CHANGES IN POLYPHENOLICS AND RESULTANT ANTIOXIDANT CAPACITY IN 'TOMMY ATKINS' MANGOS (*Mangifera indica* L.) BY SELECTED POSTHARVEST TREATMENTS

By

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

UNIVERSITY OF FLORIDA

2005

This thesis is dedicated to the people I love: my parents, my teachers, and my friends.

ACKNOWLEDGMENTS

First of all, I would like to express my heartfelt thanks to my supervisory committee chair, Dr. Stephen Talcott who is an amazing mentor and teacher. His warm personality, professional guidance, and profound knowledge encouraged me to complete this work. Continuing as a doctorate student with him is a glorious opportunity and I feel extremely fortunate to have this opportunity.

I would also like to express my gratitude to my supervisory committee members, Dr. Jeffrey K. Brecht and Dr. Susan Percival for their thoughtful advice, consideration, and support. I am grateful for all their help and encouragement in completing this work.

I thank you my beloved parents from the bottom of my heart for supporting me intellectually, psychologically and physically throughout my life. I will keep trying to be a son they can be proud of. I also want to thank my special fellow laboratory members Dr. Joonhee Lee, Chris, David, Flor, Jorge, Kristine, Lanier, and Lisbeth. I really appreciate their advice, help, and time. I want to give my special thanks to Dr. Lee for her valuable advice and instruction. I am grateful to Sunghee for her encouragement, patience, understanding, and love. I love you so much.

Finally, I would like to thank my country, South Korea, for allowing me to complete my master's degree here at University of Florida by financially supporting me for two years.

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August 2005

Chair: Stephen T. Talcott
Major Department: Food Science and Human Nutrition

Mango (*Mangifera indica* L.) is one of the most important tropical fruits worldwide and is gaining popularity in the US. The demand of mangos far exceeds domestic supply thus extending markets for fruit import, whereby a hot water treatment (HWT) is required against invasive pests. Additionally, controlled atmosphere (CA) storage is sometimes used to preserve quality and extend shelf-life of mango fruit during transportation and storage. Postharvest studies with fresh mangos have primarily focused on quality characteristics and limited data exist for fruit phytochemicals and antioxidant content and their resultant changes during postharvest handling and ripening. Therefore, these studies were conducted to investigate phytochemical and antioxidant changes in fresh mangos as influenced by the stage of fruit ripeness for the application of CA in combination with a HWT.

The first objective was to quantify phytochemical changes and resultant antioxidant capacity in mangos during 4 days storage by varying lengths of hot water immersion

treatment before storage. In this study, unripe mangos (Tommy Atkins) were first subjected to three different HWT (0, 70, 90, and 100 min all at 46˚C) and then stored at 25˚C for 4 days. Phytochemical and antioxidant content was monitored after each HWT and throughout storage. Hot water treatment at 46°C for 70 min increased total hydrolyzable tannin (30%) but did not induce significant changes in gallic acid, total soluble phenolics and antioxidant content. Hot water treatments of >70 min did not affect fruit antioxidant content but resulted in decreases of gallic acid (40%), total hydrolyzable tannins (35%) and total soluble phenolics (25%). No remarkable phytochemical changes associated with ripening were detected during the 4 days storage.

The second objective was to identify and quantify polyphenolics and resultant antioxidant capacity in mango following controlled atmosphere (CA) storage combined with HWT. In this study, unripe mangos were held under three CA conditions with or without a HWT (46°C for 75 min). Controlled atmosphere treatments included CA1 (21% O_2 + 79% N_2), CA2 (3% O_2 + 97% N_2,) and CA3 (3% O_2 + 10% CO_2 + 87% N_2) for 2 weeks at 10˚C. Half of the fruit was immediately collected for analysis after CA storage while remaining fruits were held at 25°C until fully ripe. Analyses for this study included individual and total phenolics by HPLC, total soluble phenolics (Folins assay), antioxidant capacity (ORAC assay), soluble solid content (SSC), titratable acidity (TA), and flesh color. Hot water treatment at 46°C for 75 min before CA storage did not change the phytochemical and antioxidant content of mangos. Fruit in CA1 and CA2 contained equivalent phytochemical and antioxidant levels while CA3 fruit contained significantly higher amounts of gallic acid (12%), hydrolyzable tannins (15%), total soluble phenolics 18%) and antioxidant content (13%) compared to CA1 ($P < 0.05$).

CHAPTER 1
INTRODUCTION

Mango (*Magnifera Indica*.L) is a popular tropical fruit all over the world due to its luscious color, characteristic taste, and excellent nutritional value. Since mango imports to the US have grown due to greatly increased demand and mangos have come down in price, the mango market has shown a rapid growth. For example, between 1996 and 2004, there was 40% increase in mango imports to the US (Saúco, 2004). Even though several cultivars such as Tommy Atkins, Keitt and Kent are commercially cultivated in Florida and Hawaii, the production covers less than 1% of domestic consumption (2,800 MT) in 2004 (FAO, 2004). Now, more than 99% of mangos (278,422 MT) are imported mainly from Mexico (about 70% in 2000) followed by Brazil, Ecuador, Peru, Haiti and Guatemala (Saúco, 2004).

Mango is a valuable source of phenolic compounds that have bitter and astringent taste that improve the characteristic taste of foods and develop food quality such as color and flavor (Grundhofer et al., 2001; Haard and Chism, 1996). Phenolic compounds are the most widely distributed secondary metabolites in all higher plant, which have a phenol with one or more hydroxy substitutions (Hagerman et al., 1998; Robbins, 2003). Several studies reported phenolic compounds found in mango such as quercetin, iso querectin, kaempferol, mangiferin, gallic acid, m-digallic acid, m-trigallic acid, ellagic acid, and hydrolyzable tannin such as gallotannin (El-Ansari et al., 1969; El-Sissi and Saleh, 1965). Especially, gallic acid and hydrolyzable tannins are known as major phenolic compounds found in mango.

Oxidation is a fundamental process for the cells in the body. However, there is a critical side effect of oxidation, generation of free radicals and other reactive oxygen species (ROS) such as peroxyl radical (ROO·), hydroxyl radical (HO·), superoxide ion (O_2^{-}·) and singlet oxygen ($^1O_2$) (López et al., 2003). Free radicals involving oxygen are considered ROS and most ROS are classified as free radicals (Karakaya et al., 2001). Since free radicals have unpaired electron(s) and continuously find another electron to be stable, important cellular components in the body such as DNA and cell membrane could be damaged because cellular respiration is inhibited (Antolovich et al., 2001). However, antioxidant compounds mainly found in fruits and vegetables protect the body from free radicals and other ROS. Polyphenolic compounds abundantly found in mango not only provide various functionalities in foods but also act as a strong antioxidant in the body. Phenolic compounds have a strong antioxidant capacity in quenching and/or neutralizing free radicals by donating hydrogen to reactive free radicals (Karakaya et al., 2001; Fernández-Pachón et al., 2004).

Since mango is a climacteric fruit, which implies a highly perishable nature, several postharvest treatments have been used to keep the quality and extend shelf-life. Mangos from other countries can be infested by fruit flies such as Tephripid fruit flies, so all the imported mangos must go through a thermal quarantine treatment such as a hot water treatment (HWT) to eliminate invasive pests before importing to the US (Jacobi et al., 2001). Controlled Atmosphere (CA) storage is used for fruits and vegetables to prolong their shelf-life and preserve them from quality deterioration (Sanchez-Mata et al., 2003). Since most of mangos consumed in the US (99%) are imported and transportation usually takes a few days to a few weeks, application of CA storage must be considered.

Limited study has shown the relationship between postharvest treatments and polyphenolics in mango, but mostly physiological changes such as color, firmness, shelf-life and ripening rate. Thus, two studies were designed to investigate how postharvest treatments affect polyphenolics and antioxidant capacity in mango. The first study was conducted to figure out the effect of HWT on mango polyphenolics and resultant antioxidant capacity when mangos were subjected to HWT with three different treatment times. The second study was conducted to find out phytochemical changes and resultant antioxidant capacity by CA storage of mango combined with HWT. Controlled atmosphere storage having two different gas compositions was applied to the mangos with a control for 2 weeks. The hypothesis was hot water immersion treatment with varying lengths of time and CA storage with different gas composition combined with HWT will induce phytochemical and antioxidant capacity changes in mango.

The specific objectives in this study were as follows:

1. To quantify phytochemical changes and resultant antioxidant capacity in mangos during 4 days storage by varying lengths of hot water immersion treatment (0, 70, 90 and 110 min) applied before storage.
2. To identify and quantify polyphenolics and resultant antioxidant capacity in mangos following different CA treatments combined with or without hot water immersion treatment.

CHAPTER 2
LITERATURE REVIEW

2-1. Mango Market

Mango (*Magnifera indica*. L) is widely considered as the "king" of tropical fruits due to its world-wide popularity, production, and acreage. Additionally, it is a valuable and economically important tropical fruit throughout the world due to its vibrant colors, characteristic taste, and nutritional composition. Fresh fruit imports to the US and Europe have increased due to increased consumer demands for fresh and processed mango products. In turn, mangos have become an affordable tropical fruit that has found favor with consumers in a variety of foods and beverages.

Mango has been cultivated for about 4,000 years and its production and consumption has gradually increased as its popularity grows. Originating over 4,000 years ago in India and Burma, its cultivation has spread to Malaysia, Eastern Asia, and Eastern Africa (Mitra and Baldwin, 1997). According to history records in the US, mangos began to be cultivated in Cape Sable, Florida in 1833 (Crane and Campbell, 1994; CRFG, 2001). Now, at least 87 countries are known to grow over 26,286,255 MT in 2004 throughout the world (Food and Agricultural Organization [FAO], 2004; Sauco, 2004). Mango production is highest in India, the leading mango producing country at 41% of the world's production (10,800,000 MT), followed by China, Thailand, Mexico, Pakistan, Indonesia, the Philippines, Nigeria, and Brazil (FAO, 2004) (Table 2-1). Other mango producing countries such as Australia, Peru, Venezuela, Haiti, and Dominican Republic also produce and export mangos (DA-AMAS., 2003; Jacobi et al., 2001;

Mossler et al., 2002; Sauco, 2004) (Table 2-1). Asia produces 76.9% of the total world production followed by the Americas (13.8%) and Africa (9%) (Sauco, 2004). Mexico is the leading mango exporter country at 41% of the world market (102,500 MT), followed by Philippines (7.8%) and Pakistan (7.6%) (Sauco 2004). The world's largest mango importing country is the US (Table2-2) and the mango market in the US has steadily grown in response to increasing demand. Mexico is also the leading mango exporter to the US market followed by Brazil, Ecuador, Peru, Haiti and Guatemala (Sauco, 2004). Even though the history of mango production and consumption in the US is relatively short compared to other countries such as Europe and China, now the US is the largest mango importing country due to great demand in the domestic mango market.

Table 2-1. Main mango producing countries in 2004 (www.fao.org)

Rank	Country	Production (MT)
1	India	10,800,000
2	China	3,400,000
3	Thailand	1,750,000
4	Mexico	1,503,010
5	Pakistan	1,072,000
6	Philippines	890,000
7	Brazil	845,000
8	Indonesia	800,000
9	Nigeria	730,000
10	Vietnam	337,000
11	Egypt	327,000
12	Haiti	261,000
13	Bangladesh	243,000
14	Cuba	235,000
15	Madagascar	210,000
16	Democratic Republic of the Congo	200,000
17	Sudan	195,000
18	United Republic of Tanzania	195,000
19	Guatemala	187,000
20	Dominican Republic	180,000

Table 2-2. Global imports of mangos (MT) (Sauco 2004)

Country	1998	1999	2000
USA	197,000	219,000	235,000
Europe	115,000	170,000	172,000
Hong Kong	47,000	33,000	33,000
UAE	39,000	38,000	38,000
Malaysia	21,000	25,000	25,000
Saudi Arabia	14,000	14,000	14,000
Singapore	11,000	14,000	15,000
Japan	9,000	9,000	10,000

Since mangos are grown in tropical climate, they are also cultivated in the southern states in the US. However, Florida is the only state in the United States where agricultural statistics are reported for mangos even though mangos are also cultivated to various extents in Hawaii, California, Texas, and Puerto Rico (Mossler and Nesheim, 2002). More than 80% of Florida mangos are cultivated in Miami Dade County with the remaining acreage in Lee, Palm Beach, and scattered other counties where adequate conditions for growth exist (Mossler et al., 2002). Recently, new mango trees are being planted in Merritt Island in Florida (Mossler and Nesheim, 2002).

Before Hurricane Andrew in 1992, mango production in Florida was over 10,000 MT from 1172 ha (Crane and Balerdi, 1997). However, the production was reduced to 1,250 MT from 648 ha. Recovery from damage was poor and slow because many remaining trees continued dying, damaged bark was uncovered from excessive sunlight and roots of trees got damaged (Crane and Balerdi, 1997). As a result of poor recovery, Florida production is still less than 1% of total consumption in the US (HAP, 2002; Sauco, 2004).

2-2. Mango Cultivars

Among over 1,000 different mango cultivars throughout the world, about 800 mango cultivars have been named (Pandey, 1986). Since each mango growing country possesses different climate, geological feature, harvest time, and marketing season, each country generally has its own major cultivars for commercial use (Nakasone and Paul, 1998; Sauco, 2004) (Table 2-3). For example, Australian production is dominated >95% by a single variety “Kensington” (also known as ‘Kensington pride’) and ‘Tommy Atkins’ and ‘Keitt’ are the major varieties grown in Florida (Crane and Campbell, 1994; Jacobi et al., 1998). Since many cultivars have their own characteristics, each mango is cultivated according to their regional and climatic conditions in their respective countries.

Mango may be divided into two types, which are Indian and Indo-Chinese. Since two types have quite different features, this is a good indicator to classify mangos. For example, Indo-Chinese type mangos have polyembryonic seeds that have multiple embryos while Indian type mangos have monoembryonic seeds (Crane and Campbell, 1997). Indo-Chinese type mango has better resistance to anthracnose caused by *Colletotrichum gloeosporioides Penz*, which is the most important fruit disease in Florida (Crane et al., 1997; Gamagaea et al., 2004; Vivekananthan et al., 2004). Since ‘Tommy Atkins’ mango is an Indo-Chinese type, it is relatively resistant to anthracnose.

Even though it has been reported that about eighty mango cultivars are found in Florida, only a few varieties are commercially cultivated. 'Tommy Atkins', 'Keitt', 'Van Dyke', 'Palmer', 'Kent', 'Haden', 'Sensation', and 'Parvin' are commercially grown varieties in Florida (Crane and Campbell, 1994; Sauco, 2004). Especially, 'Tommy Atkins' and 'Keitt' accounts for >50% of the total production in Florida (Campbell, 1992; Morton, 1987). 'Tommy Atkins' is oval in shape (8 to 15 cm in diameter) and its size ranges from

medium to large at 450 to 750g with a thick, tough adherent skin that is yellow to orange in color (Gilman and Watson, 1993). Even though 'Tommy Atkins' has relatively poor eating quality (rated fair to good) and has fibrous pulp, it is the most widely distributed and commercially variety produced in Florida due to great resistance to anthracnose, tolerance to handling damage and endurance to hot water immersion treatment (Campbell and Campbell, 1993).

Table 2-3. Producing countries, selected cultivars, and main marketing season (Nakasone and Paull, 1998)

Country	Selected Cultivar	Marketing Season
USA-Florida	Keitt, Irwin, Tommy Atkins, Kent, Van Dyke, Palmer	Jul-Aug
Australia	Kensington Pride, Keitt, Kent, Palmer, Irwin	Oct-Mar
India	Alphonso, Banganpalli, Dashehari, Bangalora, Langra, Mulgoa, Neelum, Pairi	Apr-Jul
Brazil	Haden, Tommy Atkins, Kent, Keitt, Palmer, Bourbon, Espada, Itamarco, Caco, Rosa, Carlota	Oct-Feb
Indonesia	Arumanis, Dodol, Gedong, Golek, Cengkir	Sep-Jan
Israel	Keitt, Tommy Atkins, Kent, Maya, Haden	Jul-Aug
Malaysia	Harumanis, Golek, Maha 65, MA 200 (Malgoa)	Jun-Aug
Mexico	Haden, Manila, Esmeralda, Kent, Keitt, Tommy Atkins, Jan Dyke, Palmer	Apr-Oct
The Philippines	Carabao, Pico, Julie	Jun-Sep
South Africa	Peach, Zill, Fascell, Sensation, Tommy Atkins, Keitt	Nov-Jan
Spain	Tommy Atkins, Keitt, Lippens, Osteen	Jul-Aug
Taiwan	Irwin, Yellow No.1, Haden	Jul-Oct
Thailand	Nan Dok Mai, Rad, Tongdum, Okrong	Mar-May

2-3. Mango Ripening

Ripening is a complex process that involves not only many physiological changes but many chemical changes due to the fact that postharvest fruits continue to respire. Mango is a climacteric fruit, which means that its respiration rate rises as fruit ripening proceeds in response to ethylene (Alexander and Grierson, 2002; Silva et al., 2003).

Physiological changes occur as a result of chemical alterations in climacteric fruits during ripening such as a change in pulp and skin color (loss of chlorophylls and synthesis of carotenoids), loss of weight and volume (loss of moisture), decline of firmness (softening caused by pectin breakdown), altered taste (loss of acidity and increased soluble solids due to conversion of starch to sugar) and synthesis of more ethylene (a ripening hormone) (Jacobi et al., 1998; Kader, 1997; Lizada, 1991; Modi and Reddy, 1967).

Since mangos are harvested when they are still green as a climacteric fruit, they are expected to withstand postharvest handling, and then complete their ripening (riper stages generally preferred by consumers) at the fresh market, determining exact ripening indices are important for market consideration. Chemical parameters may include soluble solids contents, acidity, starch content, and phenolic constituents while physical parameters may include shape, size, surface color, and shoulder growth (Mitra and Baldwin, 1997). The most definitive indicator of a mango's maturity is to determine shoulder growth (from green to mid-ripe) and color development (almost all yellow color pulp) (Holmes et al., 1990; Jacobi et al., 1998). Even if mangos are of the same variety and harvested on the same day, they could be different from each other, for example, their shape, color development, and weight. This is because the amount of sunlight, rain, and other environmental factors are different, depending on location on a tree even though fruit was grown on the same tree. In conclusion, accurate standards to distinguish the degree of maturity as well as the degree of ripeness are important for mangos to adjust time for the fruit market.

2-4. Mango Postharvest Handling

2-4-1. Hot Water Immersion Treatment (HWT)

Thermal quarantine treatment is legally required for fresh mangos to be accepted by several importing countries such as the United States and Japan because imported mangos are highly susceptible to infection by fruit flies in the form of adults, larvae, or eggs. In the US, mangos from other countries must go through a thermal quarantine treatment to eliminate invasive pests (e.g. *Ceratitis capitata* (Mediterranean fruit fly) and *Anastrepha spp*., *Anastrepha ludens* (Mexican fruit fly)) before importing to the US (APHIS, 2002). Several methods for quarantine treatments are available for fruits including irradiation, vapor heat, forced hot-air, and hot water immersion treatments (APHIS, 2002). However, only hot water immersion treatment (HWT) is approved in the US to disinfect mango; not only is HWT the most effective treatment, but it has several direct advantages (Jacobi et al., 2001). Advantages include ease of handling and strict control of treatment environments, which allows for exact temperature control during treatment, and less cost as an additional benefit of HWT (about 90% less than vapor heat treatment) (Sharp and Hallman, 1994; Fallik, 2004).

Required HWT conditions such as treatment time, temperature, and water purity are rigidly and specifically developed and controlled by USDA-APHIS (Animal and Plant Health Inspection Service) because fruit fly and/or its larvae and eggs may remain under a variety of storage conditions. The target disinfestation probability in eliminating pests in fruit is over 99.9968% (“probit 9”) in many countries (Jacobi et al., 2000). Since the profit value is so high, every condition for thermal quarantine must be controlled strictly. Legally required time and temperature for HWT in the US is 70, 90, or 110 min immersion at 46°C, so the guidelines published by USDA-APHIS sets these times based

on cultivar, weight, size, shape, and country of origin (USDA-APHIS 2002) (Table 2-4). However, hot water treatment may damage the quality of mangos and cause more internal and external injury than vapor treatment if the treatment temperature is out of optimum range (Jacobi and Wong, 1992).

Table 2-4. Determined dip time based on origin, shape, and weight of fruit. (USDA-APHIS, 2002)

Origin of fruit	Fruit shape	Fruit weight (grams)	Dip time (minutes)
Puerto Rico, U.S. Virgin Islands, or West Indies	Flat, elongated varieties[1]	Up to 400	65
		400 to 570	75
	Rounded varieties[2]	Up to 500	75
		500-700	90
		701-900	110
Mexico or Central America (North of and including Costa Rica)	Flat, elongated varieties[1]	Up to 375	65
		400 to 570	75
	Rounded varieties[2]	Up to 500	75
		500 to 700	90
		701 to 900	110
Panama, South America or West Indies islands of Aruba, Bonaire, Curacao, Margarita, Tortuga, or Trinidad and Tobago	Flat, elongated varieties[1]	Up to 375	65
		375 to 570	75
	Rounded varieties[2]	Up to 425	75
		425 to 650	90

*[1]Cultivars: ‘Frances’, Carrot’, ‘Zill’, ‘Ataulfo’, ‘Carabao’, ‘Irwin’, and ‘Manila’.
*[2]Cultivars: ‘Tommy Atkins’, ‘Kent’, ‘Heyden’, and ‘Keitt’.

An organized process for HWT was also set as a standard by USDA-APHIS. According to the Hot Water Treatment Schedule (APHIS, 2002), mangos must be sorted by origin, weight and shape before HWT. Water quality for washing, dipping, or showering the fruit should be considered before treatment and the water must be chlorinated (50-200 ppm). During the treatment, the fruit must be submerged at least 4 inches (10.16 cm) below the water level at the target temperature. In addition, the water must be constantly circulated and the temperature of the water should be kept at $46^{o}C \pm 0.3^{\circ}C$ throughout the treatment. After HWT, mangos should stand for at least 30 minutes

prior to refrigeration to ensure that all fruit fly larvae would be completely killed during this time. Recommended temperatures for refrigeration are 55-57°F (12.8-13.9°C) at 85 to 90% RH. This storage condition will delay softening and extend storage life to 2 to 3 weeks.

