time could be kept constant for further investigations when HW was combined with the 1-MCP treatment.

Preliminary Study 2 - Effect of 1.5 Mg/L 1-MCP on Ripening of MF 'September Sun' Peaches alone Or combined with HW Treatment

Ethylene was significantly reduced by both the HW and 1-MCP treatments and the combined HW x 1-MCP treatment fruit had the lowest ethylene level (Fig. 5-1A). The ethylene production at the climacteric peak (Day 3) was reduced by 20%, 60%, and 80% for 1-MCP, HW, and HW x 1-MCP fruit respectively. The average initial ethylene production (before treatment) for MF 'September Sun' was around 7 μL/kg•h, approximately 17% of the peak value. Therefore, 1.5 mg/L of 1-MCP was enough to saturate the ethylene receptors when the fruit were at the beginning of the climacteric. Respiration rates were generally similar among the treatments and appeared to be post-climacteric after Day 3 (Fig. 5-1B), indicating that the treated fruit were functioning normally, like the control fruit, in terms of the climacteric. HW and 1-MCP significantly reduced ethylene production throughout the ripening period, but did not affect respiration rate (Table 5-3).

Flesh firmness was significantly maintained at higher levels than the control by the HW and 1-MCP treatments (Table 5-4). HW and HW x 1-MCP fruit had greater flesh firmness than the control fruit on Day 0. The effect of 1-MCP application on softening inhibition was observed on Day 1 when the control fruit started the 'melting' process and this effect of 1-MCP lasted throughout the ripening period. The melting phase for the HW, 1-MCP, and HW x 1-MCP fruit was delayed until Day 3. Interestingly, the ANOVA indicated that HW was the dominant factor regulating flesh firmness in the beginning of the ripening period whereas 1-MCP became dominant during the melting phase. An interaction effect of HW × 1-MCP was only observed on last day of storage.

The HW, 1-MCP, and HW x 1-MCP treatments did not inhibit peel color development (GCh°) even though the fruit ethylene production was significantly suppressed (Table 5-5, Fig. 5-1A). This suggests that peel color development may be induced by very low levels of ethylene and that the process may be irreversible once triggered. All of the pre-conditioned fruit had significantly lower FCh° than the non-treated fruit on Day 3 and Day 5, indicating that both HW and 1-MCP may have favored either loss of chlorophyll or synthesis of carotenoids, or both, in the flesh of MF 'September Sun' peaches. The per cent WL increased throughout storage and the values were similar among the treated and non-treated fruit (Table 5-5). Therefore, WL probably had little effect on fruit firmness.

Two Year Study on Ripening of NMF 'UFSun' and 'Delta' Peaches Pre-conditioned with HW, 1-MCP, or HW x 1-MCP at 20 ºC

Ethylene production and respiration rate

Ethylene synthesis is reversibly inhibited in fruit exposed to high temperatures (Paul and Chen, 2000). This was evident in this study for both HW treated NMF cultivars. Ethylene production was first suppressed by HW then recovered to similar (Fig. 5-2A, 5-3A) or higher (Fig. 5-2B, Day 5) levels than the control for 'UFSun' fruit. The reversible nature of the ethylene biosynthesis was probably related to the restoration of enzymes involved in the ethylene biosynthesis pathway such as ACC oxidase (ACO) and S-adenosylmethionine synthetase-2 (SAMS) (Lara et al., 2009). Ethylene production by HW-treated 'Delta' peaches was suppressed throughout the entire ripening period (Fig. 5-3B).

The respiration rate of HW-treated 'UFSun' fruit was higher than that of the control fruit on Day 1, dropped to a lower level on Day 3, and recovered to a similar level on

Day 5 in 2009 (Fig. 5-2C). In 2010, the respiration rates of HW-treated and control ‘UFSun’ fruit were similar until Day 5 (Fig. 5-2D). The higher respiration rate of ‘UFSun’ peaches immediately following HW treatment could be attributed to the cyanide insensitive pathway (Inaba and Chachin, 1989; Kruse et al., 2011). The respiration rate of HW-treated ‘Delta’ fruit was reduced throughout the ripening period in both years (Fig. 5-3C and 5-3D).

In 2009, 1-MCP treatment (100 µg/L) significantly reduced ethylene production and respiration rate of ‘UFSun’ fruit until Day 5, but the same effect was not observed on ‘Delta’ peaches although the latter cultivar was still on the climacteric rise (Fig. 5-2A, 5-3A). Thus, ‘UFSun’ peaches at “commercial ripeness” were more sensitive to 1-MCP treatment than ‘Delta’ peaches at “commercial ripeness”.

In 2010, the 1-MCP concentration was increased to 1.5 mg/L or 1.5 ppm for ‘UFSun’ because the 100 µg/L 1-MCP concentration did not inhibit fruit softening and 1.0 ppm (the current registered concentration for apples in U.S. it was reported to have only a transient effect on peaches (Dal Cin et al., 2006). The initial ethylene production (before HW or 1-MCP treatment) for ‘UFSun’ peaches was around 35 µL/kg•h, approximately 17% of the peak value determined in 2009. The initial ethylene production of ‘Delta’ peaches was about 42 µL/kg•h, which was similar to the maximum level detected in 2009. Therefore, the 1-MCP concentration was increased to 5.0 mg/L or 5.0 ppm for ‘Delta’ peaches, a concentration that has been identified as optimal for extending shelf life of early season peaches and nectarines (Liguori et al., 2004). 1.5 mg/L 1-MCP significantly lowered the climacteric peaks of both ethylene production and respiration rate for ‘UFSun’ peaches (Fig. 5-2B, 5-2D) instead of delaying the peak as

observed for 1-MCP treated MF ‘Elberta’ peaches (Fan et al., 2002). 5 mg/L 1-MCP was able to reduce ethylene production of ‘Delta’ peaches but not respiration rate during ripening (Fig. 5-3C, 5-3D). The response of peach respiration rate to 1-MCP has been previously reported to be cultivar dependent (Liguori et al., 2004).

It has been shown that the transcription of ethylene receptor transcripts in peaches and nectarines resumed within a short period of time after a single 1-MCP application (Mathooko et al., 2001; Mathooko et al., 2004; Ziliotto et al., 2008). ‘Delta’ peaches may be able to regenerate ethylene receptors more rapidly than ‘UFSun’ peaches, possibly resulting in restoration of the ability of the fruit to respond to ethylene when the same concentration of 1-MCP was applied. Another possibility is that ‘Delta’ fruit may have more ethylene receptor sites than ‘UFSun’ naturally. Since the receptors are functionally redundant (Klee, 2002), normal ripening can still occur if the concentration of 1-MCP applied is too low to saturate all the receptor sites. Greater production of ethylene during recovery from 1-MCP treatment was not observed in this study in contrast to a previous report for peach (Rasori et al., 2002).

HW x 1-MCP treatment was effective in suppressing ethylene production by ‘UFSun’ and ‘Delta’ peaches in both years and the levels in this were generally the lowest (Fig. 5-2A, 5-2B, 5-3A, 5-3B). This was possibly due to interactions between HW and 1-MCP (Table 5-6, 5-7) such that ethylene biosynthesis was suppressed by HW (Lurie, 1998; Lurie and Crisosto, 2005) and ethylene action was blocked by 1-MCP. The respiration rate of HW x 1-MCP-treated fruit for both NMF cultivars was affected more by 1-MCP treatment because the respiratory patterns of the fruit from the 1-MCP and

HW x 1-MCP treatments closely resembled each other during storage regardless of the concentrations applied.

Flesh firmness

HW treatment was more effective than 1-MCP treatment in promoting firmness retention for both NMF cultivars (Table 5-8, 5-10). ‘UFSun’ peaches were more sensitive to HW treatment because softening was delayed throughout the ripening period whereas the effect only lasted until Day 3 for ‘Delta’ peaches. HW treatment was effective in delaying softening of peaches that were already producing autocatalytic ethylene, contrary to the results reported by Budde et al. (2006) who found that heat treatments have no influence on fruit firmness when ethylene production is already triggered. . The temporarily suppression of ethylene production in ‘UFSun’ peaches along with prolonged softening inhibition (Fig. 5-2A, 5-2B and Table 5-8) suggests that HW stress may inhibit cell wall catabolism by regulating both ethylene-dependent (i.e., 1-MCP-responsive) and ethylene-independent pathways (Hayama et al., 2006b).

The low concentration of 1-MCP (100 µg/L) applied in 2009 did not inhibit softening of ‘UFSun’ peaches although the rate of ethylene production was reduced throughout the ripening period (Table 5-8, Fig. 5-2A). However, ‘UFSun’ fruit treated with 1.5 mg/L 1-MCP solution retained their flesh firmness for only 1 day after the treatment, suggesting that a higher concentration or repeated application of 1-MCP may need to be used to prolong the effect (Liu et al., 2005) (Table 5-8).

The transitory firmness retention in 1-MCP treated peach fruit could be attributed to the level of internal ethylene before 1-MCP treatment. For example, 1.5 mg/L 1-MCP was very effective in delaying the melting phase of MF ‘September Sun’ peaches that were at the beginning of the climacteric rise. ‘UFSun’ peaches at commercial ripeness

were further along on the climacteric rise when the same concentration of 1-MCP was applied in 2010 (Fig. 5-4) and the fruit firmness was maintained for only 1 day (Table 5-8). On the contrary, Fan et al. (2002) reported that early harvested MF fruit showed little response to 1-MCP treatment compared to late harvested fruit although the latter had higher ethylene production than the former. 'Delta' fruit treated with 100 µg/L 1-MCP did not maintain initial flesh firmness, which could be attributed to the non-suppressed ethylene production (Table 5-10, Fig. 5-3A). Treatment with 5 mg/L 1-MCP significantly suppressed the rate of ethylene production, but the treated 'Delta' fruit softened more rapidly than the control fruit by Day 3 (Table 5-11), demonstrating that the effect of cultivar was greater than that of 1-MCP.

Softening inhibition in HW x 1-MCP-treated fruit was mainly attributed to the influence of HW treatment since similar softening patterns were observed in the fruit from the HW and HW x 1-MCP treatments (Table 5-8, 5-10). This effect did not last as long as anticipated. As soon as the effect of 1-MCP appeared, fruit quickly softened to the same degree as the control fruit. 1-MCP treatment seemed to counteract the effect of HW treatment without increased ethylene production.

Enzyme assays

Cell wall degradation during fruit ripening is intricately coordinated by different enzyme activities. PME and PG activity are crucial to modification of pectin molecules during peach fruit softening (Muramatsu et al., 2004). It has been shown that the maximum PG activity of HW-treated Mei fruit (*Prunus mume* cv. 'Daqinghe'), commonly known as Japanese apricot, was delayed instead of being repressed when softening was inhibited (Luo, 2006). The peak PME activity of HW-treated Mei fruit was also delayed and with a reduced magnitude than in the control fruit. In this study, delayed

peak endo-PG activity was only observed in HW-treated ‘UFSun’ peaches on Day 1 in 2009 (Table 5-9) and HW-treated ‘Delta’ peaches on Day7 in 2010 (Table 5-11) and the PG activity of both was higher than that of the control fruit. Interestingly, significantly higher PME activity was detected in HW-treated ‘UFSun’ fruit on Day 0 during 2009 and HW-treated ‘Delta’ peaches on Day 3 in 2010. HW-treated ‘UFSun’ fruit in 2010 (Day 1) and HW-treated ‘Delta’ fruit in 2009 (Day 0) had maximum endo-PG activity that occurred on the same day as the control fruit and with similar magnitudes. Maximum PME activity of HW-treated ‘UFSun’ fruit in 2010 (Day 1) and HW-treated ‘Delta’ fruit in 2009 (Day 3) occurred in advance of the control fruit maximum PME and the levels were slightly reduced.

Higher PME activity has been reported in fresh-cut peaches treated with HW a few hours before processing (Koukounaras et al., 2008; Steiner et al., 2006). It is believed that the excess carboxyl groups of heat-treated fruit generated by the higher PME activity bond with endogenous calcium to form Ca-pectates This hypothesis was supported by the lower decline in pectin (% of Ca-pectate) found in ripe, heat-treated fruit (Bakshi and Masoodi, 2010). The loss of neutral sugar side chains during the heat treatment may also lead to closer packing of the pectin strands (Ben-Shalom et al., 1993). Consequently, the rigidity of the cell wall and middle lamella would be increased and in turn restrict access by cell wall degradation enzymes. Therefore, the peak endo-PG activity would be delayed and the endo-PG activity would be higher, just as observed in HW-treated ‘UFSun’ peaches in 2009 and HW-treated ‘Delta’ peaches in 2010, possibly due to accumulation of enzyme that could only gain access to substrate when the fruit recovered from the HW treatment. Alternatively, the rigidity of the cell wall

and middle lamella can be built up more rapidly if the maximum PME activity is shifted to earlier stages of ripening by HW treatment. This could explain the firmness retention observed for HW-treated ‘UFSun’ fruit in 2010 and HW-treated ‘Delta’ fruit in 2009. In addition, there were no significant differences in exo-PG activity between the control and HW-treated fruit of both NMF cultivars in 2009 and the exo-PG levels were constant throughout ripening. Therefore, exo-PG was probably not directly related to softening of NMF peaches (Table 5-9, 5-11).

1-MCP-treated ‘UFSun’ had its highest PME activity immediately after the 1-MCP treatment in 2010 (Day 0), which was 3 days in advance of that of the control fruit. The peak endo-PG activity occurred on the same day as the control fruit (Day 1) (Table 5-9). 1-MCP-treated ‘Delta’ fruit had its highest PME activity (Day 3) and endo-PG activity (Day 5) on the same day as the control fruit (Table 6b). The endo-PG activity was similar, but PME activity was significantly higher. The higher PME activity induced by 1-MCP was associated with greater softening observed on Day 3 (Table 5-10). High 1-MCP concentration (5 mg/L) completely suppressed endo-PG activity in ‘Delta’ peaches until Day 3 (Fig. 5-3B, Table 5-11), indicating a positive relationship between ethylene production and endo-PG activity as reported by Hayama et al. (2006a). However, the absence of endo-PG activity did not correspond to inhibition of fruit softening. Morgutti et al. (2006) reported the NMF phenotype does not seem to be caused by a large deletion of the endo-PG gene. Furthermore, analysis of cell wall polysaccharides showed that pectins might be solubilized during ripening of NMF peaches without substantial de-polymerization (Yoshioka et al., 2011). These results suggest that NMF cultivars do not depend on endo-PG activity for cell wall degradation.

Statistical analysis indicated that interaction of HW and 1-MCP effects had a major influence on PME and PG activities for HW x 1-MCP-treated fruit of both cultivars. Therefore, no consistent trends could be delineated when HW x 1-MCP-treated fruit were compared to the control fruit. The opposite effect of HW and 1-MCP on endo-PG activity was observed on Day 1 in 2010 (Table 5-11). The endo-PG activity of HW x 1-MCP-treated 'Delta' fruit was in between that of HW and 1-MCP fruit.

Ground color and flesh color

Compared with the control fruit, 'UFSun' peaches treated with HW, 1-MCP, or HW x 1-MCP had significantly higher or similar GCh° by the end of the ripening period in 2009 and 2010, respectively (Table 5-12). The higher GCh° indicated that the peels of the pre-conditioned fruit were less yellow or greener than the control fruit. Thus, the treatments were either effective in delaying the breakdown of chlorophyll or delayed the synthesis of carotenoids, or both. The FCh° of 'UFSun' peaches was generally similar among the treatments (Table 5-12).

HW and HW x 1-MCP had similar effects on GCh° and FCh° changes during ripening of 'Delta' peaches in 2010 (Table 5-13). Fruit from both treatments had significantly lower GCh° by Day 3 and lower FChº by the end of the ripening period compared with the control fruit. Therefore, HW treatment was able to accelerate either the breakdown of chlorophyll or the development of carotenoids in both the peel and the flesh of 'Delta' peaches. 1-MCP-treated 'Delta' fruit had similar GCh° as the control when a low 1-MCP concentration was applied, but had lower GCh° by the end of the ripening period when a high 1-MCP concentration was applied.

