GUAVA (*Psidium guajava* L.) FRUIT PHYTOCHEMICALS, ANTIOXIDANT PROPERTIES AND OVERALL QUALITY AS INFLUENCED BY POSTHARVEST TREATMENTS

By

FLOR DE MARIA NUNEZ RUEDA

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

DEDICATION

To the Lord Jesus, for giving me the strength and motivation to keep pursuing my dreams.

To Sabina, my grandmother, backbone of my family and shimmering light in our lives.

To my nephew Diego, with lots of love from your always spoiling aunt.

ACKNOWLEDGMENTS

I wish to extend my special thanks to my major advisor, Dr. Stephen Talcott, for his support, advice, friendship and for being a leading example of motivation and hard work to us. I could not have asked for more. I thank my advising committee, Dr. Susan Percival and Dr. Donald Huber, for their invaluable assistance and time for my project.

I thank my lab partners and friends, Kim, Kristine, Jorge, Lanier, Chris, Lisbeth, and Stacy, for their never-ending assistance in my project and for making the work in the lab such an enjoyable and nurturing experience. Special thanks go to Dr. Joonhee Lee and soon Dr. to be David del Pozo, for investing their valuable time, sharing their knowledge and providing guidance, as well as a friendly shoulder to lean on. Graduate school would definitely not have been as much fun without David's friendship and craziness.

I would have not reached where I am without the unconditional love, support, and advice of my mother Nora, the biggest example of strength I have seen in a person. I love her so much. I thank my sister and best friend Tania, for her advice and big sis support during all these years. Big thanks go to my brother Jesus and my sister-in-law Yalile, for their warm support and example of perseverance and love, as well as to my little brother Guille, for having patience with me. Special thanks go to my family in Honduras, Nunez, Rueda, and Maier, for all their support and best wishes through the distance. Last but not least, big thanks go to Gary, for sharing his love, patience, jokes and for just adding much happiness to my graduate school days.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

GUAVA (*Psidium guajava* L.) FRUIT PHYTOCHEMICALS, ANTIOXIDANT PROPERTIES AND OVERALL QUALITY AS INFLUENCED BY POSTHARVEST TREATMENTS

By

Flor de Maria Nunez Rueda

August 2005

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

Guava (*Psidium guajava* L.) fruit, appealing for its unique tropical flavors, is considered an excellent source of nutrients and antioxidant phytochemicals, especially ascorbic acid. Guava's extremely perishable nature and quarantine issues surrounding its importation somewhat limit its fresh fruit marketability within the US. Additionally, limited studies have reported resultant changes of postharvest processes on guava's phytochemicals and antioxidant capacity. The objectives of this study were to evaluate the effects of two postharvest treatments on phytochemical content, antioxidant capacity, and overall quality of pink guavas. A hot water (HW) immersion technique was applied as a potential quarantine treatment, whereas 1-methylcyclopropene (1-MCP), an ethylene blocker, was applied as a treatment to extend the shelf-life of guavas.

For the HW immersion study, guavas were segregated into three groups according to their ripeness level-Stage I (yellowish-green skin, firm texture), Stage II (25-50% yellow skin, semi-firm texture), and Stage III (>75% yellow skin, soft texture)-and

subjected to four HW immersion times (0 (control), 15, 30, and 60 min) at 46ºC. Fruits were held at 15°C until fully ripe and collected for analysis. For the 1-MCP study, unripe guava fruit were separated into two groups: Control and 1-MCP (1000 nL/L); both were held for 24 hours at 10 °C during treatment application. They were stored at 15˚C until full ripeness and collected for analysis. Chemical analyses for both studies included moisture, total soluble phenolics (TSP), ascorbic acid (AA), antioxidant capacity (AOX), lycopene, yellow carotenoids, and polyphenolics by HPLC. Quality analysis included soluble solids, pH, titratable acidity (TA) (1-MCP), and firmness (1-MCP).

A HW treatment up until 30 min at 46˚C insignificantly affected moisture (92.2%), TSP (20,600 mg/kg, dry weight (DW)), lycopene (501 mg/kg DW), ascorbic acid (10,800 mg/kg DW), soluble solids (7.7°Brix), pH (4.1), yellow carotenoids (46.7 mg/kg DW) and AOX (133 μM TE/g DW) within each ripening stage. Stage I fruits treated for 60 min presented an enhancement of certain polyphenolics and a decrease in lycopene content as a response to heat stress. 1-MCP application effectively extended guava shelf-life for at least 5 days at 15 ˚C, delaying skin yellowing and retaining firmness. Moisture content (86.4%), TSP (19,700 mg/kg DW), AOX (133 μM Trolox equivalents/g DW), pH (4.1), soluble solids (7.8 °Brix), and TA (0.29 % citric acid) were unaffected by 1-MCP. Although ascorbic acid (6,145 mg/kg DW) and lycopene content (419 mg/kg DW) were significantly higher in 1-MCP treated fruit, similarly to other phytochemicals, effects were independent of ethylene inhibition. A 1-MCP and HW quarantine treatment up to 30 min can be applied to fresh guava without detrimental effects on phytochemical content, antioxidant capacity, and fruit quality, therefore extending its fresh fruit market window.

CHAPTER 1
INTRODUCTION

The consumption trend of fresh tropical fruits and their products is increasing steadily due to consumer's education on their exotic flavors, nutritive value, and phytochemical content with potential health effects (Food and Agriculture Organization [FAO], 2004). Guava fruit (*Psidium guajava* L.), an exotic from the tropics characterized by its appealing flavor and aroma, has been catalogued as one of the most nutritious fruits due to its high content of phytochemicals, specially ascorbic acid (United States Department of Agriculture [USDA], 2004). Guava's importation as a fresh fruit is somewhat limited within the US for two main reasons: quarantine issues surrounding its importation and its highly perishable nature.

Guavas are considered excellent sources of antioxidant phytochemicals, which include ascorbic acid, carotenoids, antioxidant dietary fiber, and polyphenolics. After acerola cherries, guava has reported the second highest concentration of ascorbic acid (ranging from 60-1000 mg/100 g) of all fruits (Mitra, 1997). Carotenoids, which are yellow, red, and orange pigments, have demonstrated many beneficial health effects related to their antioxidant properties (Wilberg and Rodriguez-Amaya, 1995). Guava's major carotenoid, lycopene, is responsible for the pink coloration in pink guava's flesh (Mercadante et al., 1999). Polyphenolics from fruits and vegetables are widely investigated because of their role as chemoprotective agents against degenerative diseases, antimutagenic effects, and antiviral effects, among others (De Bruyne et al., 1999; Gorinstein et al., 1999; Robbins, 2003). Currently, research on identification and

quantification of ripe guava polyphenolics is very limited, and information is still unclear as to the type and concentration of individual compounds present in the fruit.

Various postharvest chemical and heat applications exist as quarantine treatment for fresh fruit importation into the US, which may also preserve appearance and table quality of various fruits (Lurie, 1998). Thermal applications are gaining more popularity due to consumer's demand to ameliorate the use of chemicals. In the case of guava, an established quarantine treatment still does not exist for importing it into the US (Gould and Sharp, 1992; USDA-Animal and Plant Health Inspection Service [USDA-APHIS], 2004), potentially due to the lack of studies that demonstrate a beneficial effect. Additionally, few studies report resultant changes that these processes may have on phytochemical content, nutrient stability, and antioxidant capacity of the fruit, either in a beneficial or detrimental way.

Perishability is one the main issues in postharvest handling and marketing of fresh fruits and vegetables. In the case of guava fruit, its short shelf life (7 to 10 days) limits somewhat its marketability. Numerous technologies have been developed as means to extend their shelf-life and eating quality, some of which include modified atmospheres, polymeric films, irradiation, or chemical treatments (Mitra, 1997). A recently developed shelf-life extension tool is the application of a gaseous organic compound, 1-methylcyclopropene (1-MCP), as an ethylene blocker, delaying or inhibiting ripening on ethylene-sensitive commodities (Blankenship and Dole, 2003). Currently, limited studies exist on the impact of 1-MCP on phytochemical content of fruits and vegetables in general, and their relationship with ethylene inhibition responses.

This study evaluated the effects of two post-harvest treatments, a thermal quarantine hot water treatment and a 1-1-MCP application, on phytochemical content, antioxidant properties, and fruit quality. It was hypothesized that HW treatment at 46 °C for short periods would not stress guava fruit or affect phytochemical content, antioxidant properties, and quality. A 1-MCP treatment would affect guava ripening and characteristics associated with it, however phytochemicals and antioxidant properties would remain unaffected. The specific objectives of this study were

- Post-harvest treatment-Hot Water Immersion: To evaluate the effects of a thermal quarantine treatment using a hot water immersion technique on the phytochemical content, antioxidant capacity, and overall quality of guava fruits.
- Post-harvest treatment-1-MCP: To evaluate the effects of 1-MCP as a postharvest treatment for extention of fresh guava shelf-life and to determine these effects on the phytochemical content, antioxidant capacity, and overall quality of guava fruits.

CHAPTER 2
LITERATURE REVIEW

2.1 Guava Market and Industrial Applications

Exotic or minor tropical fruits, which include guava, carambola, durian, lychee, mangosteen, passionfruit and rambuttan have undergone a significant increase in both volume and value in recent years. Their production continues to steadily increase and is estimated to have reached 14.9 million metric tons (23% of total global output of tropical fruits) in 2002 and US total import volumes were 176,000 tons for 2003 (FAO, 2004). Fresh fruit market in general is growing in the US chiefly due to an increase in consumption demand and the development of technologies to preserve fruit eating quality and prolong shelf-life (Kipe, 2004).

Guava as an import is divided into four categories according to the National Agriculture and Statistics Service [NASS]: preserved or prepared, paste and puree, jam, and dried. Brazil was the leader for guava imports into the United States in 2003, followed by Dominican Republic, Mexico, India, and Costa Rica. Within the US, commercial producers are Hawaii, southern Florida (Gould and Sharp, 1992), and southern California. Hawaii is the main grower, with 530 harvested acres and a utilized production volume of 6.7 million pounds in 2003. The local production in Florida and Hawaii is hampered by the Caribbean fruit fly, causing serious economic losses if not controlled adequately (NASS, 2004). Currently, external or internal import of fresh guava fruit is not possible; this is mainly attributed to the tropical fruit fly and guava's very short shelf life.

For industrial applications, guava is one of the easiest fruits to process, since the whole fruit may be fed into a pulper for macerating into puree (Boyle, 1957). It is physically and biochemically stable in relation to texture or pulp browning during processing (Brasil et al., 1995). It can be processed into a variety of forms, like puree, paste, jam, jelly, nectar, syrup, ice cream or juice. Within the United States processing industry, it is gaining popularity in juice blends due to its exotic flavor and aroma.

2.2 Guava Fruit

2.2.1 Origin

Guava (Psidium guajava) is an exotic fruit member of the fruit family Myrtacea. Guava, goiaba or guayaba are some of the names given to the "apple of the tropics", popular for its penetrating aroma and flavor. Its place of origin is quite uncertain, extending in an area from southern Mexico through Central and South America. Currently, its cultivation has been extended to many tropical and subtropical parts of the world, where it also thrives well in the wild (Morton, 1987;Yadava, 1996; Mitra, 1997).

2.2.2 Morphology

Guava shape ranges from round, ovoid, to pear-shaped, and with an average diameter and weight ranging from 4-10cm and 100-400g respectively (Mitra, 1997). Classified as a berry, guava is composed by a fleshy mesocarp of varying thickness and a softer endocarp with numerous small, hard yellowish-cream seeds embedded throughout it (Malo and Campbell, 1994; Marcelin et al., 1993). Guava pulp contains two types of cell-wall tissues: stone cells and parenchyma cells. Stone cells are highly lignified woody material responsible for a characteristic sandy or gritty feeling in the mouth when the fruit is consumed; due to their nature, they are resistant to enzymatic degradation. They account for 74% of the mesocarp tissue, while the endocarp is rich in parenchyma cells,

which give it a softer texture. (Marcelin et al., 1993). Exterior skin color ranges from light green to yellow when ripe and its pulp may be white, yellow, pink, or light red. Unripe guava fruit are hard in texture, starchy, acidic in taste and astringent, due to its low sugar and high polyphenol content. Once it ripens, the fruit becomes very soft, sweet, non-acidic, and its skin becomes thin and edible (Malo and Campbell, 2004; Mitra, 1997). Many guava cultivars exist today, however they can be broadly classified as pink or white. Seedless cultivars are available in many countries, which have a great potential to become popular in the US in the future (Yadava, 1996).

2.2.3 Postharvest Physiology

Ripening and factors associated with it in climacteric fruits is regulated by ethylene synthesis. Ethylene (C_2H_4) is a naturally-produced, gaseous growth regulator associated with numerous metabolic processes in plants (Mullins et al., 2000). It is produced from L-methionine via 1-aminocyclopropane-1-carboxylic acid (ACC) synthase in a complex signal transduction pathway, which is still widely researched today (Salveit, 1998; Mullins et al., 2000). All plants produce ethylene, but only climacteric fruits and wounded or stressed tissue produce sufficient amounts to affect other tissues. In climacteric fruits, ethylene stimulates its own biosynthesis at the start of ripening, enhancing its production until reaching saturation levels (Salveit, 1999). Stresses such as chill injury, heat shock (Cisneros-Zevallos, 2003) or disease (Mullins et al., 2000), can induce ethylene production and therefore enhance fruit ripening, and the factors associated with it.

Studies evaluating respiratory patterns of guava demonstrated a climacteric response as increased carbon dioxide corresponded to increased ethylene production (Akamine and Goo, 1979; Mercado-Silva et al., 1998; Bashir and Abu-Goukh, 2002).

Guavas have a rapid rate of ripening, therefore a relatively short shelf life ranging from 3 to 8 days depending on the variety, harvest time, and environmental conditions (Reyes and Paull, 1995; Basseto et al., 2005). Ethylene production and respiration (CO_2 production) increases after the first day of harvest, at the start of ripening. Guava reaches its climacteric peak between day 4 and 5 post-harvest (mature-green harvested fruits) and then declines (Akamine and Goo, 1979; Bashir and Abu-Goukh, 2002).

As a guava ripens, total soluble solids and total sugars increase in both the peel and pulp, whereas titratable acidity declines after reaching its climacteric peak of respiration. In general, climacteric fruits undergo considerable changes in sugar content during ripening, where starch and sucrose are broken down into glucose (Bashir and Abu-Goukh, 2002). Moisture loss in guava, especially in tropical climates, can also be substantial resulting in up to 35% weight loss (Mitra, 1997) that corresponds to loss of postharvest quality and consumer acceptability. Ascorbic acid content is at its maximum level at the mature-green stage and declines as the fruit ripens in both white and pink guavas (reviewed by Bashir and Abu-Goukh, 2002), and may also be a function of postharvest handling. Lycopene synthesis in pink guavas is enhanced during ripening. In the case of tomatoes, once lycopene is accumulated, the respiration rate decreases (Thimann, 1980). Total fiber content decreases significantly during ripening, from 12 to 2g/100g, and it is hypothesized that is closely be related to the activity of certain enzymes (El-Zoghbi, 1994). Abu-Goukh and Bashir (2003) studied the activities of some cell wall degrading enzymes in both pink and white guava and showed that pectinesterase (PE) activity increased until reaching its climacteric and latter decreased, whereas polygaracturonase (PG) and cellulase increased as the fruit ripened in correspondence to

fruit softening. Increase in polyphenoloxidase (PPO) activity was also reported with ripening and a decrease in polyphenolics, which be the responsible for the reduction of astringency (Mowlah and Itoo, 1982).