2-4-2. Controlled Atmosphere (CA) Storage

During transport from an exporting country to an importing country by trucks and/or ships, fruits can easily perish unless they are held under proper storage conditions because transport usually takes from a few days to a few weeks (usually 2-3 weeks). Since the main mango exporter for the US market is Mexico followed by Brazil, Ecuador, Peru, Haiti and Guatemala, shipping mangos may vary according to the targeted countries. In order to protect quality, delay ripening, reduce ethylene production and respiration rate, prevent fruit disease, and extend shelf-life during shipping, CA storage is sometimes employed (Abd. Shukor et al., 2000; Rattanapanone and Watada, 2000). Domestic mango production satisfies a small portion (about 1%) of total mango consumption while the mango market is getting larger in the US. Since about 99% of mangos are imported, CA storage should easily be applied to the US mango market as a means to extend shelf-life and keep the quality during transportation and storage.

CA storage is a common method used to preserve fruits and vegetables from quality deterioration and to extend shelf-life. CA storage, an artificially increased CO_2 and decreased O_2 level environment at reduced temperatures, decreases ethylene (ripening hormone) production, reduces sensitivity to ethylene and lessens respiration rate. Thus, it can prolong shelf-life of fruits, postpone chlorophyll degradation, and keep the texture of fruit (Mitra and Baldwin, 1997; Kader et al., 1989; Kader, 2002). Since fruits are still

'alive' even after harvest by continuing to respire (consuming O_2 and producing CO_2), CA storage conditions are effective to inhibit respiration. CA storage also has additional benefits such as maintaining titratable acidity, preserving fruit firmness, and reducing pitting (Wang and Vestrheim, 2002; Chen et al., 1981; Sanchez et al., 2003). Therefore, CA storage can help maintain fruit quality deterioration and it also gives additional advantages to fruits such as extent postharvest shelf-life and delayed ripening.

The optimum O_2 and CO_2 composition and temperature in a CA chamber varies with the cultivar and growing region of fruits. In general, optimum atmosphere composition for mature-green mangos is 3 to 5% of O_2 and 5 to 8% of CO_2 at 12 to 13°C for up to 3 weeks and for tree-ripe mangos is 3 to 5% of O_2 and 5 to 8% of CO_2 at 8°C or 5% O_2 and 10% CO_2 at 5°C for 3 weeks (Bender et al., 2000). These recommended conditions vary due to natural variability of fruit and dynamic response to storage conditions (Saltveit, 2003). Too low O_2 and too high CO_2 levels in CA storage can induce anaerobic respiration and fruit injury (Kader et al., 1989). Anaerobic respiration can cause shortening of shelf-life, increasing susceptibility to decay, producing off-flavors, and leading to physiological disorders (Brecht, 1980; Kader et al., 1989). Furthermore, the symptoms of CO_2 injury (too high CO_2) are development of abnormal and grayish color, prevention of normal aroma development, production of off-flavors, and generation of grey spots. The symptom of O_2 injury (too low O_2) is irreversible inhibition of ripening (chlorophyll decline) (Bender et al., 2000.; Mitra and Baldwin, 1997). Keeping the atmosphere concentration in the optimum range in the CA chamber is important to prevent anaerobic respiration and fruit injury. Thus, atmosphere composition has to be monitored and adjusted to maintain optimum gas concentration.

2-5. Mango Polyphenolics

Mango is a source of phenolic compounds that improve food quality, and prevent food deterioration. Those are gallic acid, gallotannin, p-OH-benzoic acid, and ferulic acid, which are found in mango pulp and skin (Schieber et al., 2000). Phenolic compounds are the most widely distributed plant secondary metabolites and are found in all higher plants (Hagerman et al., 1998). Basically, they have one common structural feature, which is a phenol (an aromatic ring possessing at least one hydroxyl substituent) (Robbins, 2003). Phenolics may be divided into two categories that include polyphenols and simple phenols based on the number of phenol subunits present, for example, polyphenolics have at least two phenol subunits and tannins have at least three phenol subunits. Phenolic acid is a phenol that has one carboxylic acid functional group and is related to color, sensory quality, and antioxidant capacity of foods (Robbins, 2003). Polyphenolics are abundantly found in fruits and vegetables and are important compounds in reducing risks of human diseases and oxidative damage to biological membrane (Kelly et al., 2002; Robbins, 2003). Thus, it is important for human to increase consumption of polyphenolics with antioxidant properties by eating fruits and vegetables (Kang and Saltveit, 2002). Moreover, polyphenolics provide various functionalities, such as color, flavor, and astringency in foods, and polyphenolics are likely to be involved in the plant defense mechanism as a chemical barrier, helping the plant to recover from injured surface (Grundhofer et al., 2001; Haard and Chism, 1996).

Technically, phenolics that have three or more phenol subunits and have an ability to precipitate proteins are categorized as “tannin” and are categorized as either “condensed” or “hydrolyzable” tannins (Hagerman, 2002; Robbins, 2003). Tannins are a specific class of polyphenolics that have been identified, but poorly characterized in

mangos. Generally, tannins have high molecular weights (>1000 amu) and can be found in abundance in oak, tea, sumac, nuts, and some fruits. Several valuable functions of tannin include protein precipitation, cell wall stabilization, insecticides, herbicides, and anti-carcinogenic agents due to their antioxidant capacity (Hartzfeld et al., 2002; Osslpov et al., 1997; Werner et al., 1999). Tannin has about 15-30 times better peroxy radical quenching capacity than simple phenol and Trolox because it has relatively many hydroxyl groups and it is highly polymerized (Hagerman et al., 1998). Condensed tannins are polymeric forms of (+)-catechin and (-)-epicatechin and/or vary from dimers to considerably larger polymeric procyanidins, due to their ability to form cyanidin or delphinidin (prodelphinidins) in the presence of acid and heat. There are two kinds of hydrolyzable tannins: gallotannin and ellagic acid which are derivatives of gallic acid (Hagerman, 2002).

Gallic acid (3,4,5-trihydroxybenzoic acid) is an abundant polyphenolic compound found in mango with gallotannins and it is derived from quinic acid via the shikimic acid pathway (also known as the phenylpropanoid pathway). Gallotannin is the simplest form of hydrolyzable tannin. As a polygalloyl ester of glucose, gallotannin has five hydroxyl groups attached to the core sugar (glucose) (Hagerman, 2002) (Fig 2-1). Gallotannin is divided into two parts, glucose and gallic acid, upon hydrolysis with strong acid and is extended by adding galloyl esters via meta-depside bonding to glucose (Hagerman, 2002; Niemetz et al., 1999) (Fig 2-3).

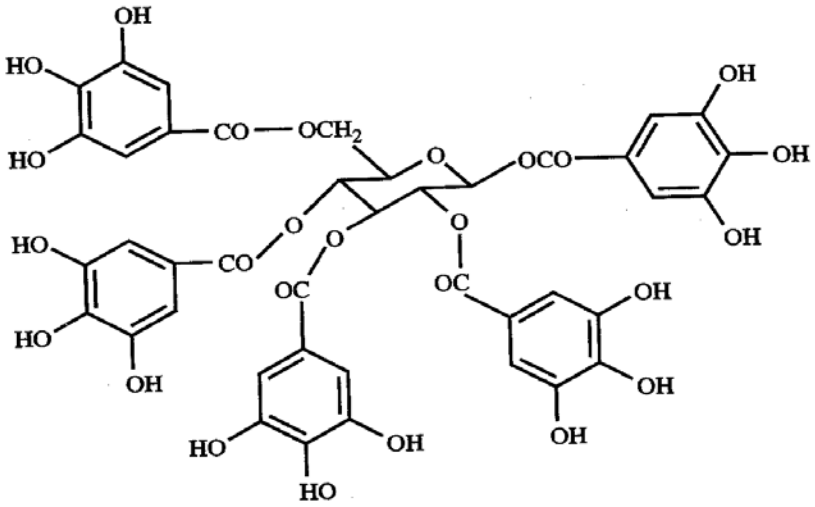


Figure 2-1. Structure of gallotannin (β-1,2,3,4,6-pentagalloyl-O-D-glucose) (Zalewska et al. 1995)

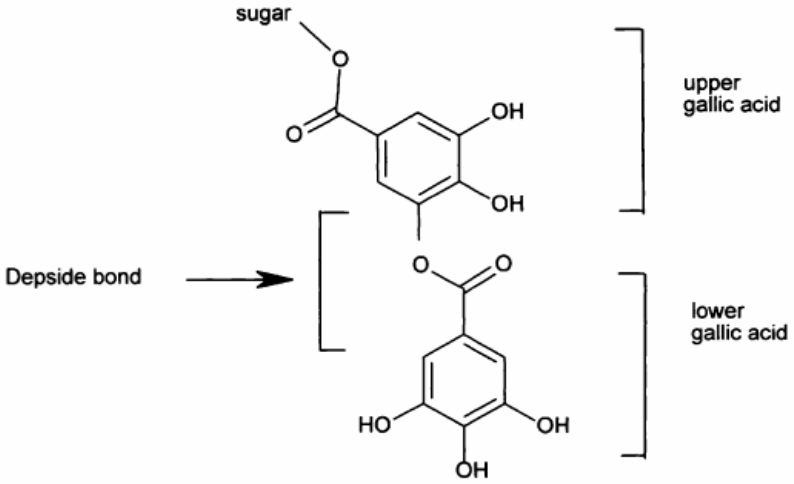


Figure 2-2. A depside bond which is formed between the phenolic group of the upper and the acid group of the lower gallic acid units (Mueller-Harvey 2001)

Many polyphenolics in mango were previously identified. Quercetin and kaempferol were the first polyphenolics to be tentatively identified in mango using 2-dimensional paper chromatography (El- Ansari et al., 1969). Subsequently, El-Ansari et al. (1969) reported the presence of ellagic acid, gallic acid (GA), *m*-digallic acid, *m*-trigallic acid, isoquercetin, mangiferin, quercetin and gallotannin in mango. Gallic acid and gallotannin are known as the major polyphenolic compounds found in mango, which are important antioxidants to quench harmful free radicals (Saleh and El-Ansari,1970).

2-6. Polyphenolics as Antioxidants

Oxygen is necessary for human beings and other living organisms to function and the oxidative mechanism is necessary for the cells to survive in the body. However, when oxygen is transformed to free radicals and ROS (reactive oxygen species), which then act as oxidants that have a tendency to donate their oxygen to other materials, oxygen plays a harmful role in our body (Karakaya et al., 2001). Many ROS are free radicals. Free radicals and other ROS such as peroxy radical, hydroxyl radical, singlet oxygen derived from normal essential metabolic process or other external sources can harm all kinds of biological materials such as proteins, carbohydrates, lipids, and nucleic acid (Karakaya et al., 2001; Fernández-Pachón et al., 2004). In the human body, ROS and free radicals can affect the formation of biological membranes, change enzyme activity, induce abnormal organ metabolism, and influence the generation of cataracts, atherosclerosis, and degenerative diseases (Karakaya and Nehir, 1999; Leitao and Mensor, 1999). ROS and free radicals cause oxidation that induces deterioration of food, resulting in rancidity, changes in color, and declines in nutritional quality, flavor, texture and safety (Antolovich et al., 2002).

Fortunately, antioxidants such as ascorbic acid, tocopherol, carotenoid, and polyphenolics can quench and neutralize free radicals (Shadidi and Naczk, 1995). Consequently, antioxidant capacity also reduces the possibility of several diseases like cancer, stroke, atherosclerosis, and cardiovascular diseases (Karakaya et al., 2001; Shadidi and Naczk, 1995). Antioxidant capacity of phenolic compounds comes from their redox properties in acting as a reducing agents, hydrogen donator, metal chelator and singlet oxygen quencher (Pyo et al., 2004). Even though there are other antioxidant compounds in fruits and vegetables such as ascorbic acid, tocopherol, carotenoids, and

lycopenes, phenolic compounds have stronger radical quenching capacity (Pyo et al., 2004; Toit et al., 2001). For example, flavonoid including multiple functional phenolic groups has 2 to 5 fold stronger radical quenching capacity than ascorbic acid and tocopherol (Toit et al., 2001).

CHAPTER 3
PHYTOCHEMICAL CHANGES AND RESULTANT ANTIOXIDANT CAPACITY IN MANGOS DURING 4 DAY STOGRAGE AFFECTED BY VARYING LENGTHS OF HOT WATER IMMERSION TREATMENT

3-1. Introduction

Mango (*Magnifera indica* L.) has been a popular and economically important tropical fruit throughout the world due to its excellent eating quality (bright color, sweet taste and luscious flavor) and nutritional composition (diverse amount of vitamins, minerals, fiber and various antioxidant compounds). Fresh fruit imports to the US, Europe, and Japan have increased due to increased consumer demand for fresh and processed mango products. In turn, mangos have become an affordable tropical fruit that has found favor with consumers in a variety of foods and beverages. In the US, although mangos are commercially produced in Florida and Hawaii, the amount of production is not enough to meet domestic demands. Thus, mango importation in the US has gradually increased in the market. Since imported mangos can easily be a host for *Ceratitis capitata* (Mediterranean fruit fly) and *Anastrepha spp*., *Anastrepha ludens* (Mexican fruit fly) causing quarantine risks in the form of adults, larvae, or eggs (Jacobi et al., 2001; USDA-APHIS, 2002), all mangos imported to the US must be subjected to a thermal quarantine treatment to eradicate invasive pests. Several methods for quarantine are available such as irradiation, fumigation, vapor heat, forced hot-air, and hot water immersion treatments. However, only the hot water immersion treatment is legally allowed in the US because it is the most effective quarantine method for mangos (Jacobi et al., 2001).

Numerous studies have addressed physiological changes (e.g. heat injury, heat tolerance, ripening velocity, and shelf-life) caused by hot water immersion treatment (HWT). Polyphenolic compounds such as gallic acid and hydrolyzable tannin are found in mango and are important because these phytochemicals have a strong antioxidant capacity and therefore serve to improve food quality. However, limited information concerning phytochemical changes after HWT is available. This study concentrated on phenolic compound changes caused by HWT with varying length of treatment time and resultant antioxidant capacity. The objective of this study was to identify and quantify polyphenolic compounds and antioxidant capacity in fresh mangos after three HWT times for two ripeness stages and its affect on fruit quality.

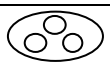
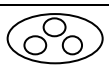
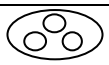
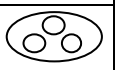
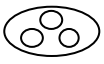
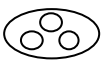
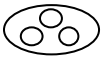
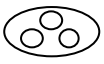
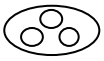
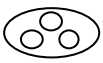
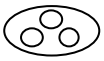
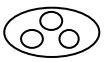
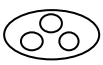
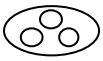
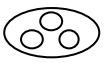
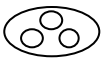
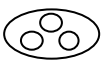
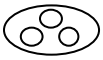
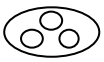
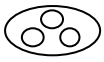
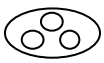
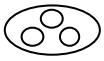
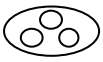
3-2. Materials and Methods

3-2-1. Fruit Preparation and Treatment

Mangos for this study were obtained from Lyons Farms in Homestead, FL in June 2002. Forty mature-green mangos were chosen based on the uniformity of color, size, and weight. Fruits were transported on the day of harvest to the University of Florida and subjected to HWT with a laboratory scale fruit heating system (Model HWH-2, Gaffney Engineering, Gainesville, FL). All mangos were stored for 2 days at 14°C prior to the treatment. The mangos were randomly divided into four groups and the first three groups were immersed into a hot water bath for 70 min (HW70), 90 min (HW90), or 110 min (HW110) at 46°C. The fourth group remained untreated to use as a control (HW0). Since median weight of sample mangos was 497.5g, required treatment time was 75 min in accordance with the direction of APHIS treatment manual for mangos (USDA-APHIS, 2002). In this study, 70 min, the nearest time to 75 min, was determined as required treatment time for a regular 20 min interval between treatments (70, 90 and 110 min).

Because treatment time usually varies within a range of 10 min according to hydrocooling rate and the median was less than 500g, 5 min difference might not influence on the result caused by HWT. Samples were acquired for immediate analysis after HWT while remaining fruit was held at 23^{o}C for an additional 4 days prior to obtaining analysis samples. A total of eighteen mangos were evaluated for each of the four treatments and included a control, divided into five groups with the edible flesh of three mangos pooled for analysis. The sixth group was utilized to measure fruit core temperature and they were not included in the dataset. Core temperature was obtained by placing a thermocouple inside the fruit during treatment.

Table 3-1. HWT with four different lengths of time (0, 70, 90, and 110 min) at 46°C with different ripeness stages (day 0 and day 4) at 23°C

Treatment / Period	HW 0 (control)		HW 70		HW 90		HW 110	
Day 0 & Day 4	C-1		H70-1		H90-1		H110-1	
	C-2		H70-2		H90-2		H110-2	
	C-3		H70-3		H90-3		H110-3	
	C-4		H70-4		H90-4		H110-4	
	C-5		H70-5		H90-5		H110-5	
	C-6		H70-6		H90-6		H110-6	
Total	**D0-18, D4-18**		**D0-18, D4-18**		**D0-18, D4-18**		**D0-18, D4-18**	

Mango skins were manually peeled and flesh blended using a laboratory blender (Waring commercial, Model 31BL91) for 5 min to obtain a consistent puree and held at -20°C until analysis whereby the samples were thawed for chemical analysis. A 5g puree was treated with 20µl of pectinase (Pectinex Ultra SP-L from *Aspergillus aculeatus*. SIGMA chemical Co.), then incubated at 35°C for 3 hours, and centrifuged until a clear

supernatant (clarified mango juice) was obtained from which phytochemical analyses were conducted.

3-2-2. Identification and Quantification of Individual Polyphenolics

Individual polyphenolics were identified and quantified using HPLC as described by Talcott et al. (2000). Clarified juice isolates were filtered through a 0.45 µm PTFE filter (Whatman, Clifton, NJ) and then injected into a Waters 2695 Alliance chromatography system. Compounds were separated on a Waters Spherisorb ODS 2 column using a gradient elution program. Mobiles phases consisted of Phase A (98% H_2O**:** 2% Acetic acid) and Phase B (68% H_2O**:** 30% Acetonitrile**:** 2% Acetic acid) run at 0.8 mL/min. Polyphenolics were separated using a gradient elution system that kept mobile phase B 0% for 5 min and then changed phase B 0% to 10% in 20 min; 10% to 25% in 30 min; 25% to 50% in 40 min; 50% to 75% in 50 min; 75% to 100% in 70 min and returned to original condition in 2 min for the next injection. Polyphenolics were detected and quantified at 280 nm using a Waters 996 photodiode array (PDA) detector against an external standard of gallic acid. Unknown compounds were characterized based on retention time and UV spectral similarities to authentic standards (Sigma Chemical Co., St. Louis, MO) using Millennium 32® workstation.

3-2-3. Quantification of Total Soluble Polyphenolics

Total soluble phenolic (TSP) concentration (a measure of total metal ion reducing capacity) including contributions from ascorbic acid was determined by Folin-Ciocalteau assay (Swain and Hillis, 1959). For each clarified juice isolate, 50 µL mango juice was added to 1 ml of 0.25 N Folin-Ciocalteu reagent and mixed by vortex for 30 sec. After 3 minutes reaction time, 1 ml of 1N sodium carbonate was added to form a water-soluble

chromophore for a distinguishable blue color. After standing for 7 minutes, all the extractions were transferred to a Spectramax 96-well and absorbance (Softmax PRO, Sunnyvale, CA) was read at 726 nm after 2 hours. TSP was quantified in mg/L gallic acid equivalents (GAE).

3-2-4. Quantification of Antioxidant Capacity

Total antioxidant capacity of mango phytochemicals (from polyphenolics and ascorbic acid) was measured using the ORAC assay (oxygen radical absorbance capacity) run according to Talcott et al. (2002) and adapted to work with a 96-well Molecular Devices fmax® fluorescent microplate reader (485 nm excitation and 538 nm emission). This method is based on the principle of the inhibition of oxidation of a fluorescent compound (fluorescence) in the presence of the peroxyl radical generator 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). A stock solution of fluorescein was produced by mixing 10µl of stock fluorescent solution and 50 ml of phosphate buffer (61.6:38.9 v/v, 0.75 M K_2HPO_4 and 0.75M NaH_2PO_4, pH 7.2) as described by Ou et al. (2001) and the peroxyl radical generator was made by mixing 350mg of AAPH in 5 ml of phosphate buffer (pH 7.0). The rate of fluorescence decay was recorded every 2 min for 70 min by calculating the area under the fluorescence decay curve for a standard curve (0-50 µM), a blank (ORAC = 0 µM), and mango juice following appropriate dilution in phosphate buffer. Antioxidant capacity affected by treatments was quantified against a standard curve of Trolox (6–hydroxy–2,5,7,8–tetramethylchroman–2–carboxylic acid), a water-soluble analog of vitamin E (ORAC = 1 µM Trolox) with potent antioxidant properties (Javanovic et al., 1994). Trolox was used at the concentrations of 1.65, 3.125, 6.25, 12.5, 25, and 50 µM by serial dilution with the phosphate buffer. Each extract was diluted 50

fold in pH 7.0 phosphate buffer before pipetting into a 96-well microplate. Data was represented in μM Trolox equivalents per mL of clarified mango juice.

3-2-5. Statistical Analysis Method

Changes in phytochemicals and antioxidant capacity affected by HWT time and fruit ripening were analyzed by analysis of variance (ANOVA) using JMP 5 statistical software (SAS Institute, 2001). Mean separation was conducted using the LSD test, $P<0.05$.