Soluble solids content, titratable acidity, and pH

Fruit SSC was generally not affected by HW treatment for both NMF cultivars (Table 5-14 and Table 5-15), which is in agreement with the reported results (Budde et al., 2006; Malakou and Nanos, 2005). It was suspected that the higher respiration rate immediately following the HW treatment as observed in HW-treated 'UFSun' fruit in 2009 (Fig. 5-2C) could have resulted in breakdown of organic acids, which would lead to lower levels of malate and citrate in the fruit tissue (Lara et al. 2009). Since there were no significant differences in TA between HW and control fruit in 2009 (Table 5-14), the higher respiration of HW 'UFSun' fruit was apparently either not due to increased organic acid metabolism or the amount of acids consumed was insignificant compared to the losses during storage. HW-treated and HW x 1-MCP-treated 'UFSun' fruit maintained higher TA and lower pH than control fruit by Day 5 in 2010 (Table 5-14) HW-treated 'Delta' peaches had significantly lower TA than the control fruit on both Day 0 and Day 5 in 2010 (Table 5-15), indicating that regeneration of organic acids was inhibited or slowed. The reduction in TA caused by HW stress may be a favorable effect since consumer acceptance is reportedly always greater for low-acid than for high-acid peach cultivars regardless of fruit maturity (Iglesias and Echeverria, 2009).

1-MCP treatment had no effect on the SSC of both NMF cultivars (Table 5-14, 5-15). 1-MCP was most effective in delaying the breakdown of organic acids as indicated by the highest level of TA on Day 5 found in both NMF cultivars after 1-MCP treatment in 2010 (Table 5-14). Similar results were observed for MF peaches treated with gaseous 1-MCP (Liguori et al., 2004) and for plums treated with aqueous 1-MCP (Liguori et al., 2004; Manganaris et al., 2007). Since 'UFSun' fruit were more responsive to 1-MCP, the TA of HW x 1-MCP-treated 'UFSun' fruit resembled that of 1-MCP fruit

(Table 5-14). ‘Delta’ peaches were less sensitive to 1-MCP. Thus, HW x 1-MCP ‘Delta’ fruit had a similar level of TA as the HW-treated fruit (Table 5-15).

Weight loss and decay

The WL of control and HW-treated ‘UFSun’ peaches was generally similar (Table 5-12), confirming the results reported by Obenland and Carroll (2000). Moreover, HW-treated fruit of both NMF cultivars in this study did not have greater WL than the control fruit, which does not support the assertion that HW treatment increases WL in peaches due to removal of trichomes at higher temperature (Bakshi et al., 2006; Phillips and Austin, 1982). In fact, 1-MCP-treated ‘Delta’ fruit (immersed in 25 ºC water) had more WL by the end of the storage period in 2009 than the HW-treated ‘Delta’ fruit (Table 5-13).

NMF ‘Delta’ peaches pre-conditioned with HW or HW x 1-MCP had less incidence of decay than the control fruit in both years (Table 5-16), but ‘UFSun’ fruit with the same treatments had higher incidence of decay in some cases. It is possible that the natural openings and barely-visible cracks in the epidermis of HW ‘Delta’ peaches were partially or entirely sealed with rearranged natural wax components present on the cuticle, thus limiting sites of fungal penetration into the fruit. The sealing of cracks or natural openings by HW treatment has been shown to lead to significantly reduced WL (Fallik et al., 1999; Fallik et al., 2000) and may also reduce entry routes for pathogenic microorganisms (Fallik, 2004). Similar results have been reported on HW-treated citrus, sweet peppers, Galia melons, tomatoes, and cactus pears (Fallik, 2004; Schirra et al., 1999). HW treatment can also act directly on the pathogen (cell damage) and indirectly on the fruit host (induction of resistance mechanisms) to suppress incidence of decay (Casals et al., 2010b).

The control and 1-MCP-treated fruit of both NMF cultivars had similar WL, usually by the end of the ripening period (Table 5-12, 5-13). 1-MCP-treated fruit of both NMF cultivars had lower incidence of decay in 2009 and similar incidence as the control fruit in 2010 (Table 5-16). The high concentration of 1-MCP applied in 2010 did not increase the severity of fruit decay as seen in strawberries (Ku et al., 1999) and avocados (Adkins et al., 2005). It has been reported that brown rot incidence, sporulating area, and production of conidia per fruit are higher on commercially mature peach fruit as compared with immature fruit (Holb and Schnabel, 2008). Maturity differences could not explain the lower decay that occurred in 2009 because the fruit were riper at the beginning of the storage period in 2009 than in 2010 based on the timing of the ethylene climacteric rise immediately after the treatment (Fig. 5-2A, 5-3A).

Chapter Conclusion

HW treatment alone is a more effective pre-storage conditioning method than 1-MCP or HW x 1-MCP in terms of delaying normal fruit softening of NMF peaches. Aqueous 1-MCP concentration greater than 5 mg/L may be required to prolong firmness retention in NMF peaches. The cultivar effect greatly dominated the response of the peaches used in this study towards HW and 1-MCP treatment. PME activity may have a more dominant role than PG in cell wall modification during ripening of NMF fruit.

Preliminary Study 1 - Determining Optimum HW Temperature, 1-MCP, and Exposure time for Pre-storage Conditioning Treatments

Table 5-1. Effect of temperature x exposure time on flesh firmness (N) of 'Last Tango' peaches after 3 days at 20 ºC.

Temp/Time	1 min	5 min	10 min	20 min	30 min
25°C	34.66	39.03a	44.60	36.88	32.81c
43°C	36.02	26.42b	34.40	44.90	42.88b
46°C	32.54	27.05b	36.46	44.54	43.27b
50°C	26.87	32.70ab	37.77	42.13	53.39a

Temp = temperature

Table 5-2. Effect of 1-MCP concentration x exposure time on flesh firmness (N) of 'LastTango' peaches after 3 days at 20 ºC

Conc/Time	1 min	5 min	10 min	20 min	30 min
0 µg/L	31.01	21.79b	34.31	34.73	21.05c
100 µg/L	42.59	41.82a	39.89	38.43	43.09ab
500 µg/L	42.81	42.47a	44.77	43.15	46.49a
1000 µg/L	39.73	39.10a	41.59	41.89	37.47b

Conc = concentration

Preliminary Study 2 - Effect of 1.5mg/L 1-MCP alone or combined with HW treatment on ripening of MF 'September Sun' peaches

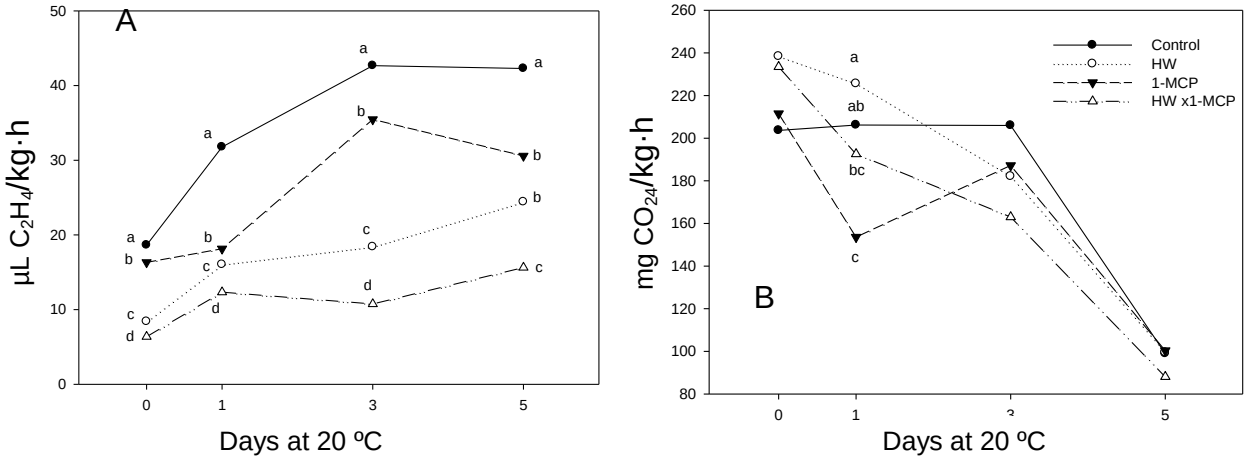

Figure 5-1. Effect of pre-storage conditioning treatments on ethylene production (A) and respiration rate (B) of 'September Sun' peaches.

Table 5-3. Effect of pre-storage HW and 1-MCP treatments on ethylene production and respiration rate of 'September Sun' peaches during ripening at 20 ºC

	Ripening period (d)			
	0	1	3	5
Ethylene production ($\mu L\ C_2H_4/kg \cdot h$)				
Factor A (HW)	**	**	**	**
Factor B (1.5 mg/L 1-MCP)	**	**	**	**
A x B	NS	**	NS	NS
Respiration rate ($mg\ CO_2/kg \cdot h$)				
Factor A (HW)	NS	NS	NS	NS
Factor B (1.5mg/L 1-MCP)	NS	**	NS	NS
A x B	NS	NS	NS	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 5-4. Effect of pre-storage HW and 1-MCP treatments on flesh firmness of 'September Sun' peaches during ripening at 20 º C

	Ripening period (d)			
	0	1	3	5
Firmness (N)				
Control	13.92b	6.92b	4.19b	3.79b
HW	23.79a	18.89a	4.73ab	4.26a
1-MCP (1.5 mg/L)	17.58b	14.16a	5.42a	4.58a
HW x 1-MCP	24.04a	13.76a	5.36a	4.43a
Significance				
Factor A (HW)	**	*	NS	NS
Factor B (1-MCP)	NS	NS	**	**
A x B	NS	*	NS	*

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 5-5. Physical characteristics of 'September Sun' peaches during ripening at 20 ºC after pre-storage conditioning treatments

	Ripening period (d)			
	0	1	3	5
GCh°				
Control	85.63	84.98	82.71	79.62
HW	87.42	85.25	82.29	78.90
1-MCP	86.90	83.47	80.79	81.05
HW x 1-MCP	86.78	82.50	82.23	79.55
Significance	NS	NS	NS	NS
FCh°				
Control	89.78	90.34	90.43a	89.81a
HW	90.34	89.19	87.96b	85.34b
1-MCP	90.43	88.75	87.14b	86.17b
HW x 1-MCP	89.81	89.61	88.18b	86.45b
Significance	NS	NS	*	*
WL (%)				
Control	0	3.59	4.91	6.31
HW	0	2.47	3.61	5.13
1-MCP	0	1.64	1.94	5.31
HW x 1-MCP	0	2.41	2.74	3.36
Significance	NS	NS	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Two Year Study on Ripening of NMF ' UFSun' and 'Delta' peaches pre-conditioned with HW, 1-MCP, or HW x 1-MCP at 20 ºC

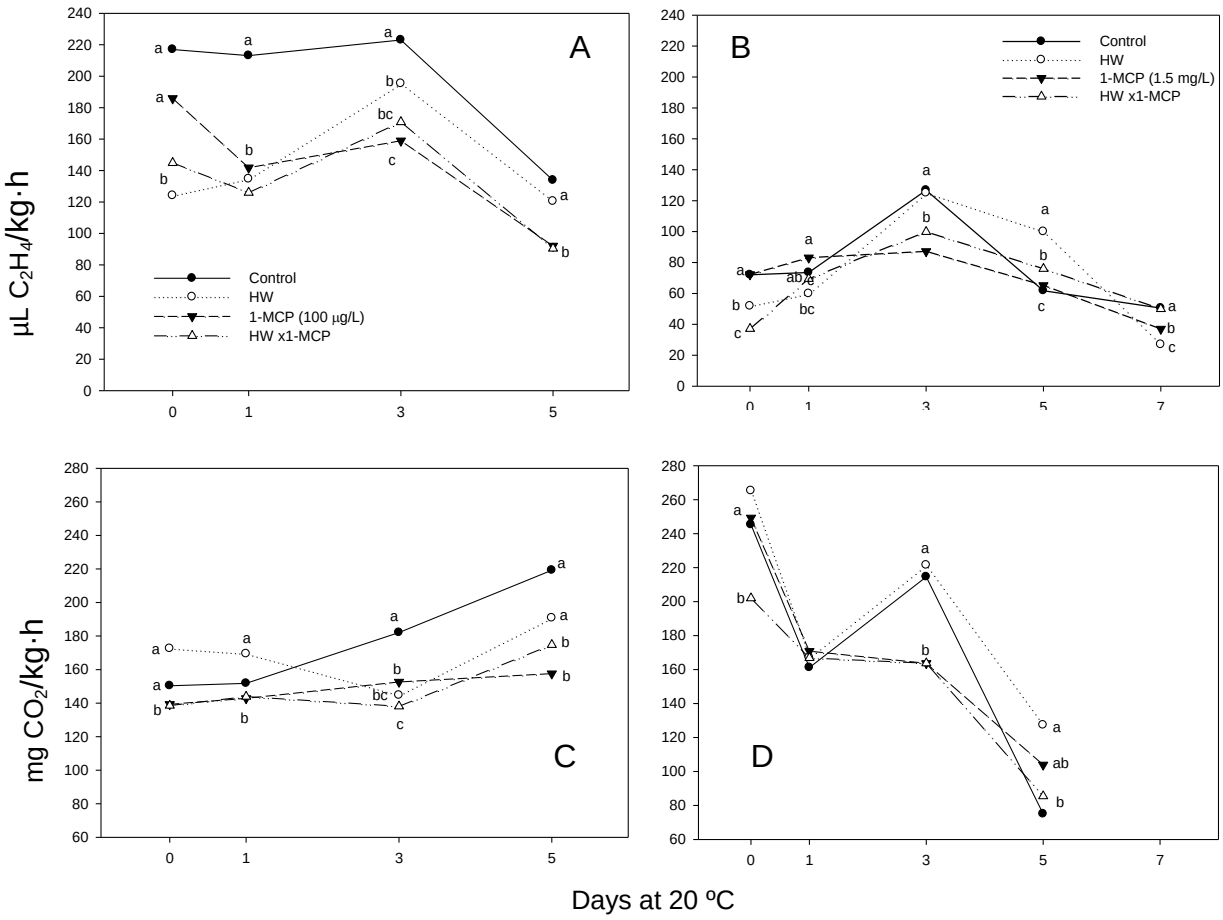

Figure 5-2. Ethylene production of 'UFSun' in 2009 (A) and 2010 (B); Respiration Rate of 'UFSun' in 2009 (C) and 2010 (D) during ripening at 20 ºC after pre-storage conditioning treatments.