Visually, the ripeness level of guava can be characterized by its skin color ranging from a dark green when unripe to a bright yellow or yellow-green at full ripeness. However, determination of ripeness can be misleading for some varieties and may be combined with a simple test for specific gravity, by placing fruit in water to determine if it sinks (unripe) or floats (ripe) to obtain a clearer picture of the degree of fruit ripeness (Reyes and Paull, 1995). Objective determination of skin color has also been used to predict ripeness, with L*, a* and hue angles of 65.93, 15.92, and 110.92° respectively indicating a mature, yellow fruit (Mercado-Silva et al., 1998). In combination with fruit texture, these simple assays can provide an adequate estimation of the stage of fruit ripeness.

2.3 Guava Phytochemicals

2.3.1 Phytochemicals

Phytochemicals may be defined as biologically active compounds present in foods, nutritive or non-nutritive, which prevent or delay chronic diseases in humans and animals. They may also be defined as food ingredients which provide health benefits beyond their nutritional value (reviewed by Ho et al., 1992). The importance of phytochemicals has grown in recent years due to consumers increased awareness of health beneficial effects. The main phytochemicals found in guava are ascorbic acid, antioxidant-containing dietary fiber, carotenoids, and polyphenolics.

2.3.2 Ascorbic Acid and Other Antioxidant Vitamins

Guavas are considered an outstanding source of ascorbic acid (AA), three to six times higher than the content of an orange and after acerola cherries it has the second highest concentration among all fruits. The AA content in guava varies from 60 to 100 mg/100 g in some cultivars, and from 200 to 300 mg/100g in others, while higher reports range from 800 to 1000 mg/100g. Mitra (1997) mentions that AA content is more influenced by the fruit's variety than by its ripening stage and storage conditions. Within the fruit, AA is concentrated in the skin, followed by the mesocarp and the endocarp (Malo and Campbell, 1994). As a water-soluble vitamin, it is highly susceptible to oxidative degradation and is often used as an index for nutrient stability during processing or storage (Fennema, 1996). Guava was also found to contain alpha-tocopherol (vitamin E) at nearly 1.7 mg/100g (Ching and Mohamed, 2001), which is an important fat-soluble dietary antioxidant.

2.3.3 Dietary Fiber

Dietary fiber in fruits and vegetables has been associated with a reduction in colon and other cancer risks. Soluble fiber content is generally associated with a reduced risk of cardiovascular disease. In a study done to a number of tropical fruits guava showed the highest content of total and soluble dietary fibers with values of 5.60 and 2.70g/100g respectively (Gorinstein et al., 1999). Total and soluble fiber present in guava is extraordinarily high in concentration as compared not only to tropicals, but all fruits and vegetables. Fiber from guava pulp and peel was tested for antioxidant properties and found to be a potent source of radical-scavenging compounds, presumably from the high content of cell-wall bound polyphenolics (2.62-7.79% w/w basis) present in each fiber isolate (Jimenez-Escrig et al., 2001).

2.3.4 Carotenoids and Lycopene

Carotenoids are yellow, red, and orange pigments abundant in a wide variety of fruits and vegetables. Due to their antioxidant properties, carotenoids have shown beneficial health effects in cancer inhibition, immuno-enhacement, and prevention of cardiovascular diseases (Wilberg and Rodriguez-Amaya, 1995). The most important carotenoids which provide oxidative protection are α-carotene, β-carotene, lutein, lycopene, zeaxanthin, and β-cryptoxanthin (VERIS, 2000). A well-established function is the vitamin A antioxidant activity of some of carotenoids, including α-carotene, β-carotene, β-cryptoxanthin. Carotenoids are a class of structurally related 40-carbon compounds (two 20-carbon tails) which consist of eight repeating isoprene units (Van de Berg et al., 2000). Lycopene, the major carotenoid present in guava (Mercadante et al., 1999), is a 40-C open chain hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds arranged linearly (Figure 2-1). Currently, High Performance Liquid Chromatography (HPLC) is the preferred procedure for carotenoid analysis.

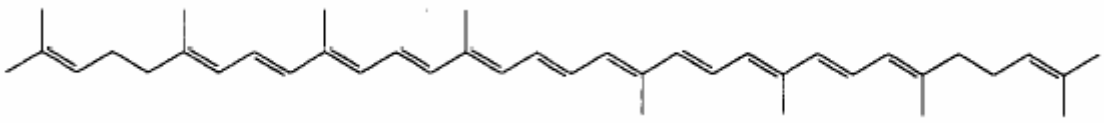


Figure 2-1. Chemical structure of lycopene, a 40-C open hydrocarbon chain.

Lycopene has received considerable attention recently due to diverse in-vivo and in-vitro studies reporting the effect of dietary lycopene in reduction in the risk of prostate cancer and coronary heart disease (Rao and Agarwal, 1999). Lycopene has reported a superior antioxidant activity in relation to lutein or β-carotene, due to its conjugated double bonds (reviewed by Lin and Chen, 2003). Currently, tomatoes and tomato-based

products are the main source of dietary lycopene. Ripe fresh tomatoes have a lycopene content ranging from 4 to 8 mg/100g (Abushita et al., 2000; Leonardi et al., 2001; Seybold et al., 2004). During tomato processing, some authors have reported lycopene and other carotenoid reduction (Takeoka et al., 2001; Sahlin et al., 2004), while others report an enhancenment, increased bioavailability and antioxidant capacity of these compounds (Dewanto et al., 2002; Seybold et al., 2004).

Lycopene content in guava 'Beaumont' variety has been found to be about 5-7 mg/100g fruit. Mercadante and partners (1999) isolated sixteen carotenoids from guava, of which thirteen were reported as guava carotenoids for the first time. In another study made to Brazilian guavas, the β-carotene concentration in ripe fruits ranged from 0.3 mg/100g to 0.5 mg/100g; while the lycopene concentration ranged from 4.8 mg/100g to 5.4 mg/100g (Wilberg and Rodriguez-Amaya, 1995).

2.3.5 Guava Polyphenolics

Polyphenols are the most abundant phytochemicals in our diets, and fruits are the main contributors (Jimenez-Escrig et al., 2001). Currently, limited studies exist on the identification and quantification of guava polyphenolics. Gorinstein et al. (1999) conducted a comparative study between several tropical and subtropical fruits and found guava to be among the top three investigated for concentrations of gallic acid (.374 mg/100g), total phenolics (4.95 mg/100g), and the highest total and soluble dietary fiber of the fruits investigated. Guava are somewhat unusual in their flavonoid polyphenolic content as well, with significant levels of myricetin (55 mg/100g) and apigenin (58 mg/100g) present in edible tissues, but do not contain the more commonly found flavonoids quercetin and kaempferol (Miean and Mohamed, 2001) that are abundant in

other fruits and vegetables. Misra and Seshadri (1967) identified procyanidins, or condensed tannins in both white and pink cultivars, concentrated in the skin and seeds, but very little in the pulp. Also, free ellagic acid was isolated in both varieties (0.2 mg/100g in pink, 0.05 mg/100g in white). In the whole guava, total phenolics are concentrated on the peel, followed by the pulp (Bashir and Goukh, 2002). For processed products, though, location of polyphenolics does not matter since the entire fruit with peel is fed into a pulper.

Although limited information is existent, it has been confirmed that guava polyphenolics decrease and undergo considerable changes during maturation and subsequent ripening (Mowlah and Itoo, 1982; Itoo et al., 1987; Bashir and Goukh, 2002). According to work conducted by Itoo et al. (1987) immature, underdeveloped guava contains approximately 65% condensed tannins of its total polyphenols, which decrease dramatically as the fruit grows and develops. According to Mowlah and Itoo (1982) in both pink and white varieties both “non tannin phenolics’ (simple phenolics, monomeric anthocyanins, catechins, and leucoanthocyanins) and “tannin phenolics” (hydrolysable and condensed tannins) decrease during ripening. However, at full-ripeness non-tannin phenolics (76 and 80% of total phenolics for pink and white respectively) contents are higher than tannin phenolics (24 and 20%). The decrease in astringency during guava ripening has been attributed to an increase in polymerization of condensed tannins to form an insoluble polymer and hydrolysis of a soluble/astringent arabinose ester of hexahydroxydiphenic acid, a precursor of ellagic acid (Goldstein and Swain, 1963; Misra and Seshadri, 1967; Mowlah and Itoo, 1982; Itoo et al., 1987). Confirming these results, an increase in free ellagic acid during ripening has been reported (Goldstein and Swain,

1963; Misra and Seshadri, 1967). Currently, limited information on individual polyphenolic compounds found in ripe fruits is existent.

2.4 Postharvest Treatments

2.4.1 Guava Postharvest Handling and Storage

Depending on its further use (fresh or processed) postharvest conditions for guava may vary; however its short shelf life is a recurring pressure for growers, packers, and processors. Due to its delicate nature, it is carefully hand-harvested while still green, and immediately stored at cool temperatures. In Florida, guavas are usually stored at temperatures between 9 to 12 ºC (personal communication, Sardinia, 2004) due to their sensivity to chill injury. They are typically shipped from packing houses in a mature-green stage (yellowish-green skin, firm), after harvesting at optimum fruit size. Reyes and Paull (1995) reported less disease incidence in mature green guavas stored at 15°C as compared with fruit that were quarter- and half-yellow under the same conditions. Additionally, 15°C was determined to be an optimum holding temperature prior to processing, since it allowed gradual ripening of mature-green fruit while delaying deterioration of quarter-yellow and half-yellow fruit. Fruit stored at 5°C did not ripen and developed skin bronzing after two weeks in storage, as a consequence of chill injury.

2.4.2 Quarantine Heat Treatments

Various thermal and chemical quarantine treatments exist for fresh tropical fruits entering the US established by US Department of Agriculture-Animal and Plant Health Inspection Service-Plant Protection and Quarantine (USDA-APHIS-PPQ). They are set to ensure disinfectations from pests, insects, larvae, eggs or fungus for fresh produce importation from other countries and other US states or territories. During the past years,

there has been an increasing interest in the use of thermal treatments as a measure of control, due to consumer demand to ameliorate the use of chemicals. Currently, there are three methods to heat commodities: hot water, vapor heat, and hot air (reviewed by Lurie, 1998). Hot water dips are effective for both fungal pathogen control and for disinfestations of insects, needing a longer time for the latter one, since the internal core of the fruit and not just the surface needs to be brought up to the required temperature. Procedures have been developed to disinfest a number of tropical and subtropical fruits from various species of fruit fly (reviewed by Paull, 1994). The USDA-APHIS-PPQ treatment manual includes treatment schedules that must be followed to import fruit into the US. In the case of mango, this includes a 46 °C hot water dip that disinfects mangoes with possible fruit fly contamination. Currently, no established treatment schedule exists for guava by the US government (USDA-APHIS, 2004).

Guava is major host for many tephritid fruit fly species, including the Caribbean Fruit fly, *Anastrepa suspensa*, which has been present in Florida for several years. Local guavas therefore, cannot be exported from Florida to other citrus-producing states, somewhat limiting their market as fresh fruit (Gould and Sharp, 1992). Gould and Sharp (1992) conducted studies to determine the suitability of hot-water (HW) immersion as a quarantine treatment to disinfest pink guavas of Caribbean fruit flies and to asses its effect on overall fruit quality. As compared to other tropicals, such as mangos, a shorter immersion time was required to kill larvae in guava due to the size of the fruits used (approx. 90g). The storage temperature was apparently more important than a HW treatment to retain fruit quality. Guavas held at 24 ºC ripened within 7 days and guavas held at 10 ºC ripened within 11 to 18 days regardless of the length of the HW treatment.

Probit statistical analysis estimated a probit 9 (99.9968%) mortality at 31 min at 46.1 ± 0.5 ºC for quarantine security, which did not affect fruit quality. This has been one of few studies done on guava HW treatment application. Further investigations are needed in order to obtain a quarantine schedule for guava.

2.4.3 Shelf-life Extension Treatments

Various treatments exist to extend the shelf-life of horticultural commodities. Storage under modified atmosphere (MA), packaging (MAP) or coating in polymeric films (cellulose or carnouba-based emulsions) have been shown to be effective on many commodities, including guava. In most cases, respiration and ethylene production are reduced, delayed or inhibited, inhibiting ripening and characteristics associated with it (Mitra, 1997). Other shelf-life extensors which act directly on ethylene binding sites are called ethylene inhibitors or ethylene blockers. Some compounds employed as ethylene inhibitors for both floricultural and horticultural commodities include: carbon dioxide, silver thiosulfate (STS), aminoethoxyvinylglycine (AVG). 2,5-norbornadiene (2,5-NBD), and diazocyclopentadiene (DACP) (Blankenship and Dole, 2003). 1-Methylcyclopropene is an ethylene blocker which is gaining popularity because of its action in a broad range of produce and its practicality of use.

2.5 1-Methylcyclopropene

2.5.1 1-Methylcyclopropene

1-Methylcyclopropene (1-MCP) is a recently developed tool used to extend the shelf life and quality of ethylene-sensitive plant produce and research the role of ethylene responses. It is an active organic compound ($C_4H_{6)}$ which is thought to interact with ethylene (C_2H_4) receptors so that ethylene cannot bind and take action. Its affinity for the receptor site, ethylene binding protein (EBP) (Mullins et al., 2000), is about ten times

greater than that of ethylene. Its origin comes from background work done by Sisler and Blankenship on cyclopropenes, breakdown products of diazocyclopentadiene (DACP), a known ethylene inhibitor. 1-MCP development resulted in good practical use because it is less volatile than cyclopropene itself and is able to act lower concentrations (ppb range). Commercialization of 1-MCP for ornamentals is sold under the trade name EthylBloc® by Floralife, Inc., whereas for edible crops it is sold under the trade name SmartFresh® by AgroFresh, Inc. Both products are generally regarded as safe, non-toxic, and environmentally friendly by the Environmental Protection Agency [EPA]. In 2000 it was approved for use in edible crops, while in 2002 it was exempted from the requirement from tolerance from residues (EPA, 2004).1-MCP is usually employed as a powder that forms a gas when mixed with water (reviewed by Blankenship and Dole, 2003).

2.5.2 1-MCP Application Conditions

Temperature, treatment duration, concentration, and type of commodity are key variables affecting the efficacy of a 1-MCP treatment. Many studies have demonstrated a direct relationship between them. At standard pressure and temperature, 1-MCP is released in approximately 20 to 30 min; however, at lower temperatures release might take longer (reviewed by Blankenship and Dole, 2003). DeEll et al. (2002) demonstrated that treatment applied at higher temperatures in apples required less exposure time; it has been hypothesized that lower temperatures might lower the affinity for the binding site of 1-MCP in apples (Mir et al., 2001). Effective concentrations vary widely, depending primarily on the commodity. Concentrations of between 1 and 12 μL/L have been effective in blocking ethylene in broccoli. For green tomatoes, higher concentrations for short durations have been effective. In most studies, treatment duration has ranged from 12 to 24 h, in order to achieve full response (reviewed by Blankenship and Dole, 2003).

Multiple or single applications during a might be experimentally significant or not, depending on the commodity. Multiple applications on 'Red Chief' apples were more beneficial (Mir et al., 2001). Plant maturity and time of harvest must be also considered, whereas the more perishable the crop, the more quickly after harvest 1-MCP should be applied (reviewed by Blankenship and Dole, 2003).