3-3. Results and Discussion

3-3-1. Individual Polyphenolic Concentration

Free gallic acid and hydrolyzable tannins were the major polyphenolics identified in mango and quantified against an authentic standard of gallic acid (Talcott et al., 2005). Average gallic acid concentration during the 4 days storage was changed as a result of HWT (Fig 3-1). Gallic acid concentration at HW70 did not show significant difference from the control while concentrations at HW90 and HW110 were significantly lower (41% and 42% less, respectively) than the control. Synthesis of gallic acid was inhibited by HWT only when treatment time is longer than that required for quarantine treatment depending on cultivar, weight, shape, and origin (APHIS, 2002; Lurie, 1998).

When fruits were treated with hot water for extended time, heat-shock protein might have been induced. Heat shock proteins (HST) are unique proteins produced in response to heat-shock treatment (high temperature and/or long time) (Saltveit, 1998). Heat-shock inhibited synthesis of phenylalanine ammonia-lyase (PAL), which is the first enzyme of primary phenolic pathway (phenylpropanoid pathway) (Loaiza-Velarde et al., 2003; Rivero et al., 2001; Saltveit, 1998; Saltveit, 2000a). Since PAL activity induces accumulation of phenolic compounds, gallic acid reduction might be affected by

decreased PAL activity when mangos were treated with hot water for extended time (Saltveit, 2000b).

Average gallic acid concentration for all HWT increased by 24% during 4 days storage (Fig 3-1). During the 4-day storage, gallic acid concentrations of control and HW70 did not change while the concentrations of HW90 and HW110 significantly increased. At both day 0 and day 4, they showed a similar result, in which gallic acid significantly decreased only when they were treated for longer times (HW90 and HW110). Reduced gallic acid concentration of HW90 and HW110 by extended time treatment might continue to recover during 4 day storage.

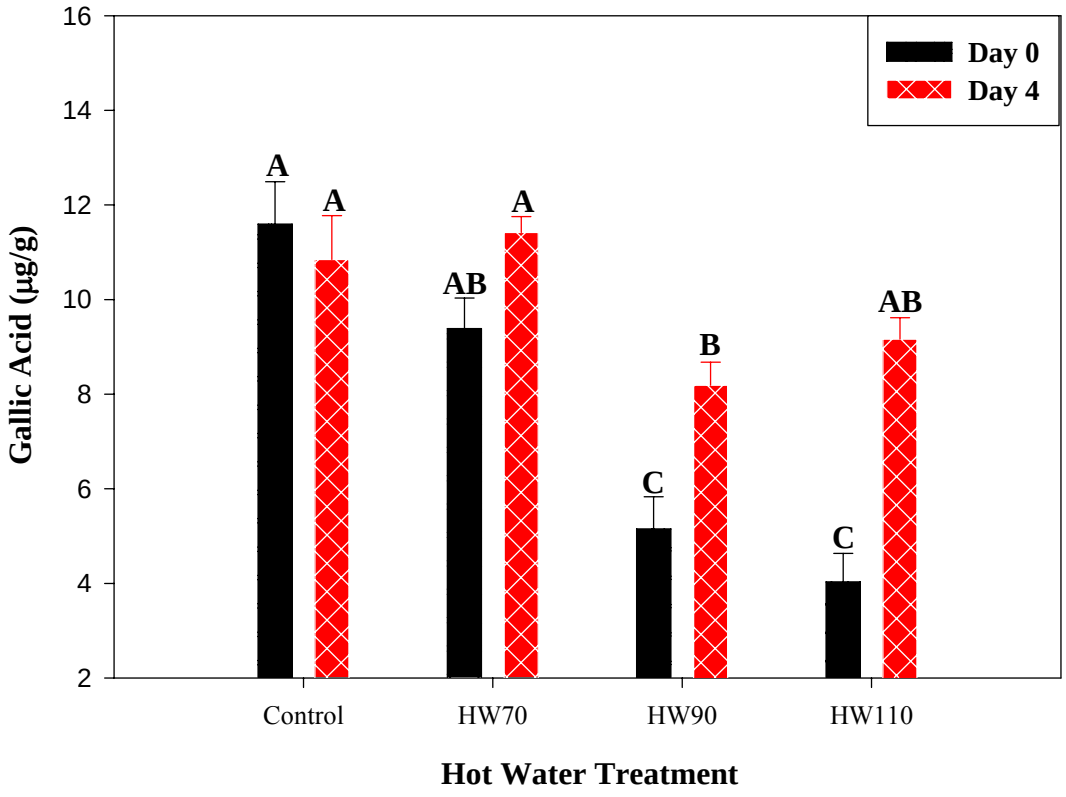


Figure 3-1. Changes in average gallic acid concentration (µg/g GAE) for two ripeness stages (day 0 and day 4) affected by HWT with varying length of treatment times (0, 70, 90, and 110 min). Average values and standard error bars of triplicate samples for all treatments are represented.

Since extended hot water treatment not only reduced phenolic compounds, but also induced heat injury, it might be an undesired process for fruit and vegetables storage. Reported symptoms of heat injury are abnormal color development, odd softening, the lack of starch breakdown, and the development of internal hollows in mango (Jacobi and Wong, 1992; Lurie, 1998).

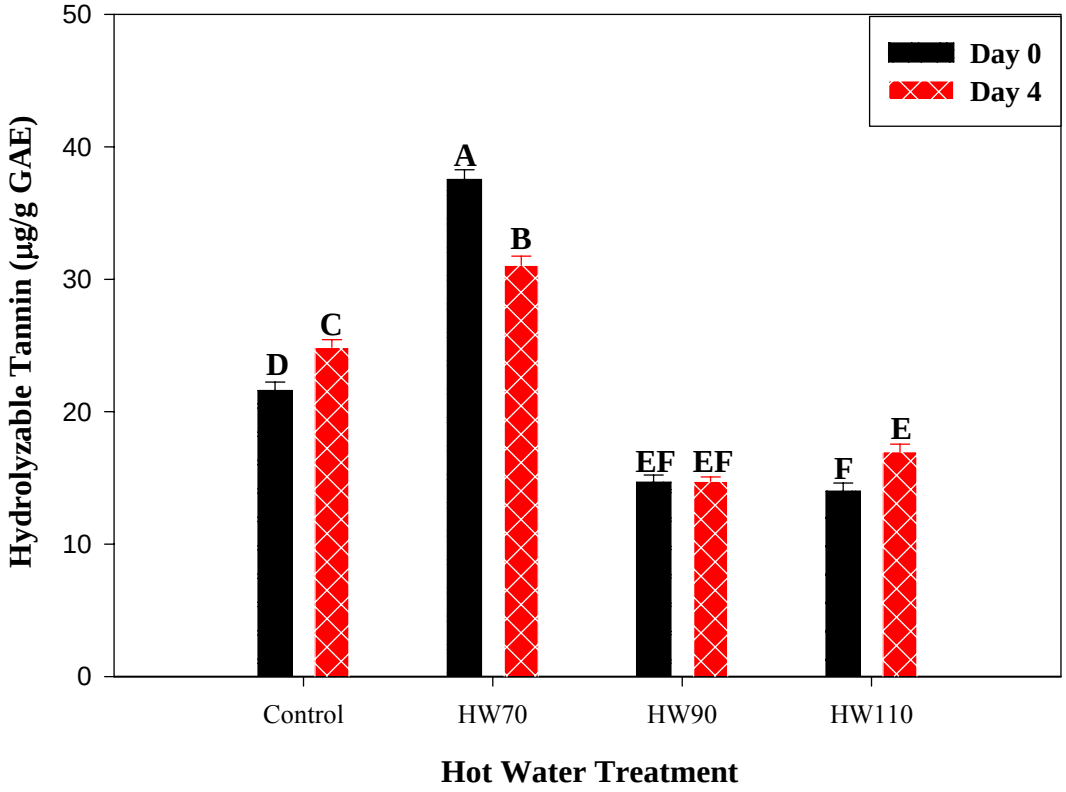


Figure 3-2. Changes in total hydrolyzable tannin concentration (µg/g) for two ripeness stages (day 0 and day 4) as a result of different lengths of HWT (0, 70, 90, 110 min). Average values and standard error bars of triplicate samples for all treatments are represented

Four hydrolyzable tannins were tentatively identified based on UV spectral similarities to gallic acid due to the diversity of hydrolyzable tannin present (Talcott et al., 2005). As a result of the four different durations of HWT, the concentration of hydrolyzable tannins increased (33% more) only at HW70, but the concentrations at

HW90 and HW110 were lower (37 and 34% less, respectively) than control (Fig 3-2). It was a similar result as gallic acid except the concentration of hydrolyzable tannins at HW70 increased and was highest among all the HWT. Since heat stress causes PAL activity that is a core enzyme of phenylpropanoid pathway in catalyzing synthesis of phenolic compounds, hydrolyzable tannin concentration could be increased by thermal stress (Rivero et al., 2001). Reduction of hydrolyzable tannin concentration at HW90 and HW110 may be induced by heat-shock as explained in gallic acid reduction.

Phenolic compounds of mango were found to decrease during ripening with a loss of astringency related to loss of phenolic content (El-Ansari et al., 1971; Lakshminarayana et al., 1970; Mitra and Baldwin, 1997; Saleh and El-Ansari, 1970). However, in this study, total hydrolyzable tannin concentration did not change while gallic acid concentration significantly increased during the 4 day storage. No change of hydrolyzable tannin concentration could be explained from the fact that since HWT inhibits ethylene synthesis, ripening might be delayed (Lurie, 1998). Thus, delayed ripening may be the reason for the lack of change in hydrolyzable tannin concentration in mango even though there was a 4-day storage time difference.

As shown in Fig 3-3, identified gallic acid and four hydrolyzable tannin peaks were eluted by HPLC at Day 0 and Day 4. According to peak area, about 30% of gallic acid increased during the 4-day storage while on average the four hydrolyzable tannins did not show any difference. Changes of gallic acid and four hydrolyzable tannins were shown in Table 3-2.

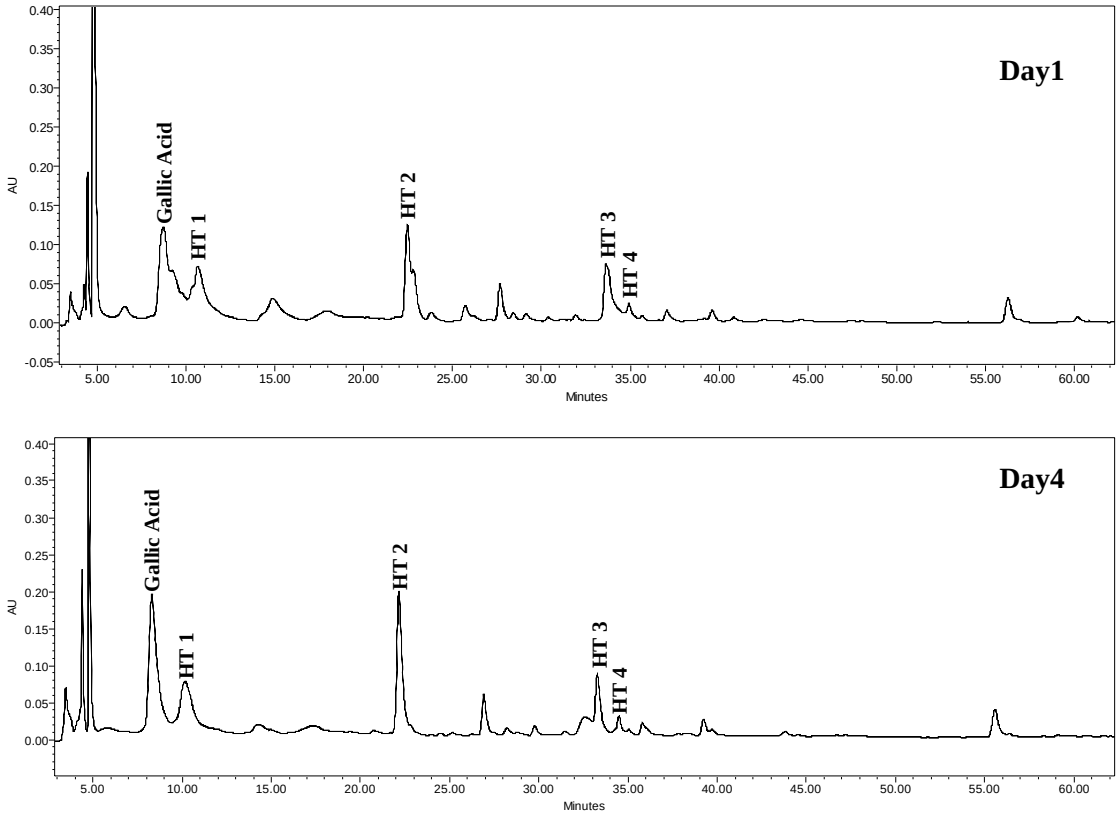


Figure 3-3. HPLC chromatogram of polyphenolics, gallic acid and four hydrolyzable tannins (HT), present in mango flesh at Day 0 and Day 4. Peaks were tentatively identified based on retention time and spectral similarities against an authentic standard of gallic acid

Table 3-2. Gallic acid (µg/g) and average of four hydrolyzable tannins (µg/g GAE) identified and quantified using HPLC as a result of different duration of HWT (0, 70, 90, 110 min) at two ripeness stages (Day 0 and Day 4)

Ripeness Stages	HotWater Treatment	Gallic Acid	HT 1	HT 2	HT 3	HT 4	Total HT
Day 0	**HW0**	11.59a	4.88b*	8.69b*	6.76a	1.27b	21.6
	HW70	9.39a	7.27a*	21.7a	7.42a*	1.13b	37.5
	HW90	5.15b	5.26b	6.04c	1.10b	2.27a	14.7
	HW110	4.70b	5.17b	5.92c	1.87b	1.04b	14.0
Day 4	**HW0**	10.84a	3.49a	12.84b	7.03a	1.50c	24.9
	HW70	11.42a	3.79a	22.42a	2.18c	2.66a*	31.1
	HW90	8.18b*	3.77a	6.01d	2.81bc*	2.14b	14.7
	HW110	9.16ab*	3.89a	7.59c	3.86b*	1.64c*	17.0

1. *Indicates a significant difference between varying length of treatment times at each day of ripeness.
2. Different letters within column indicate significant difference at each day of ripeness..
3. HT represents hydrolyzable tannin.

3-3-2. Total Soluble Polyphenolics (TSP)

TSP concentration including contribution from ascorbic acid, reducing sugar and likely small amount of soluble protein in mango decreased in response to HWT. The average TSP concentrations in HWT mangos (70, 90 and 110 min) for both ripeness stages (225 µg/g GAE) showed about 25% decline compared to control (294 µg/g GAE) (Fig 3-4). At day 4, non-HWT mangos showed higher TSP concentration (36% more) than HWT mangos. Although no significant difference was found between control and HWT mangos at day 0, the concentration of control was still higher than those of other mangos.

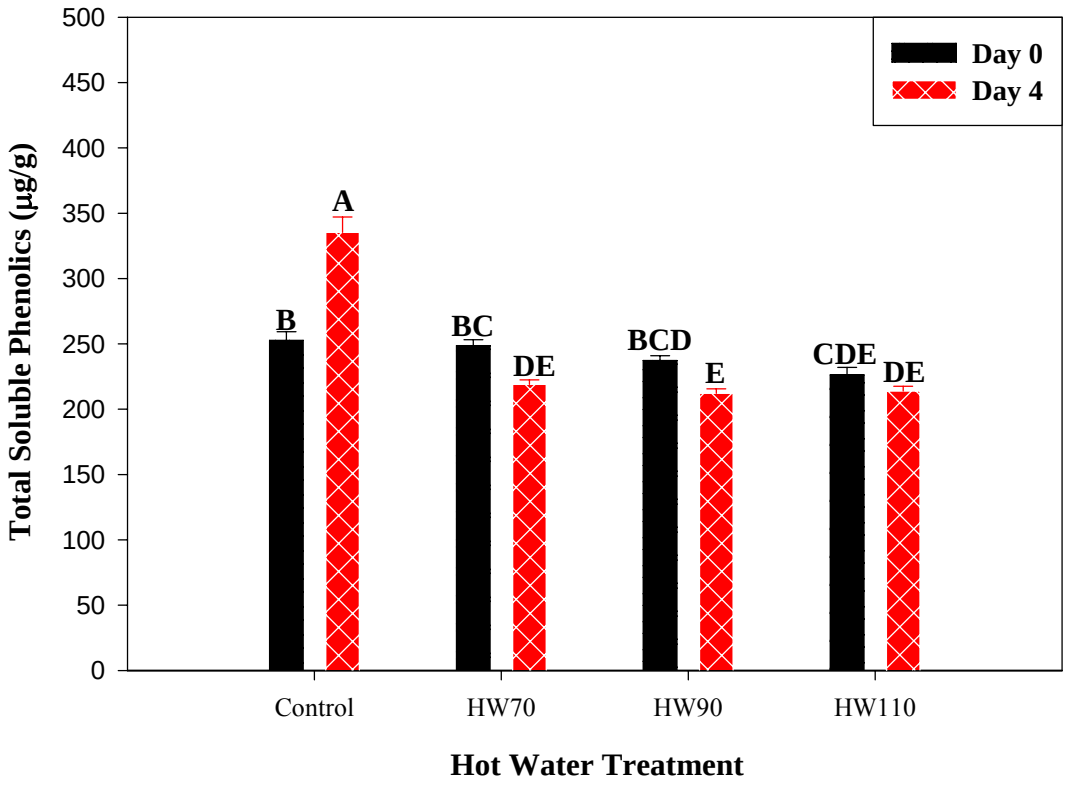


Figure 3-4. Changes in TSP (Total Soluble Phenolic) concentration (µg/g GAE) for two different ripeness stages (day 0 and day 4) effected by four different durations of HWT (0, 70, 90, and 110 min). Average values and standard error bars of triplicate samples for all treatments are represented

Varying the length of HWT was not a significant factor influencing the TSP concentration in mango. Comparing TSP concentrations between three different treatments excluding control, the average TSP concentration for two ripeness stages was not significantly different (Fig 3-4). Since polyphenolics were shown to decrease during ripening, lower TSP concentration was expected at day 4 than day 0 (Haard and Chism, 1996). However, no significant difference was observed between day 0 and day 4. Technically, 6~9 days are required to transform an unripe mango to a physiologically ripe mango (developed color and flavor) at 25°C, which was the same temperature used for mango storage in this study (Jacobi et al., 2001). Moreover, ascorbic acid which has reducing capacity is unstable to heat. Even required duration of HWT could decrease ascorbic acid content in mango. Therefore, the 4 day storage was a somewhat short period to expect ripening changes such as TSP reduction and reduced ascorbic acid content by thermal stress might affect TSP in HWT mangos.

3-3-3. Antioxidant Capacity

Antioxidant capacity, evaluated based on peroxyl radical scavenging activity of water-soluble mango constituents, was not affected by varying lengths of HWT. Although average antioxidant capacity of clarified mango juice for two ripeness stages tended to increase as duration of heating increased, it was not significantly different compared to control (Fig 3-5).

During the 4-day storage, average antioxidant capacity for different duration of HWT significantly decreased (7%) ($P < 0.05$). The radical scavenging ability of mango did not correlate with TSP in this study because TSP did not change during the 4-day storage. These observations were unexpected since gallic acid, which was the major

polyphenolic compound and a strong antioxidant in mango, increased during ripening although TSP and hydrolyzable tannins were not significantly changed. Since ascorbic acid, which is another contributor to antioxidant capacity was reduced by heat and during ripening, this might influence the retention of antioxidant capacity (Bashir and Abu-Goukh, 2003; Soto- Zamora et al., 2005; Yen and Hung, 2000)

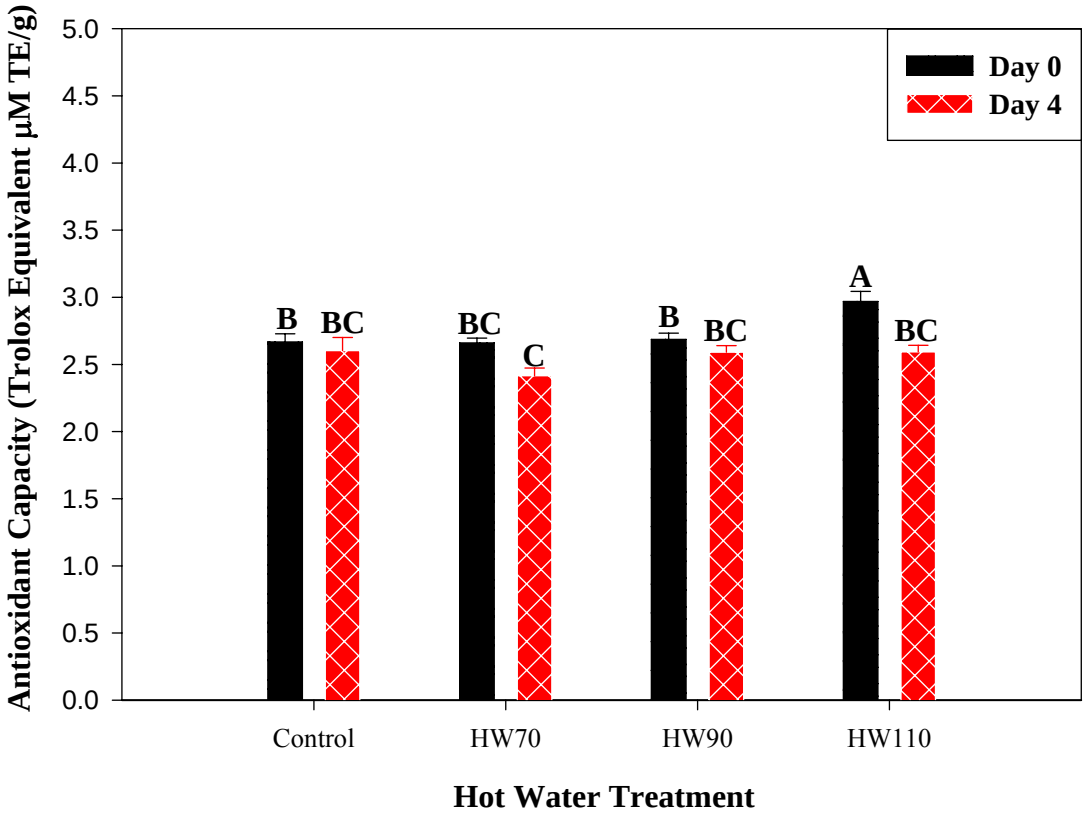


Figure 3-5. Changes in total antioxidant capacity (µM TE/g) for two different ripeness stages day 0 and day 4) affected by four different lengths of HWT (0, 70, 90, and 110 min). Average values and standard error bars of triplicate samples for all treatments are represented

3-4. Conclusion

Gallic acid concentration significantly decreased as a result of extended HWT (HW90 and HW110) while the treatment duration required for quarantine treatment

(HW70) did not change gallic acid concentration compared to control. Consequently, total hydrolyzable tannin concentration was elevated by HWT only with required treatment time by the water treatment manual while extended time of treatment decreased HT concentration. Average TSP decreased as a result of HWT regardless of duration of HWT while antioxidant capacity was not affected by HWT (Table 3-3). Since mangos imported from other countries must go through a HWT, finding optimum conditions to improve and/or keep the quality of the fruit is important. According to the results of this study, the required hot water immersion treatment time for mango quarantine showed beneficial effects such as retaining gallic acid and increasing hydrolyzable tannins.