Table 5-6. Effect of pre-storage HW and 1-MCP treatments on ethylene production and respiration rate during ripening of 'UFSun' peaches

Year	2009 Ripening period (d)					2010 Ripening period (d)				
	0	1	3	5		0	1	3	5	7
Ethylene Production ($\mu L\ C_2H_4/kg•h$)					Ethylene Production ($\mu L\ C_2H_4/kg•h$)					
Factor A (HW)	**	**	NS	NS	Factor A (HW)	**	**	NS	**	*
Factor B (100 µg/L 1-MCP)	NS	**	**	**	Factor B (1.5 mg/L 1-MCP)	*	*	**	**	*
A x B	NS	**	NS	NS	A x B	*	NS	NS	**	**
Respiration Rate ($mg\ CO_2/kg•h$)					Respiration Rate ($mg\ CO_2/kg•h$)					
Factor A (HW)	NS	NS	*	NS	Factor A (HW)	NS	NS	NS	*	NA
Factor B (100 µg/L 1-MCP)	**	*	NS	**	Factor B (1.5 mg/L 1-MCP)	**	NS	**	NS	NA
A x B	NS	NS	NS	*	A x B	**	NS	NS	**	NA

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant
NA = not available

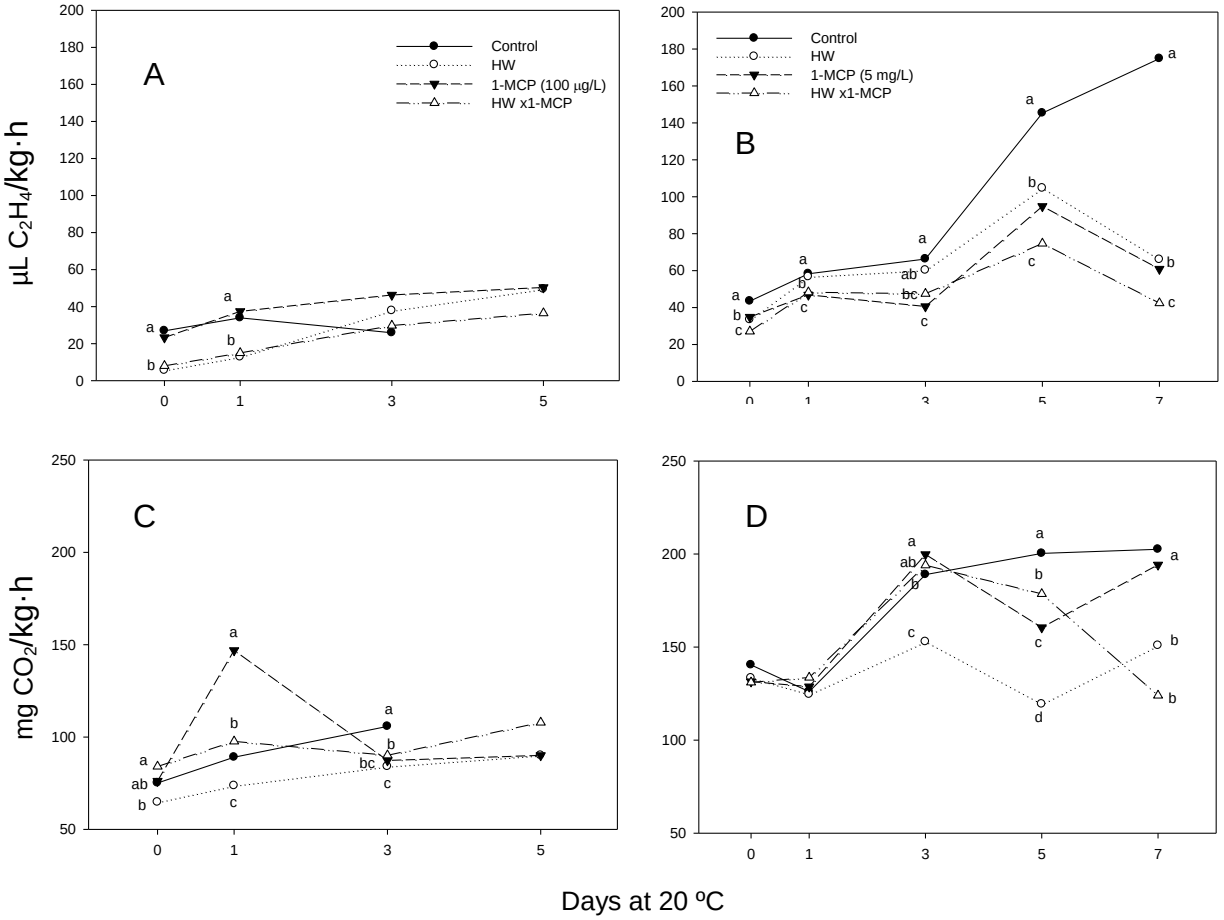

Figure 5-3. Ethylene production of 'Delta' in 2009(A) and 2010 (B); Respiration Rate of 'Delta' in 2009 (C) and 2010 (D) during ripening at 20 ºC after pre-storage conditioning treatments.

Table 5-7. Effect of pre-storage HW and 1-MCP treatment on ethylene production and respiration rate during ripening of 'Delta' peaches

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
Ethylene production (µL C_2H_4/kg•h)					Ethylene Production (µL C_2H_4/kg•h)					
Factor A (HW)	**	**	NS	NS	Factor A (HW)	**	NS	NS	**	**
Factor B (100 µg/L 1-MCP)	NS	NS	NS	NS	Factor B (5.0 mg/L 1-MCP)	**	**	**	**	**
A x B	NS	NS	NS	NS	A x B	NS	*	NS	NS	**
Respiration rate (mg CO_2/kg•h)					Respiration rate (mg CO_2/kg•h)					
Factor A (HW)	NS	**	NS	NS	Factor A (HW)	NS	NS	**	**	**
Factor B (100 µg/L 1-MCP)	*	**	NS	NS	Factor B (5.0 mg/L 1-MCP)	NS	NS	**	**	**
A x B	*	**	*	NS	A x B	NS	NS	**	**	**

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 5-8. Effect of pre-storage HW and 1-MCP treatments on flesh firmness (N) of 'UFSun' peaches during ripening

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
Firmness (N)					Firmness (N)					
Control	8.23b	9.90a	7.30b	6.83bc	Control	26.08	17.34c	14.28b	12.61c	13.69b
HW	10.90a	9.33a	9.76a	8.97a	HW	27.89	25.10a	19.63a	18.19a	19.77a
1-MCP (100 µg/L)	9.23b	8.02b	8.37b	5.67c	1-MCP (1.5 mg/L)	27.22	21.29b	15.38b	14.94b	14.58b
HW x 1-MCP	11.38a	9.42a	10.16a	7.20b	HWx1-MCP	30.56	23.39ab	19.02a	17.13a	16.44b
Significance					Significance					
Factor A (HW)	*	NS	*	*	Factor A (HW)	NS	*	*	*	*
Factor B (1-MCP)	NS	*	NS	*	Factor B (1-MCP)	NS	NS	NS	NS	NS
A x B	NS	NS	NS	NS	A x B	NS	*	NS	*	*

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 5-9. PME and PG activities of 'UFSun' peaches during ripening at 20 ºC after pre-storage conditioning treatments.

Year	2009				2010					
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
PME Activity (Unit) [m]					PME Activity (Unit) [m]					
Control	0.17	0.35	0.44	0.43	Control	0.38b	0.96	1.60a	0.62ab	0.59ab
HW	0.37	0.36	0.30	0.71	HW	0.34b	1.13	0.50b	0.19c	0.78a
Significance	*	NS	NS	NS	1-MCP (1.5 mg/L)	1.70a	0.74	0.72b	0.32bc	0.49b
					HW x 1-MCP	0.23b	0.84	1.34a	0.93a	0.40b
Endo-PG Activity (Unit) [y]					Significance	*	NS	*	*	*
Control	0.78	0.57	0.32	0.70						
HW	1.41	2.07	1.36	1.91	Endo-PG Activity (Unit) [y]					
Significance	NS	*	*	NS	Control	0.34b	0.77	0.58	0.47	0.50
					HW	0.36b	0.62	0.16	1.10	0.18
Exo-PG Activity (Unit) [y]					1-MCP (1.5 mg/L)	0.32b	0.52	0.45	0.38	0.21
Control	0.62	1.23	0.98	0.99	HW x 1-MCP	1.13a	0.80	0.22	0.55	0.20
HW	1.27	1.71	1.14	1.41	Significance	*	NS	NS	NS	NS
Significance	NS	NS	NS	NS						

[m] = 1 unit of PME activity = ΔA_{620} mg^{-1} protein min^{-1}

[y] =1 unit of PG activity = 1 µg galacturonic acid mg-^{1} protein h^{-1}

* = Significant at $p \leq 0.05$

NS = non-significant

Table 5-10. Effect of pre-storage HW and 1-MCP treatments on flesh firmness of 'Delta' peaches during ripening at 20 ºC

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
Firmness (N)					Firmness (N)					
Control	33.21	21.36b	17.50b	14.91	Control	44.99b	41.23b	25.22b	18.92	15.52
HW	33.47	33.27a	28.44a	16.43	HW	48.43ab	47.21a	28.42a	20.01	15.48
1-MCP (100 µg/L)	35.89	24.16b	17.69b	14.33	1-MCP (5 mg/L)	45.13b	33.34c	22.57c	17.49	14.48
HW x 1-MCP	29.24	33.36a	16.79b	14.74	HW x 1-MCP	50.82a	43.76b	26.51ab	20.78	17.15
Significance					Significance					
Factor A (HW)	NS	*	*	NS	Factor A (HW)	*	*	*	NS	NS
Factor B (1-MCP)	NS	NS	*	NS	Factor B (1-MCP)	NS	*	*	NS	NS
A x B	NS	NS	*	NS	A x B	NS	*	NS	NS	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 5-11. PME and PG activities of 'Delta' peaches during ripening at 20 ºC after pre-storage conditioning treatments

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
PME Activity (Unit) [m]					PME Activity (Unit) [m]					
Control	0.60	0.83	0.25	1.28	Control	1.51	1.50	1.66b	1.48	2.66
HW	0.82	0.80	0.89	0.26	HW	1.20	0.71	2.35ab	2.17	2.12
Significance	NS	NS	*	*	1-MCP (5 mg/L)	1.86	1.60	3.15a	0.55	0.40
					HW x 1-MCP	1.36	2.42	2.02b	1.42	2.37
Endo-PG Activity (Unit) [y]					Significance	NS	NS	*	NS	NS
Control	1.93	0.38	0.36	1.35						
HW	1.62	0.86	0.65	0.71	Endo-PG Activity (Unit) [y]					
Significance	NS	*	NS	*	Control	0.13	0.63	0.38	0.82	0.28b
					HW	NA	0.37	0.73	1.02	1.19a
Exo-PG Activity (Unit) [y]					1-MCP (5 mg/L)	NA	NA	0.00	0.48	0.26b
Control	1.09	0.72	0.58	0.48	HW x 1-MCP	NA	0.16	0.44	0.50	0.64ab
HW	1.04	1.06	1.06	1.43	Significance			NS	NS	*
Significance	NS	NS	NS	NS						

[m] = 1 unit of PME activity = ΔA_{620} mg^{-1} protein min^{-1}

[y] =1 unit of PG activity = 1 µg galacturonic acid mg^{-1} protein h^{-1}

* = Significant

NS = non-significant

NA = no enzyme activity detected

Table 5-12. Physical characteristics of 'UFSun' peaches during ripening at 20 ºC after pre-storage conditioning treatments

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
GCh°					GCh°					
Control	70.32	66.41c	65.82b	65.18b	Control	86.07	83.54b	78.13	71.10	72.09
HW	70.94	68.45bc	70.42a	68.21ab	HW	85.91	86.34ab	78.04	74.88	72.73
1-MCP (100 µg/L)	68.77	70.09ab	71.10a	70.05a	1-MCP (1.5 mg/L)	88.58	89.26a	78.47	75.55	69.53
HW x 1-MCP	68.89	71.89a	69.67a	71.16a	HW x 1-MCP	89.62	85.20b	74.17	72.55	69.51
Significance	NS	*	*	*	Significance	NS	*	NS	NS	NS
FCh°					FCh°					
Control	77.90	77.45	74.05c	78.21	Control	80.97	79.93b	80.40a	80.33	83.89
HW	76.62	76.76	76.94b	75.28	HW	83.08	82.14a	79.17a	78.27	81.51
1-MCP (100 µg/L)	77.92	75.85	77.57ab	76.46	1-MCP (1.5 mg/L)	84.10	79.27b	74.58c	78.38	80.49
HW x 1-MCP	76.98	78.18	79.90a	75.28	HW x 1-MCP	80.74	80.28b	76.87b	81.76	82.95
Significance	NS	NS	*	NS	Significance	NS	*	*	NS	NS
WL (%)					WL (%)					
Control	0.00	2.24	7.94	8.79	Control	0.00	1.76c	4.94	8.48	13.3
HW	0.00	2.34	6.96	8.05	HW	0.00	1.85b	4.72	8.22	13.6
1-MCP (100 µg/L)	0.00	2.34	6.96	8.05	1-MCP (1.5 mg/L)	0.00	1.96a	5.32	9.00	13.1
HW x 1-MCP	0.00	2.32	6.62	10.40	HW x 1-MCP	0.00	1.80bc	5.34	8.15	13.1
Significance	NS	NS	NS	*	Significance	NS	*	NS	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 5-13. Physical characteristics of 'Delta' peaches during ripening at 20 ºC after pre-storage conditioning treatments

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
GCh°					GCh°					
Control	88.17	87.49	81.55	77.65	Control	89.17b	88.67a	87.04a	79.77	80.92a
HW	86.70	86.36	81.11	75.99	HW	89.64b	85.73b	82.64c	81.42	81.04a
1-MCP (100 µg/L)	86.39	85.78	81.66	78.90	1-MCP (5.0 mg/L)	92.39a	88.57a	86.13ab	82.76	77.68b
HW x 1-MCP	88.29	83.92	79.66	73.93	HW x 1-MCP	89.79b	88.63a	83.83bc	80.76	80.44a
Significance	NS	NS	NS	NS	Significance	*	*	*	NS	*
FCh°					FCh°					
Control	76.22	78.89bc	77.67	77.37	Control	88.39	88.40	88.41	87.47	87.13a
HW	70.81	87.65a	80.79	80.28	HW	88.53	87.76	88.00	86.77	85.26c
1-MCP (100 µg/L)	71.90	80.85b	83.03	84.91	1-MCP (5.0 mg/L)	88.73	87.93	88.62	88.09	86.46ab
HW x 1-MCP	80.85	73.56c	85.60	79.92	HW x 1-MCP	88.40	88.15	87.69	86.54	85.81bc
Significance	NS	*	NS	NS	Significance	NS	NS	NS	NS	*
WL (%)					WL (%)					
Control	0.00	1.28a	6.47a	NA	Control	0.00	NA	3.15a	5.07	11.80
HW	0.00	0.94b	3.52b	6.55b	HW	0.00	NA	1.31b	3.95	6.55
1-MCP (100 µg/L)	0.00	1.16ab	4.08b	8.88a	1-MCP (5.0 mg/L)	0.00	NA	1.20b	4.06	9.32
HW x 1-MCP	0.00	1.30a	7.44a	10.36a	HW x 1-MCP	0.00	NA	1.34b	2.81	4.47
Significance	NS	*	*	*	Significance	NS		*	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant
NA = not available

Table 5-14. Chemical characteristics of 'UFSun' peaches during ripening at 20 ºC after pre-storage conditioning treatments

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
SSC (%)					SSC (%)					
Control	13.33	12.83	13.67	12.58	Control	10.60	10.53a	10.00	10.73	10.70
HW	13.90	12.90	12.53	12.67	HW	10.97	10.27ab	9.77	10.27	9.70
Significance	NS	NS	NS	NS	1-MCP (1.5mg/L)	10.47	9.27b	9.97	10.73	10.80
					HW x 1-MCP	10.70	10.93a	9.40	10.85	10.70
					Significance	NS	NS	NS	NS	NS
TA (%)					TA (%)					
Control	0.31	0.30	0.21	0.21	Control	0.64	0.60ab	0.48b	0.39c	0.32
HW	0.29	0.28	0.26	0.25	HW	0.64	0.58b	0.47b	0.48b	0.36
Significance	NS	NS	NS	NS	1-MCP (1.5mg/L)	0.64	0.62a	0.54a	0.53a	0.34
					HW x 1-MCP	0.65	0.64a	0.46b	0.51ab	0.36
					Significance	NS	*	*	*	NS
pH					pH					
Control	4.77	4.93	5.27	5.19	Control	4.08	4.16	4.35	4.54a	4.87
HW	4.88	4.98	5.05	5.13	HW	4.07	4.11	4.30	4.34b	4.74
Significance	NS	NS	NS	NS	1-MCP (1.5mg/L)	4.14	4.12	4.24	4.31b	4.77
					HW x 1-MCP	4.02	4.09	4.31	4.30b	4.80
					Significance	NS	NS	NS	*	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 5-15. Chemical characteristics of 'Delta' peaches during ripening at 20 ºC after pre-storage conditioning treatments