2.5.3 1-MCP on Climacteric Fruits

Various studies have been conducted on the effects of 1-MCP on climacteric fruits, including commodities such as apples, pears, stonefruits, bananas, melons, citrus, and mangos. Reports are variable, depending on the commodity or even on the species. In general, as a response on ethylene inhibition, increases in respiration rates have been reduced or delayed. In avocado, a highly perishable commodity, 1-MCP treatment reduced significantly the rate of softening by suppressing enzyme activities and helped retain green coloration at full ripeness stage (Jeong et al., 2002). Soluble solids content (SSC) has been reported higher in 1-MCP- treated pineapples, papaya, and apples; while in mangos, oranges, apricots, and plums it was unaffected. Reports on the effect of 1-MCP on titratable acidity, have been very mixed (reviewed by Blankenship and Dole, 2003). In experiments with apples, peaches, and nectarines an inhibition in ethylene production, softening, and titratable acidity was reported (Fan et al., 1999; Liguori et al., 2004). Jiang et al. (2001) found that 1-MCP applied preharvest to strawberries, a non-climacteric commodity, lowered ethylene production and maintained fruit color, but it lowered anthocyanin production. In greenhouse tomatoes, 1-MCP delayed the onset of ripening-associated changes but it did not alter significantly final values of lycopene, firmness, color, and PG activity (Mostofi et al., 2003).The effects of 1-MCP on fruit disorders and diseases has been varied, depending on the species. In some, cases, it has

alleviated disorders, like reducing superficial scald in apples (Fan et al., 1999) or decreasing internal flesh browning in apricots and pineapples (Dong et al., 2002, Blankenship and Dole, 2003). In other instances, a lower phenolic content in 1-MCP treated strawberries accounted for increased disease incidence (Jiang at al., 2001). In papaya and custard apple, 1-MCP has been related to a higher incidence of external blemishes (reviewed by Blankenship and Dole, 2003). Limited studies, however, exist on the impact of 1-MCP on phytochemical content of fruits and vegetables in general.

2.5.4 Guava and 1-MCP

Literature on guava and 1-MCP is currently very limited. Basseto and partners (2005) demonstrated the effectiveness of application of 1-MCP to 'Pedro Sato' guavas as well as a direct relation between concentration and exposure time. Fruit were subjected to different concentrations (100, 300, 900 nL/L) of 1-MCP and exposure times (3, 6, 12h) at 25º C, to improve the shelf-life of guavas marketed at room temperature. In general, treated fruit had a storage life twice as long as non-treated fruit (5 vs. 9 days respectively). Positive effects on skin color retention and respiration rates were observed. Quality parameters such as SSC, ascorbic acid, and firmness were not influenced by 1-MCP in all treatments. However, fruit treated with 900 nL/L for more than 6h did not ripen at all and treatments at 100 nL/L were ineffective. Treatments at 300 nL/L for 6 or 12 h and at 900 nL/L for 3 showed the best results, and were equally effective.

2.6 Polyphenolics

2.6.1 Polyphenolics

Phenolic compounds are bioactive substances synthesized as secondary metabolites by all plants connected to diverse functions such as nutrient uptake, protein synthesis, enzyme activity, photosynthesis, and as structural components (reviewed by Robbins,

2003). They are considered very important in foods not only because of their influence in sensory properties, but also for their potential health benefits related to their antioxidant activity (Fennema, 1996). Recent studies have shown that polyphenolics of fruits and vegetables improve lipid metabolism and prevent the oxidation of low-density lipoprotein cholesterol (LDL-C), which hinders the development of artherosclerosis (reviewed by Gorinstein et al., 1999).

The term 'phenolic' or 'polyphenol' may be identified chemically as a substance which possesses an aromatic ring attached to one or more hydroxy substituents, and may include functional derivatives such as esters, methyl esters, glycosides or others (reviewed by Ho et al., 1991). Approximately 8,000 naturally occurring phenolic compounds have been identified. Phenolic plant compounds, including all aromatic molecules from phenolic acids to condensed tannins, are products of a plant aromatic pathway, which consists of three main sections: the shikimic acid pathway which produces the aromatic amino acids phenylalanine, tyrosine and tryptophan that are precursors of phenolic acids; the phenylpropanoid pathway which yields cinnamic acid derivatives that are precursors of flavonoids and lignans; and the flavonoid pathway which produces various flavonoid compounds (reviewed by De Bruyne et al., 1999). Phenolic acids like caffeic, gallic, coumaric, chlorogenic and ferulic acids occur widely in the shikimic acid pathway of plant tissues, which begins with the condensation of phosphoenolpyruvate and erythrose 4-phosphate (reviewed by Fennema, 1996).

2.6.2 Polyphenolic Classification

Phenolics can be broadly classified in simple phenols and polyphenols, based on the number of phenol subunits present. Simple phenols, known as phenolic acids, may be classified according to their carbon frameworks into two groups: 1) Hydroxylated

derivatives of benzoic acid (C_6-C_1), which are very common in free state, as well as combined as esters or glycosides. This group includes gallic acid, the main phenolic unit of hydrolysable tannins. 2) Hydroxylated acids derived from cinnamic acid (C_6-C_3), which occur mainly sterified and are very rare in free state. This group includes coumaric, caffeic, and ferulic acid (reviewed by Robbins, 2003; reviewed by Skerget, 2005). Both hydroxybenzoic and hydroxycinnamic acids are derived primarily from the phenylpropanoid pathway (Brecht et al., 2004). Polyphenols possessing at least two phenol-phenol subunits include the flavonoids, whereas compounds possessing three or more subunits are referred to as tannins (Robbins, 2003).

Plant polyphenolics are commonly referred to as "vegetable tannins" (Fennema, 1996). Tannins are high molecular weight ($M_r > 500$) compounds containing many phenolic groups (Hagerman et al., 1998), and are classified according to their chemical structure into condensed and hydrolysable tannins (Fennema, 1996). Condensed tannins are oligomers or polymers composed of flavan-3-ol-nuclei, and have a lower molecular weight than hydrolysable tannins, which are polyesters of gallic and hexahydroxydiphenic acid (gallotannins and ellagitannins, respectively). There is an additional class of polyphenols called "complex" tannins, in which a flavan-3-ol unit is connected to a gallo- or ellagitannin through a C-C linkage (reviewed by De Bruyne et al., 1999).

Condensed tannins are commonly known as procyanidins or polyflavonols. Procyanidins are widespread in nature and more researched than hydrolysable tannins. They consist of chains of flavan-3-ol-units, which are commonly sterified, mainly with gallic acid units (ex: epigallocatechin gallate in tea). Specifically, the flavan-3-ols which

are condensed tannin building blocks are (+)-catechin (2,3-trans) and (-)-epicatechin (2,3-cis). Flavan-3-ols are derived from a branch of the anthocyanin and other flavonoids pathway, of which elucidation is still unclear (reviewed by Xie and Dixon, 2005). Structural variability among proanthocyanidins depends on hydroxylation, stereochemistry at the three chiral centers, the location and type of interflavan linkage, and terminal unit structure. A classical assay for proanthocyanidins consists of an acid hydrolysis, where the terminal units of the molecules convert to colored anthocyanidins. Condensed tannins can be classified into many subgroups, of which the procyanidins is the most common one (reviewed by De Bruyne et al., 1999). In guava, it has been found procyanidins to compose the major portion of guava polyphenolics (Mowlah and Itoo, 1982), however further identifications have been limited.

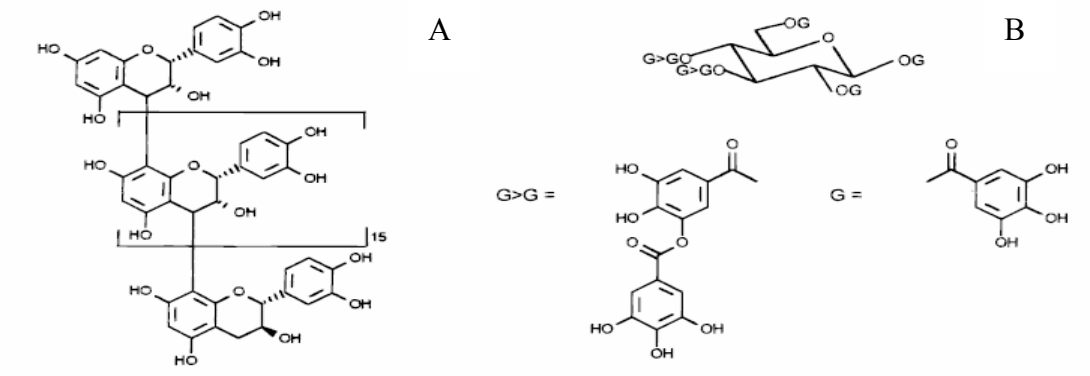


Figure 2-2. Chemical structures of a condensed tannin (A) and hydrolysable tannin (B). A is a typical condensed tannin composed of catechin and epicatechin; B is a polygalloyl glucose composed of a glucose core esterified with gallic acid residues

Flavonoids are a diverse group of polyphenolics which can polymerize to form condensed tannins. They are low-molecular weight compounds, with the characteristic flavan nucleus and composed of three phenolic (pyrane) rings. The major flavonoid classes include flavones, flavanones, flavonols, catechins (flavanols), anthocyanidins,

isoflavones, and chalcones. Most flavonoids occur naturally as flavonoid glycosides. Quercetin, rutin, and robinin are the most common glycosides in the diet, which are then hydrolyzed by intestinal flora to produce the biologically active aglycone (sugar-free flavonoid) (reviewed by Cook and Samman, 1996). In guava, considerable amounts of the flavonoids apigenin and myricetin have been found (Miean and Mohamed, 2001).

Figure 2-3. Chemical structures of flavonoids apigenin and myricetin, which have previously reported in guava. They are composed of three pyrane rings.

Hydrolysable tannins are characterized mainly by containing a varied number of gallic acids, their major phenolic unit (Grundhofer et al, 2001). Structural variation among them is caused by oxidative coupling of neighboring gallic acid units or by oxidation of aromatic rings. Some species of hydrolyzable tannins produce either gallotannins or ellagitannins, while others produce mixtures of gallo-, ellagi- and condensed tannins. Pentagalloylglucose has been identified as the precursor for many complex tannin structures. Gallotannins consist of a central polyol, such as glucose, surrounded by several gallic acids units. The ellagitannins are a more complex group of tannins also derived from pentagalloylglucose by oxidative reactions between gallic acid units (reviewed by Mueller-Harvey, 2001). The biosynthetic pathway to hydrolysable tannins may be divided in three smaller sections: The initial route encompases reactions that start from free gallic acid unit, which esterifies with a glucose and undergoes further

esterification to form the end product pentagalloylglucose. Pentagalloylglucose is the starting point for the two subsequent routes. The gallotannin route is characterized by the addition of galloyl residues to pentagalloylglucose. The ellagitannin route are oxidation processes that yield C-C linkages between galloyl groups of pentagalloylglucose (reviewed by Grundhofer et al., 2001)

2.6.3 Polyphenolics as Antioxidants

Polyphenols have shown potential health benefits, chiefly relating to antioxidant capacity. Antioxidants prevent free radicals from harming host tissues and thus are thought to reduce the risk of certain degenerative diseases such as cancer or cardiovascular disorders. Polyphenolics behave as antioxidants, mainly due to the reactivity of the hydroxyl substituents in the aromatic ring. There are several mechanisms, but the predominant role of antioxidant activity in polyphenols is believed to be radical scavenging via hydrogen atom donation or singlet oxygen quenching (reviewed by Robbins, 2003). In order for a polyphenol to be defined as an antioxidant it must satisfy two basic conditions: first, when present in low concentration relative to the substrate to be oxidized it can delay, retard, or prevent autoxidation or free radical-mediated oxidation; second, the resulting radical formed must be stable (reviewed by Shahidi and Wanasundara, 1992). Structurally, on monomeric phenolics, the ability to act as antioxidants depends on extended conjugation, number and arrangement of phenolics substituents, and molecular weight. Tannins, which are highly polymerized with many phenolic hydroxyl groups, may be 15-30 times more effective in quenching peroxyl radicals than simple phenolics (Hagerman et al., 1998). Various *in-vitro* studies have demonstrated the helpful antioxidant effects of polyphenolics in fruits and vegetables, however evidence in *in-vivo* studies remains unclear. Flavonoids have shown to inhibit

LPO *in-vitro* by acting as scavengers as superoxide anions and hydroxyl radicals, however in-vivo, the evidence is quite unclear (reviewed by Cook and Saman, 1996). The analytical procedures most widely used today to separate and identify polyphenolics are HPLC, Gas Chromatography (GC), and HPLC-Mass Spectrometry (Robbins, 2003).

CHAPTER 3
EFFECTS OF HOT WATER IMMERSION TREATMENT ON GUAVA FRUIT PHYTOCHEMICALS, ANTIOXIDANT PROPERTIES AND QUALITY

3.1 Introduction

Guava (*Psidium guajava* L.*)*, the "apple of the tropics", is an exotic fruit from tropical and subtropical regions. Since its introduction in Florida in the late 1960's a market for fresh guava fruit and its products has been slowly growing in the US (personal communication, Sardinia, 2004). However, guava fresh fruit marketability is somewhat limited, mainly due to quarantine issues and the highly perishable nature of the fruit. Unlike other tropicals, a quarantine schedule for guava has not been approved by the USDA (USDA-APHIS, 2004), probably due to the prevalence of Caribbean fruit fly contamination (Gould and Sharp, 1992) and lack of studies demonstrating beneficial effects.

Thermal quarantine treatments in particular are increasing in industrial use due to consumer demand and governmental regulations concerning the use of chemical treatments (Lurie, 1998). Currently, limited studies exist on thermal quarantine treatments for guava. Additionally, information on resultant changes of postharvest handling on guava's phytochemicals and antioxidant properties are very limited. The objective of this study was to evaluate the effects of a thermal quarantine treatment, using a simulated hot water immersion technique, on the phytochemical content, antioxidant capacity, and overall quality of guava fruits at three stages of ripeness.

3.2 Materials and Methods

3.2.1 Materials and Processing

3.2.1.1 Fruit preparation and HW treatment

Mature (pink) guavas at various stages of ripeness from a single harvest were procured from C-Brand Tropicals, Homestead, FL in August 2003. Fruit were transported overnight via a courier service to the Food Science and Human Nutrition Department of the University of Florida. Upon arrival, fruit were washed and stored at 15 ºC for 18 hrs. Approximately 200 fruit were selected for ripeness uniformity and freedom from surface damage. Based on differences in skin color, firmness, and whether the fruit floated or sank in water (Reyes and Paull, 1995) three groups were segregated. Stage I fruit were mature, green fruit with a yellowish-green skin, firm texture, and floated in water. Stage II fruit contained 25-50% yellow skin, semi-firm texture, and sank in water. Stage III fruit were 75-100% yellow, soft texture, and sank in water. A hot water (HW) immersion treatment was applied according to the conditions set by Gould and Sharp (1992). Within each ripening stage, fruit were randomly separated into four groups for each treatment time, placed into nylon bags and completely immersed in 46°C water for 0 (control), 15, 30, and 60 min. Control guavas were immersed in water at 23 °C, in order to have water immersion conditions. Due to the limited availability of Stage III fruits, only a control and 30 min immersion times were evaluated. After each respective immersion time, fruit were cooled by immersion in 23°C water for 60 min. Stage I and II fruit were held in a 15 °C storage room and held until fully ripe based on color and texture as observed for the State III fruit. Stage III fruit, since they were already at a full ripe stage, were held at 15°C for an additional two days to determine short-term treatment effects. Therefore, this

study focused on Stage I and II guavas where the effects of HW treatment and changes during ripening could be evaluated.