Table 3-3. Total soluble phenolics (µg/g GAE) and total antioxidant capacity (µM TE/g) of mango treated with hot water with varying length of treatment times (0, 70, 90, and 110 min) at two different ripening stages (Day 0 and Day 4)

Ripening Stages	Hot Water Treatment	Total Soluble Polyphenolics	Total Antioxidant Capacity
Day 0	**HW0**	252.6a	2.67b
	HW70	248.7a*	2.66b*
	HW90	237.2ab	2.69b
	HW110	226.2b	2.97a*
Day 4	**HW0**	335.4a*	2.60a
	HW70	218.9b	2.59a
	HW90	212.1b*	2.59a
	HW110	213.8b	2.41a

1. * Indicates a significant difference between varying length of treatment times at each day of ripening.
2. Different letters within column indicate significant difference at each day of ripening.

CHAPTER 4
TO EVALUATE POLYPHENOLICS, ANTIOXIDANT CAPACITY, AND PHYSIOLOGICAL CHANGES IN MANGOS (*MANGIFERA INDICA L.*) FOLLOWING CONTROLLED ATMOSPHERE STORAGE COMBINED WITH HOT WATER IMMERSION TREATMENT

4-1. Introduction

Even though several states such as Hawaii, California, and Texas grow mangos Florida is the only state to report mango production in the US (Mossler and Mosheim, 2002). However, according to latest records, domestic mango production in Florida (2,800 MT) accounted for less than 1% of total consumption in the US in 2004, so more than 99% of mangos are imported from other countries such as Mexico, Brazil, Peru, Haiti, and Guatemala to meet the consumer demands (FAO, 2004; Sauco, 2004).

Since many mangos are imported from other countries (278,422 MT in 2003) and it takes a few days up to a few weeks for the imported mangos to reach retail markets, CA storage is sometimes used to maintain the quality of fruit during transportation and storage (FAO, 2004). Especially, mangos imported from other countries must be treated with hot water to eliminate invasive pest insects. Studies relating CA storage and HWT of fruits have focused on physiological changes such as firmness, color, moisture content, acidity and sugar content, so limited information is available relating phytochemical changes during CA storage with or without HWT to postharvest quality factors during fruit ripening. Therefore, this study focused on the changes in polyphenolics and resultant antioxidant capacity during CA storage associated with HWT.

4-2. Materials and Methods

4-2-1. Fruit Preparation and Treatment

For this study, 300 ‘Tommy Atkins’ mangos were procured from Fresh King. Inc., Homestead, Florida in June, 2004 at the peak of the harvest season for ‘Tommy Atkins’. Mango fruits with uniform size, oval shape, and green color were pre-selected to reduce variation among treatments. Fruits were held at 10°C during the 6-hour transport to Gainesville. Mangos were stored at 10°C at the Horticultural Sciences Department in University of Florida for 2 days until hot water treatment and CA storage were applied. Of the 300 mangoes originally obtained, 234 mangos were selected based on their uniform size, shape (oval), and weight (average weight >500g).

Prior to treatments, eighteen mangos were selected as controls. This initial set of fruit, representative of the population was analyzed for phytochemical and antioxidant content as previously described in chapter 3. Additionally, soluble solids content (SSC) and titratable acidity (TA) values were quantified as additional indices for fruit quality. USDA-APHIS guidelines were followed, which involve immersing fruit in water at 45.6~ 46.1°C for 75 min. The remaining 216 mangos were divided into two groups, half for HWT and the remaining half immersed in water at 25°C as a non-heated control. Following these treatments, the fruits were held at 25°C until the skin was completely dry (about 60 min) and the center of the heated mangos returned to 25°C. The fruit were then transferred to their respective CA chambers with the following gas contents:

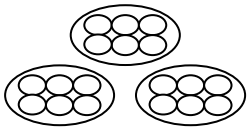
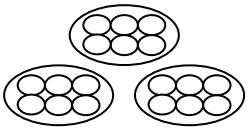
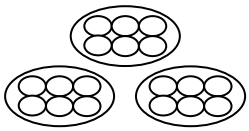
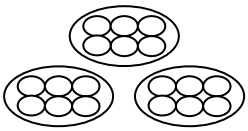
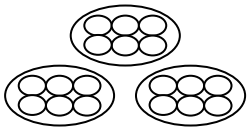
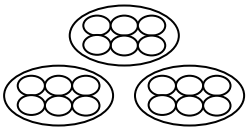
1. CA 1 (Control): Air (about 21% O_2 + 79% N_2)

2. CA 2: 3% O_2 + 97% N_2

3. CA 3: 3% O_2 + 10% CO_2 + 87% N_2

Fruits were placed in the chambers and gas composition regulated by four external gas tanks (air, O_2, CO_2, and N_2) for 2 weeks at 10°C. The gas composition in each chamber was checked twice daily and regulated to desired composition. After 2 weeks, half of the mangos were removed and immediately analyzed for phytochemical attributes, antioxidant capacity, and quality index such as titratable acidity, soluble solids content, and fruit pulp color. The remaining fruits were moved into a 25°C environment to complete their ripening in an air (21% O_2) environment. All the mangos stored in CA chamber were analyzed as described in Chapter 3. Additionally, the internal flesh color of the peeled fruits (CIE color values L*, a*, b*) was measured for all the mangos.

Table 4-1. Mango CA storage with 1 control (air) and 2 different air compositions (CA2 and CA3) (CA1=Air, CA2=3% O_2, CA3=3% O_2 + 10% CO_2) at 10°C for 2 weeks. One half of the mangos were treated with hot water and the other half was treated with 25°C water.

CA storage chamber	No HWT	HWT	Total
1. Air (Control)			**36**
2. 3% O_2 + N_2			**36**
3. 3% O_2 + 10% CO_2 + N_2			**36**

4-2-2. Phytochemical Changes and Antioxidant Capacity

Total soluble phenolic concentration and antioxidant capacity were determined as previously described in Chapter 3. Identification and quantification of individual

polyphenolics were conducted using Waters 2690 HPLC as previously outlined in Chapter 3.

4-2-3. Evaluation of Flesh Color

The internal flesh color of peeled fruit expressed as CIE color values (L*, a*, and b*) was measured by Minolta Chroma Meter CR 200 Series with a 8mm aperture (Minolta Co., Ltd., Osaka, Japan). Hue angle and chroma values were calculated from a* and b* using the method described by López and Gómez (2004). The colorimeter was calibrated by a standard flat white tile before measuring the flesh color of mangos. The following equations were used to calculate hue angle and chroma values of mango flesh color.

Hue Angle = $\left[\left(Tan^{-1}(b^*/a^*)\right)\times(180/3.14)\right]$+180 (180 is needed only if a* is negative)

Chroma = $\sqrt{a^{*2}+b^{*2}}$

4-2-4. Quantification of Titratable Acidity (TA)

Titratable acidity was measured by titrating clarified juice against 0.1N NaOH to pH 8.2 using phenolphthalein indicator and a Corning model 140 digital pH meter as described by Jacobi et al. (2000). The acidity was expressed as anhydro citric acid equivalents, the predominant organic acid in mango. Mango juices (3g) were diluted 5-fold in a small beaker on a stir plate. The sample was titrated using 0.1M NaOH until pH 8.2 was obtained. Finally, the amount of 0.1M NaOH used for the titration was recorded. Acid content (%) of each sample was calculated based on the following formula. In the formula, 0.064 was the miliequivalent factor for anhydrous citric acid

$$\%acid = \frac{ml \cdot NaOH \times 0.1 \cdot (NaOH) \times 0.064}{juice(g)} \times 100$$

4-2-5. Quantification of Soluble Solids Content (SSC)

Soluble solids contents (°Brix) were quantified by clarifying fruit puree by centrifuging using a Beckman Optima TL 100 tabletop ultracentrifuge (Beckman Instrument Inc. Palo Alto, CA) at the speed of 15,000 rpm for 15 min. The supernatant was measured using a digital Leica Mark Ⅱ Abbe refractometer (Leica Inc. Buffalo, NY) at 20°C. Before using the instrument, it was calibrated using distilled water. Approximately 100μL of juice was placed on the prism of the refractometer, and the refractive index was read.

4-2-6. Statistical Analysis Method

Statistical analysis was performed as previously described in Chapter 3 using JMP 5 statistical software (SAS Institute, 2002). Analysis of variance (ANOVA) and LSD ($P<0.05$) test were used to determine the effect of CA storage combined with or without HWT on phytochemicals and other quality changes of fruit during storage and ripening. Pearson correlation test was used for relation between TSP concentration and antioxidant capacity.

4-3. Result and Discussion

4-3-1. External Fruit Quality

Hot water treatment was effective in preventing physiological disorders on the fruit. Darkened lenticels (black spots) are the symptoms of anthracnose, the most common surface postharvest disease on mangos, and appeared only on the skin of the non-HWT mangos after 2 weeks of CA storage. Consequently, the deterioration of non-HWT mangos was faster than that of HWT mangos during ripening. Non-HWT mangos started to deteriorate at 25°C 3 days after CA storage was finished. Their flesh started to sink and

more darkened lenticels were detected on the skin while development of anthracnose on HWT mangos was delayed until 1 week after CA storage was complete. On average, one of six repetitions was rotten at 25°C. Thus, one mango was removed from each set of mangos before analyzing.

CA storage could prevent anthracnose on mangos as well. Even though black spots are also symptom of CO_2 injury, the spots on the mango skin were probably not induced by CO_2 in this study because less black spots were shown in CA3 (the only CA storage that contained CO_2). Mangos stored in CA1 (air) developed more anthracnose compared to mangos in CA2 ($3\%O_2$) and CA3 ($3\%O_2 + 10\%CO_2$) after CA storage. Consequently, when comparing mangos between CA2 and CA3, more anthracnose was detected on the mangos stored in CA2 than CA3 because CA storage with low O_2 and high CO_2 was more effective in preventing fruit decay than with only low O_2 (Tian et al., 2004). After all the mangos were moved to 25°C, non-HWT mangos started to deteriorate 3 days after transfer. The mangos without HWT showed anthracnose on the skin without regard to CA storage conditions after 3 days at 25°C. With the exception of mangos stored in CA1, no fruit disorders were found on HWT mangos. Through this result, it was hypothesized that a synergistic effect occurred in inhibiting anthracnose when CA storage was combined with HWT.

4-3-2. Individual Polyphenolic Concentration

Two major polyphenolics (gallic acid and hydrolyzable tannin (HT)) and four minor polyphenolics (p-OH-benzoic acid, *m*-coumaric acid, *p*-coumairc acid, and ferulic acid) in mango were identified and quantified by HPLC. Gallic acid and six HTs were found about 98% more than four minor polyphenolics found in mango. Since the minor

polyphenolic concentrations were somewhat negligible, details of their changes were not analyzed.

Two CA storages containing different gas compositions and one air control were applied to mangos. CA1 was consisted of 21% O_2 and 79% N_2 (normal air composition) and was considered the control for the study. Technically, CA storage is based on lowered O_2 and increased CO_2 levels at low temperature and optimum O_2 and CO_2 levels for mango are 3-5 and 5-10 % depending on degree of ripeness (Kader, 2002; Mitra and Baldwin, 1997). Therefore, CA2 was 3% O_2 and 97% N_2 in an effort to monitor the effect of CA storage with reduced O_2 (usually less effective than O_2+CO_2) (Tian et al., 2004). CA3 was 3% O_2, 10% CO_2, and 87% N_2 to determine the effect of CA storage with lowered O_2 and elevated CO_2 in the optimum range for mango CA storage. The temperature for CA storage of fully mature mangos must be at least 10°C to prevent chilling injuries such as discoloration, pitting, poor color, and uneven ripening (Mitra and Baldwin, 1997). Thus, storage temperature for this study was fixed as 10°C regardless of storage gas compositions.

Gallic acid concentrations for CA1, CA2 and CA3 were measured at three different stages of ripening (green, mid, and full) in effort to determine treatment effect during ripening. It was reported that phenolic compounds such as gallic acid decreased as fruit ripened (Haard and Chism, 1996; Mitra and Baldwin, 1997), and likewise in this study gallic acid concentrations of CA1, CA2 and CA3 significantly decreased by 16, 17 and 8% during ripening from green to full ripeness stage (Fig 4-1). Gallic acid concentration significantly decreased only between green and mid ripe at CA1 while the concentrations at CA3 decreased only between mid and full ripe. Gallic acid concentration at CA2

gradually declined throughout fruit ripening. Even though it was a relatively short time between mid and full ripeness stages, there were several types of evidence to support that mangos ripened during this period such as titratable acidity decrease and color development occurred between mid and full ripeness stage in this study. Thus, it could be described as a "ripening related change" of gallic acid.

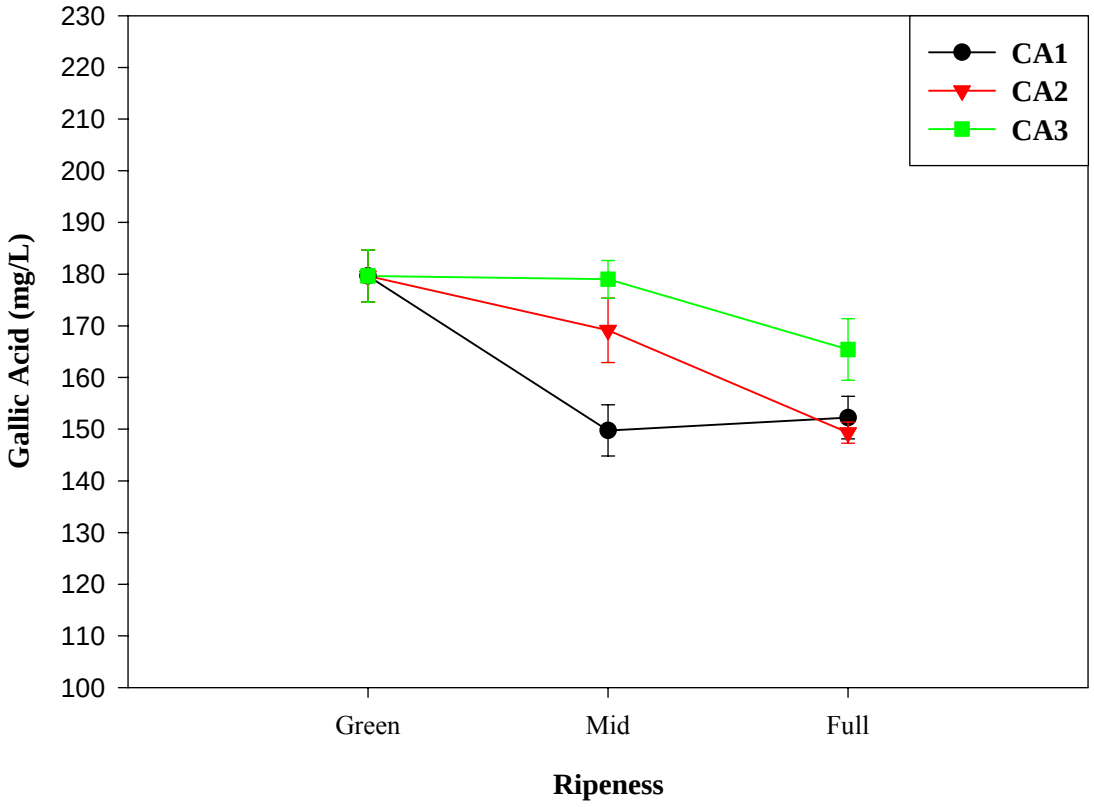


Figure 4-1. Changes in average gallic acid concentration (mg/L) for CA1, CA2 and CA3 during ripening for fruit samples at green, mid, and full ripeness. The ripeness stages were green (on the day of harvest), mid (after 2 week CA storage), and full (the time fruit started deterioration) ripe.

In conclusion, gallic acid concentration significantly decreased during storage ($P < 0.05$), and then the concentration was held constant at CA1. This was supported by several studies, in which polyphenolic compounds in mango were reported to gradually

decrease then maintain fairly steady during ripening (Lakshininarayana et al., 1970; Mitra and Baldwin, 1997). However, CA storage, especially CA3, inhibited reduction of gallic acid. After CA storage, the concentration continued to decreased, but it was still higher than that of CA1 and CA2.

Gallic acid concentration was not affected by HWT. In Chapter 3, gallic acid concentration was unaffected only when HWT was applied with required temperature developed by USDA-APHIS for the sample mangos while the concentration decreased as a result of extended treatment times. Since HWT time for this study was 75 min and it was the required treatment time for the mangos used according to the USDA-APHIS treatment manual, no effect by HWT showed a similar result with the first study. In conclusion, HWT did not affect gallic acid concentration in this study because mangos were subjected to a HWT for the required time for insect quarantine found in the USDA-APHIS treatment manual.

Each storage contained different gallic acid and total HT concentrations. Average gallic acid concentration of mango in CA2 was not higher compared to concentration in CA1. The average concentration in CA3 (172 mg/L) was significantly higher than that of CA1 (159 mg/L) and CA2 (151 mg/L) (Fig 4-2) and it was 9% higher in CA3 compared to that of CA1 ($P < 0.05$). According to Agrawal and Deepak (2002), Castells et al. (2002), Davey et al. (2004), Penuelas et al. (1996) and Saxon et al. (2004), elevated CO_2 increases synthesis of phenolic compounds in plants. Furthermore, high CO_2 concentration could induce abiotic stress, which increases phenolic compounds in plants. Even though low O_2 could be a factor to induce abiotic stress as well, it was not enough to increase polyphenolics in this study. Therefore, it was hypothesized that combination

of O_2 and CO_2 significantly increased gallic acid concentration in mango by an effect of CO_2 and abiotic stress while O_2 did not increase the concentration. In studies conducted by Sanchez-Mata et al. (2003) and Tian et al. (2004), CA storage was more effective in preventing or reducing fruit decay and prolonging shelf-life if reduced O_2 and elevated CO_2 levels were applied together when compared with normal atmospheric conditions. In conclusion, increasing phenolic compounds in mango could be an additional benefit of CA storage with reduced O_2 and elevated CO_2 as was found through this study.

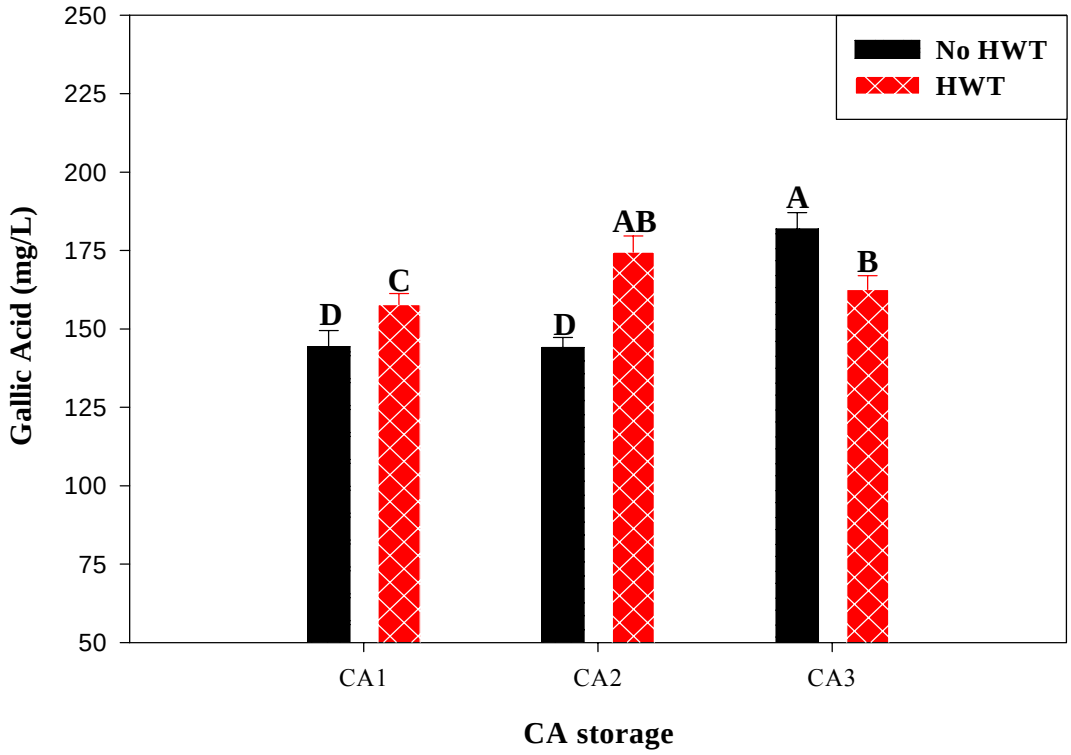


Figure 4-2. CA storage induced effects in average gallic acid concentration (mg/L) with or without HWT. Control was expressed as CA1 (21% air + 97% N_2) and two CA storages were shown as CA2 (3% O_2 and 97% N_2), and CA3 (3% O_2, 10% CO_2 and 87% N_2).