Year	2009					2010				
	Ripening period (d)					Ripening period (d)				
	0	1	3	5		0	1	3	5	7
SSC (%)					SSC (%)					
Control	10.53	9.93	8.87	10.67	Control	10.17ab	10.63	10.13	10.33	9.65
HW	10.80	9.57	10.43	7.80	HW	9.47b	9.97	10.47	9.97	9.83
Significance	NS	NS	NS	NS	1-MCP (5 mg/L)	10.23ab	10.10	10.27	10.20	9.63
					HW x 1-MCP	10.63a	9.87	10.03	10.30	9.73
					Significance	*	NS	NS	NS	NS
TA (%)					TA (%)					
Control	0.66	0.48	0.43	0.47	Control	0.45b	0.47	0.52	0.49a	0.45
HW	0.60	0.59	0.47	0.42	HW	0.40c	0.45	0.46	0.44ab	0.42
Significance	NS	NS	NS	NS	1-MCP (5 mg/L)	0.54a	0.48	0.48	0.51a	0.45
					HW x 1-MCP	0.48b	0.46	0.43	0.41b	0.42
					Significance	*	NS	NS	*	NS
pH					pH					
Control	3.97	4.10	4.28	4.37	Control	4.12b	4.07	4.09	4.08c	4.28
HW	4.01	3.96	4.30	4.42	HW	4.26a	4.17	4.15	4.21ab	4.29
Significance	NS	NS	NS	NS	1-MCP (5 mg/L)	4.03c	4.06	4.08	4.09bc	4.22
					HW x 1-MCP	4.08bc	4.15	4.21	4.28a	4.29
					Significance	*	NS	NS	*	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 5-16. Incidence of decay of pre-conditioned NMF peaches after 7 days of storage at 20 ºC

Year	2009			2010	
Cultivar	'UFSun'	'Delta'		'UFSun'	'Delta'
Decay (%)			Decay (%)		
Control	11.7	52.1	Control	11.0	22.0
HW	17.0	18.8	HW	24.0	4.0
1-MCP (1.5 mg/L)	5.0	29.2	1-MCP (5 mg/L)	12.2	24.0
HW x 1-MCP	22.0	6.3	HW x 1-MCP	18.3	0.6

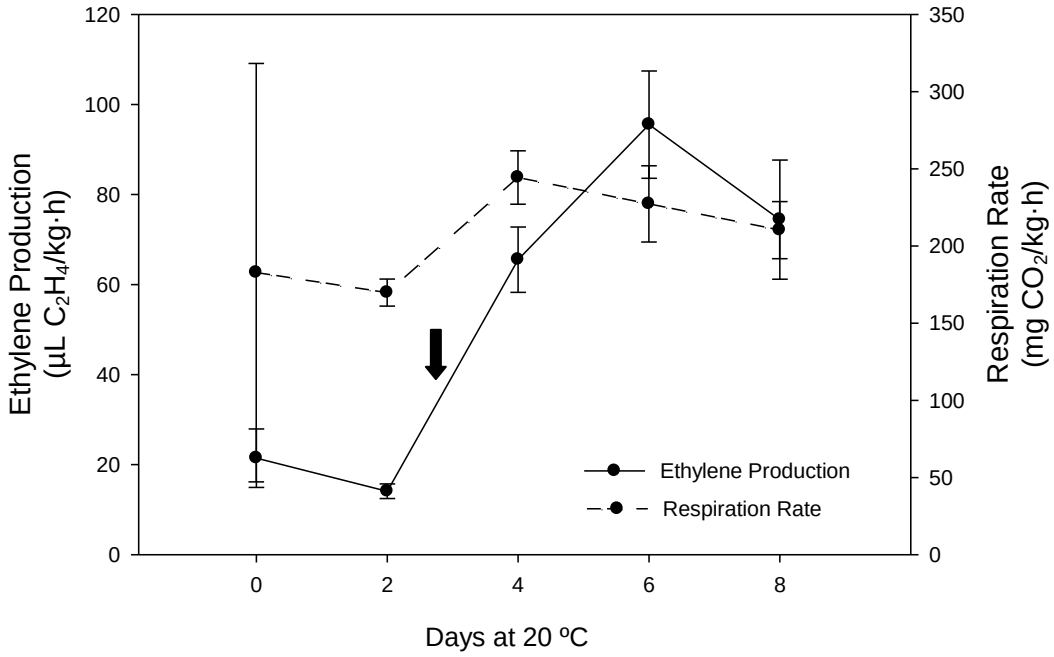

Figure 5-4. Preclimacteric to climacteric ethylene production and respiration rate of NMF ‘UFSun’ peaches. The arrow indicates the ethylene production rate at the time when HW and 1-MCP treatments were applied in 2010.

CHAPTER 6
EFFECT OF PRE-STORAGE HOT WATER TREATMENT ALONE OR COMBINED WITH AQUEOUS 1-METHYCLCOPROPENE ON RIPENING OF NON-MELTING FLESH PEACHES AFTER LOW TEMPERATURE STORAGE

Overview

Peaches can be separated into different types based on their texture. Melting flesh (MF) peaches soften excessively toward the end of ripening. Thus, they have to be harvested at 'firm-mature' stage (AMS, 2004; Cascales et al., 2005; Delwiche, 1987). Non-melting flesh (NMF) peaches soften relatively slowly and are characterized by the absence of the "melting" phase when they are full ripe. Thus, NMF peaches can be left on the tree longer to develop maximum flavor while still possessing sufficient flesh firmness for storage and shipping (Sherman et al., 1990).

Changes in flesh firmness are associated with extensive modification in cell wall structure and composition during fruit ripening, which is achieved by synergistic actions of related enzymes (Toivonen and Brummell, 2008). Pectin methylesterase (PME) activity peaks early in peach ripening and remains constant or decreases throughout the mid- and full ripe stages (Brummell et al., 2004; Glover and Brady, 1995). PME removes methylester groups from pectin molecules, which are subsequently depolymerized by the action of polygalacturonase (PG) during the melting phase (Orr and Brady, 1993; Wakabayashi, 2000).The limited softening of NMF cultivars is related to their deficiency of endo-PG mRNA accumulation and enzyme activity compared to MF cultivars (Callahan et al., 2004; Lester et al., 1994; 1996; Pressey and Avants, 1978).

Peaches ripen quickly at room temperature. Thus, immediate storage at 0 ºC after harvest is necessary. However, peaches can develop chilling injury (CI) when they are exposed to 0°C for 3 or more weeks (Crisosto et al., 1999). However, CI symptoms

develop most rapidly and intensely when peaches are stored between 2.2°C and 7.6°C (Lurie and Crisosto, 2005). Symptoms of CI in peaches vary among genotypes and typically include flesh discoloration, such as internal bleeding and flesh browning, abnormal softening, reduced aroma, and dry or grainy texture (i.e., leatheriness or mealiness). Mealiness is the most frequently reported symptom of CI in MF peaches (Brovelli et al., 1998c; Murray et al., 2007). NMF peaches with CI are reported to have rubbery texture, higher incidence of flesh bleeding, and off-flavors such as astringency, bitterness, and fermentative taste (Cantin et al., 2010; Karakurt et al., 2000a; Robertson et al., 1992b). CI in peaches has been associated with reduced endo-PG activity and enhanced or stable PME activity during low temperature storage (Ben-arie and Sonego, 1980; Brummell et al., 2004; Nilo et al., 2010; Zhou et al., 2000a).

Pre-storage heat treatment has been shown to reduce CI in many fruits and vegetables, which may be related to induction of heat shock proteins (Lurie, 1998). The term ‘heat treatment’ has been used to describe exposure to temperatures higher than 33 °C (Li and Han, 1998). Heat treatment is also capable of suppressing postharvest decay (Casals et al., 2010b; Malakou and Nanos, 2005; Obenland and Carroll, 2000) and texture change in MF peaches through down-regulation of enzymes involved in ethylene biosynthesis (Budde et al., 2006; Martinez and Civello, 2008; Steiner et al., 2006). As a result, firmness retention in heat-treated fruit may be due to inhibited synthesis of ethylene-dependent cell wall hydrolytic enzymes such as endo-PG (Jin et al., 2009; Lurie, 1998). The most common application methods are hot water (HW), hot water vapor, and hot air (Zhang et al., 2007). The internal temperature of peaches treated with HW increases more rapidly than in fruit treated with hot air, due to the

higher convective heat transfer coefficient for water compared with air (Zhou et al., 2002).

Applying 1-methylcylopropene (1-MCP), an ethylene action inhibitor, to MF peaches has been shown to be effective in suppressing deterioration of fruit quality during ripening although the impact is usually transient (Watkins, 2006). 1-MCP is traditionally applied in gaseous form, but its usage has been limited by low temperature storage. In one report, gaseous 1-MCP lost its efficacy in nectarines stored at 4°C for 3 days (Bregoli et al., 2005). Furthermore, 1-MCP can induce greater incidence of CI in peaches and nectarines stored in low temperature for more than 3 weeks (Bregoli et al., 2005; Dong et al., 2001; Fan et al., 2002). An aqueous 1-MCP formulation has been recently developed and is relatively efficient in controlling fruit ripening (Choi and Huber, 2008). The effect of aqueous 1-MCP treatment may not be influenced by low temperature as much as the gaseous method, since treated 'Harrow Sun' plums were found to maintain better fruit quality after ripening following 10 days of 0°C or 5°C storage (Manganaris et al., 2007).

Currently, no studies have been conducted to investigate the quality of NMF peaches pre-conditioned with either HW or aqueous 1-MCP and ripened after low temperature storage. Since both pre-conditioning methods have been shown to be effective on MF peaches, the combined treatment (HW x 1-MCP) may be more beneficial in preventing CI and regulating fruit ripening. The objective of this study is to evaluate the effect of combining HW and aqueous 1-MCP treatments with low temperature storage on shelf life extension of NMF peaches.

Materials and Methods

Plant Material

In May of 2009 and 2010, NMF 'UFSun' peaches were commercially harvested from Punta Gorda, FL when fruit were considered as 'tree-ripe' (peel GC was yellow; diameter ≥ 57mm). In June of 2009 and 2010, NMF 'Delta' peaches were commercially harvested from Mershon, GA when the peel GC started to transform from green to yellow and the diameter was greater or equal to 64mm. Peaches of both cultivars were transported to the University of Florida in Gainesville, FL by air-conditioned vehicle. In 2009, 280 fruit per cultivar were separated into 4 treatments. Fruit were immersed for 30 min in water at 25°C (Control) or 46°C (HW), or immersed in 25°C water containing 100 µg/L of aqueous 1-MCP (1-MCP), or 46°C water containing 100 µg/L of aqueous 1-MCP (HW x 1-MCP). Zipper-lock bags were used in all treatments to trap off-gas released by 1-MCP when it was introduced to water. The temperature x exposure time and 1-MCP concentration x exposure time combinations were chosen based on their effect on firmness retention in a preliminary study (Chapter 5). Following the pre-conditioning treatments, fruit were stored at 0°C for 14 days before ripening at 20°C for 5 days. For 'UFSun' peaches, data from the last day of low temperature storage was not collected. Data collection on ripening fruit started 12 h after they were transferred from 0°C to 20°C.

In 2010, the experiment was repeated with 840 fruit per cultivar. Results in 2009 showed that 100 µg/L 1-MCP did not inhibit fruit softening of both cultivars. Furthermore, the inhibitory effect of 1.0 ppm (1,000 µg/L; the current registered concentration for apples in U.S.) was reported to be transient (Dal Cin et al., 2006). Therefore, the aqueous 1-MCP concentration for 'UFSun' was increased to 1.5 mg/L

(1.5 ppm). The initial ethylene production (before treatment) in 2010 for ‘UFSun’ peaches at 20°C was around 35 µL/kg•h, approximately 17 % of the peak value determined at 20°C in 2009. The initial ethylene production in 2010 for ‘Delta’ peaches at 20°C was about 42 µL /kg•h, which was similar to the peak level detected at 20°C in 2009. Since ‘Delta’ peaches were further along on the climacteric rise than ‘UFSun’ peaches, the 1-MCP concentration was increased to 5.0 mg/L or 5.0 ppm, a concentration that has been identified as optimal for extending the shelf life of early season peaches and nectarines (Liguori et al., 2004). Following the pre-conditioning treatments, all of the fruit in 2010 were stored at 0°C for 14 days before ripening at 20°C for 7 days.

Respiration rate, ethylene production, and physical characteristics (ground color, flesh color, weight loss) were measured in both years. Since 1-MCP treatment was unable to inhibit fruit ripening in 2009, chemical characteristics (soluble solids content, titratable acidity, and pH) and activities of PME and endo-PG were only measured in 2010. Incidence of decay was determined at the end of the ripening period in both years. Incidence of pitting was only recorded in 2010.

Ethylene Production and Respiration Rate Determination

In 2009, a gas analyzer ETH-1010 (Fluid Analytics, Inc., West Linn, Oregon) with an infrared detector for CO_2 measurement and a gold catalyst detector for ethylene measurement was used to measure respiration rate and ethylene production. 10 Fruit were equally divided into five replications and fruit of each replication were sealed in a 2.735L container that was connected to the device. In 2010, ethylene production and respiration rate for each treatment were monitored using a static system consisting of three 18.9-L glass jars each containing 10 fruit. The jars were sealed for 15 min before

5 mL headspace gas samples were withdrawn. The concentrations of gases were determined using a Varian gas chromatograph (GC) (CP-3800, Middelburg, The Netherlands) equipped with a Valco valve system (Houston, Texas, USA). Ethylene was separated on a molecular sieve column (Ultimetal, 1.5 m*1/8”; 13x80-100 mesh) and a Pulse Discharge Helium Ionization Detector (PDHID) was used for detection. Measured concentrations of ethylene were converted into rates of production based on the mass of fruit in a jar, the void volume, and the duration of sealing.

Physical Characteristics

Ground color (GC) and flesh Color (FC) determination

GC and FC were determined using a reflectance colorimeter that measured in C.I.E. L*, a*, b* values (Minolta CR-400, Konica Minolta, Japan). The shade of color, which is best described by hue angle (h°; arctangent of b*/a*), can often change after postharvest treatment (McGuire, 1992). Therefore, GCh° and FCh° were presented in this study. GC was measured on the greenest portion of the peel. FC was measured after removing a small (circa 2 cm diameter) patch of peel.

Flesh firmness determination

Flesh firmness was determined with an Instron (Model 1132) that applied a compressive force from a 50 kg load cell. A convex tip probe (Magness-Taylor type), 7.9 mm in diameter, was attached to the load cell and the force applied with the probe moving at a speed of 12 cm/min. Flesh firmness was measured on the cheeks of the fruit at the fruit equator on both sides with peel removed and was expressed as the bioyield force (N). Following color and firmness measurements, fruit samples were placed in quart size (17.7cm x 20.3cm) zipper locking, plastic freezer bags and stored at -30 ºC for later compositional analyses.

Weight loss (WL) determination

WL was calculated by subtracting the final (after storage) fresh weight of the fruit from the initial fresh weight and dividing the difference by the initial fresh weight. The resulting values were converted to percentage by multiplying by 100.

Chemical Characteristics

Soluble solids content (SSC), titratable acidity (TA), and pH determination

Frozen fruit tissues were pureed in a Waring blender for 1 min. The resulting slurry was centrifuged (20 min; 15,000 × g_n; 4°C) and the clear supernatant was used to determine SSC and TA. The SSC was measured with a temperature compensated digital refractometer (model ABBE Mark II, Cambridge Instruments Inc, U.S.A) and expressed as percent FW. TA was determined by titration (model 719 S. Titrino, Metrohm, Switzerland) of 6.0 g of juice plus 50 mL of water with 0.1N sodium hydroxide solution until pH 8.2 was reached and the TA was expressed as percent malic acid. The pH of the diluted juice was determined automatically by the Titrino equipped with a pH electrode.

Enzyme Assays

Preparation of cell-free protein extract

Enzyme extracts were prepared similarly to the method of Jeong et al. (2002). Partially thawed mesocarp tissue (15 g) was homogenized with 25 mL of ice-cold 95% ethanol for 1 min in an Omnimixer (Model GLH-01, New-town, CT, USA) and centrifuged at 15,000 × g_n for 10 min at 4°C. The supernatant was discarded and the pellets were resuspended in 25 mL of ice-cold 80% ethanol for 1 min and centrifuged again at 15, 000 × g_n for 10 min at 4°C. The pellets were transferred to 10 mL of 50 mM Na-acetate buffer, pH 5, containing 0.5 M NaCl, for 30 min in an ice-cold water bath

followed by centrifugation 15, 000 × g_n for 10 min at 4°C. The resulting supernatant was analyzed for enzyme activities. Total soluble protein in the supernatant was measured using the bicinchoninic acid method with bovine serum albumin as the standard (Smith et al., 1985).