3.2.1.2 Guava fruit processing

Ripe guava fruit were collected from storage and immediately processed into a puree. After crown and peduncle were removed, whole fruits were manually chopped into smaller cubes and processed into a puree using a kitchen-scale juice extractor (Braun, MP80) which removed excess skin and seeds. “Guava composites” or replicates were formed by joining 4 random fruits, making up 4 replicates for each treatment. Puree was packed in 0.1 mm thick sample bags and held frozen at -20 ºC until analysis.

Samples were taken for moisture determination, soluble solids content, pH, and for chemical extraction of polyphenolics, ascorbic acid, carotenoids, and antioxidant capacity (AOX). For all of the analyses except moisture, pH, and carotenoids a clarified guava juice was evaluated. To obtain this isolate, 10 g of guava puree was treated with 5 µL of pectinase (Pectinex ® Ultra, SP-L, Novozymes), incubated at 32 ºC for 30 min, and centrifuged until a clear supernatant was obtained. The clarified juice was then filtered through cheesecloth, treated with sodium azide (0.01% w/v) to prevent microbial spoilage, and held frozen at -20 ºC until further analysis.

3.2.2 Chemical Analysis

3.2.2.1 Moisture content determination

Moisture content was determined on the guava puree by placing 3 g into a pre-weighed aluminum pan and drying to a constant weight in a convection oven (Precision Economy) at 135 °C for 2 hrs (AOAC Method 920.149149(c)).

3.2.2.2 Quantification of total soluble phenolics

Total soluble phenolics were determined by the Folin-Ciocalteu assay (Swain and Hillis, 1959). Briefly, guava juice diluted 10-fold was pippeted into a test tube and 1 mL of 0.25 N Folin-Ciocalteu reagent added. The mixture was allowed to react for and letting stand 3 minutes to allow for the reduction of phosphomolybdic-phosphotungstic acid by phenolic compounds, ascorbic acid, and other reducing agents in the juice. Subsequently, 1 mL of 1N sodium carbonate was added as an alkali to form a blue chromophore. After 7 minutes, 5 mL of distilled water was added and thoroughly mixed. Absorbance was read using a microplate reader (Molecular Devices Spectra Max 190, Sunnyvale, CA) at 726 nm. Concentration of total soluble phenolics was quantified based on a linear regression against a standard of gallic acid with data was expressed in gallic acid equivalents (GAE).

3.2.2.3 Analysis of ascorbic acid by HPLC

Ascorbic acid was determined by reverse-phase HPLC using a Waters Alliance 2695 HPLC system equipped with a Waters 996 PDA detector (Waters Corp, Milford, MA), using a Supelcosil LC-18 column (Supelco, Bellefonte, PA) with detection at 254 nm. An isocratic running program was established using a 0.2 M potassium phosphate ($K_2H_2PO_4$) buffer solution at pH 2.4 (adjusted with phosphoric acid) as the mobile phase run at 1 mL/min. Ascorbic acid was identified by comparison to a standard (Sigma Chemical Co., St. Louis, MO) and based on UV spectroscopic properties. Samples for analysis were prepared by diluting the guava juice 10-fold with a 3% citric acid solution prior to HPLC analysis.

3.2.2.4 Quantification of antioxidant capacity

Antioxidant capacity was determined by the oxygen radical absorbance capacity (ORAC) assay as modified by Ou et al. (2001). The assay monitors the decay of fluorescein as the fluorescent probe in the presence of the peroxyl radical generator 2,2'-azobis (2-methylpropionamidine dihychloride) and is evaluated against Trolox, a synthetic, water-soluble vitamin E analog. Assay conditions were described by Talcott et al. (2003) for the use a Molecular Devices fmax 96-well fluorescent microplate reader (485 nm excitation and 538 nm emission). For analysis, GJ samples were diluted 100-fold in pH 7.0 phosphate buffer prior to pipetting into a microplate. Additionally, a Trolox standard curve (0, 6.25, 12.5, 25, 50 μM Trolox) and a phosphate buffer blank were prepared. Fluorescence readings were taken every 2 min over a 70 min period at 37°C. The rate of fluorescence decay over time was determined by calculating the area under the fluorescent decay curve and the antioxidant capacity quantified by linear regression based on the Trolox standard curve. Final ORAC values were expressed in μM of Trolox equivalents per gram (μM TE/g).

3.2.2.5 Analysis of lycopene by HPLC

Lycopene was quantified by HPLC using a Dionex HPLC system equipped with a PDA 100 detector and separations made using a YMC Carotenoid column (250mm x 4.6 mm). An isocratic solvent delivery of 70% methyl-tert-butyl-ether and 30% methanol was run at 2 mL/min with detection at 470 nm. A standard of lycopene was isolated from from guava puree by extracting 5 g of guava puree with 20 mL of acetone and ethanol (1:1) and filtering through #4 Whatman paper. This isolate contained a mixture of non-lycopene carotenoids. The extraction process was repeated until the filtrate lost nearly all of its non-lycopene yellow color, whereby the addition of 100% acetone was added to

extract lycopene. The lycopene was then partitioned into hexane that contained 100 mg/L BHT as an antioxidant. Lycopene (MW 536.9) was quantified using an extinction coefficient of 3,450 at 470 nm in hexane. Purity was estimated at >98% as determined by the presence of extraneous carotenoid compounds by HPLC at 470 nm.

Lycopene was extracted from guava puree using modified conditions of Martinez-Valverde et al. (2002) used for tomato lycopene extraction. Approximately 0.5 g of puree was extracted with 5 mL of 100% acetone and vortexed vigorously. Quantitatively, 3 mL of hexane was added to the mixture, mixed, and water added to ensure adequate bi-layer separation. Aliquots of hexane extracts were filtered through 0.45 µm filter prior to HPLC injection.

3.2.2.6 Quantification of non-lycopene carotenoids

Non-lycopene or "yellow" carotenoids were quantified in total using a spectrophotometer at 470 nm. Yellow carotenoids were extracted from 5g of guava puree with a known volume of acetone:ethanol (1:1) and subsequently filtered through Whatman #4 filter paper. Absorbance was recorded using a Beckman DU 60 spectrophotometer (Beckman, Fullerton, CA) between 350 and 500 nm. Carotenoid concentration was calculated based on the extinction coefficient for ß-carotene.

3.2.2.7 Analysis of polyphenolics by HPLC

Individual polyphenolic compounds were analyzed by reverse-phase HPLC using a Waters Alliance 2695 HPLC system (Waters Corp, Milford, MA) equipped with a Waters 996 PDA detector and a 5 µm Waters Spherisorb ODS2 column (250 x 4.6 mm) using modified HPLC conditions described by Talcott et al. (2002). Mobile phases for gradient elution (Table 3-1) consisted of 98:2 water and acetic acid (mobile phase A) and 68:30:2 water, acetonitrile, and acetic acid (mobile phase B) accordingly, at a flow rate of 0.8

mL/min, and detected at 280 nm. Major polyphenolic compounds were characterized by spectroscopic interpretation, retention time, and comparison to authentic standards (Sigma Chemical Co., St. Louis, MO). Following filtration through at 0.45 μM filter, the guava juice was injected into the HPLC without further modification.

Table 3-1. Gradient elution running program for HPLC analysis of polyphenolics.

Running time (min)	% Mobile phase A	% Mobile phase B
0.00	100	--
20.00	70	30
30.00	50	50
50.00	30	70
70.00	--	100
72.00	100	--

3.2.3 Quality Analysis

The quality parameters soluble solids and pH were measured on the guava puree using a digital Leica Abbe Mark II refractometer (Model 10480, Buffalo, NY) and Corning pH meter (Model 140, NY) respectively. Overall fruit quality were subjectively evaluated during storage to detect any effects related to HW treatment or fruit decay.

3.2.4 Statistical Analysis

For Stage I and Stage II fruits, the experiment consisted of a 4 x 2 x 4 full-factorial design. The factors studied were HW immersion time (0, 15, 30, and 60 min) and ripening stage (I and II), with a mean of 4 replications represented in each data point. Stage III fruits were analyzed (2 x 1 x 4 design) and discussed separately, since they were only subjected to two HW treatment times (0 and 30 min). Statistical analysis were conducted in JMP (SAS, Cary, NC) and consisted of analysis of variance, Pearson Correlation, and mean separation by LSD test ($P< 0.05$).

3.3 Results and Discussion

3.3.1 Chemical Analysis

For chemical analyses performed, the effect of HW immersion time as compared to control within each ripening stage was evaluated, as well as the effect of ripening stage within each HW immersion time (0, 15, 30, and 60 min), and the interaction of both factors. Results for chemical analysis, excepting polyphenolics by HPLC, were reported in dry weight basis (DW), in order to eliminate difference between samples due to varying water loss fruits experienced during ripening.

3.3.1.1 Moisture content

Moisture content within each ripening stage was insignificantly affected by HW treatment time duration up until 60 min. Average moisture content for Stage I and Stage II fruit was 91.8 and 92.6% respectively (Figure 3-1). Total solids, determined by difference, was also insignificantly affected by HW treatment duration. Uniformity in total solids was an indicator of uniformity in the pool of guavas, since fruit were not affected by HW treatment duration. Due to differences in initial degree of ripeness, duration of HW treatment, and duration of storage as the fruit ripened, some change in moisture content was expected. However, no significant differences in final moisture contents and subsequently total solids due to ripening stage were observed from 0 to 30 min. At 60 min in HW, Stage II fruit presented significantly higher moisture content (1.3 %) than Stage I fruit, which is more attributed to fruit variation, since HW treatment at 60 min did not differ from control within both stages. It is concluded that fruits at either ripening stage could be heated up to 60 min without affecting their moisture content or total solids. To report values for fresh fruit and have more uniformity, moisture content was used to calculate chemical analysis results in dry weight basis (DW).

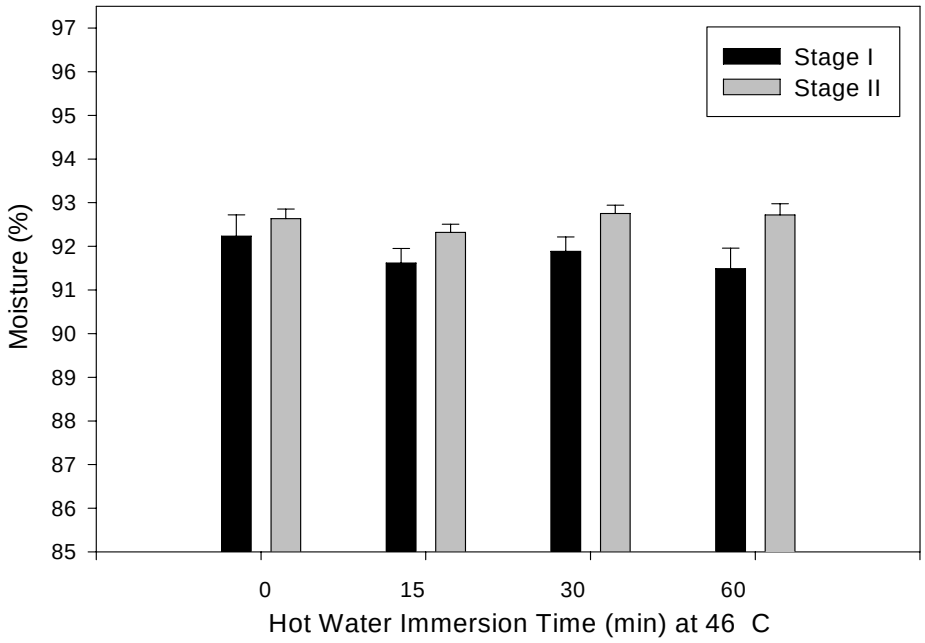


Figure 3-1. Moisture content (%) of ripe guavas as affected by a hot water immersion treatment (0,15, 30, and 60 min at 46 °C) and ripening stage (Stage I and II). Error bars represent standard error of the mean, n =4.

3.3.1.2 Total soluble phenolics

HW treatments up to 30 min did not affect concentrations of total soluble phenolics (TSP) in relation to the untreated fruit within each ripening stage (Figure 3-2). At 60 min in HW, however, Stage I fruit presented lower TSP content than untreated fruits, as opposed to Stage II fruit, which remained unaffected. This lower content might be related to other metal-reducing compounds, which may contribute to the TSP value, due to the nature of the Folin's assay. Within each HW immersion time, control fruit presented differences due to ripening stage; However within HW treatments (15 to 60 min) no significant differences in TSP were found due to ripening stage, confirming no significant effects attributed to ripening stage and its interaction with HW treatment. Differences found between stages in the untreated control were probably due to fruit variability and contribution of other metal-reducing compounds, including ascorbic acid. Stage I fruit,

therefore, can be subjected to up until 30 min in HW without affecting its TSP content, while Stage II fruit can be held up until 60 min.

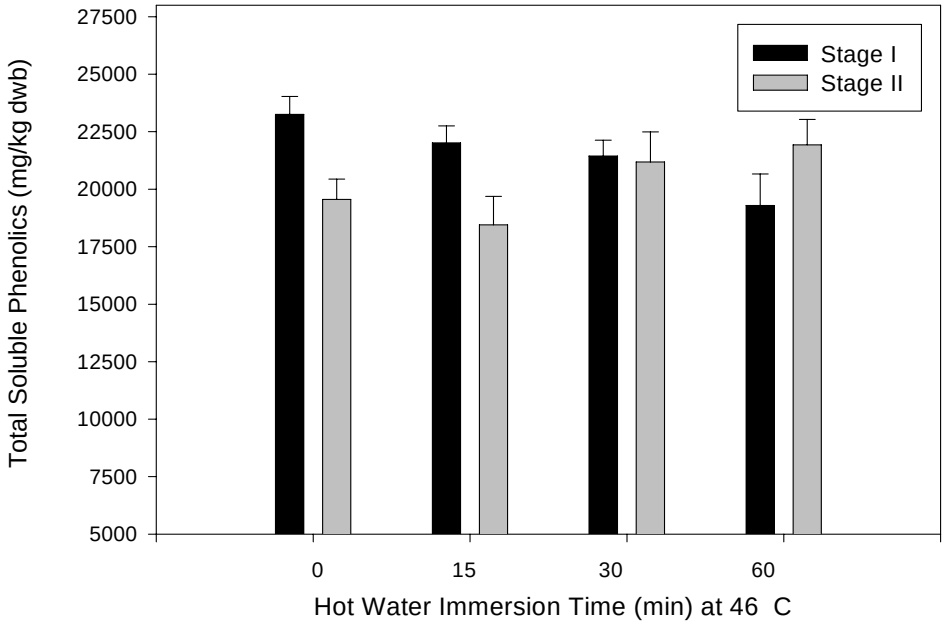


Figure 3-2. Total soluble phenolics (mg/kg DW) in guava as affected by a hot water immersion treatment (0, 15, 30, and 60 min at 46 °C) and ripening stage (Stage I and II). Error bars represent standard error of the mean, n = 4.

Studies have demonstrated guava TSP content decreases during ripening (Bulk et al., 1997; Bashir and Goukh, 2002). Pink guavas in particular, have a less-marked reduction in TSP from mature green stage (Stage I) until full ripeness as compared to white guavas (Bashir and Goukh, 2002). For the present study, guavas from both ripening stages, treated and not treated, were able to ripen and attain TSP levels comparable to other reports in ripe guavas. Average TSP values for the different ripening stages (I-III) ranged from 19,588 to 21,502 mg/kg GAE, DW (1,360 to 1,760 mg/kg GAE, FW). Bashir and Abu-Goukh (2002) reported similar values of TSP (1200-1800 mg/kg FW) for white pulp guavas, but a lower content in pink pulp guavas. Kondo et al. (2005) reported

a TSP content of 1852 μmol/kg FW. Variety, origin, and even harvest season play an important make an impact in differences in TSP.