Hydrolyzable tannin is a polyphenolic compound mainly found in mango and total HT decreased by 46, 49 and 42% at CA1, CA2 and CA3 (green through full) during

ripening (Fig 4-3). Total HT concentrations at CA1 and CA2 did not change significantly while the concentration at CA3 increased during storage at 10°C. However, total HT concentrations at all the storages significantly decreased in air at 25°C following CA storage, and the average reduction was about 45%. According to this result, it was hypothesized that storage condition (low temperature) prevented or increased total HT reduction because total HT concentration during storage at 10°C (green to mid) was fairly stable and then its concentration significantly decreased in air at 25°C after CA storage ($P < 0.05$).

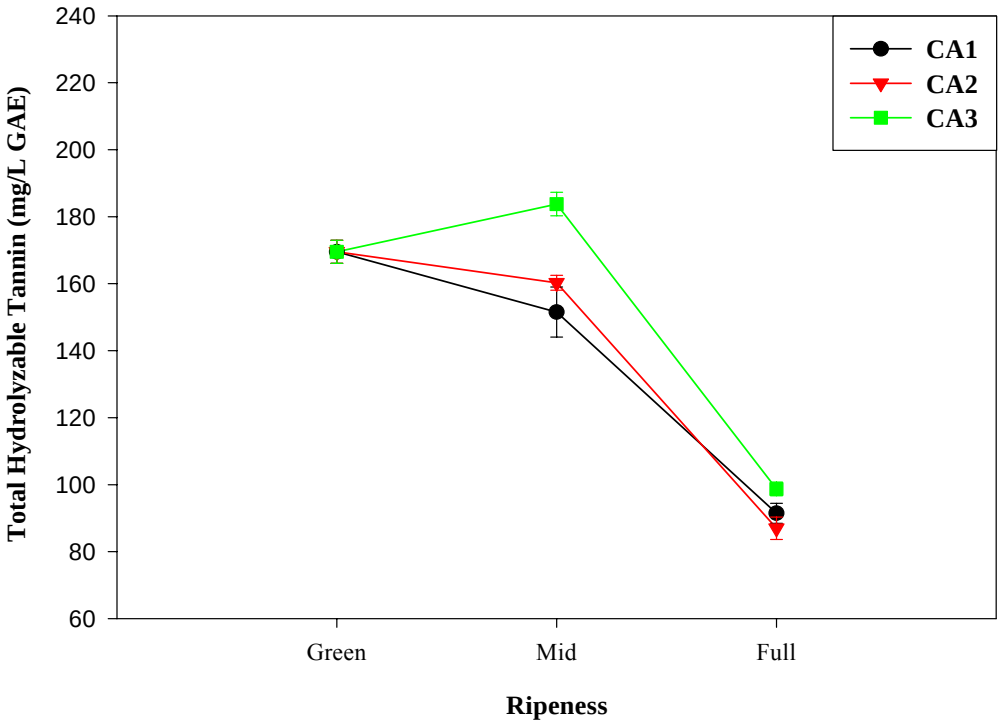


Figure 4-3. Changes in average total hydrolyzable tannin concentration (mg/L GAE) for CA1, CA2, and CA3 during mango ripening. Each stage represents green (on the day of harvest), mid (after 2 week CA storage), and full (the time fruit started deterioration) ripe.

Average total HT concentration increased as a result of HWT (Fig 4-4). HWT induced an increase of 17% HT compared to NHW mangos. In this study, only

hydrolyzable tannin concentration was affected by HWT. Other parameters like gallic acid, total soluble phenolics and antioxidant capacity were not affected by hot water treatment. In Chapter 2, HT concentration was elevated by required duration of HWT, and likewise HT significantly increased in response to heat treatment in this study. Thus, it could be hypothesized that HT concentration increased when mangos were treated with required treatment time. Furthermore, CA2 and CA3 also contained more HT in mango than CA1 (Fig 4-4). CA3 induced a significant increase of 15% total HT in mango compared to control while the concentrations in CA2 and CA3 were not significantly different from each other ($P < 0.05$). It was a similar result with gallic acid change by CA storage.

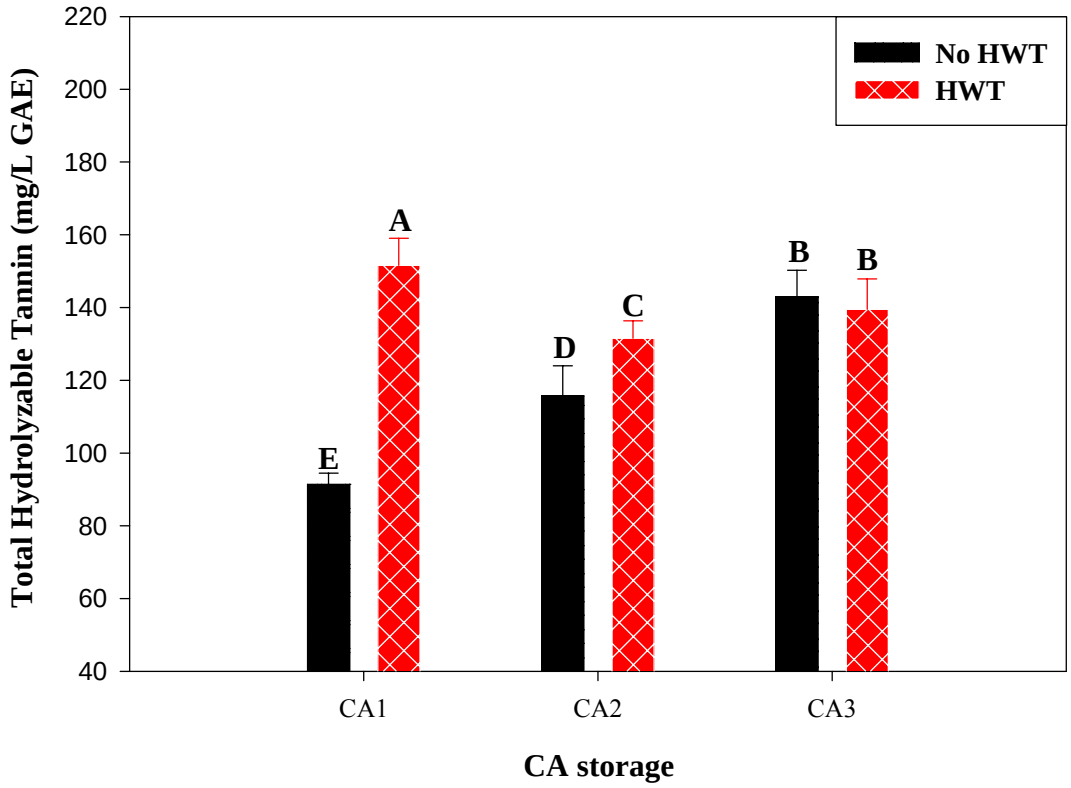


Figure 4-4. CA storage induced change in average HT concentration (mg/L GAE) in mango with or without HWT. Control was expressed as CA1 (21% air + 97% N_2) and two CA storages were shown as CA2 (3% O_2 and 97% N_2), and CA3 (3% O_2, 10% CO_2 and 87% N_2).

Gallic acid attaches to glucose via meta-depside bond by esterification, and it becomes B-Glucogallin with one gallic acid which is the simplest gallotannin (one of HTs). Depening on the number of gallic acid to glucose, gallotannin have different structure (Fig 4-5). Thus, more free gallic acids are synthesized, more gallotannins could be produced. In conclusion, it could be hypothesized that increase of total HT was caused by elevated CO_2 that resulted in increased synthesis of phenolic compounds in the fruit. Elevated CO_2 affects synthesis of phenolic compounds by increasing PAL (phenylalanine ammonia lyase) which is the first step of the phenylpropanoid pathway to be related to the synthesis of phenolic compounds such as lignin and tannins (Assis et al., 2001; Castells et al., 2002; Davey et al., 2004).

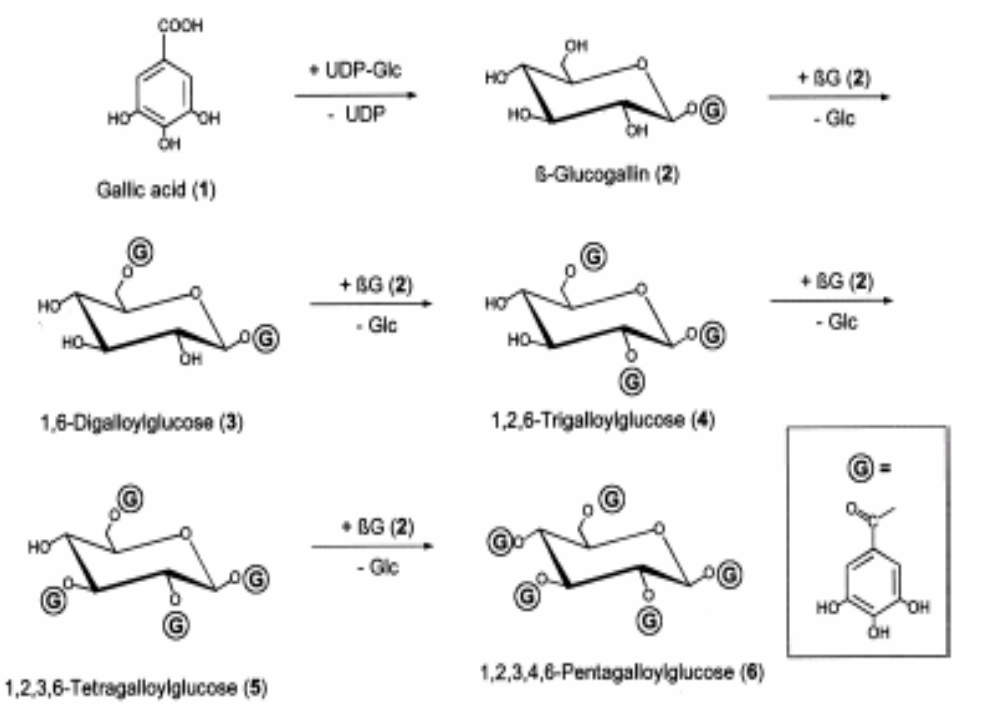


Figure 4-5. Gallotannin biosynthesis via meta-depside bond by esterification. Depending on the number of gallic acid to glucose, gallotannin have 5 different structure such as β-glucogallin, 1-6-Digalloylglucose, 1,2,6-Trigalloylglucose, 1,2,3,6-Tetragalloylglucose, and 1,2,3,4,6-Pentagalloylglucose. (Grundhöfer et al., 2001).

4-3-3. Total Soluble Phenolics (TSP)

Total soluble phenolics determined by the Folin-Ciocalteau assay decreased by 58, 53 and 41% at CA1, CA2 and CA3 during fruit ripening (Fig 4-6). In general, it has been reported that polyphenolic compounds decrease in many climacteric fruits such as mangos, bananas, tomatoes and guavas during ripening (Haard and Chism, 1996; Lakshminarayana et al., 1970; Mitra and Baldwin, 1997; Selvaraj and Kumar, 1989), and likewise in this study TSP concentration significantly declined as fruit ripened ($P < 0.05$). Since major phenolic compounds (gallic acid and total HT) in mango also significantly decreased during ripening (12 and 46%, respectively), reduction of TSP might be influenced by changes of those two phenolic compounds.

Controlled atmosphere storage delayed the reduction of average TSP concentration for HWT during ripening (Fig 4-6). When CA storage was finished (Mid point at Fig 4-6), the average concentrations in CA2 and CA3 were both 11% higher than that in CA1. After 2 week CA storage, it was found that both CA storage conditions effectively delayed TSP reduction in mango. Both CA2 and CA3 delayed 12% of average TSP reduction for HWT compared to control during 2 weeks CA storage. After storage, when the fruits were moved to air at 25°C to complete ripening, TSP concentration was significantly higher (30%) in mangos previously stored in CA3 than in CA1. This was because the CA storage mangos were less ripe than the air control when the analysis was performed. In conclusion, CA2 as well as CA3 were effective in slowing the reduction of average TSP concentration as a result of delayed ripening. However, more appropriate gas composition for mango storage (CA3) to maintain the quality of the fruits and vegetables better and longer was more effective at decreasing the reduction rate than CA2

because CA3 combination delayed 30% of TSP reduction while CA2 prolonged only 12% of TSP concentration after CA storage.

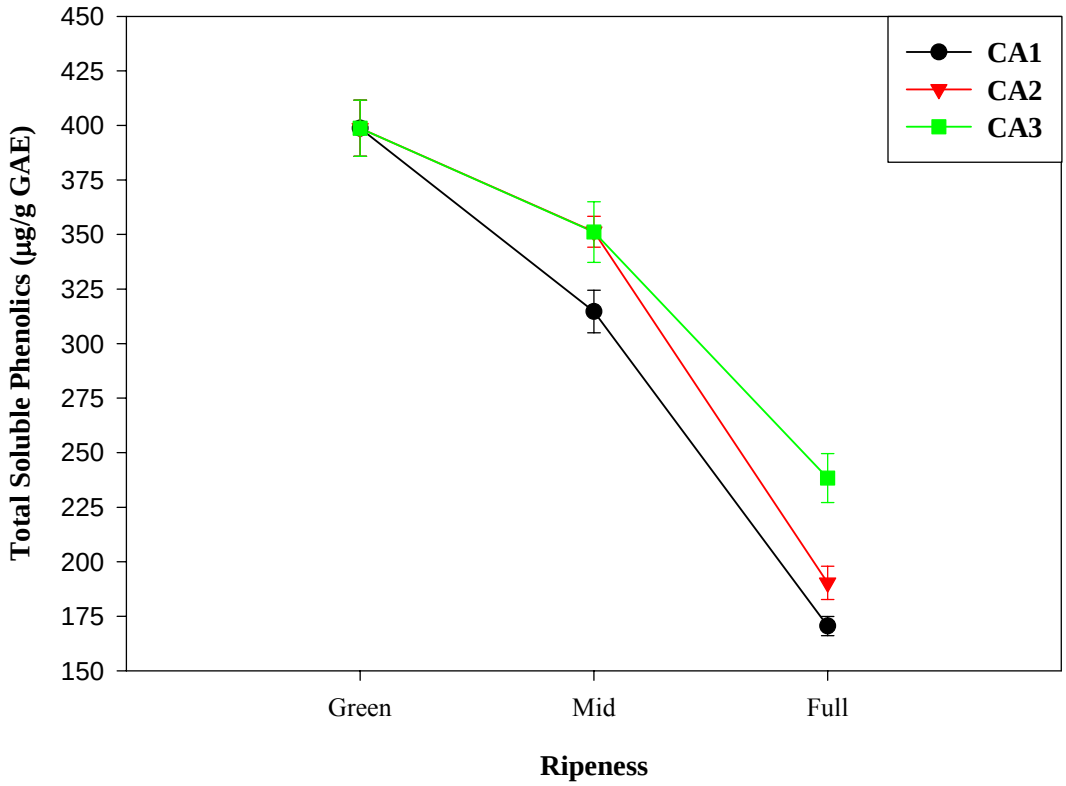


Figure 4-6. Change in average total soluble phenolics (TSP) (µg/g GAE) in mango for CA1, CA2 and CA3 during ripening. Each stage represents green (on the day of harvest), mid (after 2 week CA storage), and full (the time fruit started deterioration) ripe.

Average TSP for all CA treatments did not change as a result of HWT (Fig 4-7). As previously shown in Chapter 3, the required duration of HWT did not have an effect on average TSP in mango. Otherwise, average TSP concentration for HWT was affected by CA storage (Fig 4-7). In CA2 and CA3, average TSP was 11 and 18% higher than CA1, respectively. Even though average TSP in CA2 and CA3 were significantly higher than control ($P < 0.05$), the concentration in CA3 was 9% higher than in CA2. Since gallic acid and total HT concentrations for HWT increased due to an effect of CO_2, increase of

average TSP concentration for HWT might be directly affected by changes of gallic acid and total HT.

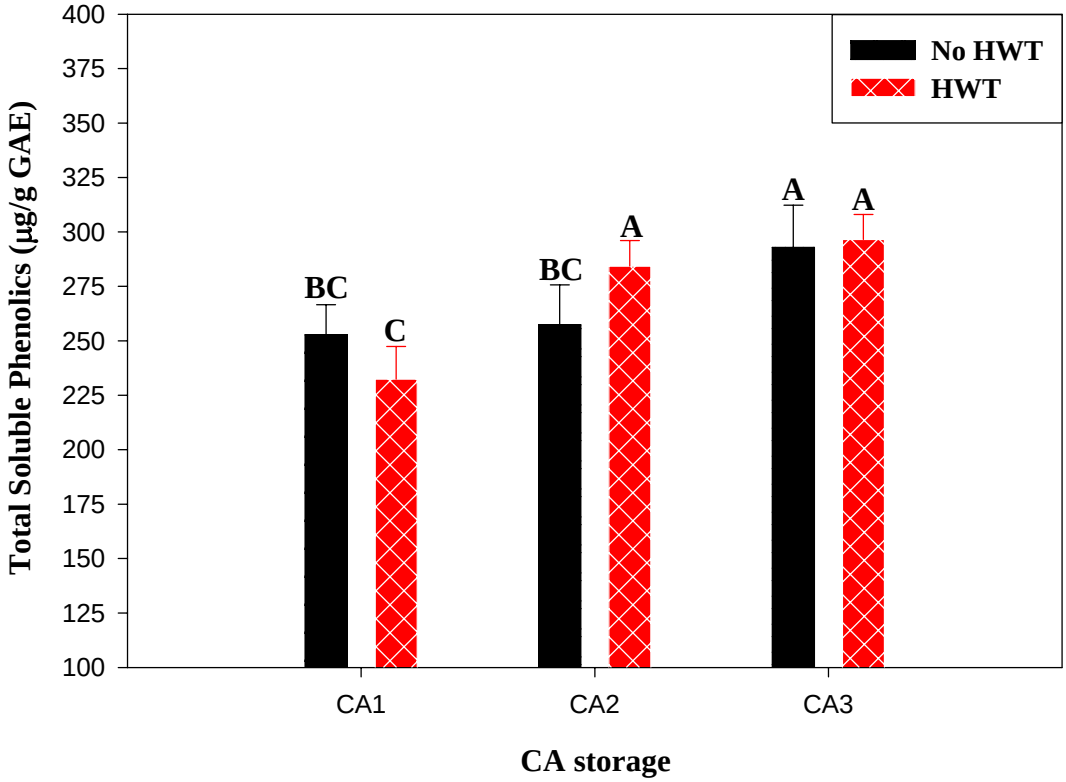


Figure 4-7. Changes in average total soluble phenolics (µg/g GAE) as a result of CA storage (Air, O_2, and O_2+CO_2) with or without HWT. Control was expressed as CA1 (21% air + 97% N_2) and two CA storages were shown as CA2 (3% O_2 and 97% N_2), and CA3 (3% O_2, 10% CO_2 and 87% N_2).

4-3-4. Antioxidant Capacity

Overall, antioxidant capacity changes of mango, which were thought to originate from polyphenolics and ascorbic acid showed a very good correlation with changes in TSP (r=0.98). Even though other antioxidant compounds such as carotenoids and tocopherol were found in mango, the ORAC assay does not detect antioxidant capacity from carotenoid and tocopherol. During fruit ripening, average antioxidant capacity

decreased from 5.43 to 3.26 (µM TE/g) (Fig 4-8). The reduction in antioxidant capacity was 45, 43 and 32% during ripening (from the mature green stage to the final ripe stage).

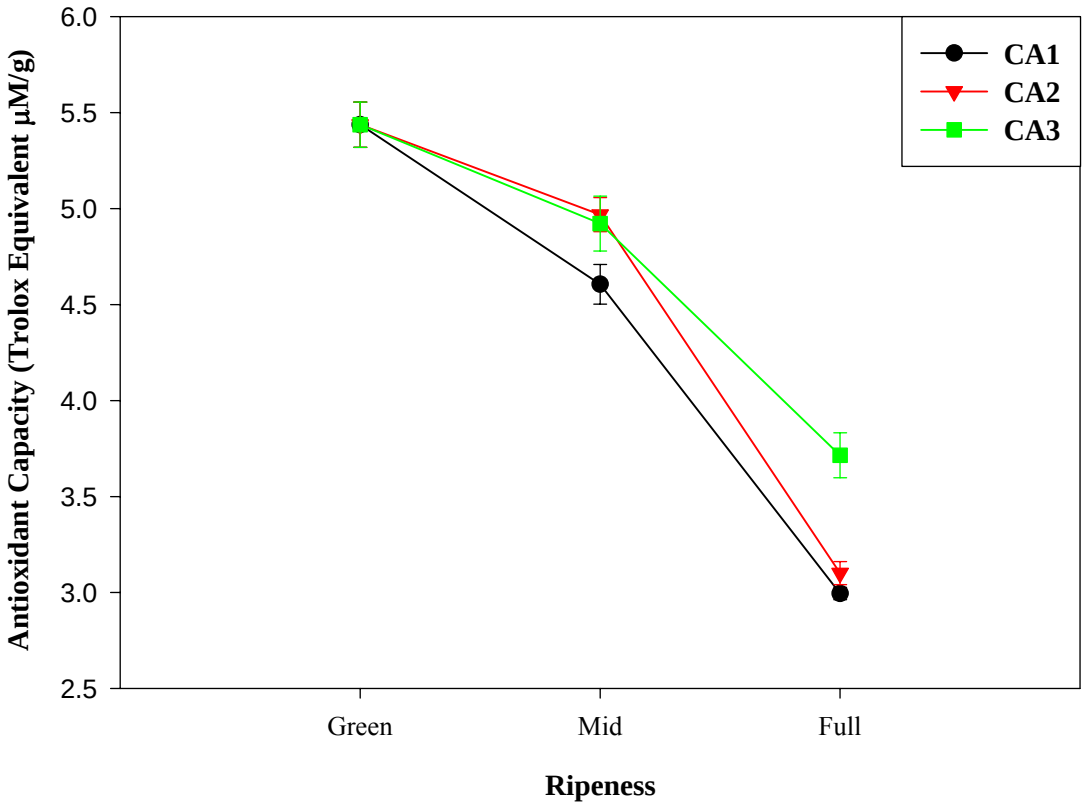


Figure 4-8. Change in average antioxidant capacity (Trolox Equivalent µM TE/g) for CA1, CA2 and CA3 determined by ORAC (Oxygen Radical Absorbance Capacity) assay during fruit ripening. Each stage represents green (on the day of harvest), mid (after 2 week CA storage), and full (the time fruit started deterioration) ripe.