Pectinmethylesterase (PME) activity determination

PME (E.C. 3.1.1.11) activity was measured using modifications of the method of (Jeong et al., 2002). A 1% (w/v) solution of 93% esterified citrus pectin (Sigma Chemical Co., St. Louis, MO, USA) was prepared in 0.1M NaCl and adjusted to pH 7.5 with dilute NaOH. A 0.01% solution of bromothymol blue was prepared in 0.003M potassium phosphate buffer, pH 7.5. A 166 µL volume of 1% citrus pectin was mixed with 12 µL of 0.01% bromothymol blue and 70 µL of water on a microplate, and the pH adjusted to 7.5 with dilute NaOH. The reaction was initiated by adding 2 µL of the cell-free protein extract adjusted to pH 7.5 with dilute NaOH. The decrease in A_{620} over a 10 min reaction time was recorded and PME activity was expressed as ΔA_{620} mg^{-1} protein min^{-1}.

Endo-polygalacturonase (Endo-PG) activity determination

Endo-PG (E.C. 3.2.1.15) activity was assayed by mixing 100 µL of enzyme extract with 400 µL of 0.5% polygalacturonic acid (from orange peel, Sigma Chemical Co., St. Louis, MO, USA) in 50 mM Na-acetate buffer (pH 4.4) and incubating at 30°C for 16 h (Pressey and Avants, 1973). Uronic acid (UA) reducing groups released were measured using the method of Milner and Avigad (1967) with mono-D-galacturonic acid as the standard. One unit of activity was defined as 1 µg galacturonic acid produced mg^{-1} protein h^{-1}.

Incidence of Decay and Pitting

Incidence of decay was calculated by dividing the total number of decayed fruit by the total number of fruit. The resulting value was converted to percentage by multiplying by 100. The same calculation was used to determine the percentage of pitted fruit.

Statistical Analysis

The study was conducted by randomized complete design with a factorial arrangement of treatments. The data were analyzed by two-way ANOVA. Significant influence of HW (factor A), 1-MCP (factor B), and A x B interaction at both $p \leq 0.01$ and $p \leq 0.05$ were only shown for ethylene production, respiration rate, and flesh firmness. Differences among the treatments were determined by LSD at $p \leq 0.05$.

Results and Discussion

Ethylene Production and Respiration Rate

Ripening of all fruit was slowed during low temperature storage as evident by reduced ethylene biosynthesis and respiration rate. The impact of HW treatment was immediate. HW suppressed ethylene biosynthesis in ‘UFSun’ and ‘Delta’ fruit in 2009 (Table 6-1, 6-3) or induced ethylene biosynthesis of ‘Delta’ fruit in 2010 (Table 6-4) on Day 0. Ethylene suppression in HW fruit may be due to reduced ACC oxidase (ACO) activity more than ACC synthase (ACS) activity (Lurie 1998; Giradi et al., 2005). HW ‘Delta’ fruit generally exhibited higher ethylene production than the control fruit of the same cultivar during low temperature storage (Table 6-3, 6-4), which could be a protective mechanism against CI. Inhibition of ethylene can increase severity of CI and application of ethylene during low temperature storage can decrease the percentage of woolly fruit, as shown for nectarines (Dong et al., 2001). Immediate suppression of ethylene production following 1-MCP treatment was only observed in 2010 when higher

1-MCP concentrations were applied to both NMF cultivars (Table 6-2, 6-4). Higher concentrations of 1-MCP were also more persistent in inhibiting ethylene production throughout low temperature storage. The persistent effect of 1-MCP may be due to irreversible binding to ethylene receptors and inhibition of ACO activity (Mathooko et al., 2001; Sisler and Serek, 1997).

Since response to 1-MCP has been related to the internal ethylene level of fruit (Zhang et al., 2009), the internal ethylene levels of both NMF cultivars might be too high to be overcome by the concentration of 1-MCP applied in 2009. Interestingly, HW x 1-MCP fruit followed a similar pattern of ethylene production as 1-MCP fruit (Table 6-1, 6-2, 6-3). Therefore, 1-MCP treatment was more likely responsible for inhibiting ethylene production than HW treatment at low temperature.

An initial increase in the respiration rate of HW-treated ‘UFSun’ fruit was observed on Day 0 in 2010, but respiration declined to the level of the non-treated fruit by the first week of low temperature storage (Table 6-2). The temporary increase in respiration rate induced by heat stress may be attributed to the cyanide insensitive pathway (Inaba and Chachin, 1989). Since the fruit were able to recover, it was assumed that the temperature x exposure time of the HW treatment did not cause irreversible damage. Temporary increase in respiration rate during low temperature storage was detected in 1-MCP ‘UFSun’ fruit on Day 14 (Table 6-2) and ‘Delta’ fruit on Day 7 in 2010 (Table 6-4), which could be related to the high 1-MCP concentrations applied. Liguori et al., (2004) showed that ‘Oded’ peaches had slightly higher CO_2 production when fruit were treated with 5 or 20 µL/L 1-MCP at 0°C for 20 h relative to those treated with 0.5 or 1 µL/L 1-MCP. Respiration rate of HW x 1-MCP ‘UFSun’ and ‘Delta’ fruit resembled that of

the 1-MCP fruit during low temperature storage in both years regardless of the concentrations of 1-MCP applied (Table 6-1, 6-2, 6-3, 6-4).

Ethylene biosynthesis quickly resumed when the peaches were transferred from 0°C to 20°C for ripening. In 2009 and 2010, peak ethylene production of HW-treated 'UFSun' fruit occurred on the same day as the control fruit although the magnitudes were 15% and 7% lower respectively (Table 6-1, 6-2). Peak ethylene production of HW-treated 'Delta' fruit was delayed in 2009 and was 30% lower than that of the control fruit in 2010 (Table 6-3, 6-4). Low concentration of 1-MCP did not affect ethylene production of 'UFSun' peaches during ripening, but delayed the peak of ethylene production for 'Delta' peaches by 2 days after that of the control (Table 6-1, 6-3). 1-MCP treatment suppressed peak ethylene production by 'UFSun' and 'Delta' fruit relative to the control fruit when higher concentrations were applied (Table 6-2, 6-4). HW x 1-MCP treatment was most promising in inhibiting ethylene biosynthesis of both NMF cultivars during fruit ripening at 20°C following low temperature storage in both years.

Respiration rate of the control 'UFSun' fruit rapidly rose upon removal from low temperature storage (Table 6-1, 6-2). HW-treated 'UFSun' fruit had lower respiration rate than the control fruit upon transfer to 20°C as well as a delayed respiration peak in 2009 (Table 6-1). The climacteric respiration peak of HW-treated 'Delta' fruit occurred on the same day as that of the control fruit in both years but the magnitudes were approximately 40% lower (Table 6-3, 6-4). According to Lurie (1998), the climacteric respiration peak can be decreased or increased as well as advanced or delayed depending on the temperature and length of exposure of a heat treatment.

1-MCP treatment generally lowered the peak respiration of both cultivars (Table 6-2, 6-3, 6-4). Similar behaviors were observed in 1-MCP-treated plums and apricots ripened following 10 days of storage at 0°C (Dong et al., 2002; Manganaris et al., 2007). HW x 1-MCP fruit followed a similar respiration pattern as HW-treated fruit during ripening, when the concentration of 1-MCP applied was too low to have an impact on both NMF cultivars (Table 6-1, 6-3). Respiration rate of HW x 1-MCP treated 'UFSun' fruit was similar to that of 1-MCP fruit (Table 6-2) or at an intermediate level between 1-MCP and HW fruit as shown in HW x 1-MCP treated 'Delta' fruit (Table 6-4), when higher 1-MCP concentrations were applied.

Flesh Firmness, PME and endo-PG Activities

In 2009, HW-treated 'UFSun' fruit did not soften, while fruit from the control, 1-MCP, HW x 1-MCP treatments softened to a similar degree by the first week of low temperature storage (Table 6-5). Firmness retention in HW-treated fruit might be attributed to the persistent reduction of ethylene biosynthesis since temporary reduction of ethylene by 1-MCP (Day 7) did not correlate with a firmer texture (Table 6-1, 6-5). Upon removal from low temperature storage, HW-treated fruit initially maintained better firmness than fruit from the 1-MCP and HW x 1-MCP treatments. However, HW x 1-MCP treated fruit retained the same firmness until Day 17 when the HW-treated fruit had already softened to the level of the control fruit (Table 6-5). Softening inhibition was more prominent when a higher concentration of 1-MCP was applied to 'UFSun' peaches (Table 6-6). This could be the reason for the prolonged firmness retention that occurred in HW x 1-MCP treated 'UFSun' fruit in 2010.

For 'Delta' peaches, all the treatments had no effect on firmness retention during low temperature storage in 2009, while HW treatment was most effective in 2010 (Table

6-8, 6-9). Furthermore, HW treatment significantly inhibited softening of ‘Delta’ peaches during ripening in 2010, but not in 2009 (Table 6-8, 6-9). This could be related to the difference in maturity of fruit in those two years (Budde et al., 2006). 1-MCP was relatively ineffective in delaying softening of ‘Delta’ peaches because even the high concentration of 1-MCP was only capable of retaining firmness for 1 day after removal from low temperature storage (Table 6-9). In 2010, the inhibitory effect of 1-MCP lasted 4 days longer for ‘UFSun’ peaches than for ‘Delta’ peaches (Table 6-6, 6-9), demonstrating that sensitivity to 1-MCP is cultivar dependent. Repeated 1-MCP applications on peaches may be able to maintain suppression of ripening longer (Liu et al., 2005).

Firmness retention may be explained by changes in PME and PG activities. PME and PG activity were affected differently in the two NMF cultivars. Similar to the results reported in Zhou et al. (2000) and Ben-Arie and Sonego (1980), PME activity increased in ‘UFSun’ peaches (Table 6-7) but was maintained at the same level in ‘Delta’ peaches by the first week of low temperature storage (Table 6-10). Proteomic analysis indicated that PME protein increased to a higher level after peaches were stored at 4°C for 3 weeks than when the fruit were ripened immediately after harvest (Nilo et al., 2010). In this study, PME activity dropped to a lower level by the second week of low temperature storage for both cultivars (Table 6-7, 6-10).

PME activity of control ‘UFSun’ peaches peaked on Day 15 (Table 6-7), decreased on Day 17 to Day 19 and increased on Day 21. Control ‘Delta’ peaches had peak PME activity during mid-ripening (Day 17) and decreased thereafter (Table 6-10). It has been shown that the PME activity of juicy peach fruit after 2 weeks of storage at 5 ºC and

subsequent ripening is low compared to mealy fruit, which tend to maintain the same level of PME activity (Brummell et al., 2004). In this study, the increased PME activity of control 'UFSun' fruit on Day 21 coincided with an unusual, firmer texture that developed by the end of ripening period (Table 6-6). The abnormal texture may be attributed to leatheriness since NMF peaches are less likely to develop mealiness (Brovelli et al., 1998c; Cantin et al., 2010; Ju and Duan, 2000).

High PME activities of HW-treated and HW x 1-MCP treated 'UFSun' fruit were detected during ripening (Table 6-7). PME has been demonstrated to be more active at higher temperatures (Koukounaras et al., 2008; Steiner et al., 2006). It is hypothesized that after methoxy groups are released from galacturonic acid residues by the action of PME, the carboxyl groups may complex with free cations, particularly endogenous Ca^{2+} (Steiner et al., 2006). The subsequent increase in Ca-pectate may result in a more rigid middle lamella and cell wall. The PME activity of HW 'UFSun' fruit dropped on Day 19 then increased again. It was 2-fold higher than that of the control fruit by Day 21 (Table 6-7). It was observed that 'Baifeng' peaches exposed to 38°C for 12 h had higher flesh mealiness index after 5 weeks of storage at 0°C (Jin et al., 2009). The abnormal increase in flesh firmness of HW-treated 'UFSun' fruit by the end of storage in 2010 could have reflected a change in texture from normal to rubbery (Table 6-6). Since HW x 1-MCP treated 'UFSun' fruit softened normally, peak PME activity was simply delayed to Day 21 (Table 6-6, 6-7). Higher PME activities were not detected in HW-treated and HW x 1-MCP treated 'Delta' fruit although softening was suppressed (Table 6-9, 6-10). The data suggests that the mechanisms underlying the preservation of flesh firmness deviated greatly between these two NMF cultivars.

Surprisingly, high PME activity was also detected in 1-MCP-treated ‘UFSun’ fruit during ripening (Table 6-7). Hence, regulation of cell wall modification during ripening of peaches may have been similar between 1-MCP and HW treatments. 1-MCP-treated ‘UFSun’ fruit softened normally during ripening and had significantly lower PME activity than the control fruit by Day 21 (Table 6-6, 6-7), suggesting that those fruit did not develop rubbery texture like the control and HW-treated fruit (Giradi et al., 2005). Therefore, 1-MCP treatment may be capable of preventing certain chilling-related disorders, as observed in ‘Fantasia’ nectarines (DeEll et al., 2008).

Low temperature storage has a more pronounced effect on protein synthesis and activity of endo-PG than it does on PME in peaches (Girardi et al., 2005; Nilo et al., 2010). Endo-PG activity of ‘UFSun’ fruit was inhibited completely by the first week of low temperature storage (Table 6-7). Endo-PG activity in ‘UFSun’ fruit from the control, HW, and HW x 1-MCP treatments recovered by Day 17 while endo-PG in 1-MCP-treated fruit rapidly recovered on Day 15 (Table 6-7). Endo-PG activity of the control ‘Delta’ fruit was detectable, but decreased consistently during low temperature storage (Table 6-10). Endo-PG activity in HW-treated and HW x 1-MCP treated ‘Delta’ fruit was suppressed completely by Day 7 and Day 15 (Table 6-10). Endo-PG activity was detected on Day 15 in 1-MCP-treated fruit, but was slightly lower than that of the control fruit. Delaying the increase in activity of endo-PG could be the mechanism responsible for firmness retention in HW-treated and HW x 1-MCP treated ‘Delta’ fruit. Since PME activity was not increased, the HW and HW x 1-MCP treatments may have regulated endo-PG at the transcriptional level via ethylene inhibition. Interestingly, endo-PG activity of control ‘Delta’ fruit gradually decreased throughout ripening, a trend that is the opposite of MF

peaches (Brummell et al., 2004). The rapid recovery of endo-PG activity in 1-MCP-treated fruit upon removal from low temperature storage for both cultivars might be associated with the minor (Table 6-2) or no (Table 6-4) suppression of ethylene biosynthesis in those fruit. It has been shown that ACS1 expression and activity are not affected by 1-MCP in ripening peaches (Dal Cin et al., 2006) and ACS1 may be a key factor in the modulation of responses to 1-MCP application (Ziliotto et al., 2008).

Ground Color, Flesh Color, and Weight Loss

HW and HW x MCP treatments had no effect on GChº of 'UFSun' peaches during low temperature storage in 2009 (Table 6-11) but significantly delayed the changes by Day 14 in 2010 (Table 6-12). A decrease in GChº in the range measured in these experiments denotes an increase in orange or red coloration of the skin. The control fruit gradually became more orange during ripening while the HW-treated and HW x 1-MCP treated 'UFSun' fruit appeared to be more yellow by Day 15 in 2009 (Table 6-11) and Day 19 in 2010 (Table 6-12). 1.5 mg/L 1-MCP was required to inhibit GCh° change of 'UFSun' fruit during low temperature storage and ripening (Table 6-12). GCh° of all pre-conditioned 'Delta' fruit was similar to that of control fruit in low temperature storage (Table 6-13, 6-14). During ripening of 'Delta' peaches, GCh° changes were transiently inhibited by HW, 1-MCP, and HW x 1-MCP treatments in 2009 (Table 6-13) or not affected in 2010 (Table 6-14).