3.3.1.3 Ascorbic acid

Ascorbic acid is an effective nutrient stability index during food processing and storage operations. It has been generally observed that if AA is well retained, the other nutrients are also well retained (Fenema, 1996). Ascorbic acid was insignificantly affected by HW immersion time within Stage I fruits (Figure 3-3). In a HW treatment at 38 ºC for 30 min applied to guavas and subsequent exposure to chill injury conditions (5 ºC), these processes did not affect its ascorbic acid content, although chilling injury symptoms were present. (Regalado-Contreras and Mercado-Silva, 1998). Stage II fruit at 30 min however, presented lower values as compared to control, which does not seem to be a treatment effect, but rather fruit variation effect, since fruit treated at 60 min was not different from untreated fruit. Fruit variation may be observed by the large error rates presented in Figure 3-3 (statistical analysis, however, for all parameters was done using a pooled standard error). Even within the same variety, large variability in ascorbic acid contents has been reported (Mitra, 1997). There were no differences in AA due to ripening stage at each treatment time. The fact that guava was unaffected by increasing HW times may not only indicate its stability during a postharvest treatment, but the stability of other guava phytochemicals. Additionally, since ascorbic was unaffected by most HW treatments, it may also indicate a uniformity of the pool of guavas used, most of them achieving similar contents.

The average AA content for Stage I and Stage II fruit was 10,846 mg/kg DW (843.5 mg/kg FW) (Figure 3-3), comparable to other studies on ripe guavas. Bashir and Abu-Goukh (2002) reported an ascorbic acid content of 800 mg/kg and 670 mg/kg FW

for white and pink guavas respectively, while Leong and Shui (2002) reported 1,310 mg/kg FW , and Bulk et al. (1997) reported values between 882 and 1113 mg/kg FW.

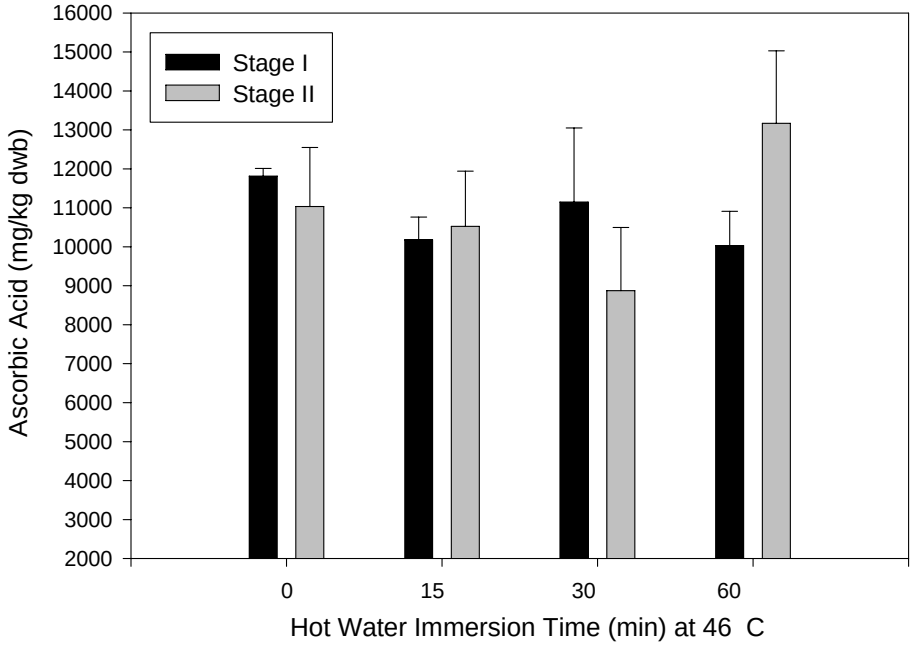


Figure 3-3. Ascorbic acid content (mg/kg DW) in guava as affected by a hot water immersion treatment (0, 15, 30, and 60 min at 46 °C) and ripening stage (Stage I and II). Error bars represent standard error of the mean, n = 4.

Fruit variety is one of the main factors affecting AA content in guava, influencing more than ripening stage or storage conditions (Mitra, 1997).

3.3.1.4 Antioxidant capacity

Antioxidant capacity in fresh guava was insignificantly affected by increasing times of HW treatment within Stage I and Stage II fruits (Figure 3-4), which presented values of 146 and 126 μM TE/g DW respectively. Additionally, ripening stage at the time of treatment application did not affect antioxidant capacity within each HW treatment. In a study conducted in Mexico, guavas were subjected to chill injury conditions (5 °C), where no changes in antioxidant capacity (ferric reducing antioxidant power; FRAP) and TSP were reported as compared to control, even when the fruit presented external chilling

injury symptoms (Edmundo et al., 2002). The biosynthesis of certain antioxidant compounds in guava might not be affected by certain temperature changes, even though they generate stress and injuries on the commodities. However, this is extremely dependent on the degree of stress, which largely depends on the temperature differential, atmospheric conditions, and exposure duration (Lurie, 1998; Paull and Chen, 2000).

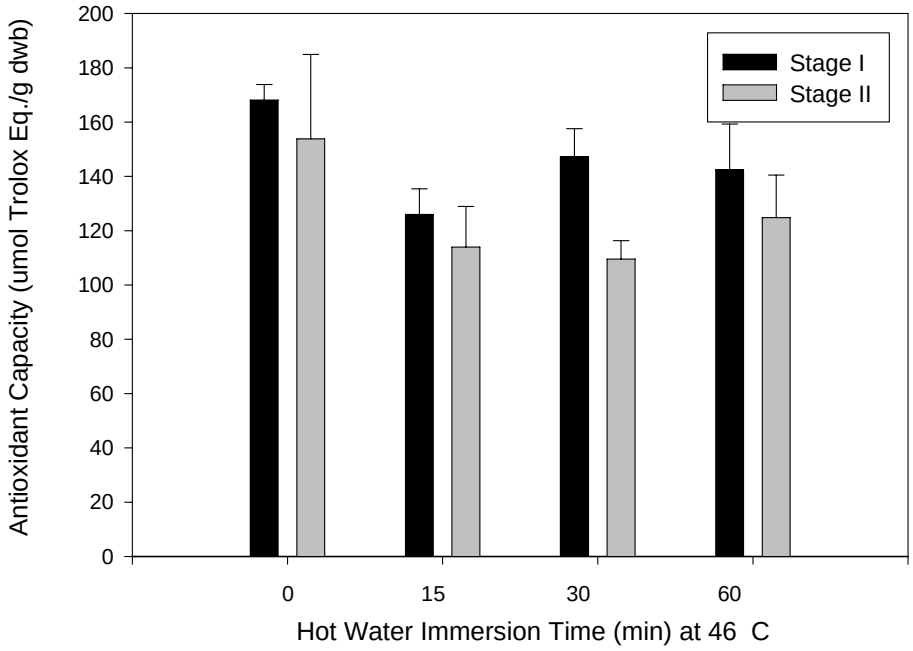


Figure 3-4. Antioxidant capacity (µmol Trolox Equivalents/g DW) in guava as affected by a hot water immersion treatment (0, 15, 30, and 60 min at 46 °C) and ripening stage (Stage I and II). Error bars represent standard error of the mean, n = 4.

The major contributors to water-soluble antioxidant activity in guava are ascorbic acid (AA) and polyphenolics. Ascorbic acid reportedly contributes to approximately 50% guava's antioxidant activity (Leong and Shui, 2002). The correlation between antioxidant capacity and ascorbic acid was low ($r = 0.30$, $P < 0.05$), probably due to the high degree of variability in ascorbic acid values. There was also a low correlation with TSP ($r = 0.27$, $P < 0.05$), since TSP content presented significant differences with increasing HW treatment time. Due to the use of GJ, however, overall antioxidant capacity in guava was

probably underestimated, since lycopene, carotenoids, and other lypophilic compounds were not taken into consideration.

3.3.1.5 Lycopene and yellow carotenoids

Average lycopene values for Stage I and Stage II guavas were 558 and 472 mg/kg DW (45.1 and 34.8 mg/kg FW) respectively (Figure 3-5). Studies conducted on Brazilian guavas reported similar values, ranging from 47 to 53 mg/kg FW (Padula and Rodriguez-Amaya, 1986; Wilberg and Rodriguez-Amaya, 1995). Lycopene content in ripe tomatoes, which ranges from 30 to 80 mg/kg FW (Abushita et al., 2000; Thompson et al.,2000; Leonardi et al. 2000; Martinez-Valverde et al., 2002; Seybold et al., 2004), is comparable to ripe guavas. Subjective determination of guava ripeness, based on skin color observations and texture, may have created some uncertainty about the uniformity of ripening stage. These effects were evident when guava fruit were cut and variation in pulp color intensity was observed within the same ripeness stage. However, fruits were grouped in composites, joining fruits with similar pulp colorations, in order to have more uniformity and reduce variation within treatments.

Guava lycopene was unaffected by HW treatment up until 30 min within both ripening stages (Figure 3-5). At 60 min, Stage I fruits exhibited lower lycopene content as compared to untreated fruits, whereas Stage II fruit remained unaffected. This reduction might be related to an inhibition in lycopene biosynthesis due to a longer exposure to heat stress. Studies of HW treatments on tomatoes have reported this inhibition mainly due to an inhibition of the transcription gene for lycopene synthesis, which recovers after the removal of heat (Cheng at al., 1988; Lurie et al., 1996; Paull and Chen, 2000). Following the removal of the heat stress for 60 min, Stage I fruit recovered and synthesized lycopene, but not to the extent of the other treatments. However, this difference between

untreated fruit and 60 min, might also be due to the significantly higher content of Stage I fruit at both 0 and 15 min, as compared to Stage II. These differences might be attributed to pre-treatment storage conditions, were fruits of the different ripening stages ripened different. Within the HW treatments, there were no differences due to ripening stage from 15 to 60 min; however, Stage I fruit presented a higher content in untreated fruit.

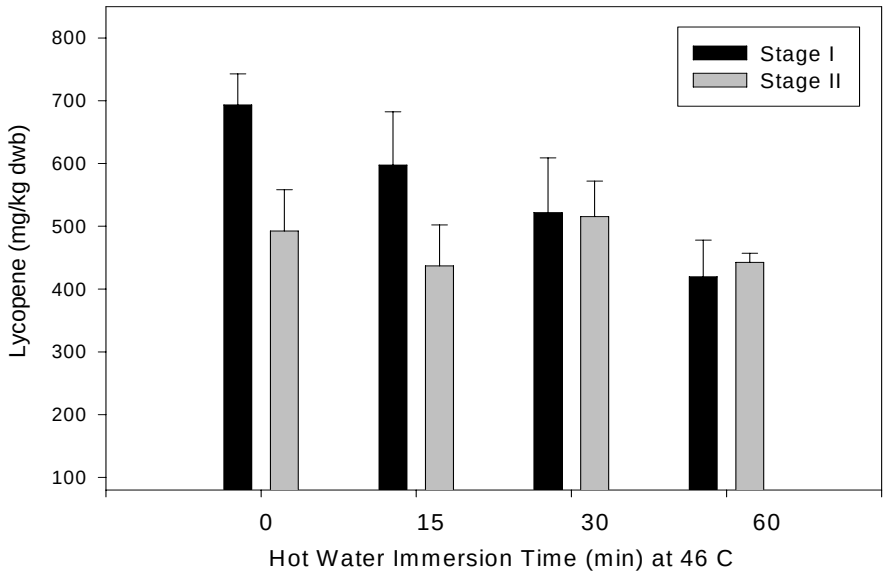


Figure 3-5. Lycopene content (mg/kg DW) in guava as affected by a hot water immersion treatment (0, 15, 30, and 60 min at 46 °C) and ripening stage (Stage I and II). Error bars represent standard error of the mean, n = 4.

Non-lycopene, or yellow, carotenoids are referred to guava carotenoids other than lycopene. Yellow carotenoids were unaffected by increasing HW treatment time and by ripening stage within each HW treatment, included untreated fruits (Figure 3-6). A hot water treatment applied to papaya (Carica papaya L.) did not cause any detrimental effects on the content of β-carotene and lycopene (Perez and Yahia, 2004). Lycopene and other carotenoids stability in tomatoes has associated to the influence of the tomato matrix itself and its adhesion to membranes (Seybold et al., 2004). A whole guava at its early stages of ripening contains appreciable amounts of cellulose, hemicellulose, lignin

(stone cells) and insoluble pectin in its cell walls, which creates a strong matrix that may protect well guava carotenoids and other phytochemicals. Yellow carotenoids content of these carotenoids ranged from 42.9 to 49.9 mg/kg DW (3.27 to 4.11 mg/kg FW), which are comparable to β-carotene contents of 3.7 and 5.5 mg/kg FW found in Brazilian guavas (Padula and Rodriguez-Amaya, 1986; Wilberg and Rodriguez-Amaya, 1995). Stability of carotenoids, especially lycopene, in tomatoes during food processing operations has been widely discussed by various authors, and sometimes inconsistent results are found, where lycopene content might be enhanced or reduced (Abushita et al., 2000; Leonardi et al. 2001, Seybold et al., 2004; Sahlin et al., 2004). A HW immersion treatment, at a lower temperature than cooking or other processing operations, and for a shorter period of time, might be milder, in order to cause significant effects in the biosynthetic pathways of lycopene and other carotenoids.

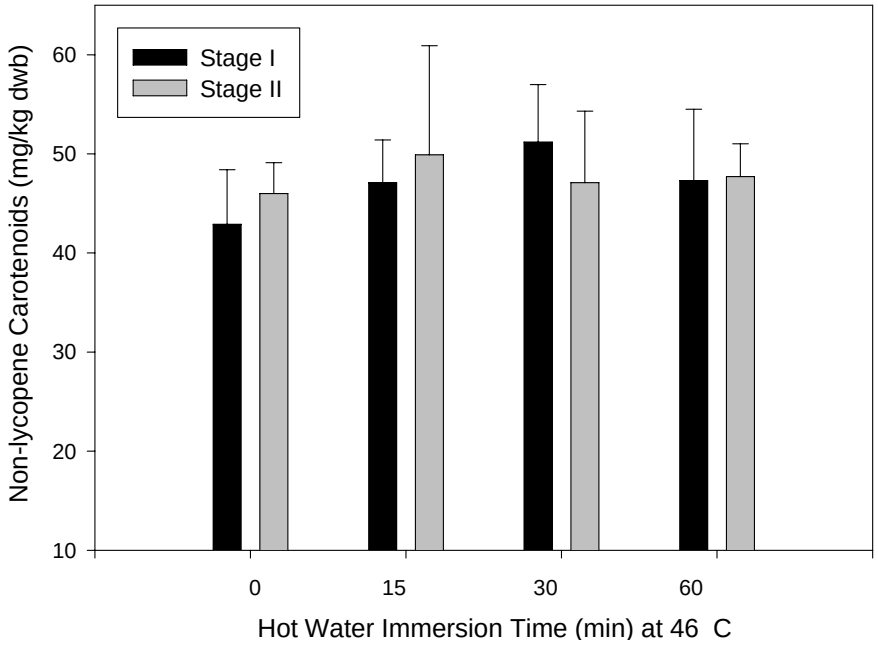


Figure 3-6. Non-lycopene carotenoids (mg/kg DW) in guava as affected by a hot water immersion treatment (0, 15, 30, and 60 min at 46°C) and ripening stage (Stage I and II). Error bars represent standard error of the mean, n = 4.