During ripening, antioxidant capacity of mango decreased as a result of loss of phenolic compounds. Since antioxidant capacity is a desirable process in quenching harmful free radicals, the method to prevent or slow loss of compounds to be related to antioxidant capacity should be determined. In this study, it was found that CA storage slowed the reduction of antioxidant capacity as shown in TSP concentration during fruit ripening. After CA storage, both CA2 and CA3 showed higher antioxidant capacity (4.96

and 4.92 µM TE/g, respectively) compared to CA1 (4.60 µM TE/g). At 25°C, antioxidant capacity was 20% higher in CA3 than in CA1 while antioxidant capacity in CA1 and CA2 was not different (Fig 4-8).

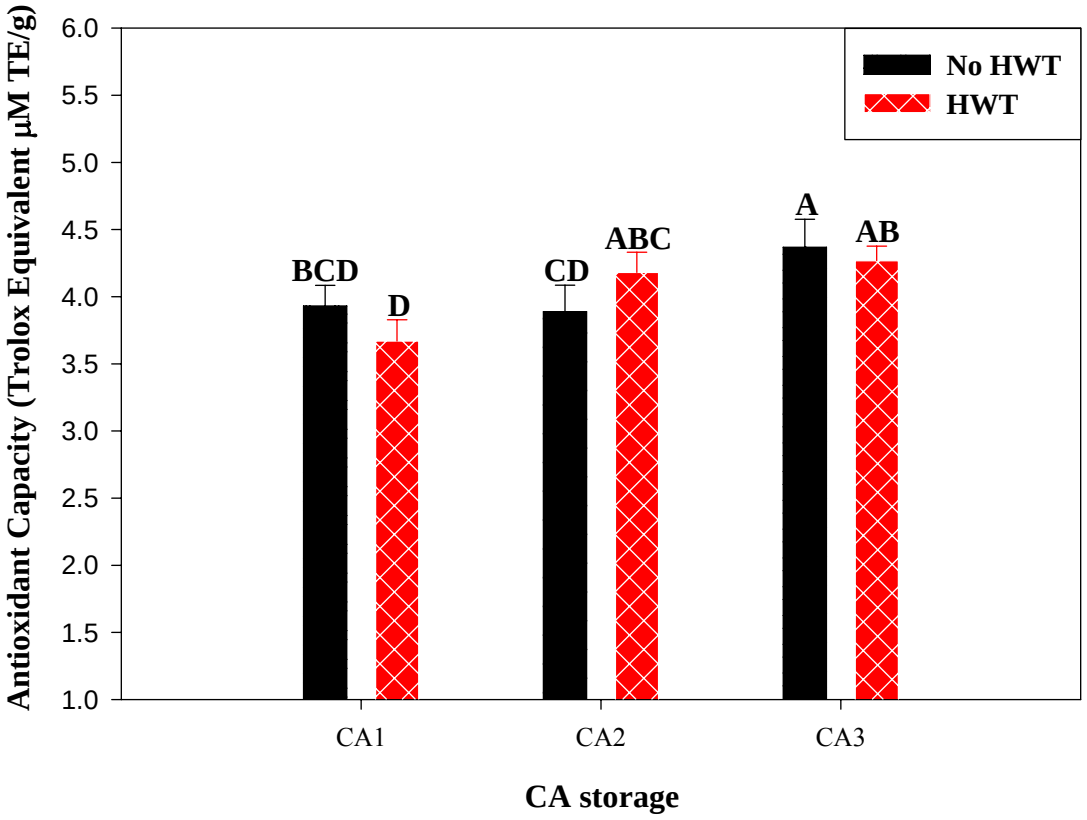


Figure 4-9. Antioxidant capacity (Trolox equivalent µM TE/g) effected by CA storage with or without HWT. It is measured by ORAC (Oxygen Radical Absorbance Capacity) assay.

Hot water treatment did not affect antioxidant capacity of mangos while antioxidant capacity was affected by CA storage as shown in TSP concentration. However, the mangos kept in CA3 showed 13% higher antioxidant capacity than that in CA1, and antioxidant capacity was not different in CA1 and CA2 (Fig 4-9). Antioxidant capacity was highest (4.31 µM TE/g) in CA3 while the lowest value was CA1 (3.79 µM TE/g).

Since phenolic compounds and TSP concentration was higher at CA3 than CA1 and CA2, antioxidant capacity might be influenced by those concentrations.

4-3-5. Fruit Flesh Color

The color change of fruit is a reliable parameter to determine the extent of fruit ripening (Ninio et al., 2003). The internal flesh color of peeled mango fruit was determined based on CIE color values (L*, a* and b*). L* denotes relative darkness (0) and lightness (100), a* represents green or red, and b* means blue or yellow of the samples (López and Gómez, 2004) (Fig 4-10). Hue angle is the numeric description of how yellow (90°), or green (180°) (in the case of mango fruit) the object is and chroma is the value to describe the vividness to dullness of a color and (Jeong et al., 2003; López and Gómez, 2004). In this study, color value was presented as L*, hue angle, and chroma by conversion from L*, a* and b*.

Ethylene is a naturally occurring compound related to many biological changes such as growth, development, and ripening in fruits and vegetables (Saltveit, 1999). Since ethylene synthesis increases in climacteric fruits during ripening, fruit ripening is accelerated. Synthesis of carotenoids increases as fruits ripen because ethylene stimulates synthesis of pigments such as anthocyanin and carotenoids and degradation of chlorophyll (Hatlen et al., 1998; Saltveit et al., 1999; Vilavicencio et al., 2001). As a result of increased carotenoid concentration during ripening, the L* value and hue angle decrease, but a* (redness), b* (yellowness), and chroma increase (Hatlen et al., 1998).

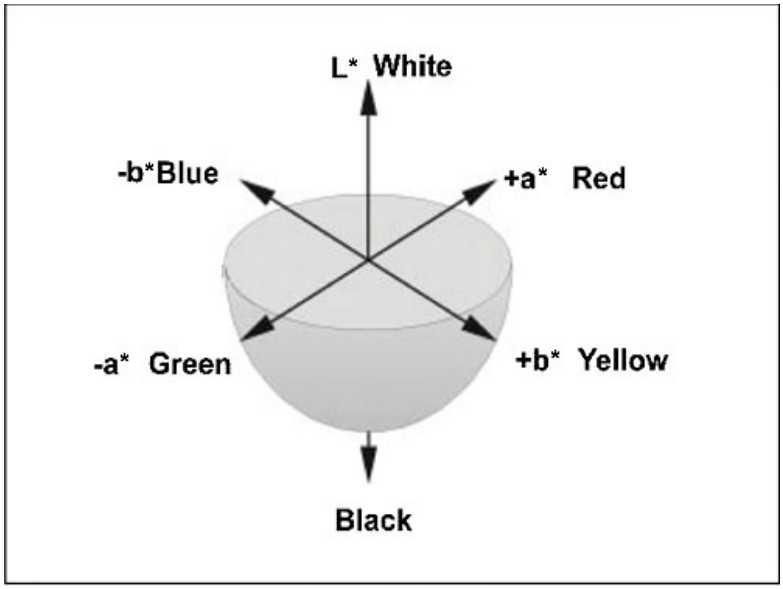


Fig 4-10. The CIELAB color space system. L* represents lightness and a*, and b* show the strength of own colors (a: red to green and b: yellow to blue) (López and Gómez, 2004)

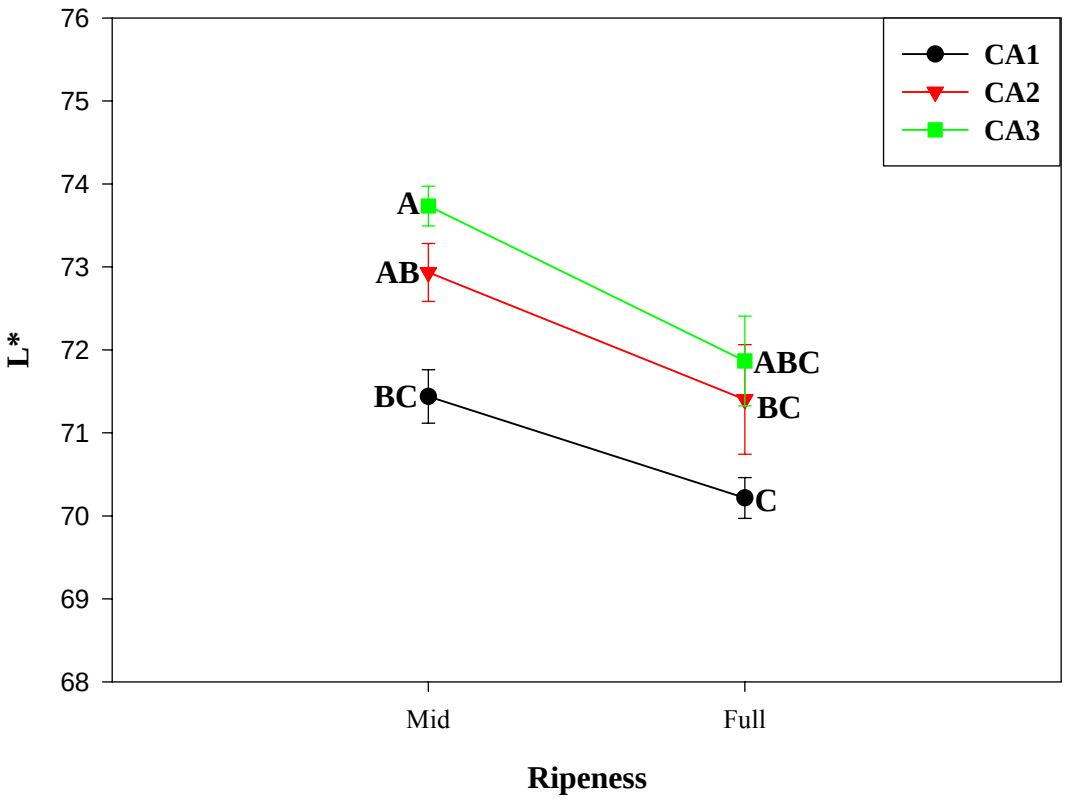


Figure 4-11. Average rate of reducing lightness (L*) for HWT during fruit ripening. Each stage represents mid (after 2 week CA storage) and full (the time fruit started deterioration) ripe.

Since color of yellow pulp fruit such as mango develops from greenish-white to yellow during ripening, pulp color changes from lighter (higher L* value) to deeper color (lower L* value). As shown in Fig 4-11, L* was 4% higher in CA3 than in CA1 after CA storage, but L* values in all CA storage did not show differences at 25°C. According to this result, ripening was inhibited when the fruit was under CO_2 environment (during CA storage at CA3) and then no difference was found at 25°C after CA storage.

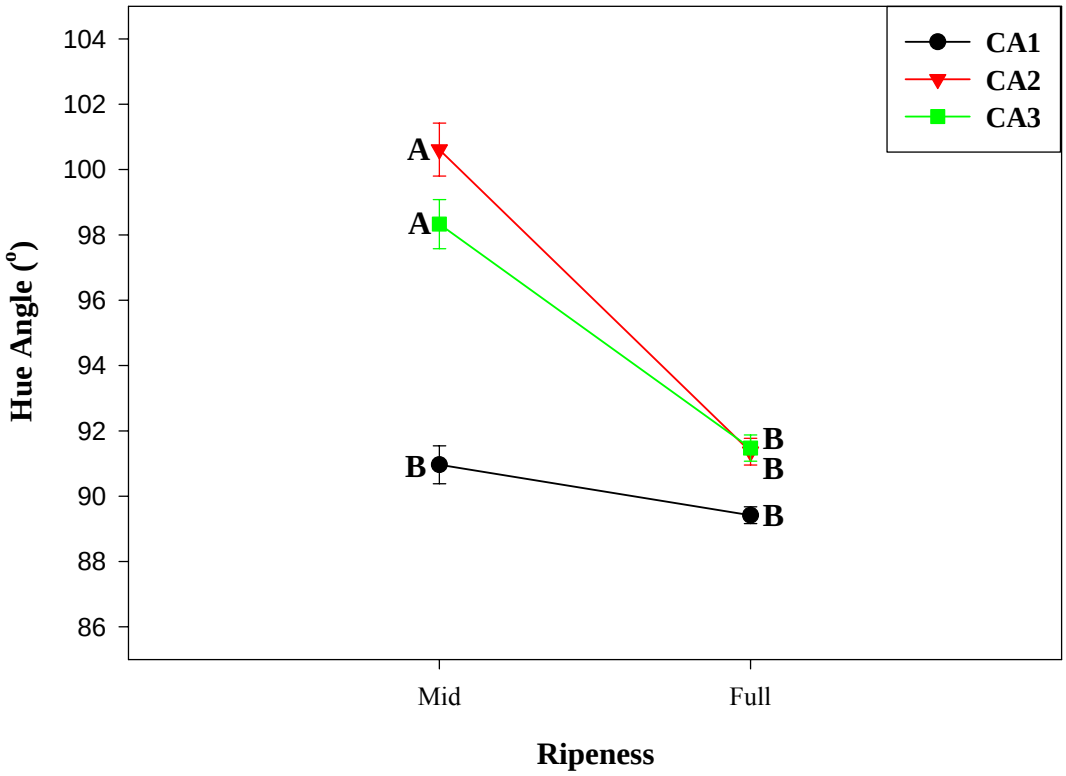


Figure 4-12. Reduction rate of average hue angle (°) for HWT depends on CA storage air composition during ripening. Each stage represents mid (after 2 week CA storage) and full (the time fruit started deterioration) ripe. Hue angle was defined by the coordination a* and b*.

Average hue angle and chroma for HWT tended to decrease without regard to CA storage during mango ripening (Fig 4-12, 13). The decline in hue angle represented the change from green (180°) to yellow (90°) as fruits lost their chlorophyll (green pigment

in the skin) and synthesis of carotenoid (yellow pigment in the flesh) was stimulated by ethylene during ripening (Guevara and Pardo-González, 1996; Saltveit, 1999). After CA storage, hue angles were higher in CA2 and CA3 (8 and 10%, respectively) than in CA3. However, all hue angles were not significantly different at 25°C. This result showed that ripening was delayed and/or carotenoid synthesis was inhibited as a result of CA storage, but there was no residual effect after transfer to air at 25°C. Since CA storage condition (lowered O_2 and elevated CO_2) inhibits respiration of fruits (consuming O_2 and producing CO_2) and decreases ethylene activity, higher hue angle in CA2 and CA3 was an evidence of delayed ripening.

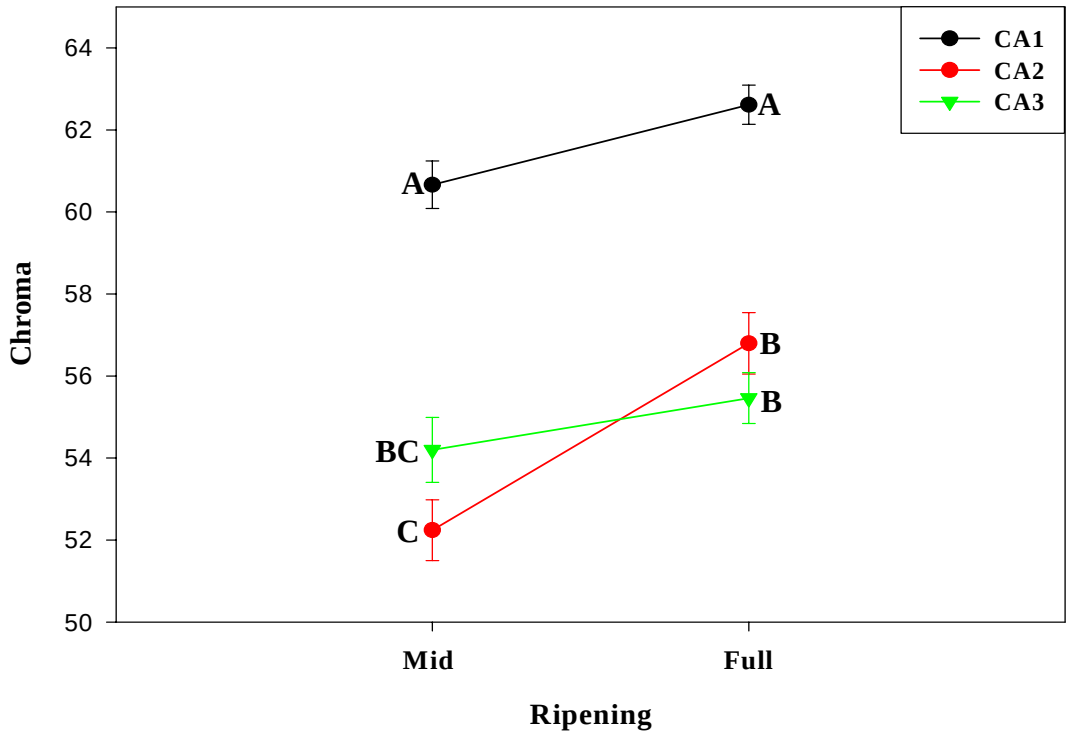


Figure 4-13. Increase in average chroma for HWT depends on CA storage air composition during ripening. Each stage represents mid (after 2 week CA storage) and full (the time fruit started deterioration) ripe. Chroma was defined by the coordination a* and b*.

As yellow pulp fruits such as mango, star fruit, and pepino ripen, pulp color changes from green to yellow and this appears as an increase in chroma and mixture of color is a reason of low chroma (Prono-Widayat et al., 2003). Consequently, chroma was highest at CA1 through mango ripening because mangos in CA1 were riper than in CA2 and CA3 that inhibited mango ripening. Chroma in CA2 rapidly decreased after storage while chroma in CA3 did not change even after storage.

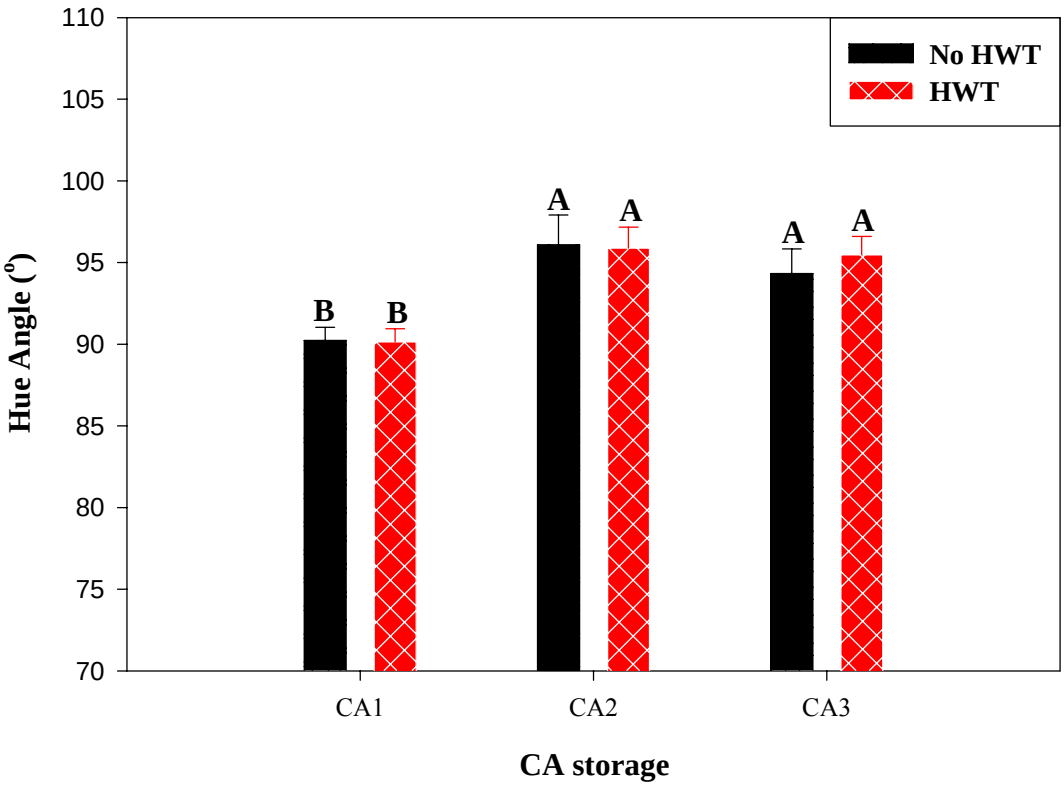


Figure 4-14. Changes of average hue angle (°) affected by CA storage with or without HWT. Control was expressed as CA1 (21% air + 97% N_2) and two CA storages were shown as CA2 (3% O_2 and 97% N_2), and CA3 (3% O_2, 10% CO_2 and 87% N_2).

Neither CA storage nor HWT affected the change of L* value of mango flesh (data not shown). However, hue angle significantly increased by (7 and 5%, respectively) as a result both CA storages (CA2 and CA3) compared to control (CA1), and chroma was

higher only in CA2 and CA3 (8 and 4%, respectively) compared to control (CA1) (Fig 4-15, 16).