Both cultivars developed localized slight red coloration naturally in the flesh as fruit senescence proceeded. HW treatment increased the intensity and area of red coloration but no browning was observed in the flesh. This alteration was reflected by the decreased FCh° of HW fruit by the end of the ripening period of both cultivars in 2009 (Table 6-11, 6-13). Flesh reddening has been shown in heat-treated fruit ripened

directly at room temperature; therefore, this feature has been proposed to be associated with alteration of ethylene biosynthesis (Budde et al. 2005; Murray et al., 2007). 1-MCP-treated ‘UFSun’ peaches developed excessive red coloration in the flesh by Day 21 in 2010 (Table 6-12), a symptom associated with inhibition of ethylene production after low temperature storage (Dong et al., 2001). It has been shown that PAL activity and accumulation of anthocyanin are generally reduced by 1-MCP (Jiang et al., 2001; Manganaris et al., 2007). The induction of red coloration in this study might be attributed to the recovery of ethylene biosynthesis. Therefore, it was only evident toward the end of ripening. HW x1-MCP was the most significant treatment in inhibiting FCh° changes in ‘UFSun’ peaches in 2010 (Table 6-12). The FCh° values were significantly different among the treatments during low temperature storage and ripening of ‘Delta’ peaches in 2010, but the differences in flesh color were most likely too small to be detected visually. It has been reported for peaches and nectarines that a h° difference of 2.5 units or greater is required for consumers to be able to perceive a color difference (Obenland et al., 2005).

HW treatment increased WL in both NMF cultivars. HW-treated and HW x 1-MCP treated ‘Delta’ fruit lost about 2% more weight than the control and 1-MCP-treated fruit by Day 19 in 2009 (Table 6-13). Transiently higher WL of HW x 1-MCP treated ‘Delta’ peaches was observed in 2010, but the WL difference quickly disappeared as ripening progressed (Table 6-14). In contrast, HW-treated ‘UFSun’ fruit exhibited lower WL from Day 14 to 17 in 2010 but eventually reached the same level of WL as the control fruit by Day 19 (Table 6-12). The WL characteristics of each NMF cultivar after HW treatment may have been caused by different rearrangements of epicuticular wax (Lopez–

Castañeda J. et al., 2010). 1-MCP-treated fruit had WL that was similar to the control fruit regardless of the concentration of 1-MCP applied (Table 6-11, 6-12, 6-13, 6-14), in agreement with the results of Manganaris et al. (2008) for 'Harrow Sun' plums.

Soluble Solids Content, Titratable Acidity, pH

In 2009, HW-treated ‘UFSun’ fruit had slightly higher SSC than the control fruit by Day 19 (Table 6-15), but a similar effect was not observed for HW-treated ‘Delta’ fruit (Table 6-17). Others have also reported that HW treatment can either have no effect or slightly increase the SSC of ripened peaches (Malakou and Nanos, 2005; Obenland et al., 2005). In 2010, HW-treated and HW x 1-MCP treated ‘UFSun’ fruit had lower SSC than the control fruit on Day 15 (Table 6-16) while ‘Delta’ fruit from the same treatments showed a reverse trend (Table 6-18). Higher SSC may be attributed to increases in glucose and fructose because the sucrose level of peaches is not affected by heat treatment (Lara et al., 2009). No consistent trend was observed for SSC of 1-MCP-treated ‘UFSun’ during low temperature storage (Table 6-16). Upon removal from low temperature storage, 1-MCP-treated ‘UFSun’ fruit exhibited no accumulation of SSC, similar to HW-treated and HW x 1-MCP treated fruit. 1-MCP did not affect SSC in ‘Delta’ peaches during low temperature storage or ripening (Table 6-18).

TA of the control fruit was maintained throughout low temperature storage, then decreased gradually during ripening for both cultivars (Table 6-15, 6-16, 6-17, 6-18). HW-treated ‘UFSun’ fruit had significantly lower TA than the control fruit by Day 19 in 2009 (Table 6-15), but the opposite trend was observed in 2010 (Table 6-16). The higher SSC and lower TA that developed as a consequence of HW treatment may have improved the flavor of ‘UFSun’ peaches although the control fruit already had very high SSC/TA (51.68) on Day 19 in 2009 (Table 6-15). The SSC/TA for minimum acceptability

of MF peaches at the eating ripe stage is 15 or greater (Beckman and Krewer, 1999; Malakou and Nanos, 2005). The decrease in TA and accumulation of SSC suggest that organic acids may be preferred over sugar as respiratory substrate in heat-treated fruit (Lara et al., 2009). In 2009, HW had no effect on TA of 'Delta' fruit during low temperature storage and ripening (Table 6-17). In 2010, TA of HW-treated and HW x MCP treated 'Delta' fruit gradually decreased during low temperature storage, which corresponded with an increase in pH, while no changes occurred in both control and 1-MCP-treated fruit (Table 6-18). Although the TA of HW-treated and HW x 1-MCP treated 'Delta' fruit was statistically higher than that of control fruit by Day 21, the differences were probably too small to be noticed by the consumer.

1-MCP was the most effective treatment in delaying the changes of TA and pH of both cultivars during ripening (Table 6-16, 6-18); however, the effect was more persistent in 'UFSun' peaches. Reduced catabolism of organic acids in 1-MCP-treated fruit may be attributed to the suppressed ethylene production and, consequently, reduced respiration rate (Table 6-2, 6-4) (Girardi et al., 2005). As ripening progresses, the pH of the fruit generally increases as the TA decreases. The rise in pH was accelerated by HW x MCP treatment in both cultivars (Day 17) (Table 6-16, 6-18). This could be related to the higher respiration rates in those treatments that were observed during low temperature storage (Table 6-2, 6-4).

Incidence of Decay and Pitting

HW treatment was effective in inhibiting decay of 'Delta' peaches in both years (Table 6-19). The rearrangement of epicuticular waxes under high temperature conditions partially or entirely seals the natural openings and barely-visible cracks in the epidermis, thus limiting sites of fungal penetration into the fruit (Fallik et al., 1999; Fallik

et al., 2000). HW treatment can also act directly on the pathogen (cell damage) and indirectly on the fruit host (induction of resistance mechanisms) to suppress incidence of decay (Casals et al., 2010b). However, if the temperature and exposure time is not suitable for the variety, the “melted” wax can run off and cause major injuries to fruit tissues (Lopez–Castañeda J. et al., 2010). HW x 1-MCP treatment was also capable of reducing incidence of decay of ‘Delta’ peaches; however, the decay incidence was greater than that of fruit from HW treatment (Table 6-19). This data suggests that 1-MCP can induce infection when applied at high temperature.

It is possible that differences in skin composition between the two NMF peaches may contribute to the differences in responsiveness to 1-MCP that were observed. Inhibition of ripening by 1-MCP was very transient in ‘Delta’ peaches compared with ‘UFSun’ peaches, even though the 1-MCP concentration used for the former was 3 times more than for the latter. Recently, it has been shown that an intact apple absorbs gaseous 1-MCP at a relatively slow rate compared to a peeled apple (Huber et al., 2010). Secondly, hydrophobic compounds such as lignin, high methoxy pectins and oil have high sorption for 1-MCP. Hence, the hydrophobicity of the peel may naturally influence sorption rate and affect access of 1-MCP to the internal tissue (Choi and Huber, 2009). Limited diffusion across the peel tissue may also be responsible for the observed transient effect of 1-MCP for ‘Delta’ peaches. Tomato fruit partially exposed to aqueous 1-MCP had a more acute ripening inhibitory effect in the external (epidermal) tissues compared with internal tissues (Choi and Huber, 2008).

Pitting was observed only on HW-treated and HW x 1-MCP treated fruit of both cultivars (Table 6-20). Low temperature storage did not induce pitting on peaches in

these experiments since it was not found on the control fruit of either cultivar (Table 6-20). Pitting was also not observed on 1-MCP-treated fruit, indicating that it was caused by high temperature exposure. Pitting did not correlate with incidence of decay as evident on 'UFSun' peaches in 2010 (Table 6-19, 6-20). HW-treated and HW x 1-MCP treated 'UFSun' fruit had the same amount of decay but pitting was found on 57% of the HW x 1-MCP treated fruit compared with 17% of the HW-treated fruit.

It has been shown that non-chilling pitting of 'Navelina' orange and 'Marsh' grapefruit increases when the fruit are initially exposed to low humidity then transferred to a high humidity environment (Alferez et al., 2003; 2010). The low humidity environment results in negative turgor pressure in exposed fruit compared to positive turgor pressure of freshly harvested fruit. Based on these previous reports, it was likely that the HW conditioning in this study predisposed the peach fruit to lose more water after the treatment, such as the case of 'Delta' peaches in 2009 (Table 6-13), which caused a decrease in turgor pressure of the epidermal cells due to dehydration. Apparently, 1-MCP exacerbated the effect of high temperature. Thus, the combined treatment caused higher incidence of pitting (Table 6-20). Fruit pitting may be related to localized programmed cell death because a cysteine protease gene has been identified with peel pitting of navel oranges (Fan et al., 2009; Vaux and Korsmeyer, 1999; Xu and Chye, 1999). Coincidently, peach fruit treated with hot air at 39°C for 3 days also showed increased gene expression of cysteine protease (Lara et al., 2009). Overall, pitting renders fruit unmarketable, but it can be potentially reduced by adding sodium chloride in the water (Obenland and Aung, 1997).

Chapter Conclusion

Pre-storage HW and HW x 1-MCP treatments were more effective than aqueous 1-MCP application in delaying softening of NMF peaches ripened after low temperature storage. Prevention of postharvest decay by HW treatment was cultivar dependent. ‘Delta’ peaches were more suitable than ‘UFSun’ peaches for the temperature and exposure time used for the HW treatment in this study as evidenced by less pitting. Pre-conditioning ‘UFSun’ peaches with 1.5 mg/L aqueous 1-MCP may be a better postharvest practice to maintain fruit quality for this cultivar.

Table 6-1. Effect of pre-storage HW and 1-MCP treatments on ethylene production and respiration rate of 'UFSun' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	14.5	15	17	19
Ethylene production ($\mu L\ C_2H_4/kg \bullet h$)							
Control	15.26a	19.91a	NA	96.67a	162.0ab	107.7ab	79.82ab
HW	5.62c	2.95c	NA	22.65c	139.0b	122.7a	73.54b
1-MCP (100 µg/L)	16.28a	7.84b	NA	76.39b	176.2a	120.5ab	93.14a
HW x 1-MCP	11.17b	6.19b	NA	19.04c	94.04c	101.8b	87.84a
Significance							
Factor A (HW)	**	**	NA	**	**	NS	NS
Factor B (1-MCP)	**	**	NA	**	NS	*	**
A x B	*	**	NA	**	**	NS	NS
Respiration rate ($mg\ CO_2/kg \bullet h$)							
Control	29.71a	68.31a	NA	183.3a	138.3b	161.5a	234.8a
HW	22.73b	29.49c	NA	148.4b	197.5a	153.2b	168.1c
1-MCP (100 µg/L)	32.22a	43.44b	NA	187.3a	159.0b	164.9a	184.9bc
HW x 1-MCP	29.05a	52.43b	NA	145.9b	148.1b	142.2c	220.4ab
Significance							
Factor A (HW)	**	**	NA	**	**	**	NS
Factor B (1-MCP)	*	NS	NA	NS	NS	*	NS
A x B	NS	**	NA	NS	**	*	**

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = Non-significant
NA = not available

Table 6-2. Effect of pre-storage HW and 1-MCP treatments on ethylene production and respiration rate of 'UFSun' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
Ethylene production ($\mu L\ C_2H_4/kg•h$)							
Control	5.29a	5.63a	6.14	83.96a	251.6a	131.7a	101.6b
HW	5.34a	5.24b	6.07	67.88b	235.1a	129.1a	117.5a
1-MCP (1.5 mg/L)	4.38c	4.77c	5.79	61.64c	155.1b	110.9ab	86.46c
HW x 1-MCP	4.77b	4.93bc	5.97	28.40d	120.5c	91.83b	69.63d
Significance							
Factor A (HW)	*	NS	NS	**	*	NS	NS
Factor B (1-MCP)	**	**	NS	**	**	**	**
A x B	NS	*	NS	**	NS	NS	**
Respiration rate ($mg\ CO_2/kg•h$)							
Control	84.52b	86.37	88.60b	304.5a	290.3a	214.0	264.0
HW	96.13a	76.46	87.39b	302.0a	301.0a	219.3	240.8
1-MCP (1.5 mg/L)	70.82c	63.89	93.85a	264.5b	225.3b	264.5	323.5
HW x 1-MCP	82.30b	99.48	96.83a	247.7c	236.5b	250.3	283.8
Significance							
Factor A (HW)	**	NS	NS	NS	*	NS	NS
Factor B (1-MCP)	**	NS	**	**	**	NS	NS
A x B	NS	NS	NS	NS	NS	NS	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 6-3. Effect of pre-storage HW and 1-MCP treatments on ethylene production and respiration rate of 'Delta' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)		
	0	7	14	15	17	19
Ethylene production ($\mu L\ C_2H_4/kg \bullet h$)						
Control	3.30a	0.83b	0.29c	30.98a	61.99a	49.98
HW	0.86d	2.11a	1.02a	14.29b	47.78b	51.85
1-MCP (100 µg/L)	2.69b	0.83bc	0.41b	13.43b	44.94b	60.81
HW x 1-MCP	1.88c	0.63c	0.34bc	6.55c	27.49c	53.10
Significance						
Factor A (HW)	**	**	**	**	**	NS
Factor B (1-MCP)	NS	**	**	**	**	NS
A x B	**	**	**	**	NS	NS
Respiration rate ($mg\ CO_2/kg \bullet h$)						
Control	21.17a	11.88c	14.57b	78.74a	144.60a	97.57
HW	19.83a	27.49a	26.27a	78.28ab	92.98c	85.25
1-MCP (100 µg/L)	16.71b	15.03b	11.45c	49.22c	120.50b	114.20
HW x 1-MCP	16.84b	14.41b	12.12bc	72.90b	83.11c	92.71
Significance						
Factor A (HW)	NS	**	**	**	**	NS
Factor B (1-MCP)	**	**	**	**	**	NS
A x B	NS	**	**	**	NS	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 6-4. Effect of pre-storage HW and 1-MCP treatments on ethylene production and respiration rate of 'Delta' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
Ethylene production ($\mu L\ C_2H_4/kg•h$)							
Control	4.43bc	8.84a	4.50b	187.5a	97.35a	312.1a	288.1a
HW	5.28a	7.87a	5.04ab	188.9a	92.99a	222.3b	234.0b
1-MCP (5 mg/L)	4.28c	4.91b	4.73b	156.2b	83.88b	226.7b	191.9c
HW x 1-MCP	4.84ab	7.41a	5.64a	182.5a	63.93c	213.9b	185.3c
Significance							
Factor A (HW)	**	NS	**	NS	**	*	NS
Factor B	NS	**	*	**	**	**	**
A x B	NS	*	NS	*	*	NS	*
Respiration rate ($mg\ CO_2/kg•h$)							
Control	68.73b	71.92c	64.40	184.6	196.9a	217.1a	250.2a
HW	69.68b	71.04c	63.54	195.0	155.7c	162.5c	172.5b
1-MCP (5 mg/L)	74.90b	80.15b	60.17	186.3	153.2c	180.5b	206.1ab
HW x 1-MCP	86.17a	88.39a	69.04	206.7	185.0b	177.7bc	181.9ab
Significance							
Factor A (HW)	*	NS	NS	NS	NS	**	**
Factor B (1-MCP)	**	**	NS	NS	*	NS	NS
A x B	*	NS	NS	NS	**	**	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 6-5. Effect of pre-storage HW and 1-MCP treatment on flesh firmness of 'UFSun' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	14.5	15	17	19
Firmness (N)							
Control	23.47a	16.54bc	NA	11.53b	12.27b	11.84ab	9.82
HW	17.18b	27.69a	NA	20.41a	13.68ab	10.10b	10.30
1-MCP (100 µg/L)	16.25b	13.24c	NA	12.77b	12.73b	10.00b	10.46
HW x 1-MCP	20.53ab	18.61b	NA	14.29b	16.70a	14.05a	11.10
Significance							
Factor A (HW)	NS	**	NA	**	*	NS	NS
Factor B (1-MCP)	NS	**	NA	NS	NS	NS	NS
A x B	**	NS	NA	**	NS	**	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = Non-significant
NA = not available

Table 6-6. Effect of pre-storage HW and 1-MCP treatments on flesh firmness of 'UFSun' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C in 2010