Heat treatments and other postharvest processes can delay, inhibit or accelerate ripening, as part a commodity's response to abiotic (environmental) stress (Lurie, 1998; Paull and Chen, 2000; Jacobi et al., 2001; Basseto et al., 2004). This may also be closely associated with respiration rate, ethylene production, fruit softening, enzyme activities (cell-wall degrading, ethylene-related), carotenoid development, and other components related to ripening (Paull and Chen, 2000; Jacobi et al., 2001). These processes can actually be used as tools to enhance their marketability and added value of produce, for example, uniformity of skin color development in mangoes (Jacobi et al., 2001) or anthocyanin accumulation in strawberries by HW treatments (Civello et al., 1997). In other cases, they can bring detrimental effects, affecting flavor, aesthetic qualities, or inhibiting the synthesis of certain antioxidant compounds (Cisneros-Cevallos, 2003; Sahit, 2004). Stress responses were observed in Stage I fruit heated for 60 min, where there was an enhancement in total soluble phenolics and a decrease in lycopene. Especially for lycopene, its inhibition might be detrimental to the fruit's phytochemical content. However, results indicated that a HW treatment up until 30 min at 46 °C insignificant major phytochemicals in guava.

3.3.1.6 Polyphenolics by HPLC

Studies identifying ripe guava polyphenolics by HPLC are limited. Earlier studies have been done with less precise analytical procedures in which the major classes of compounds present have been identified (Misra and Seshadri, 1967; Mowlah and Itoo, 1982; Itoo et al., 1987). Immature, still developing guava are composed mainly of condensed tannins, which decrease markedly during its development and ripening, along with the rest of guava polyphenolics (Misra and Seshadri, 1967; Itoo et al., 1987). Kondo

et al. (2005) identified gallic acid, catechin, epicatechin, and chlorogenic acid in guava skin, as well as catechin at lower concentrations in its pulp. In ripe fruits, the presence of procyanidins, or condensed tannins, and free ellagic acid has been confirmed (Misra and Seshadri, 1967).

Various solvent extraction and fractionation procedures on puree and juice were attempted for HPLC analysis of guava polyphenolics with the enzyme-clarified guava juice producing the most reproducible HPLC chromatograms with maximal peak separation (Figure 3-7). In this study, HPLC analysis of polyphenolics was used to idenfity overall data trends as affected by ripeness stage and HW treatment. Among the multitude of polyphenolic compounds present, 14 were selected based on adequate separation and abundance for treatment differentiation (Table 3-2).

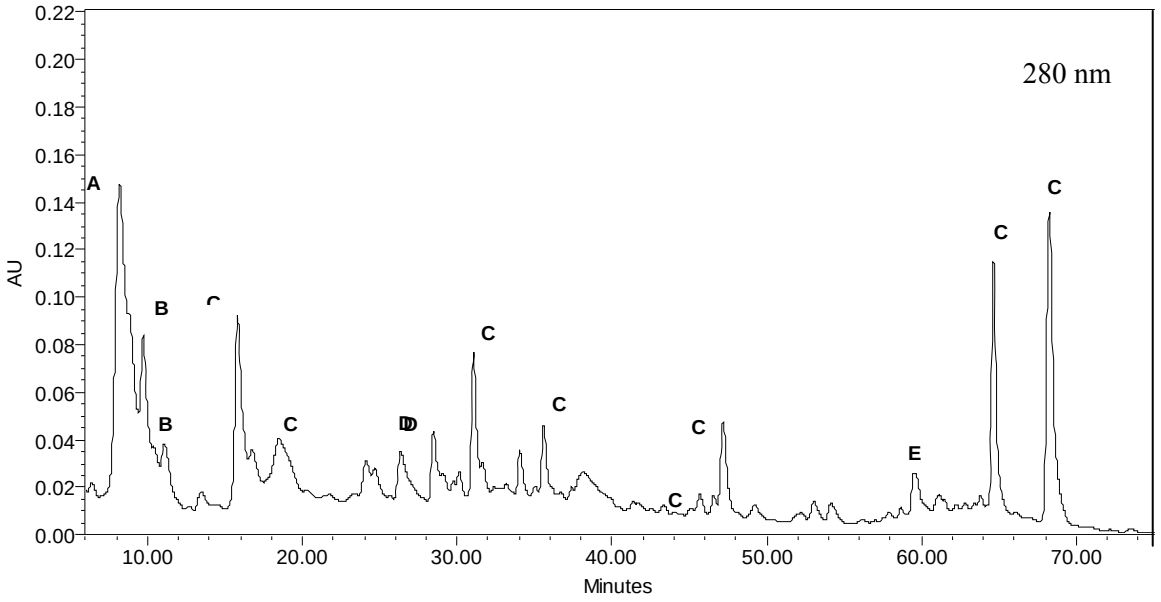


Figure 3-7. HPLC chromatogram of polyphenolic compounds found in guava juice-A) gallic acid, B) gallic acid derivatives, C) unknown-characteristic guava polyphenolics, D) procyanidins, and E) ellagic acid derivative. Identification (280 nm) was done by comparison to authentic standards and spectral properties.

Peaks were tentatively identified and/or grouped into a common class of polyphenolics based on their spectroscopic properties and comparison to authentic standards (Table 3-2). Gallic acid (Peak 1) and an ellagic acid derivative (Peak 12) were clearly identified by comparison to standards. Gallic acid derivatives (Peaks 2 and 3) were tentatively identified, as they shared similar spectral properties with gallic acid. Procyanidin compounds (Peaks 6 and 7), or condensed tannins, were identified based on their spectroscopic similarities to (+)-catechin and (-)-epicatachin, which are the bulding blocks of condensed tannins. The remaining compounds were characterized based on retention time and spectroscopic properties but were dissimilar from any known polyphenolic compounds. Further work will be needed to isolate and identify these individual polyphenolic compounds in ripe guavas. Since gallic acid was the most prevalent compound in guava, and is found in abundance in many fruits, all compounds were quantified in gallic acid equivalents (GAE).

Table 3-2. Tentative identification of guava polyphenolics at 280 nm by HPLC based on retention time, spectral properties, and comparison to authentic standards.

Peak No.	**Retention Time** (min)	**Spectral Properties**	**Tentative Identification**
1	8.12	229.3, 271.6	Gallic Acid
2	8.90	229.3, 276.3	Gallic Acid Derivative
3	9.71	233.9, 290.5 / 233.9, 276.3	Gallic Acid Derivative
4	15.8	224.5, 262.2	Unknown
5	18.5	233.9, 266.9	Unknown
6	26.3	233.9, 281.1	Procyanidin
7	28.4	233.9, 281.1	Procyanidin
8	31.1	281.1 / 219.9, 281.1	Unknown
9	35.5	215.2, 266.9	Unknown
10	45.7	224.6, 281.1	Unknown
11	47.0	219.9, 262.2	Unknown
12	59.5	224.6, 252.8, 369.9	Ellagic Acid Derivative
13	64.6	262.2	Unknown
14	68.2	224.6, 281.1	Unknown

Gallic acid (Peak 1)

Gallic acid (GA) (Peak 1) has been reported as an effective antioxidant due to its structure and positioning of hydroxyl groups. Within each ripening stage, GA was insignificantly affected by HW treatment up until 30 min (Table 3-3); however in Stage I fruit GA content was significantly higher than control at 60 min (52% increase). Stage II, however, remained unaffected. It has been discussed that the chronological age of a tissue is an important factor in response to HW treatment (reviewed by Paull and Chen, 2000). The presence of the heat stress for a longer period might have resulted in increased biosynthesis of gallic acid through the phenylpropanoid pathway, as a response to stress. Additionally, Stage I fruit probably had higher polyphenolic content than other ripening stages, especially in its peel, most prone to be affected by abiotic stress. As shown in Table 3-3, within all HW treatment times, including control, an effect of ripening stage was found, in which Stage I fruit exhibited a significantly higher GA content. The fact that Stage I fruit content was higher in all HW times, including control, indicates that it might not be attributed to a HW treatment effect, but rather pre-treatment storage conditions and post-treatment storage duration.

Gallic acid derivatives (Peaks 2 and 3)

GA derivatives, Peaks 2 and 3, eluted in the HPLC column immediately after GA. Due to their closeness to GA in spectral properties, these compounds were tentatively classified as GA derivatives (Table 3-3), and their content was summed. There was no effect due to HW treatment (15 to 60 min) as compared to control within Stage II (Table 3-3). In Stage I, GA derivatives content was lower than control at 15 min, which

Table 3-3. Guava gallic acid (GA), gallic acid derivatives, and an ellagic acid derivative as affected by a hot water quarantine treatment and ripening stage.

HW treatment (min)	Pk 1: Gallic Acid (GA) (mg/kg GAE[2])		Pks 2 and 3 : GA Derivatives (mg/kg GAE)		Pk 12: Ellagic Derivative (mg/kg GAE)	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
0	16.2 b[1]	7.70 c	12.8 a	10.5 bc	1.04 c	1.71 ab
15	16.1 b	9.16 c	9.20 c	12.0 abc	1.43 bc	1.55 b
30	15.8 b	5.64 c	11.9 abc	10.3 bc	1.35 bc	2.10 a
60	24.7 a	5.71 c	13.8 a	11.8 abc	1.61 b	1.50 b

[1]Values with different letters within columns of the same ripeness level and within rows for each hot water treatment are significantly different , and indicate the effect of either hot water treatment or ripening stage, and their interaction (LSD test, $P<0.05$). [2]GAE = Gallic Acid Equivalents

Table 3-4. Guava procyanidins, other characteristic unknown compounds, and total polyphenolics by HPLC as affected by a hot water quarantine treatment and ripening stage.

HW treatment (min)	Pks 6 and 7: Procyanidins (mg/kg GAE[2])		Unknown Guava Compounds (mg/kg GAE)		Total Polyphenolics by HPLC (mg/kg GAE)	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
0	6.33 a[1]	3.93 bc	36.3 a	24.7 d	72.7 a	48.5 c
15	4.99 abc	3.60 c	36.5 a	27.0 bcd	67.7 ab	53.3 c
30	5.42 ab	4.60 bc	28.6 bc	25.2 cd	62.2 b	47.8 c
60	4.20 bc	3.53 c	29.6 b	27.7 bcd	73.9 a	50.2 c

[1]Values with different letters within columns of the same ripeness level and within rows for each hot water treatment are significantly different , and indicate the effect of either hot water treatment or ripening stage, and their interaction (LSD test, $P<0.05$). [2]GAE = Gallic Acid Equivalents.

probably was due to variation, since at 30 and 60 min there was no HW treatment effect.Within all HW treatments and control, no ripening stage effect was observed.

Procyanidin compounds (Peaks 6 and 7)

Peaks 6 and 7 were identified as belonging to procyanidin compounds. The presence of procyanidin compounds has been confirmed in both white and pink guavas (Mowlah and Itto, 1982), and they have been reported to be composed mainly of (+) catechin and (+) gallocatechin (Itoo et al., 1987).

Procyanidins presented no HW treatment effect in Stage II fruit; however Stage I fruit presented lower content at 60 min as compared to control (Table 3-4). This difference between control and 60 min might not attributed to a treatment effect, rather to variation within control fruit, since an increase in heat stress would probably increase the content of procyanidin compounds, rather to decrease it. Additionally, within untreated controls, Stage I fruit presented higher procyanidin content than Stage II fruit, but no differences between ripening stages were found at 15, 30, and 60 min. This confirms that Stage I control fruit procyanidin content was significantly higher probably due to a variation in values of this compound in control fruit. It can be concluded that procyanidin content in HW treated fruit up to 30 min did not differ significantly from untreated fruits.

Ellagic acid derivative (Peak 12)

Peak 12 was identified as an ellagic acid derivative, most likely a glycoside (Lee et al., 2005), due to its closeness in spectral properties to ellagic acid. Free ellagic acid in ripe guava was isolated and identified by Misra and Seshadri (1967). Similarly to other compounds, Stage II fruits ellagic acid content was unaffected by increasing HW treatment up until 60 min (Table 3-3). Stage I fruit ellagic acid content at 60 min HW

treatment, however, was significantly higher than control. This trend in Stage I is comparable to gallic acid results; it must be noted though that concentrations of this compound are much lower. Very low concentrations might not give an accurate measure of a treatment effect. Within HW treatment time, Stage I fruit was significantly lower than Stage II fruit at time 0 and 30, but at time 15 and 60 min, there were no significant differences due to ripening stage.

Guava characteristic unknown compounds (Peaks, 4, 5, 8-11, 13, 14)

This group is composed of guava compounds that had characteristic and sometimes repeating spectroscopic properties (Table 3-4), but were not identifiable as any known polyphenolic compounds. Many of them shared similar spectrocospic properties to gallic acid and its derivatives or procyanidin compounds, which might relate them closer in further research. They were characterized by their consistency in spectroscopic properties among most samples, in contrast with other compounds, whose spectral properties differed mainly due to small peak areas or interference of extraneous compounds during elution.

Stage II fruit unknown compounds content presented no effect due to HW treatment. Stage I fruit content, however, decreased with an increase in HW treatment at 30 and 60 min. This HW treatment effect was particular for two compounds represented by Peaks 13 and 14, which constituted 41% of the overall unknown compounds content. Peaks 13 and 14, along with gallic acid, contributed with the largest peak areas of all guava polyphenolics. The decrease in these compounds in Stage I fruits may be due to a loss of polyphenolic compounds in response to stress. Within HW treatment, significant differences were observed due to ripening stages at control and at 15 min, but not at 30

and 60 min, which does not relate it to a HW treatment related response but rather to variation in some of the compounds.

Total guava polyphenolics by HPLC (Peaks 1 to 14)

Briefly, the overall trend of all 14 guava polyphenolic compounds will be discussed. Stage II fruit presented no significant differences in total polyphenolics due to HW treatments as compared to control (Table 3-4). Stage I fruit content at 15 and 60 min was no different from control, which accounts that insignificantly of a lower content at 30 min, in general, Stage I fruit presented no HW treatment effect. Within untreated fruit and HW treated fruit (15 to 60 min), significant differences due to ripening stage were observed.

Many postharvest stresses, including heat treatments, have shown to affect the levels of polyphenolics in plant commodities, either by inducing or inhibiting their biosynthesis. When fruits or vegetables undergo stress, cinnamic acid and benzoic acid derivatives are among the first polyphenolics to be synthesized (Sahit, 2004). The phenylpropanoid pathway, regulated by PAL, is responsible for the synthesis of hydroxycinnamic and hydroxybenzoic acids. An increase in PAL activity due to stress may result in the accumulation of many polyphenolic compounds (Cisneros-Zevallos, 2003). In the present study, gallic acid, a hydroxycinamic acid, was significantly enhanced in Stage I fruit after 60 min, which may be closely related to a response due to a longer exposure to heat stress. However, most polyphenolics were unaffected by increasing HW treatment times, especially in Stage II fruits.

3.3.2. Quality Analysis

3.3.2.1 pH and soluble solids

Quality parameters for fresh guava fruit and its industrial applications include fruit diameter and weight, percentage of seeds, puree color, skin color, acidity, flavor, soluble solids, pH, and ascorbic acid (Boyle et al., 1957). Soluble solids and pH were unaffected by HW treatment duration (15 to 60 min) within both ripening stages (Table 3-6). Heat treatment, water or hot air (38 to 48 °c for 1 h to 3 days), had no effect on tomato soluble solids or acidity (Lurie and Klein, 1991; McDonald et al., 1997). In the case of mango, soluble solids are not affected by an insect vapor heat treatment (Jacobi and Giles, 1997, Jacobi et al., 2001). Differences in pH were observed due to ripening stage at 30 and 60 min, while for soluble solids Stage I fruit present higher contents at 60 min.