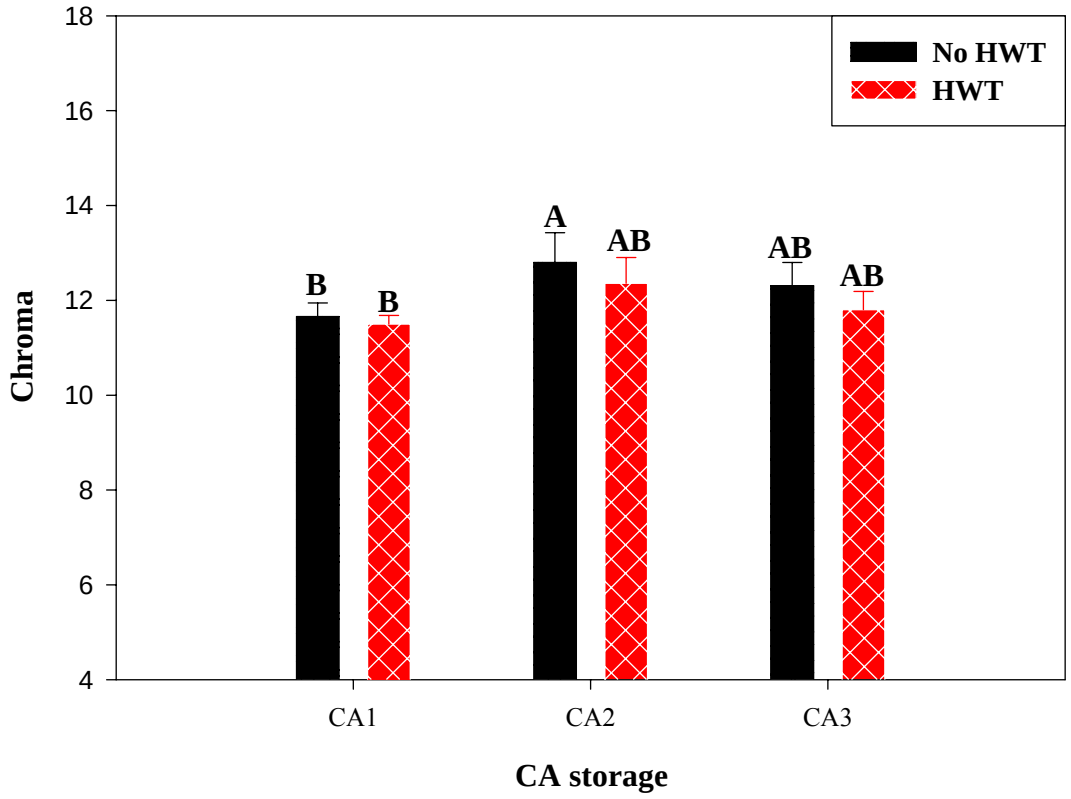


Figure 4-15. Changes of average chroma affected by CA storage with or without HWT. Control was expressed as CA1 (21% air + 97% N_2) and two CA storages were shown as CA2 (3% O_2 and 97% N_2), and CA3 (3% O_2, 10% CO_2 and 87% N_2).

4-3-6. Titratable Acidity (TA)

Titratable acidity (TA) represents the total concentration of titratable acid in a sample and is considered an important attribute in determining the taste quality of fruits and vegetables (Berezin et al., 1994). According to Jacobi et al. (2000) and Tovar et al. (2001), TA in mango decreases as mango ripens because two major organic acids (citric and malic acid) used in respiration decrease during mango ripening. Titratable acidity for CA1, CA2 and CA3 decreased during ripening (Fig 4-16). Since CA storage delays fruit

ripening, reduction rate of TA at CA2 and CA3 was inhibited during the CA storage period (green to mid ripe) compared to the period from mid to full ripe after CA treatment. However, reduction rate of TA at CA1 was not inhibited during CA storage period.

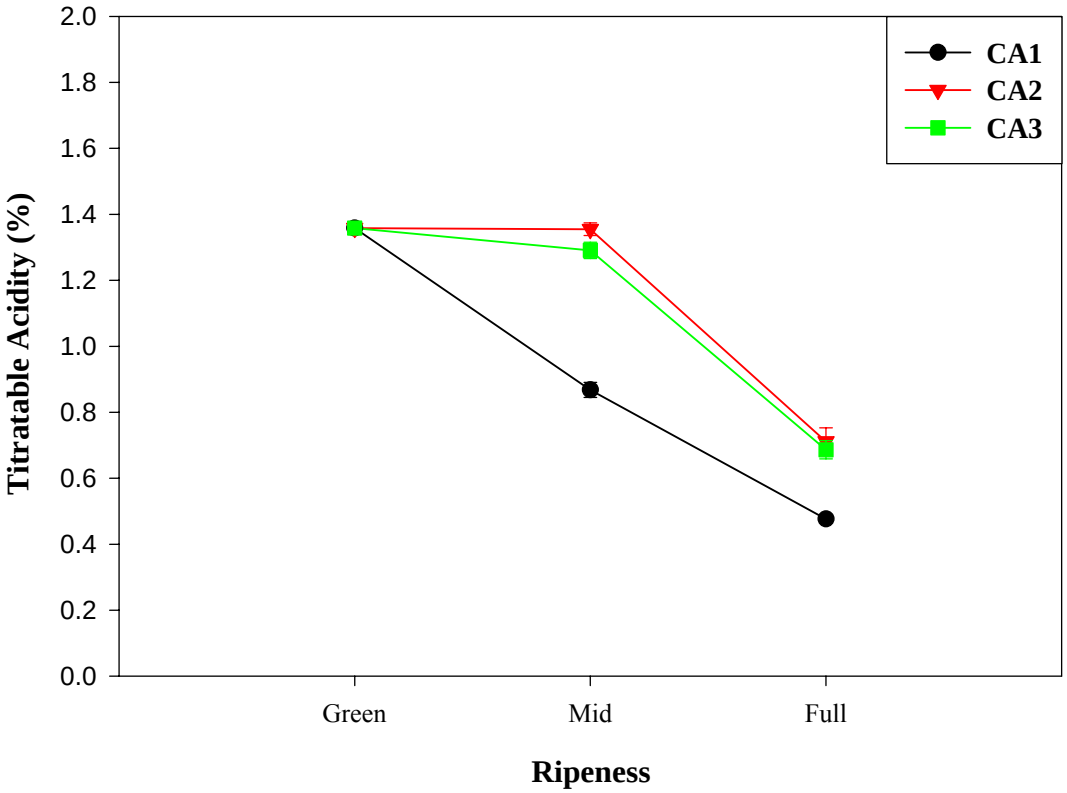


Figure 4-16. Change of average titratable acidity (%) for CA1, CA2 and CA3 during mango ripening. Each stage represents green (on the day of harvest), mid (after 2 week CA storage), and full (the time fruit started deterioration) ripe.

Average TA was higher at CA2 and CA3 as a result of CA storage (CA2-35% and CA3-32%) compared to control (CA1) and TA of mango decreased by 18% HWT (Fig 4-17). This change has been reported previously that TA of fruits including mango are reduced by heat treatment (Jacobi et al., 2000; Neven and Mitcham, 1994; Ninio et al., 2003).

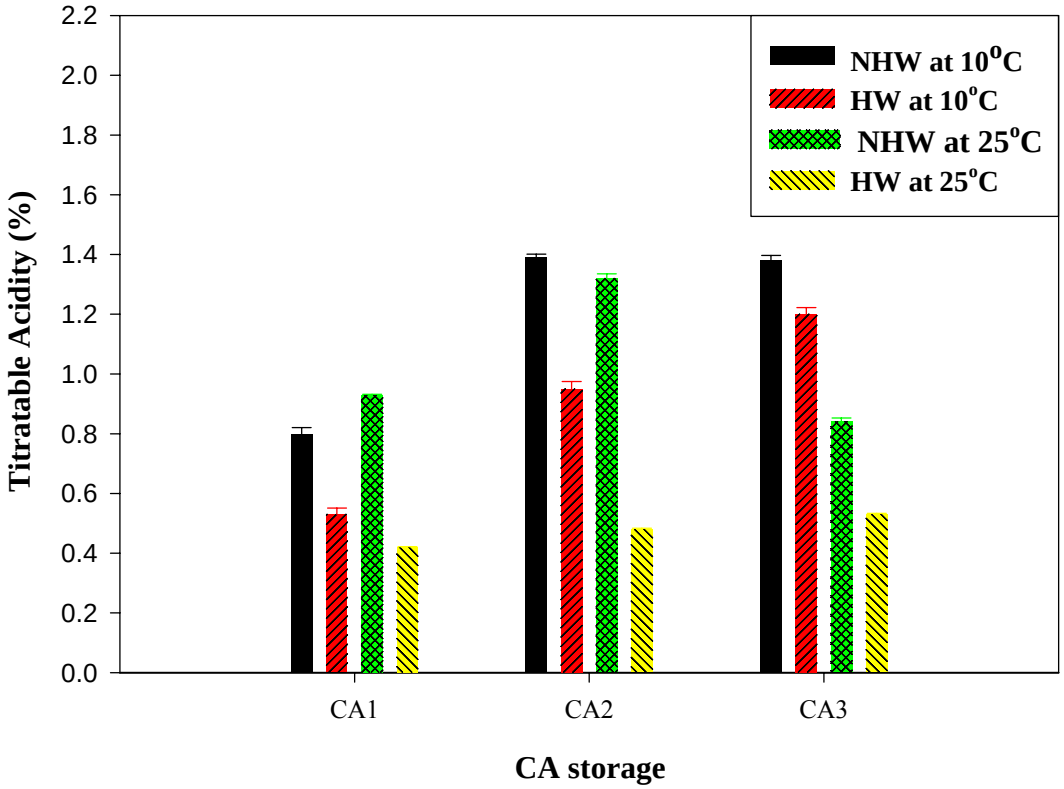


Figure 4-17. Change of titratable acidity (%) in mango affected by CA storage with or without HWT. Titratable acidity was measured twice at 10°C (mid ripe) and 25°C (full ripe).

4-3-7. Soluble Solids Content (SSC)

Soluble solids content is one of the most reliable parameters in judging fruit quality as consumers consider quality factors such as SSC and TA as much as visible quality (e.g. color, size and firmness) (Hoehn et al., 2002; Lu, 2004). In this study, average SSC decreased by 50% during CA storage at 10°C and then increased by 35% at 25°C. Especially, a degree of reduction was higher at CA2 than at CA1 and CA3 (Fig 4-18).

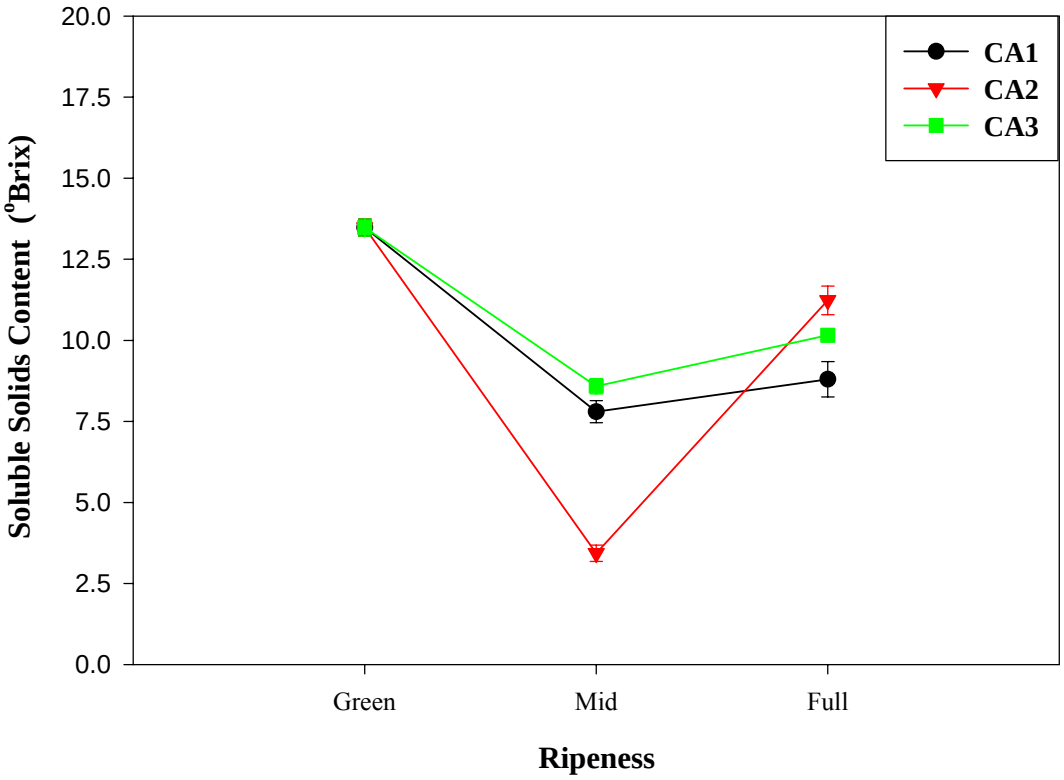


Figure 4-18. Effect of the fruit ripening on average soluble solids content (°Brix) for all CA storage and HWT in mangos. Each stage represents green (on the day of harvest), mid (after 2 week CA storage) and full (the time fruit started deterioration) ripe.

Since SSC is known to increase during fruit ripening as insoluble starch is transformed into soluble solids (Martisen and Schaare, 1998; McGlone and Kawano, 1998; Vela et al., 2003), reduction of SSC during storage was not anticipated result. However, several studies have shown several examples in decreasing SSC during ripening. Numerous studies have reported that low O_2 storage suppresses SSC increase (Hoehn et al., 2003; Lopez et al., 2000; Taylor et al., 1995). According to Taylor et al. (1995), SSC in plum, which is a climacteric fruit like mango also decreased during ripening, and when apples were stored with high relative humidity to prevent drying out, SSC decreased during ripening (Saad et al., 2004). Therefore, it could be assumed that

decrease of SSC might be influenced by low O_2 composition or naturally decreased or affected by high relative humidity during storage. After 2 weeks CA storage, SSC started to increase as shown in Fig 4-18. Comparing SSC of CA1, CA2 and CA3, SSC in CA2 at 10°C was significantly lower compared to CA1 and CA3 (Fig 4-19). This might be because lowered O_2 suppressed SSC synthesis as explained above. After storage at 10°C, SSC in CA2 became higher than CA1 at 25°C.

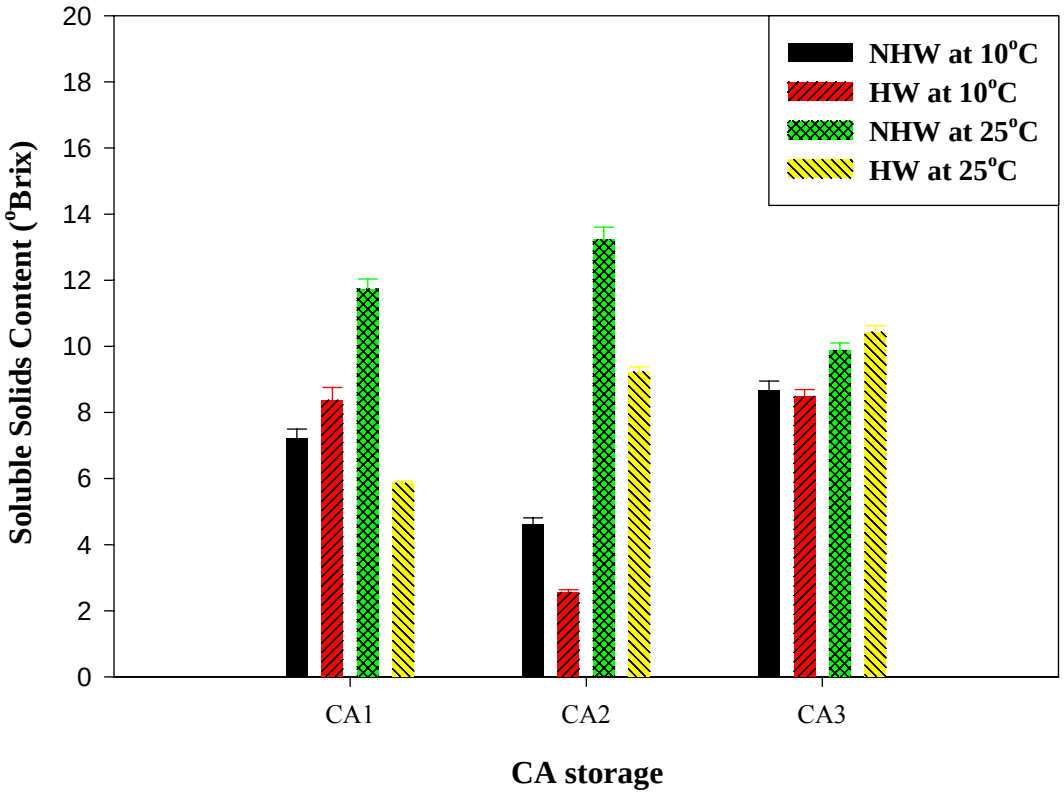


Figure 4-19. Average soluble solids content affected by CA storage with or without HWT. Soluble solids content was measured twice at 10°C (mid ripe) and 25°C (full ripe).

4-4. Conclusion

The external quality of mango fruit during ripening was affected by CA storage and HWT as both postharvest treatments inhibited external disorders such as anthracnose on

the mango skin. CA storage was effective to increase phenolic compounds and resultant antioxidant capacity in this study. The CA with reduced O_2 and elevated CO_2 concentration (CA3) was more effective than CA with low O_2 (CA2) and the former increased TSP by 18%, antioxidant capacity by 12%, gallic acid by 9%, and total hydrolyzable tannin by 15% compared to control. However, HWT increased only total HT (17%) and did not affect to the other parameters. The TSP, antioxidant capacity, gallic acid, and total HT significantly decreased by 50%, 40%, 12% and 46%, respectively during ripening (from green to full ripe). In addition, CA3 significantly inhibited the reduction rate of TSP and antioxidant capacity compared to CA1. At the end of fruit ripening, TSP and antioxidant capacity in CA3 fruits were higher by 30% and 20% than those in CA1. Strictly speaking, since CO_2 in CA3 increased synthesis of phenolic compounds, reduction of TSP and antioxidant capacity in CA3 was delayed during ripening.

Unlike imported mangos, domestic mangos grown in Florida such as ‘Tommy Atkins’ are not usually subjected to HWT because of the possibility that they were infested by dangerous tropical fruit flies and/or larvae is negligible. Through this study, it was confirmed that domestic mangos do not have any disadvantages compared to imported mangos, although they are not treated with hot water. Furthermore, since it is proved that CA storage with elevated CO_2 was effective to increase polyphenolics and resultant antioxidant capacity, using CA storage will be good choice even for domestic transportation.

CHAPTER 5
SUMMARY AND CONCLUSION

As the popularity and consumption of mango in the US have increased, the necessity of postharvest treatments for maintaining the quality and extending shelf-life of fruits during transportation and storage has also increased. Numerous studies related to mango postharvest treatment have stressed physiological changes during this time. Therefore, these studies were designed to investigate phytochemical changes and resultant antioxidant capacity in mango by hot water immersion and controlled atmosphere storage, which are most important postharvest treatments on mangos.

Hot water immersion treatment (HWT), legally required thermal quarantine treatment for imported mangos was applied to mangos with varying lengths of treatment times (0, 70, 90 and 110 min) followed by 4 days of storage at 10°C. When mangos were subjected to the required length of treatment time (70 min) for quarantine, polyphenolic compounds were stable or elevated. However, when mangos were treated for extended times (90 and 110 min), polyphenolics significantly decreased compared to control (0 min). Antioxidant capacity of mangos was not affected by HWT. Controlled atmosphere (CA) storage based on reduced O_2 and/or elevated CO_2 at low temperature was a factor to change phytochemicals in mango. CA2 (lowered O_2) did not change phytochemicals and antioxidant capacity compared to CA1 (normal air) while CA2 (lowered O_2 and elevated CO_2) increased polyphenolics and resultant antioxidant capacity as a result of CO_2 effect. HWT used in this study had no affect on changes of polyphenolics and antioxidant capacity.

In conclusion, selected postharvest treatments in this study were effective to prevent anthracnose and increase shelf-life. Moreover, CA storage with lowered O_2 and elevated CO_2 induced increases in polyphenolics and resultant antioxidant capacity in mangos. Domestically produced mangos are usually not subject to HWT and CA storage is not used due to relatively short transportation and storage compared to imported mangos. Therefore, applications of HWT and CA storage should be considered even for domestic mango varieties such as Tommy Atkins to get advantages in preserving fruit quality and extending shelf-life with beneficial effects on phytochemical contents.

LIST OF REFERENCES

Abd. Shukor, A.R.; Razali, M.; Omar, D. Poststorage respiratory suppression and changes in chemical composition of mango fruit after storage in low oxygen atmosphere. Acta Hort. **2000**, 467-470.

Agrawal, M.; Deepak, S.S. Physiological and biochemical responses of two cultivars of wheat to elevated levels of CO2 and SO2, singly and in combination. Environ. Pollut. **2003**, 121,189–197.

Alexander, L.; Grierson, D. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *J. Exp. Bot*. **2002**, 53, 2039-2055.

Antolovich, M.; Prenzler, P.D.; Patsalides, E.; McDonald, S.; Robards, K. Methods for testing antioxidant activity. *Analyst*. **2001**, 127, 183–198.

Assis, J.S.; Maldonado, R.; Munoz, T.; Escribano, M.I.; Merodio, C. Effect of high carbon dioxide concentration on PAL activity and phenolic contents in ripening cherimoya fruit. Postharvest Biol Technol. **2001**, 23, 33–39.

Bashir, H.A.; Abu-Goukh, A.A. Compositional changes during guava fruit ripening. *Food Chem*. **2003**, 80, 557–563.