Year 2010	Storage days (0°C)			Ripening days (20 ºC)			
	0	7	14	15	17	19	21
Firmness (N)							
Control	29.35	26.40b	29.26	28.77	19.35c	13.63c	14.74ab
HW	28.88	29.57a	31.25	28.02	19.48c	14.70bc	17.86a
1-MCP (1.5 mg/L)	29.94	28.87a	29.87	28.71	22.43b	15.38b	13.01b
HW x 1-MCP	28.26	29.85a	29.35	32.03	25.48a	19.91a	18.50a
Significance							
Factor A (HW)	NS	**	NS	NS	*	**	**
Factor B (1-MCP)	NS	*	NS	NS	**	**	NS
A x B	NS	NS	NS	NS	*	**	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = Non-significant

Table 6-7. PME and PG activities of 'UFSun' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C after pre-storage conditioning treatments in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
PME Activity (Unit) [m]							
Control	0.33	1.02	0.81	1.23	0.37b	0.44b	1.18bc
HW	0.46	1.28	0.90	0.85	4.30a	1.13b	2.47ab
1-MCP (1.5 mg/L)	0.63	1.53	0.34	1.32	3.95a	2.21a	0.40c
HW x 1-MCP	0.63	1.60	0.62	0.71	0.66b	0.75b	3.35a
Significance	NS	NS	NS	NS	*	*	*
Endo-PG Activity (Unit)[y]							
Control	1.15	NA	NA	NA	0.75	1.44	0.86
HW	0.74	NA	NA	NA	1.09	0.00	1.66
1-MCP (1.5 mg/L)	0.40	0.00	NA	0.87	0.80	0.94	0.73
HW x 1-MCP	0.46	NA	0.12	NA	0.50	0.80	0.65
Significance	NS				NS	NS	NS

[m] = 1 unit of PME activity = ΔA_{620} mg^{-1} protein min^{-1}

[y] =1 unit of PG activity = 1 µg galacturonic acid mg-$^{-1}$ protein h^{-1}

* = Significant at $p \leq 0.05$

NS = non-significant

NA = no enzyme activity detected

Table 6-8. Effect of pre-storage HW and 1-MCP treatments on flesh firmness of 'Delta' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)		
	0	7	14	15	17	19
Firmness (N)						
Control	34.07	28.77	34.98	27.00b	25.38	23.43
HW	36.62	33.13	35.85	36.15a	20.86	20.41
1-MCP (100 µg/L)	35.02	31.66	32.49	26.11b	25.31	20.93
HW x 1-MCP	35.51	30.38	28.34	30.30b	26.72	22.66
Significance						
Factor A (HW)	NS	NS	NS	**	NS	NS
Factor B (1-MCP)	NS	NS	NS	NS	NS	NS
A x B	NS	NS	NS	NS	NS	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 6-9. Effect of pre-storage HW and 1-MCP treatments on flesh firmness of 'Delta' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
Firmness (N)							
Control	47.17	45.66b	49.81ab	44.6c	35.50b	23.58b	16.46b
HW	50.54	47.12a	50.99a	48.82b	34.63bc	30.18a	22.48a
1-MCP (5.0 mg/L)	51.31	47.62a	46.47bc	52.1a	34.51c	23.63b	17.16b
HW x 1-MCP	48.77	45.77b	45.59c	52.51a	36.73a	25.46a	20.02a
Significance							
Factor A (HW)	NS	NS	NS	**	*	**	*
Factor B (1-MCP)	NS	NS	**	**	NS	**	NS
A x B	NS	**	NS	*	**	**	NS

* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$
NS = non-significant

Table 6-10. PME and PG activities of 'Delta' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C after pre-storage conditioning treatments in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
PME Activity (Unit) [m]							
Control	0.99	1.34	0.67	0.27	1.00	0.36b	0.32
HW	1.23	0.17	0.28	0.31	0.75	0.34b	0.49
1-MCP (5 mg/L)	1.24	0.27	0.86	0.59	0.65	0.69a	0.39
HW x 1-MCP	1.21	0.95	0.67	0.60	0.85	0.22b	0.28
Significance	NS	NS	NS	NS	NS	*	NS
Endo-PG Activity (Unit)[y]							
Control	1.44a	0.80	0.24	0.55	0.45	0.41	0.23
HW	0.87ab	NA	0.48	NA	0.06	0.25	0.21
1-MCP (5 mg/L)	0.46b	0.04	0.95	0.32	0.28	0.37	0.67
HW x 1-MCP	0.24b	0.00	0.04	NA	0.28	NA	0.32
Significance	*		NS		NS		NS

[m] = 1 unit of PME activity = ΔA_{620} mg^{-1} protein min^{-1}

[y] =1 unit of PG activity = 1 µg galacturonic acid mg-$^{-1}$ protein h^{-1}

* = Significant at $p \leq 0.05$

NS = non-significant

NA = no enzyme activity detected

Table 6-11. Physical characteristics of 'UFSun' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C after pre-storage conditioning treatments in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	14.5	15	17	19
GChº							
Control	66.62	72.06	NA	66.30c	70.31b	69.57	69.66
HW	68.57	69.63	NA	70.70ab	73.48a	70.08	68.92
1-MCP (100 µ/L)	68.00	69.62	NA	68.23bc	69.88b	68.16	67.19
HW x 1-MCP	70.33	73.77	NA	72.53a	72.57ab	70.84	68.43
Significance	NS	NS		*	*	NS	NS
FChº							
Control	79.49	77.81	NA	80.76	78.13	77.15	77.03a
HW	79.54	81.85	NA	80.47	77.19	73.07	64.04b
1-MCP (100 µ/L)	78.90	79.89	NA	77.83	78.82	76.06	75.30a
HW x 1-MCP	80.91	81.43	NA	82.09	80.49	73.05	76.02a
Significance	NS	NS		NS	NS	NS	*
WL (%)							
Control	0.00	0.21	NA	1.08	3.06	5.30	9.33
HW	0.00	0.23	NA	1.41	2.97	5.74	9.05
1-MCP (100 µ/L)	0.00	0.16	NA	0.97	2.97	5.20	9.15
HW x 1-MCP	0.00	0.34	NA	1.67	3.26	4.82	8.39
Significance	NS	NS		NS	NS	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant
NA = not available

Table 6-12. Physical characteristics of 'UFSun' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C after pre-storage conditioning treatments in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
GChº							
Control	88.10a	85.80	79.35c	75.19b	76.88bc	68.26c	69.48
HW	89.79a	85.19	83.22b	75.97b	74.87c	74.43a	72.44
1-MCP (1.5 mg/L)	89.15a	84.40	86.01a	87.02a	81.43a	70.53bc	70.63
HW x 1-MCP	84.99b	83.00	85.52a	85.11a	80.85ab	71.73b	70.90
Significance	*	NS	*	*	*	*	NS
FChº							
Control	78.47	83.41b	81.83c	81.91c	76.18b	75.48b	76.95a
HW	80.26	83.83b	85.07a	82.54bc	75.62b	74.91b	76.67a
1-MCP (1.5 mg/L)	78.80	82.22c	83.25b	83.63a	75.33b	69.24c	70.13b
HW x 1-MCP	79.83	86.21a	85.50a	82.98ab	82.37a	78.03a	75.12a
Significance	NS	*	*	*	*	*	*
WL (%)							
Control	0.00	6.82	13.74ab	15.89a	19.22a	20.56	21.36
HW	0.00	5.16	11.16b	13.10b	15.92b	18.43	20.71
1-MCP (1.5 mg/L)	0.00	7.44	14.74a	16.60a	19.67a	20.97	22.84
HW x 1-MCP	0.00	6.83	14.28a	16.17a	20.11a	22.79	26.12
Significance	NS	NS	*	*	*	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 6-13. Physical characteristics of 'Delta' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C after prestorage conditioning treatments in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)		
	0	7	14	15	17	19
GChº						
Control	88.17	86.17	85.28	81.66b	80.39	77.36a
HW	86.70	88.32	87.28	85.06ab	80.23	78.96a
1-MCP (100 µg/L)	86.39	84.10	87.07	86.17a	83.29	76.99ab
HW x 1-MCP	88.29	88.84	87.10	86.48a	80.91	74.28b
Significance	NS	NS	NS	*	NS	*
FChº						
Control	88.50	87.28	88.21ab	86.58	85.59a	78.09a
HW	85.60	88.27	88.40a	87.03	70.43c	61.55b
1-MCP (100 µg/L)	87.61	88.19	89.23a	85.95	80.51ab	74.96a
HW x 1-MCP	86.82	86.77	86.13b	85.29	78.30b	76.29a
Significance	NS	NS	*	NS	*	*
WL (%)						
Control	0.00	1.72	3.20	4.18	5.17	8.02b
HW	0.00	1.65	3.08	4.02	6.99	10.61a
1-MCP (100 µg/L)	0.00	1.56	2.97	3.60	6.41	7.81b
HW x 1-MCP	0.00	1.50	2.95	3.90	7.16	10.08a
Significance	NS	NS	NS	NS	NS	*

* = Significant at $p \leq 0.05$
NS = non-significant

Table 6-14. Physical characteristics of 'Delta' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C after pre-storage conditioning treatments in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
GChº							
Control	92.14	90.92	91.58	91.98	84.58	81.30	79.34b
HW	91.57	92.38	90.50	90.20	82.58	81.32	81.34a
1-MCP (5.0 mg/L)	91.24	90.93	91.77	89.26	84.98	79.88	79.13b
HW x 1-MCP	91.40	90.43	91.54	90.54	83.95	80.54	78.91b
Significance	NS	NS	NS	NS	NS	NS	*
FChº							
Control	87.81d	87.85	88.51b	88.64	86.44	86.24a	86.28
HW	89.36a	88.42	90.06a	88.89	85.37	85.97a	86.15
1-MCP (5.0 mg/L)	89.03b	88.26	88.50b	89.25	86.42	82.58c	85.34
HW x 1-MCP	88.66c	88.24	88.01c	89.88	86.05	84.70b	85.95
Significance	*	NS	*	NS	NS	*	NS
WL (%)							
Control	0.00	1.84	3.91b	4.54b	7.02ab	8.63	11.31
HW	0.00	1.76	3.72b	4.47b	6.08b	7.46	11.71
1-MCP (5.0 mg/L)	0.00	1.23	3.20b	4.84b	5.29b	6.48	9.69
HW x 1-MCP	0.00	2.32	5.26a	6.31a	8.01a	9.70	8.04
Significance	NS	NS	*	*	*	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 6-15. Chemical characteristics of 'UFSun' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C after pre-storage conditioning treatments in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	14.5	15	17	19
SSC (%)							
Control	11.30	10.45	NA	13.90	12.30	12.65	12.30
HW	12.90	12.80	NA	11.00	11.30	12.65	13.05
Significance	NS	NS		*	NS	NS	*
TA (%)							
Control	0.441	0.498	NA	0.330	0.302	0.262	0.238
HW	0.392	0.530	NA	0.427	0.283	0.244	0.224
Significance	NS	NS		NS	NS	NS	*
pH							
Control	4.52	4.40	NA	4.55	4.52	3.90	4.88
HW	4.54	4.46	NA	3.95	4.99	4.69	5.28
Significance	NS	NS		*	NS	*	NS

* = Significant at $p \leq 0.05$
NS = non-significant
NA = not available

Table 6-16. Chemical characteristics of 'UFSun' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C after pre-storage conditioning treatments in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
SSC (%)							
Control	9.15c	11.37a	10.45	12.50a	11.53	11.40a	12.03
HW	11.60a	10.60b	10.77	10.95b	11.47	11.50a	13.13
1-MCP (1.5mg/L)	10.27b	9.95c	11.47	10.75b	11.30	11.80a	12.67
HW x 1-MCP	11.35a	11.50a	11.30	11.23b	10.73	10.43b	12.77
Significance	*	*	NS	*	NS	*	NS
TA (%)							
Control	0.61	0.59ab	0.60c	0.70a	0.52b	0.46c	0.39b
HW	0.63	0.53b	0.63bc	0.56c	0.54b	0.51b	0.44a
1-MCP (1.5mg/L)	0.70	0.65a	0.68a	0.58bc	0.66a	0.55a	0.44a
HW x 1-MCP	0.64	0.59ab	0.67ab	0.62b	0.54b	0.49bc	0.47a
Significance	NS	*	*	*	*	*	*
pH							
Control	4.05	4.06	4.15a	4.02c	4.16c	4.40a	4.51
HW	4.07	4.15	4.15a	4.21a	4.22b	4.29c	4.55
1-MCP (1.5mg/L)	4.04	4.06	4.06b	4.14b	4.17bc	4.26c	4.52
HW x 1-MCP	4.07	4.17	4.13a	4.17ab	4.30a	4.35b	4.57
Significance	NS	NS	*	*	*	*	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 6-17. Chemical characteristics of 'Delta' peaches during 14 days of storage at 0°C plus 5 days of ripening at 20°C after pre-storage conditioning treatments in 2009

Year 2009	Storage days (0°C)			Ripening days (20°C)		
	0	7	14	15	17	19
SSC (%)						
Control	10.13	10.73	10.40	10.13	8.93	10.30
HW	10.80	10.67	10.40	10.10	9.60	11.20
Significance	NS	NS	NS	NS	NS	NS
TA (%)						
Control	0.52	0.55	0.59	0.47	0.42	0.43
HW	0.58	0.5	0.55	0.48	0.52	0.36
Significance	NS	NS	NS	NS	*	NS
pH						
Control	3.97	4.10	4.28	4.34	4.17	4.10
HW	4.01	3.96	4.30	4.37	4.14	4.18
Significance	NS	NS	NS	NS	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 6-18. Chemical characteristics of 'Delta' peaches during 14 days of storage at 0°C plus 7 days of ripening at 20°C after pre-storage conditioning treatments in 2010

Year 2010	Storage days (0°C)			Ripening days (20°C)			
	0	7	14	15	17	19	21
SSC (%)							
Control	10.30	10.70a	11.00	10.55b	10.80	10.25	10.85
HW	9.45	10.80a	10.13	11.00ab	10.70	10.60	10.20
1-MCP (5.0 mg/L)	10.00	10.65a	10.75	10.40b	9.90	10.30	10.55
HW x 1-MCP	10.47	9.67b	10.53	11.33a	10.87	10.57	10.57
Significance	NS	*	NS	*	NS	NS	NS
TA (%)							
Control	0.52a	0.52	0.57a	0.47b	0.47ab	0.44	0.46b
HW	0.44b	0.51	0.49b	0.49b	0.43b	0.46	0.47ab
1-MCP (5.0 mg/L)	0.52a	0.52	0.55a	0.55a	0.49a	0.42	0.48a
HW x 1-MCP	0.52a	0.45	0.43c	0.49b	0.45bc	0.43	0.48a
Significance	*	NS	*	*	*	NS	*
pH							
Control	4.03b	3.92b	3.95d	4.06a	4.11b	4.15	4.16
HW	4.21a	4.09a	4.08b	4.04a	4.007b	4.19	4.21
1-MCP (5.0 mg/L)	4.00b	3.97b	4.02c	3.96b	4.10b	4.15	4.27
HW x 1-MCP	4.04b	4.13a	4.22a	4.03a	4.20a	4.16	4.32
Significance	*	*	*	*	*	NS	NS

* = Significant at $p \leq 0.05$
NS = non-significant

Table 6-19. Incidence of decay of pre-conditioned NMF peaches after 14 days of storage at 0°C plus 5-7 days of ripening at 20°C

Year	2009			2010	
Cultivar	'UFSun'	'Delta'		'UFSun'	'Delta'
Decay (%)			Decay (%)		
Control	5.0	31.3	Control	8.8	8.8
HW	16.3	3.1	HW	15.0	2.1
1-MCP (100 µg/L)	7.5	25.0	1-MCP (1.5 or 5 mg/L)	8.3	10.4
HW x 1-MCP	17.5	6.3	HW x 1-MCP	12.5	5.8

Table 6-20. Incidence of pitting of pre-conditioned NMF peaches after 14 days of storage at 0°C plus 7 days of ripening at 20 ºC in 2010

Cultivar	'UFSun'	'Delta'
Pitting (%)		
Control	0	0
HW	17	10
1-MCP	0	1
HW x 1-MCP	52	24

CHAPTER 7
SUMMARY AND CONCLUSIONS

In the first study of this work, the effects of maturity, ripening and low temperature storage on peach fruit quality were explored with the aim to determine the optimum harvest maturity and maturity indices for low-chill, subtropical MF and NMF peach varieties. Two MF cultivars, ‘Flordaprince’ and ‘TropicBeauty’, and two NMF cultivars, ‘UFSun’ and ‘Gulfking’, were evaluated. Fruit were sorted into different maturity groups (MG) at harvest based on their initial ground color a* value (GCa*). Fruit quality factors were determined after ripening at 20°C for 1 week or following storage at 0°C for 2 weeks.