Table 3-5. Quality parameters, soluble solids and pH, in guava as affected by a hot water immersion treatment (0, 15, 30, and 60 min at 46 °C) and ripening stage (Stage I and Stage II). Data are expressed as fresh weight basis (mean ± standard error), n = 4.

HW treatment (min)	**Soluble Solids** (°Brix)		**pH**	
	Stage I	**Stage II**	**Stage I**	**Stage II**
0	7.77 ± 0.49 ab	7.37 ± 0.22 b	4.05 ± 0.09 ab	4.05 ± 0.03ab
15	8.39 ± 0.33 a	7.68 ± 0.18 ab	4.03 ± 0.05 b	4.15 ± 0.03ab
30	8.12 ± 0.33 ab	7.25 ± 0.19 b	4.03 ±0.02 b	4.18 ± 0.05a
60	8.51 ± 0.47 a	7.29 ± 0.26 b	4.03 ± 0.02 b	4.18 ± 0.05 a

3.3.2.2 Overall fruit quality

Observations performed during the storage period following the HW treatments reported no differences in aesthetic quality of HW-treated guavas as compared to non-treated fruit. HW treated guavas of all ripening stages did not presented visible signs of heat injury. Some damages as a consequence of heat stress on HW treated fruit include irreversible changes such as skin scalding, skin browning and failure to soften (Paull and

Chen, 1990; Jacobi and Giles, 1997), among others. Gould and Sharp (1992) reported no signs of damage to guavas after HW treatment for 35 min at 46 °C. Guavas in the present study were comparable in weight and shape to Gould and Sharp (1992) study, which are an important factor which affects the uniformity of heating (Paul and Chen, 2000) and any subsequent damages present. Additionally, Gould and Sharp (2002) reported storage temperature after treatment was more important in maintaining fruit quality than the HW treatment itself. Although storage temperatures were not assessed in the present study, duration of storage at 15 °C might have played an important role in differences observed between Stage I and II fruits in chemical properties, although aesthetic quality was not affected. All guavas attained full ripeness and showed no differences among themselves in quality attributes like firmness and skin coloration independently differences in initial fruit ripeness and storage temperatures.

3.3.3 Stage III Fruit

Stage III fruit were analyzed independently, being the main interest to determine if the hot water treatment affected phytochemical properties of ripe guava fruit or not. Fruit were treated for 30 min at 46 ° and along with a control group, they were allowed to reach full ripeness for approximately two days at 15 °C. Moisture content, total soluble phenolics, antioxidant capacity, ascorbic acid, lycopene, and yellow carotenoids were unaffected by HW treatment in Stage III guavas. Quality parameters such as pH and soluble solids were also unaffected. Probably, the fruit had already developed all its quality and phytochemical properties by the time of treatment application. Comparable to Stage I and II, the HW treatment caused no change in guava quality and phytochemistry.

Table 3-6. Phytochemical content and quality parameters in Stage III guavas as affected by a hot water quarantine treatment at 46 °C (mean ± standard error). n = 4.

Time (min)	**Moisture** (%)	**Soluble Phenolics** (mg/kg dwb)	**AOX Capacity** (μmol TE/g)	**Ascorbic Acid** (mg/kg dwb)
0	93.21 ± 0.25 a	20070 ± 1200 a	135.5 ± 19 a	13120 ± 1900 a
30	92.85 ± 0.23 a	19110 ± 740 a	110.7 ± 6.4 a	10170 ± 1000 a

	Lycopene (mg/kg)	**Yellow carotenoids** (mg/kg dwb)	**Soluble Solids** (° Brix)	**pH**
0	458.5 ± 81 a	45.98 ± 6 a	6.79 ± 0.25 a	4.225 ± 0 a
30	437.3 ± 76 a	41.98 ± 3 a	7.15 ± 0.23 a	4.100 ± 0 b

3.4 Conclusions

A quarantine hot water treatment at 46 °C for up to 30 min can be applied to guavas (Stage I-III) without affecting its phytochemicals (total soluble phenolics, ascorbic acid, lycopene, yellow carotenoids, polyphenolic compounds), antioxidant capacity and quality (pH, brix, overall fruit appearance). Uniformity of the pool of guavas was confirmed by moisture content, ascorbic acid, yellow carotenoids, and quality parameters. After 60 min in hot water, Stage I fruit presented an increase in gallic acid, which might be attributed to an increase in biosynthesis of polyphenolic compounds as a response to heat stress. Additionally, a decrease in lycopene content was observed, which is also related to reversible inhibition in its biosynthesis when stress was applied. The chronological age of the tissue plays an important role, especially when the stress is applied for a longer period of time. Many of the differences observed in most parameters were due to ripening stage and storage conditions rather than a hot water treatment effect. Although it is not feasible to treat ripe guavas, Stage III fruit phytochemicals and quality parameters were not affected by the HW quarantine treatment for 30 min. Stage I fruit guavas treated for 30

min at 46 °C are preferred for HW treatment, since they could have a longer shelf life and allow more marketability to guavas.

CHAPTER 4
EFFECTS OF 1-METHYLCYCLOPROPENE ON GUAVA FRUIT PHYTOCHEMICALS, ANTIOXIDANT PROPERTIES AND QUALITY

4.1 Introduction

Guava (*Psidium guajava* L.) and more specifically guava juice and puree have increased in popularity within US markets (NASS, 2004) due to its exotic tropical flavor and overall consumer appeal. Fresh guava is highly perishable with a retail shelf life of approximately 7-10 days, creating recurrent pressure for packers and distributors to deliver a consistent product with widespread consumer acceptability. Due to the delicacy of its skin and rapid loss in firmness, special care is taken in most postharvest handling operations such as individually paper-wrapped fruit placed into specially designed packages (personal communication, Sardinia, 2005). Numerous technologies have been developed in the past years to extend shelf life of fruits and vegetables, and at the same time preserve their table quality, allowing their marketability to distant markets. These include storage under controlled atmospheres (CA), in polybags or with modified atmosphere packaging (MAP), and coating with polymeric films have shown to prolong the shelf-life of many commodities (Mitra, 1997). In the case of Florida guavas, many of these technologies have been evaluated by packers without appreciable success (Sardinia, 2005).

A recent technology emerged from the field of ethylene inhibitors is 1-methylcyclopropene, or 1-MCP, a gaseous compound that when applied to a commodity binds to ethylene receptors causing an inhibition in ethylene action (Sisler and Serek,

1997). Due to ethylene's close relation to various ripening processes, many beneficial effects have been attributed to 1-MCP in the extension of shelf-life (Blankenship and Dole, 2003). Its practicality of use, low cost, and beneficial effects are an attractive way of increasing fresh fruit marketing competitiveness. However, despite its vast potential for the fresh fruit industry, little is known of 1-MCP's effect on phytochemicals and antioxidant properties, especially with guava. The objective of the present study was not only to evaluate quality parameters of guava as affected by a 1-MCP treatment, but also its effects on polyphenolics and antioxidant properties.

4.2 Materials and Methods

4.2.1 Materials and Processing

4.2.1.1 Fruit preparation and 1-MCP treatment

Mature, green pink fleshed guavas (a hybrid variety) procured from Sardinia Farms (Homestead, Florida) were harvested on July 7, 2004. They were transported to the Food Science and Human Nutrition Department of the University of Florida, washed, and stored at 15 ºC until treatment application. Fruit were uniform in size, shape, firmness, and skin color (green) and free from any surface damage. Fruit were then transported to the Horticultural Sciences Department, University of Florida, and approximately 320 fruit were selected and randomly separated into two groups: Control and 1-MCP treated. Both groups were arranged separately into two impermeable 174 L capacity chambers in a storage room held at 10 ºC. Calculations by regression (Huber, 2004) were performed to measure the amount of 1-methylcyclopropene (1-MCP, Smartfresh®, Agrofresh, Inc.) powder, based on total fruit weight, to yield a final concentration of 1,000 nL/L gaseous 1-MCP inside the chamber. The powder was dissolved in 40 mL of deionized water in a 125 mL flask, which was sealed, and vortexed. The flask was placed in the 1-MCP

chamber, unsealed, and the chamber door immediately sealed. The same conditions applied to the chamber containing the control group using a flask of water without the 1-MCP. The 1-MCP treatment (1000 nL/L) was maintained for 24 hours, with a second application after the first 12 hours. At the conclusion of the treatment, fruit were again transported to the Food Science and Human Nutrition Department and held at a storage room at 15 ºC until complete ripeness. Ripe fruit were removed from storage for physicochemical analysis when the outer skin became thin, completely yellow and the fruit presented a soft texture, characteristics for a "ready-to-eat", ripe fruit. Day 0 was established as the 24 hour 1-MCP application period, while Day 1 was established as first day of storage at 15 °C, less than an hour after the fruits were removed from 1-MCP treatment. Subsequent days were 24 hours apart from the preceding day. For quality analysis over time, a group of five guavas were randomly obtained from each group every 3 to 4 days for evaluation of firmness followed by measurements for pH, total soluble solids, and titratable acidity.

Additionally, a secondary experiment was simultaneously conducted to evaluate the effects of 1-MCP application on boxed guavas, in effort to assess applicability of the 1-MCP treatment on fruit ready for shipping. Approximately 180 fruit were selected and also separated into Control and 1-MCP treated. Fruits for each treatment were arranged inside four small cardboard boxes (22 to 23 guavas per box), which were stacked inside their respective chambers and treated as previously described and allowed to ripen at 15 °C. During storage, three guavas were obtained every 3 to 4 days for quality analysis during ripening.

4.2.1.2 Guava fruit processing

Procedures for guava fruit processing were followed as outlined in Chapter 3 with composites of 5 guavas evaluated within each treatment. Fruits were processed when they achieved full ripeness, according to parameters described in Chapter 3. Collection of ripe fruits was done periodically, until the last fruits achieved full ripeness.

4.2.2 Quality Analysis

4.2.2.1 Aesthetic fruit quality assessment during storage

Following treatment with 1-MCP (Day 0), fruit were assessed daily every day for changes in aesthetic quality characteristics (skin coloration, firmness, presence of damages/diseases) during the storage period at 15 °C (Days 1 to 26).

4.2.2.2 Firmness determination during storage

Firmness on fresh guava fruits, obtained every 3 to 4 days during ripening, was measured using an Instron Universal Testing Instrument (Model 4411-C8009, Canton, Mass.), equipped with a 5 kg load cell and an 8-mm diameter compressive probe, adapting conditions from Bashir et al. 2002, Reyes and Paull 1995, and Ergun and Huber 2004. The probe was positioned at zero force contact with the surface of the guava. Probe penetration was set at 10 mm (1 cm) at a crosshead speed of 50 mm/min, and readings were taken at 3 equidistant points on the equatorial region of the fruit. Firmness data was expressed as the maximum force (kg) attained during penetration.

4.2.2.3 Titratable acidity, soluble solids and pH

Titratable acidity analysis was performed the guavas obtained every 3 to 4 days during storage. Approximately 3 g of puree were combined with 10 mL of deionized water and titrated with 0.1 N NaOH to an end point of pH 8.2. TA was calculated based on the volume of NaOH used and results were expressed as % citric acid, which is the

major organic acid in guava (Wilson et al., 1982). Soluble solids (SS) and pH measurements were performed as outlined in Chapter 3; Additionally, SS and pH were performed on all final ripe samples.

4.2.3 Chemical Analysis

Chemical analysis (total soluble phenolics, antioxidant capacity, ascorbic acid, lycopene, polyphenolics by HPLC and moisture content) were conducted according to the procedures outlined in Chapter 3.

4.2.4 Statistical Analysis

The experimental design consisted of a completely randomized design with two treatments: control and 1-MCP. Statistical analysis consisted of t-test using JMP (SAS, Cary, NC) to compare differences between treatments ($P < 0.05$).

4.3 Results and Discussion

4.3.1 Quality Analysis

Detailed treatments comparisons of fruit quality were assessed during storage since one of the main focuses of 1-MCP is extending produce shelf-life and preserving many of the physicochemical and quality attributes of fresh guava. The most important aesthetic quality parameters for guava are firmness and skin coloration. In order to differentiate between stages of skin coloration during ripening, 4 color criteria were used to describe the fruit that included green (mature-green stage), yellowish-green (a brighter green color with yellow tints), turning (40 to 70% surface yellow), and yellow (>70% surface yellow).

4.3.1.1 Aesthetic fruit quality during storage.

Days 0 to 7: First identifiable differences between treatments

Perceivable differences in surface color were not apparent until 4 days in storage when approximately 20% of the control fruit were classified as yellowish-green compared to green for the 1-MCP treated fruit. On Day 5, 30% of control fruit were yellowish-green and turning, while the 1-MCP group presented 5% of its fruits at a yellowish-green stage. By Day 7, some bruises on guava skin became apparent on control fruit, probably due to a more advanced degree of ripening.

Days 9 to 15: Control guavas ripening

Day 9 was characterized by the first collection of ripe guavas from the control fruits, which accounted for approximately 35% of control fruits. Additionally, control guavas from boxed treatment were collected. The 1-MCP group remained primarily in the green color stage, including boxed guavas. A difference in fruit texture between the treatments was apparent. On Day 13, the first collection of ripe 1-MCP guavas was done, close to 13% of the 1-MCP group; while 80% of the control fruits had already been collected for ripeness. Ripe 1-MCP fruits had completely yellow skin coloration and were firmer than control fruits. The last guavas from the control group were collected on Day 15, when 80% of the original 1-MCP group was still in the process of ripening. A brief summary of changes in skin coloration during storage is presented in Table 4-1.

Days 16 to 26: 1-MCP guavas ripening

By Day 17, only about 30% of 1-MCP ripe fruits had been collected. Most fruit remained green, while 40% of the group was turning or yellow. 1-MCP guava ripening continued until Day 26, when the last batch was collected. Approximately 70% of the 1-MCP ripe guavas were collected in the period from Day 18 to Day 26. Reyes and Paull (1995) have reported that guavas stored at 15 °C usually attain full ripeness in a period

between 8 to 11 days. Although, variety, harvest time, post harvest handling and other parameters shall be considered. Comparably, most of the control guavas (82%) achieved full ripeness between Day 9 and 13 (12 to 15 days after harvest). 1-MCP was able to extend the shelf life was able to extend the shelf-life of guavas for at least 5 days.

Days in Storage at 15 °C	**Green** (%*)		**Yellowish/green** (%)		**Turning** (%)		**Yellow** (%)	
	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP
0	100	100						
5	70	100	20		10			
7	2	95	73	5	25			
9		90	60	10	10		30	
13		80	20	20	30		50	
15		60		20	5	5	100	15
18	--	20	--	30	--	30	--	20
22	--		--	50	--	20	--	30
26	--		--		--		--	100

Table 4-1. Changes in skin coloration in non-treated (control) and 1-MCP-treated guavas during 1-MCP application and storage at 15 °C.

* % of fruit from the treatment containing determined skin coloration.

The influence of 1-MCP on diseases or disorders has been specific depending on the species showing mixed results (Blankenship et al., 2003). 1-MCP treated mangos reported twice the amount of stem rot than control fruit (Hofman et al., 2001), whereas in apples it has reduced superficial scald (Fan et al., 1999) and in papaya it has shown less incidence in decay (Ergun and Huber, 2004). Along with bruising, an incidence of brown spots around the crown and other parts of the fruit was observed in some fruits during ripening, which was likely due to firm rot, a common disease in guavas usually induced by bruising (Ko and Kunimoto, 1980; Reyes and Paull, 1995). As the fruit became riper, bruises and spots became more apparent, especially in control fruits, which started to ripen earlier. Due to a slowing down in their ripening process, 1-MCP fruits did not show

these disorders as markedly, however by Days 20 to 26, they were more apparent. Approximately 15% of the original fruits from both groups were lost mainly due to incidence of firm rot. 1-MCP treatment to guavas did not ameliorated or induced this disorder.