Bender, R.J.; Brecht, J.K.; Sargent, S.A.; Huber, D.J. Low temperature controlled atmosphere storage for tree-ripe mangoes (*Mangifera indica* L.). *Acta Hort.* **2000**, 447-458

Berezin, O.Y.; Turyan, Y.I.; Kuselman, I.; Shenhar, A. Alternative methods for titiratable acidity determination. *Talanta*. **1995**, 42, 507-517.

Brecht, P.E. Use of controlled atmosphere to retard deterioration of produce. *Food Technol*. **1980**, 34, 45-49.

California Rare Fruit Growers. Inc. http://www.crfg.org (accessed Feb 2005).

Campbell, R.J. A Guide to Mangos in Florida; Fairchild Tropical Garden: Miami, FL. **1992**; pp 24-27.

Campbell, R.J.; Campbell C.W. Commercial Florida Mango Cultivars. *Acta Hort*. **1993**, 341, pp 55-59.

Carbonaro, M.; Mattera, M.; Nicoli, S.; Bergamo, P.; Cappelloni, M. Modulation of antioxidant compounds in organinc vs conventional fruit (peach, *Prunus persica* L., and pear (*Pyrus communis* L.). *J. Agric. Food Chem*. **2002**, 50, 5458-5462.

Castells, E.; Roumet, C.; Penuelas, J.; Roy, J. Intraspecific variability of phenolic concentrations and their responses to elevated CO_2 in two Mediterranean perennial grasses. *Environ. Exp.Bot.* **2002**, 47, 205–216.

Chen, P.M.; Mellenthin, W.M.; Kelly, S.B.; Facteau, T.J.; Effects of low oxygen and temperature on quality retention of 'Bing' cherries during prolonged storage. *J. Am. Soc. Hort. Sci.* **1981**, 106,533-535.

Crane, J.H. and Campbell, C.W. The Mango; University of Florida IFAS Extension: **1994**.

Crane, J.H.; Bally, I.S.E.; Mosqueda-Vazquez R.V, and Tomer E. Crop Production. In: The Mango. Botany Production and Uses; CAP Intl: Willingston, U.K. **1997**, 203-256.

Crane, J.H.; Balerdi, C.F. Effect of hurricane Andrew on mango trees in Florida and their recovery. Acta Hort. **1997**, 455, 323-330.

Davey, M.P.; Bryant, D.N.; Cummins, I.; Ashenden, T.W.; Gates, P.; Baxter, R.; Edwards, R. Effects of elevated CO_2 on the vasculature and phenolic secondary metabolism of Plantago maritime. *Phytochemistry*. **2004**, 65, 2197–2204.

El Ansari, M.A.; Reddy, K.K.; Sastry, K.N.S.; Nayudamma, Y. Polyphenolic components of mango (*Magnifera Indica*). *Leather Science*. **1969**, 16,13-14.

El Ansari, M.A.; Reddy, K.K.; Sastry, K.N.S.; Nayudamma, Y. Polyphenols of Mangifera indica. *Phytochemisty*. **1971**, 10, 2239-2241.

El Sissi, H. I.; Saleh, N. A. M. Phenolic components of *Mangifera indica* Part II. *Planta Medica.* **1965.** *13*,346-352.

Fallik, E. Prestorage hot water treatment (immersion, rinsing and brushing). *Postharvset. Biol. Technol*. **2004**, 32, 125-134.

Fernandez-Pachon, M.S.; Villano, D.; Garcia-Parrilla, M.C.; Troncoso, A.M. Antioxidant activity of wines and relation with their polyphenolic composition. *AFFnalytica Chimica Acta*. **2004**, 513, 113–118.

Ferrer, A.; Remon, S.; Negueruela, A.I.; Oria, R. Changes during the ripening of the very late season Spanish peach cultivar Calanda Feasibility of using CIELAB coordinates as maturity indices. Scientia Horticulturae, in press.

Food and Agriculture Organization. FAOSTAT Database Collections, Agricultural Data, Food and Agriculture Organization of the United Nations. **2004**. http://faostat.fao.org/faostat/collections?subset=agriculture

Friedman, M. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. *J. Agric. Food Chem.* **1997**, *45*, 1523-1540.

Gamagaea, S.U.; Sivakumara, D.; Wijesundera, R.L.C. Evaluation of post-harvest application of sodium bicarbonateincorporated wax formulation and *Candida oleophila* for the control of anthracnose of papaya. *Crop Protection*. **2004**, 23, 575–579.

Gilman, E.F. and Watson, D.G. *Mangifera indica*: Mango; University of Florida Extension: **1993**.

Gokmen, V.; Kahraman, N.; Demir, N.; Acar, J. Enzymatically validated liquid chromatographic method for the determination of ascorbic and dehydroascorbic acids in fruit and vegetables. *J. Chromatogr.* **2000**, 881, 309–316.

Grundhofer, P.; Niemetz, R.; Schilling, G.; Gross, G.G. Biosynthesis and subcellular distribution of hydrolysable tannins. *Phytochemistry*. **2001**, 57, 915-927.

Guevara, R.G.L.; Pardo-González, J.E. Evolution of color during the ripening of selected varieties of paprika pepper (*Capsicum annuum* L.). *J.Agric. Food Chem.* **1996**, 44, 2049 -2052.

Haard, N.F. and Chism G.W. *Characteristics of Edible Plant Tissues*. In: *Food Chemistry*, 3rd Ed. Fennema, O. R. Marcel Dekker Inc: New York, **1996**; pp 943-1011.

Hagerman, A.E. Hydrolyzable tannin structural chemistry; Tannin handbook. http://www.users.muohio.edu/hagermae/tannin.pdf. (accessed Mar 2005).

Hagerman, A.E.; Riedl, K.M.; Jones, G.A.; Sovik, K.N.; Ritchard, N.T.; Hartzfeld, P.W.; Riechel, T.L. High molecular weight plant polyphenolics (Tannins) as biological antioxidants. *J. Agric. Food Chem.* **1998,** *46,* 1887-1892.

Haiti Agricultural Program (HAP) Product Bulletin #1. http://www.haitihap.org. (accessed Feb 2005).

Hartzfeld, P.W.; Forkner, R.; Hunter, M.D.; Hagerman, A.E. Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *J. Agric. Food Chem*. **2002**, 50, 1785-1790.

Hatlen, B.; Jobling, M.; Bjerkeng, B. Relationships between carotenoid concentration colour of fillets of Arctic charr, *Salvelinus alpinus* (L.), fed astaxanthin. *Aquaculture Research*. **1998**, 29, 191-202.

Hoehn, E.; Gasser, F.; Guggenbuhl, B.; Kunsch, U. Efficacy of instrumental measurements for determination of minimum requirements of firmness, soluble solids, and acidity of several apple varieties in comparison to consumer expectations. *Postharvest Biol. Technol.* **2003**, 27, 27-37.

Holmes, R.; Campbell, T.; Ledger, S. Mango picking guide; Queensland Department of Primary Industries Publication: Queensland, **1990**.

Jacobi, K.K.; Wong, L.S. Quality of 'Kensington' mango (*Mangifera indica* Linn.). following hot water and vapour–heat treatments. *Postharvest Biol. Technol*. **1992**, 1, 349–359.

Jacobi, K.K.; Macrae, E.A.; Hetherington S.E. Early detection of abnormal skin ripening characteristics of 'Kensington' mango (*Mangifera Indica* Linn). *Sci Hort.* **1998**, 72, 215-225.

Jacobi, K.K.; Macrae, E.A.; Hetherington, S.E. Effects of hot air conditioning of 'Kensington' mango fruit on the response to hot water treatment. *Postharvest Biol. Technol.*. **2000**, 21, 39–49.

Jacobi, K.K.; Macrae, E.A.; Suzan, E.H. Postharvest heat disinfection treatments of mango fruit. *Scientia Hort*. **2001**, 89:171-193.

Jeong, J.; Huber, D.J.; Sargent, S.A . Delay of avocado (*Persea americana*) fruit ripening by 1-methylcyclopropene and wax treatments. *Postharvest Biol. Technology.* **2003**, 28, 247-257

Loaiza-Velarde, J.G.; Mangrich, M.E.; Campos-Vargas, R.; Saltveit, M.E. Heat shock reduces browning of fresh-cut celery petioles. *Postharvest Biol. Technology*. **2003**, 27, 305-311.

Kader, A.A.; Zagory, D.; and Kerbel, E.L. Modified atmosphe re packaging of fruits and vegetables. *CRC Crit. Rev. Food Sci. Nutr*. **1989**, 28, 1-30.

Kader, A.A. Fruit maturity, ripening, and quality relationships. *Acta Hort*. **1997**, 203-208.

Kader, A.A. Mango Produce Facts. UC-Davis Postharvest Technology Research and Information Center. **2002,** http://rics.ucdavis.edu/postharvest2/Produce/ProduceFacts/Fruit/mango.shtml (accessed Feb 2005).

Karakaya, S.; Nehir, E.L S. Quercetin, luteolin, apigenin and kaempferol contents of some foods. *Food Chem*. **1999**, 66, 289-292.

Karakaya, S.; El, S.N.; Tac, A.A. Antioxidant activity of some foods containing phenolic compounds. *Int. J. Food Sci. Nutr*. **2001**, 52, 501–508.

Kang, H.M.; Salveit, M.E. Antioxidant capacity of lettuce leaf tissue increases after wounding. *J. Agric. Food Chem*. **2002**, *50*, 7536-7541.

Kelly, M., Xu, J., Alexander, K., Loo, G. Disparate effects of similar phenolic phytochemicals as inhibitors of oxidative damage to cellular DNA. *Mutation Res*. **2001**, *485*, 309-318.

Lakshminarayana, S.; Subhadra, N.V.; Subramanyam, H. Some aspects of developmental physiology of mango fruit. *J. Hort. Sci*. **1970**, 45, 133-142.

Leitao, G.G.; Mensor, L.L. Phenolic content and antioxidant activity: A study on plants eaten by a group of howler monkeys (*Alouatta fusca*). In: Plant Polyphenols 2. Chemistry, Biology, Pharmacology, Ecology (Gross, G. G.; Hemingway R. W.; Yoshida, T., eds). Plenum Press: New York and London, **1999.** pp 883-895.

Lizada, M. C. Postharvest physiology of the mango-a review. *Acta Hort.* **1991**, 291, 437-453.

López, M.; Martinez, F.; Del Valle, C.; Ferrit, M.; Luque, R. Study of phenolic compounds as natural antioxidants by a fluorescence method. *Talanta*. **2003**, 60, 609-616.

López, C,A.F.; Gómez, P.A. Comparison of color indexes for tomato ripening. *Horticultura Brasileira*. **2004**, 22, 534-547.

Lu, R. Multispectral imaging for predicting firmness and soluble solids content of apple fruit. *Postharvest Biol. Techol*. **2004**, 31, 147-157.

Lurie, S. Postharvest heat treatment. *Postharvest. Biol. Tech*. **1998**, 14;257-269.

Martinsen, P.; Schaare, P. Measuring soluble solids distribution in kiwifruit using near-infrared imaging spectroscopy. *Postharvest Biol. Techol*. **1988**, 14, 271-281.

McGlone, V.A.; Kawano, S. Firmness, dry-matter and soluble-solids assessment of postharvest kiwifruit by NIR spectroscopy. *Postharvest Biol. Techol*. **1998**, 13, 131-141.

Medlicott, A., Bhogol, M., Reynolds, S. Changes in peel pigmentation during ripening of mango fruit (*Magnifera indica*, var. Tommy Atkin). *Annals Appl Biol.* **1986**, *109*, 651-656.

Mitra, S.K.; Baldwin, E.A. Mango. In: Postharvest physiology storage of tropical and subtropical fruit, CAB International; New York. NY. **1997**, pp 85-122.

Modi, V.; Reddy, V. Carotenogenesis in ripening mangoes. *Ind. J. Expt Biol.* **1967**, 5, 233-235.

Morton, J. Mango. In: Fruits of warm climates: Miami, FL, **1987**. pp 221–239.

Mossler, M.A.; Nesheim, O.N. Florida crop/pest management profile: Mango. University of Florida Extension; Institute of Food and Agricultural Science: CIR 1401; **2002**.

Mueller-Harvey, I. Analysis of hydrolysable tannins. *Anim.Feed sci. Tech*. **2001**, 91,3-21.

Nakasone, H.Y.; Paul, R.E. Tropical fruits. CAB Intl. Willingford, UK, **1998**, pp 208-238.

Neven, L.G.; Mitcham, E. Heat as a potential alternative quarantine treatment for codling moth in cherries: The shocking truth. Proceedings of the 68th Annual Western Orchard Pest & Disease Management Conference. **1994**, 68, 83-84.

Niemetz, R.; Niehaus, J.U.; Gross, G.G. Biosynthesis and biodegradation of complex gallotannins. In: Plant Polyphenols 2. Chemistry, Biology, Pharmacology, Ecology (Gross, G. G.; Hemingway, R. W.; Yoshida, T., eds). Plenum Press, New York and London. 63–82. **1999**.

Ninio, R.; Lewinsohn, E.; Mizrahi, Y.; Sitrit, Y. Changes in sugars, acids, and volatiles during ripening of koubo [*Cereus peruvianus* (L.) Miller] fruits. *J. Agric. Food Chem.* **2003,** 51, 797-801.

Ou, B., Hampsch-Woodill, M., Prior, R.L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**. 49, 4619-4626.

Osslpov, V.; Loponen, J.; Ossipova, S.; Haukioja, I.E.; Pihlaja, K. Gallotannins of birch *Betula pubescens* leaves: HPLC Separation and Quantification. *Biochem. Syst. Ecol*. **1997**, 25, 493-504.

Pandey, S.N. Mango cultivars: Nomenclature and Registration. ISHS Acta Hort, 182: 1 International Symposium on Taxonomy of Cultivated Plants. **1986**.

Penuelas, J.; Estiarte, M.; Kimball, B.; Idso, S.; Pinter, P.; Wall, G.; Garcia, R.; Hansaker, D.; LaMorte, R.; Hendrix, D. Variety of responses of plant phenolic concentration to CO_2 enrichment. *J. Exp. Bot.* **1996**, 47, 1463-1467.

Prono-Widayat, H.; Schreiner, M.; Huyskens-Keil1, S.; Lüdders, P. Effect of ripening stage and storage temperature on postharvest quality of pepino (*Solanum muricatum* Ait.). *Food Agric. Environ*. **2003**, 1, 35- 41.

Pyo, Y.H.; Lee, T.C.; Logendrac, L.; Rosen, R.T. Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris subspecies cycla*) extracts. *Food Chem*. **2004**. 85, 19–26.

Rattanapanone, N; Watada, A.E. Respiration rate and respiratory quotient of fresh-cut mango (*Mangifera indica* L.) in low oxygen atmosphere. *Acta Hort*. **2000**, 471-478.

Rivero, R.M.; Ruiz, J.M.; Garcia, P.C; Lopez-Lefebre, L.R.; Sanchez, E.; Romero, L. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Science*. **2001**. 160, 315–321.

Robbins, R.J. Phenolic acids in food: An overview of analytical methodology. *J. Agric. Food Chem*. **2003**, 51, 2866-2887.

Saad, F.; Khanhnizadeh, S.; Groleau, Y.; Inteaz Alli, J.D. Effect of selected chemical applications (Sevin XLR Plus, Accell) on the quality and yield of Mcintosh apples. Horticulture Research and Development Center. http://www.pgris.com/partners/sevin/index.html (assessed Apr 2005).

Saeed, A.R.; Ladu, P.; Gamar, Y.; Khattab, A.H. Preliminary investigations on mango polyphenoloxidase. *Sudan J. Food Sci. Technol.* **1976**, 8, 74-9.

Saleh, N.A.M.; El-Ansari, M.A.I. Polyphenolics of twenty local varieties of *Mangifera Indica. Planta Medica*. **1970**, 28, 124-130.

Saltveit, M.E. Heat-shock and fresh cut lettuce. Perishable Handling Quarterly. **1998**. 95, 5-6.

Saltveit, M.E. Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biol. Technol*. **1999**, 15, 279–292.

Saltveit, M.E. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biol. Technol*. **2000**a, 21, 61–69.

Saltveit, M.E. Effect of 1-methylcyclopropene on phenylpropanoid metabolism, the accumulation of phenolic compounds, and browning of whole and fresh-cut 'iceberg' lettuce. *Postharvest Biol. Technol*. **2000**b**,** 34 ,75–80.

Saltveit, M.E. Is it possible to find an optimal controlled atmosphere? *Postharvest Biol. Technol*. **2003**, 27, 3-13.

Sanchez-Mata, M.C.; Camara, M.; Diez-Marques, C. Extending shelf-life and nutritive value of green beans (Phaseolus vulfaris L.), by controlled atmosphere storage: macronutrients. *Food Chem*. ***2003***, 80, 309-315.

SAS Institute, Inc. **2002**. JMP Software Version 5, Cary, NC.

Sauco, V.G.. Mango production and world market: Current situation and future prospects. Acta Hort. **2004**, 1, 107-116.

Saxon, M.E.; Davis, M.A.; Pritchard, S.G.; Runion, G.B.; Prior, S.A.; Stelzer, H.E.; Rogers, H.H. Jr; Dute, R.R. Influence of elevated CO_2, nitrogen and *Pinus elliottii* genotypes on performance of red-headed pine sawfly, neodiprion lecontei. *Can. J. For. Res*. **2004**. 34, 1007-1017.

Selvaraj, Y., Kumar, R. Studies on fruit softening enzymes and polyphenol oxidase activity in ripening mango (Mangifera indica L.) fruit. *J. Food Sci. Technol.* **1989**, *26*, 218-222.

Schieber, A.; Ullrich, W.; Carle, R. Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. *Innovative Food Science & Emerging Technologies*. **2000**. 1, 161-166.

Shadidi, F.; Nazck, M. Food Phenolic Sources. Chemistry, Effects, Applications. CRC Press: London. **1995**.

Sharp, J.L.; Hallman, G.J. Hot water immersion. In Quarantine Treatments for Pests of Food Plants; Westview Press, Boulder, CO, USA: **1994**, 133–147.

Silva, J.A.; Costa, T.S.; Lucchetta, L.; Marini, L.J.; Zanuzo, M.R.; Nora, L.; Nora, F.R.; Twyman, R.M.; Rombaldi, C.V. Effect of heat treatment on ethylene and CO2 emissions rates during papaya (*Carica papaya* L.) fruit ripening. Rev. Sci. Inst. **2003**, 74, 703-705.

Soto-Zamoraa, G.; Yahiab, E.M.; Brechtc, J.K.; Gardeaa, A. Effects of postharvest hot air treatments on the quality and antioxidant levels in tomato fruit. LWT. **2005**. 38, 657–663.

Swain, T.; Hillis, W.E. The phenolic constituents of *Prunus domestica* I. – The quantitative analysis of phenolic constituents. *J. Food Sci. Agric*. **1959**, 10, 63-68.

Talcott, S.T.; Howard, L.R.; Brenes, C.H. Antioxidant changes and sensory properties of carrot puree processed with and without periderm tissue. *J. Agric. Food. Chem.* **2000**, 48, 1315-1321.

Talcott, S.T.; Lee, J.H. Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. *J. Agric. Food. Chem*. **2002**, 50, 3186-3192.

Talcott, S.T., Moore, J.P., Lounds-Singleton, A.J., Percival, S.S. Ripening associated phytochemical changes in mangos (*Mangifera indica*) following thermal quarantine and low temperature storage. *J. Food Sci*. in press.

Taylor, M.A.; Rabe, E.; Jacobs, G.; Dodd, M.C. Effect of harvest maturity on pectic substances, internal conductivity, soluble solids and gel breakdown in cold stored 'Songold' plums. *Postharvest Biol. Technol.* **1995,** 5, 285-294.

Tian, S.P.; Jiang, A.L.; Xu, Y.; Wang, Y.S. Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage. *Food Chem*. **2004,** 87,43–49.

Toit, R.D.; Volsteedt, Y.; Apostolides, Z. Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. *Toxicology*. **2001**, 166, 63–69.

Tovara, B.; Garcia, H.S.; Mataa, M. Physiology of pre-cut mango II. Evolution of organic acids. *Food Rev. Int*. 2001, 34,705-714.

USDA-APHIS. Treatment schedules. T102-Water treatment. http://www.aphis.usda.gov./ppq/manuals/pdf_files/Treatment%20Chapters/05-02-T100-5.pdf (accessed June 2003).

Vela, G.; Leon, D.M.; Garcia, H.S.; Cruz Unida, J.D. Polyphenoloxidase activity during ripening and chilling stress in `Manila' mangoes. *J. Hort. Sci. Biotechnol.* **2003**, 78, 104-107.

Villavicencio, L.E.; Blankenship, S.M.; Sanders, D.C.; Swallow, W.H. Ethylene and carbon dioxide concentrations in attached fruits of pepper cultivars during ripening. *Scientia Hort*. **2001**. 91, 17-24.

Vivekananthana, R.; Ravia, M.; Saravanakumara, D.; Kumarb, N.; Prakasama, V.; Samiyappan, R. Microbially induced defense related proteins against postharvest anthracnose infection in mango. *Crop Protection*. **2004**, 23, 1061–1067.

Wang, L.; Vestrheim, S. Controlled atmosphere storage of sweet cherries (*Prunus avium* L.). *Acta. Agric. Scand. Sect. B. Soil. Plant Sci*. **2002**, 52,136-142.

Werner, I.; Bacher, A.; Eisenreich. Analysis of gallic acid biosynthesis via quantitative prediction of isotope labeling patterns. In: Plant Polyphenols 2. Chemistry, Biology, Pharmacology, Ecology (Gross, G. G.; Hemingway, R. W.; Yoshida, T., eds.). Plenum Press, New York and London: **1999**, pp 43-61.

Yen, G.C.; Hung, C.Y. Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tsao (Mesona procumbens Hemsl.). *Food Res Int.*. **2000**, 33, 487-492.

Zalewska, A.; Wawer, I,; Kecki, Z. Effect of gallotannins on DPPH radical. *Radiat. Phys. Chem*. **1995**, 45, 971-986.

BIOGRAPHICAL SKETCH

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