The changes in peel GC and flesh color (FC) of both the MF and NMF cultivars from green to red or orange as harvest maturity advanced or during fruit ripening were indicated by the increases in a* values. Larger increases in the FCa* of both NMF cultivars were detected during ripening following 0°C storage than during 20°C storage. Since no browning was observed, this feature was not considered to be a symptom of chilling injury (CI).

The flesh firmness of the MF cultivars dropped markedly as the ‘melting’ process began, but the flesh firmness of the NMF cultivars decreased relatively slowly as ripening progressed. The NMF peaches were approximately 3 to 5 times firmer than the MF peaches when both were at the full ripe stage. Cell wall synthesis and degradation of ‘Flordaprince’ and ‘Gulfking’ might have overlapped when ripening was initiated because softening of these two cultivars began prior to attainment of full size. ‘UFSun’ peaches were most susceptible to CI since abnormal softening occurred in fruit of that cultivar from the lower MG after ripening following low temperature storage.

The soluble solids content (SSC) and total sugar (TS) of all the cultivars were generally little affected by harvest maturity and ripening. The SSC content varied between 10 to 13% among the cultivars, with ‘TropicBeauty’ being the highest. The SSC/TA increased as harvest maturity advanced and during ripening for all of the cultivars, primarily due to gradual decreases in titratable acidity (TA). Most of the fruit in the lower MG for all of the cultivars attained SSC/TA of ≥15, the minimum acceptable SSC/TA for “ready-to-eat” fruit, after ripening following low temperature storage (Beckman and Krewer, 1999; Kader and Mitchell, 1989). Thus, the prolonged low temperature storage period may have actually been beneficial to taste quality by increasing the perceived sweetness of those peaches that were not susceptible to CI.

Optimum harvest maturity of the MF and NMF cultivars was defined based on their initial flesh firmness and fruit quality after ripening at 20°C – either immediately after harvest or following 0°C storage. MF cultivars usually require an initial flesh firmness of at least 45 N to prevent excessive softening during shipping and to allow proper softening after transfer to ambient temperature for ripening (Beckman and Krewer, 1999; Kader and Mitchell, 1989). Initial flesh firmness levels of 14 N and 27 N were considered to be suitable for NMF peaches destined for local and distant markets, respectively (Metheney et al., 2002; Rouse et al., 2004). Flesh firmness of 8.8 to 13.2 N, TA < 0.8%, and 10 to 11% SSC or 15% SSC/TA were minimum quality standards recommended for ripe MF fruit, but also considered appropriate for ripe NMF fruit when selecting the range of ideal harvest maturity (Kader et al., 1982; Robertson et al., 1990a).

The results of this study suggest that NMF peaches for fresh (i.e., local) consumption can be harvested at more advanced stages (MG 15-20) than MF peaches (MG 5-10). ‘UFSun’ should be harvested at more advanced stages (MG 10-15) than the other three cultivars (MG 0-10) to avoid CI in low temperature storage.

The maturity indices for all of the cultivars were developed based on linear correlations between fruit maturity and fruit quality factors determined at harvest. The GCa* and TA were the most reliable maturity indicators for all of the cultivars. The FCa* can used as a secondary indicator for MF peaches while pH can be used specifically for NMF cultivars when the peel GC is not visible due to excessive peel blush coverage.

Ethylene production and respiration rates of these MF and NMF peaches harvested at different developmental stages were measured during 5 days at 20°C. The NMF peaches generally produced ethylene at higher rates than the MF peaches at harvest because the latter were mostly preclimacteric or at onset of ripening when harvested at appropriate maturity stages. Thus, the ripening process for the NMF cultivars had started prior to harvest and the fruit at more advanced stages could quickly become postclimacteric during ripening. Peaches at the postclimacteric stage are more prone to physical damages and decay than less ripe fruit because they may already be at the ‘ready-to-eat’ stage at harvest. Therefore, the optimum harvest maturity for NMF peaches intended for immediate consumption should be selected to avoid fruit that are either immature or at the full-ripe stage.

Examination of endo-polygalacturonase (endo-PG), exo-PG, and pectin methylesterase (PME) activities of both MF and NMF cultivars was carried out after 5 days of storage at 20°C. The endo-PG activities were similar for the MF and NMF

cultivars although the texture of the two types was significantly different. The exo-PG and PME activities did not correlate with the flesh firmness of either MF and NMF cultivars. Therefore, PG and PME activities may not be directly related to peach fruit softening, at least as it was measured in this study.

In a second study, I focused on the potential use of different pre-storage conditioning methods, including hot water treatment, aqueous 1-methylcyclopropene application (1-MCP), or a combination of the two, on quality maintenance of NMF peaches during ripening at 20°C or following low temperature (0°C) storage. Results from a preliminary study showed that a NMF cultivar treated with 46°C water for 30 min or 25°C water containing 100 µg/L 1-MCP for 30 min had the best firmness retention among other combinations after ripening at 20°C for 3 days. Thus, NMF 'UFSun' and NMF 'Delta' peaches were immersed for 30 min in 25°C (Control) or 46°C (HW) water, or 25°C water containing 100 µg/L 1-MCP (1-MCP), or 46°C water containing 100 µg/L 1-MCP (HW x 1-MCP).

Two trends of ethylene production were observed in the HW-treated fruit of both cultivars during ripening at 20°C for 1 week or following 2 weeks of 0°C. The ethylene production of the HW-treated fruit was initially suppressed, then recovered to a level similar to the control fruit, or it was suppressed throughout the entire ripening period. It has been reported that CI in nectarines is linked to inhibition of ethylene synthesis after prolonged low temperature storage (Dong et al., 2001). HW-treated 'Delta' peaches maintained higher ethylene production than the other cultivars during low temperature storage, which could help protect the fruit from CI. An immediate rise in respiration rate of HW-treated 'UFSun' peaches was observed at the beginning of both storage

conditions. The temporary rise in respiration rate after the HW treatment may be attributed to the cyanide insensitive respiratory pathway (Inaba and Chachin, 1989). The peak respiration rates of the HW-treated NMF peaches were delayed, reduced, or a combination of the two during ripening in both storage conditions.

Treatment with 100 µg/L 1-MCP reduced ethylene production and respiration rate of ‘UFSun’ peaches during ripening at 20°C, but it had no effect on ‘Delta’ peaches. This suggests that sensitivity to 1-MCP is cultivar dependent, which may be related to the differences in terms of ratio, expression pattern, and turnover of the ethylene receptors, and mechanisms leading to altered chemical binding of 1-MCP (Cin et al., 2006; Ziliotto et al., 2008). Since100 µg/L 1-MCP was unable to inhibit softening of both NMF cultivars during ripening at 20°C or following low temperature storage, 1.5 mg/L 1-MCP was applied to ‘UFSun’ peaches during the second year of the study, after its effect had been investigated on a MF cultivar obtained from California. In addition, 5.0 mg/L 1-MCP was applied to ‘Delta’ peaches due to the high levels of climacteric ethylene production measured in freshly harvested fruit before the treatments were applied. When those high concentrations of 1-MCP were applied, the reduction of ethylene biosynthesis during ripening of both NMF cultivars at 20°C storage or following 2 weeks of 0°C storage was generally prolonged, but the response in terms of respiration rate was cultivar dependent.

The HW x 1-MCP treatment was the most effective pre-storage conditioning method for suppressing ethylene production and respiration rate of both NMF cultivars during ripening for both storage conditions. The ethylene production and respiration rate of the HW x 1-MCP treated fruit either was similar to that of the 1-MCP-treated fruit or

was at an intermediate level between that of the 1-MCP-treated and HW-treated fruit. This suggests that the ethylene signaling pathway may be more important than the ethylene biosynthesis pathway in controlling the ripening process of peaches.

The HW treatment was more effective than the 1-MCP treatment in delaying the softening of the NMF peaches during ripening at 20°C or following low temperature storage. The effect of cultivar dominated the response of the NMF peaches to the HW treatment. For example, softening inhibition of HW-treated 'UFSun' peaches occurred throughout 5 days of storage at 20°C, whereas the effect lasted only 3 days in HW-treated 'Delta' peaches during both years of this study. The temporary suppression of ethylene production in HW-treated 'UFSun' peaches along with prolonged softening inhibition during ripening at 20°C suggests that HW stress may inhibit cell wall catabolism by regulating both ethylene-dependent (i.e., 1-MCP-responsive) and ethylene-independent pathways (Hayama et al., 2006b).

The 1.5 mg/L 1-MCP treatment delayed softening of 'UFSun' peaches by only 1 day, while the 5 mg/L 1-MCP treatment did not inhibit softening of 'Delta' peaches. Hence, repeated 1-MCP applications or even higher 1-MCP concentrations may be necessary to achieve prolonged firmness retention of these cultivars. The effect of 1-MCP was more significant when it was combined with low temperature storage. The firmness retention of 'UFSun' and 'Delta' peaches treated with 1.5 or 5mg/L 1-MCP, respectively, persisted 4 days and 1 day more during ripening after low temperature storage relative to 20°C storage.

The HW x 1-MCP treatment was more effective than the HW treatment in retaining the firmness of 'UFSun' peaches, but HW and HW x 1-MCP were equally effective in

retaining the firmness of ‘Delta’ peaches during ripening after low temperature storage. The softening inhibition of both NMF cultivars during ripening at 20°C could be mainly attributed to the influence of the HW treatment. In contrast, following low temperature storage, firmness retention was due to the strong interaction between HW and 1-MCP.

Transient reduction or promotion of weight loss (WL) by all the treatments during ripening at 20°C and by the HW and HW x 1-MCP treatments following 0°C storage were found in both cultivars. However, the differences in WL between the HW and 1-MCP treatments and the control fruit generally became non-significant as ripening progressed. Therefore, WL differences cannot be used to explain the texture differences created by those treatments in the two NMF peach cultivars.

Peak PME activity in the HW-treated fruit of both cultivars either occurred earlier or the magnitude was higher than that of the control fruit during ripening at 20°C or following low temperature storage. Those earlier peak or relatively high PME activities may be related to the firmer texture of the HW-treated fruit due to promotion of bonding between endogenous Ca^{2+} and pectin in the cell wall and middle lamella (Koukounaras et al., 2008; Steiner et al., 2006). However, a delay in peak endo-PG activity occurred in the HW fruit of both cultivars during ripening in both storage conditions. This could be attributed to inhibition of mRNA synthesis and/or restriction of the enzyme to access the reaction site. The exo-PG activities of both cultivars were not affected by the HW treatment.

Different patterns of PME and PG activities were observed for the two NMF peach cultivars treated with 1-MCP during ripening in both storage conditions. The peak PME activity of 1-MCP-treated ‘UFSun’ fruit was similar to that of the HW-treated fruit. PME

activity was either advanced or the magnitude was higher than that of the control fruit. The peak endo-PG activity in 1-MCP-treated 'UFSun' fruit occurred on the same day as in the control fruit and with same magnitude. The peak PME activity for 1-MCP-treated 'Delta' fruit was also higher than that of the control fruit during ripening at 20°C, but was delayed following low temperature storage. The peak endo-PG activity of 1-MCP-treated 'Delta' fruit during ripening was either suppressed or delayed. These results suggest that PME may be more important than endo-PG in regulating cell wall modification of 'UFSun' peaches, while a concerted action between PME and endo-PG may be involved in the texture change in 'Delta' peaches during ripening. This is supported by the HW x 1-MCP treated fruit in that the treatment only delayed the PME activity of 'UFSun' fruit, but suppressed both the PME and endo-PG activities of 'Delta' peaches during ripening following low temperature storage.

Both HW and 1-MCP treatments inhibited changes of hue in the peel (GCh°) and flesh (FCh°) of the NMF cultivars while the control fruit exhibited constant decline in h° during ripening at 20°C or following low temperature storage. However, the same treatments accelerated red color development in both the peel and flesh as shown by significant decreases in GCh° and FCh°. Red coloration in the flesh was intensified by 2 weeks of 0°C storage compared to 20°C storage in both HW-treated and 1-MCP-treated fruit although no browning occurred, which would indicate CI. Since flesh reddening has been proposed to be associated with alteration of ethylene biosynthesis (Murray et al., 2007; Budde et al. 2005; (Dong et al., 2001), the induction of red coloration observed in this study may be attributed to the recovery of ethylene biosynthesis (Jiang

et al., 2001; Manganaris et al., 2007). Surprisingly, HW x 1-MCP was the most effective treatment in inhibiting GCh° and FCh° changes during both storage conditions.

The TA and pH of both NMF cultivars were affected more significantly than the SSC by all the treatments. The pH of the NMF peaches was generally inversely related to TA. The TA exhibited no consistent response to HW treatment during ripening in either storage condition. The reduction of TA that sometimes occurred in response to HW stress may be favorable since consumer acceptance was found to be always greater for nectarine cultivars with lower TA regardless of fruit maturity (Iglesias and Echeverria, 2009). 1-MCP delayed the changes in TA and pH of both cultivars during ripening at 20°C and after removal from 0°C storage. This impact was more persistent in ‘UFSun’ peaches than ‘Delta’ peaches. The HW x 1-MCP treatment was as effective as the other treatments in delaying changes of TA of both NMF cultivars, but the influence of HW or 1-MCP was inconsistent.

The HW and HW x 1-MCP treatments reduced the incidence of decay in ‘Delta’ peaches after ripening in both storage conditions. This is in agreement with some previous reports in which it was proposed that HW treatment of peaches causes rearrangement of epicuticular waxes, and this restricts fungal penetration into the fruit by sealing the natural openings and cracks in the epidermis (Fallik et al., 1999; Fallik et al., 2000). The HW treatment might also act directly on the pathogen (cell damage) and indirectly on the fruit host (induction of resistance mechanisms) to suppress incidence of decay (Casals et al., 2010b). The 1-MCP-treated fruit of both NMF cultivars generally had levels of decay that were similar to the control fruit after ripening immediately at

20°C or at 20°C following low temperature storage. Therefore, it can be concluded that 1-MCP had no effect on preventing decay regardless of the concentration applied.

Pitting developed during ripening following low temperature storage and was mainly found on HW-treated and HW x 1-MCP treated fruit, but not on the control or 1-MCP-treated fruit; therefore, pitting was induced by HW treatment during ripening following low temperature storage. It is unclear if the pitting that was observed was damage from HW or if it was CI-related pitting exacerbated by the HW treatment. Different decay patterns in the two NMF peach cultivars treated with HW or HW x 1-MCP leads to the hypothesis that skin composition of peaches may contribute to the responsiveness to 1-MCP treatment (Choi and Huber, 2009). Limited diffusion of 1-MCP across the epidermal tissue may also account for the transient effect of 1-MCP treatment, especially on ‘Delta’ peaches (Choi and Huber, 2008).

In conclusion, HW treatment was the most effective conditioning method for both NMF when they were ripened at 20°C immediately after harvest. Both HW and HW x 1-MCP treatments were suitable for ‘Delta’ peaches destined for low temperature storage. Due to the high incidence of decay and pitting, aqueous 1-MCP was a better pre-storage condition method for ‘UFSun’ peaches, especially with ripening following low temperature storage.

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BIOGRAPHICAL SKETCH

Ming-Wei Sherry Kao was born in 1982, the third of five children of Hung-Chi Kao and Shu-Mei Kao. Sherry was born in Taiwan and moved to Canada with her family when she was 11 years old. She completed her Bachelor of Science in biochemistry at the University of Waterloo, Ontario, Canada. She got interested in postharvest physiology of fruits and vegetables during her senior year in college and thus decided to pursue a master's degree in horticultural sciences. In 2004, she received her Master of Science in horticulture from Auburn University, Auburn, Alabama. Soon after graduating from Auburn University, she began her work towards a doctorate degree in the Horticultural Sciences Department at the University of Florida under the direction of Dr. Jeffrey K. Brecht and Dr. Jeffrey G. Williamson. She received her Ph.D. from the University of Florida in the summer of 2011.