4.3.1.2 Firmness during storage

Firmness loss during climacteric fruit ripening is directly related to disassembly of cell wall components (Lohani et al., 2003) and modification of pectin fractions mainly, with an increase in pectin solubilization (Huber, 1983). These changes are resultant of an increase in activity of cell wall hydrolases, which have been closely associated to ethylene (Brummell and Harpster, 2001). Cell wall hydrolases polygalacturonase (PG), pectinesterase (PE) and cellulose in both white and pink flesh guavas have shown to increase in activity during ripening, with a correlation between increase in activity of PG and cellulose and loss of flesh firmness (Abu-Goukh and Bashir, 2003).

A 1-MCP treatment effect was observed since Day 4, were 1-MCP fruits presented significantly firmer texture as compared to control (Figure 4-1). The trend continued until Day 12, before the last procurement of control guavas was performed. Comparably, Basseto et al. (2005) reported that ‘Pedro Sato’ guavas treated at 900 nL/L (6 to 12 h at 25 °C) retained firmness as compared to a control, while fruits treated at lower concentrations presented no differences in texture. They reported, however, these guavas (900 nL/L) were not able to attain full ripeness, as opposed to the present study. Important factors to consider are the relationship between application time and temperature; in the present study guavas were exposed for a longer period of time at a lower temperature.

During guava storage, there were no significant differences in firmness between each sampling point, from Day 1 until Day 13 for control fruits and until Day 17 for 1-MCP treated fruits, as observed in Figure 4-1. A decline in firmness was expected in control fruits during storage, since softening is directly related to an increase in days of ripening in guava (Abu-Goukh and Bashir, 2003).However, there were no significant differences between sampling points. This was likely attributed to the variability of the samples procured, since they were chosen based on physiological similarities to 1-MCP fruits collected each sampling day. Mercado-Silva et al. (1998) reported a large variability in firmness between different ripening stages of guavas. 1-MCP fruits maintained their firmness throughout the entire storage period, even when they attained full ripeness.

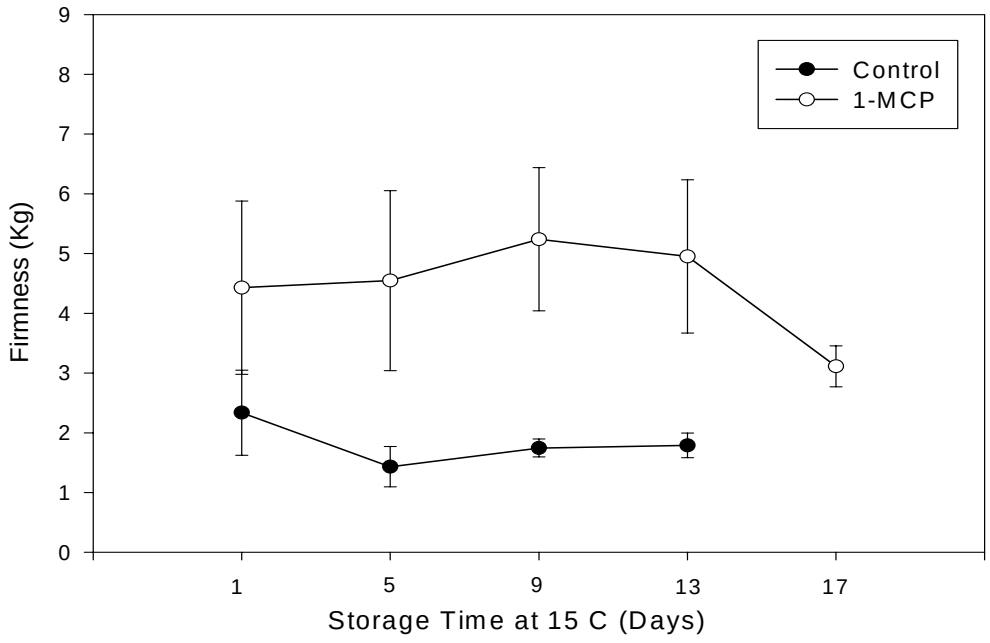


Figure 4-1. Firmness (kg) of guavas treated with 1-MCP (1000 nL/L ,10°C, 24 h) during storage at 15 °C. Error bars represent the standard error of the mean, n = 5.

Various effects of ethylene during fruit ripening are apparently associated with alterations in the properties of cellular membranes. It is thought that ethylene, along with

other growth substances found in plants, binds to some components of the cellular membrane (proteins, glycoproteins, and lipids) thereby initiating secondary responses (Noogle and Fritz, 1983). Such bindings, especially with proteins, may have immediate effects, including protein conformation or cell turgor changes, or trigger other processes which might take a longer time to become visible. It has been discussed that when ethylene binds to proteins possessing enzymatic activity, the act of binding may activate them and alters their rate of degradation (Noogle and Fritz, 1983). By inhibiting ethylene action, 1-MCP has been related to the delay or inhibition of the activity of cell wall hydrolases, responsible for tissue softening.

4.3.1.3 Titratable acidity, soluble solids, and pH during storage

Titratable acidity (TA) and pH in 1-MCP fruits was higher than control only on Day 5 of storage, but not insignificantly different at other days (Figures 4-2 and 4-3). This difference might be attributed to variation, as seen in the large error rate. Independently of Day 5, 1-MCP did not affect TA and pH during storage time. Basseto et al. (2005) reported that 1-MCP treated fruit (900 nL/L) maintained higher titratable acidity levels during entire storage, attributing it to a ripening delay. Reports on influence of 1-MCP on titratable acidity during are mixed, depending on the type of commodity or even variety (Blankenship and Dole, 2003). Additionally, pH on final ripe samples was not influenced by 1-MCP (average = 4.07). Within the 1-MCP group, TA and pH remained unaffected by increasing storage time. Within the control group, Day 13 TA was significantly higher than Day 5, but not different from the rest of the sampling points; while pH remained unaffected. According to results reported by Reyes and Paull (1995), both TA and soluble solids as a function of fruit age rather than stage of ripeness, were both quality parameters are maintained during ripening

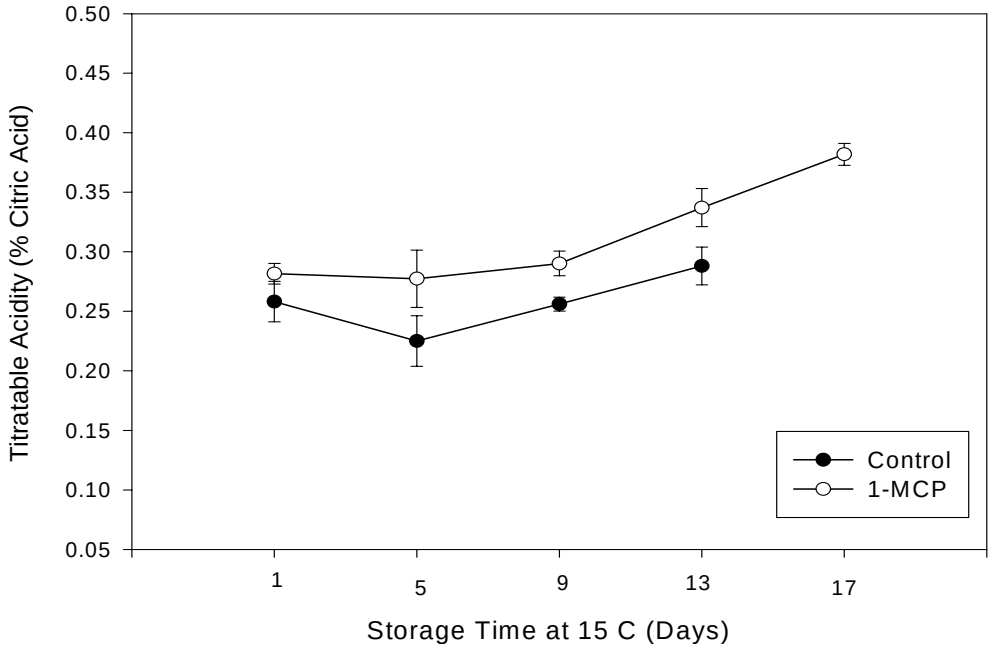


Figure 4-2. Titratable acidity (% citric acid) of guavas treated with 1-MCP during storage at 15 °C. Error bars represent the standard error of the mean, n = 5.

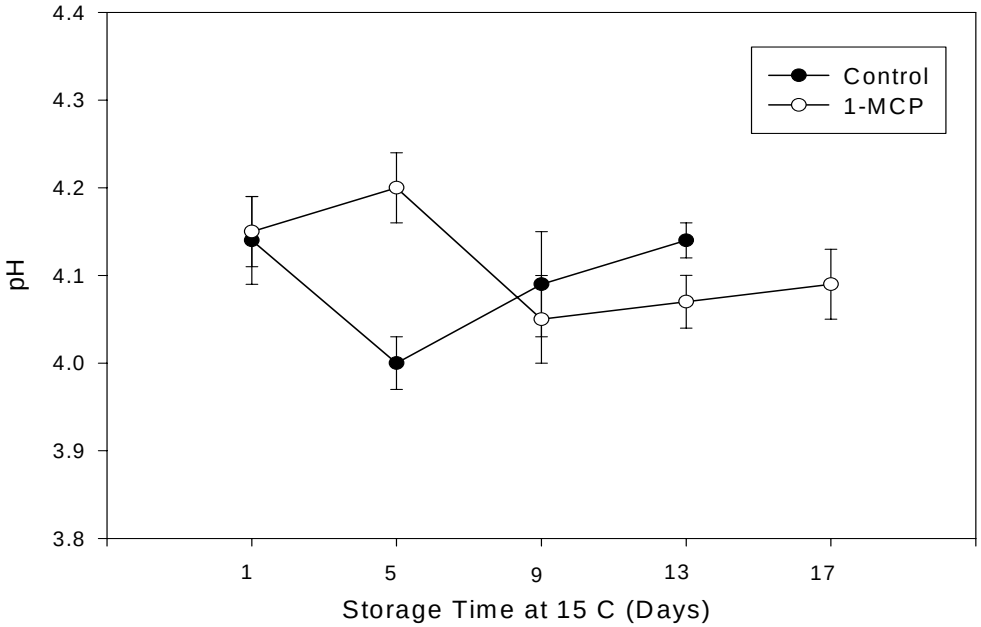


Figure 4-3. Effect of a 1-MCP treatment (1000 nL/L ,10°C, 24 h) on guava pH during storage at 15 °C. Error bars represent the standard error of the mean, n = 5.

Soluble solids were not influenced by the 1-MCP treatment both during storage (Figure 4-4) and in final ripe guavas (average = 7.81 °Brix). There has been no reported effect on soluble solids on Brazilian guava (Basseto et al., 2005), mango, custard apple

(Hofman et al., 2001), apricots, and plums (Dong et al., 2002). Guava soluble solids maintained uniformity during the storage time within both the control and 1-MCP group. The role of ethylene on starch and/or sugar conversion is still not clear, with mixed reports of whether 1-MCP affects their conversion or not (Blankenship and Dole, 2003).

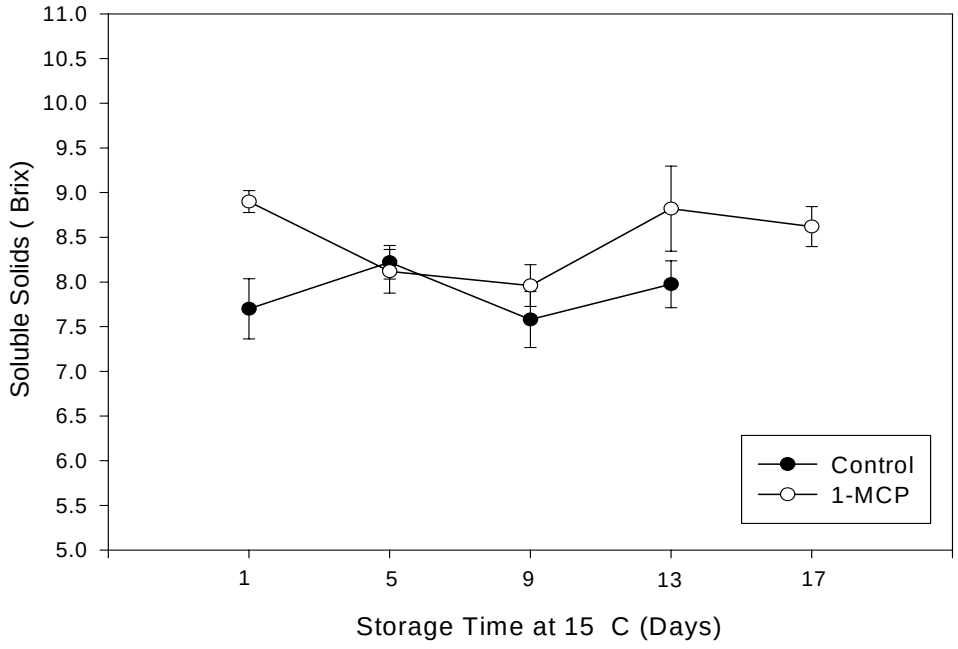


Figure 4-4. Effect of 1-MCP treatment (1000 nL/L at10°C, 24 h) on guava soluble solids (°Brix) during storage at 15 °C. Error bars represent the standard error of the mean, n = 5.

4.3.2 Chemical Analysis

For all chemical analysis performed, except for polyphenolics by HPLC, results were reported in dry weight basis (DW), in order to eliminate difference between samples due to varying water loss fruits experienced during ripening, an important factor since 1-MCP fruits were stored considerably longer than control fruit.

4.3.2.1 Moisture content

Moisture content in the final, ripe fruit was not significantly different between control and 1-MCP treated fruit at 86.6 and 85.9% respectively. Although, moisture

content determined at final ripe guava puree samples is not the same as a measure of individual fruit weight loss during ripening, it gives a clearer picture of differences in final samples due to moisture. It was expected that 1-MCP treated fruits probably had a higher weight loss due to their longer time in storage. However, no differences in final moisture values were reported for the present study.

4.3.2.2 Total soluble phenolics

Total soluble phenolics (TSP) content was unaffected by 1-MCP treatment (Figure 4-5). Although some aspects of the ripening process were delayed in 1-MCP fruits, these presented TSP values of full ripe fruit, as compared to control. Total phenolics from both groups probably decreased during ripening achieving similar values, independently of the inhibition of ethylene. However, the addition of ethylene may affect polyphenolic increase in biosynthesis (Cisneros-Cevallos, 1997). In lettuce, phenylalanine lyase (PAL) activity was induced by exogenous ethylene, causing an increase in phenolics compounds (Ke and Salveit, 1988; Dixon and Paiva, 1995; Tomas-Barberan et al., 1997). Also in lettuce, 1-MCP application (1000 nL/L) did not affect polyphenolic content as compared to control; however, when 1-MCP was applied prior to exposure to ethylene, there was a significant reduction in ethylene-induced polyphenolic synthesis (Campos-Vargas and Salveit, 2002). The addition of exogenous ethylene rather than endogenous ethylene inhibition by 1-MCP seems to affect more the levels of polyphenolics. In apples, total phenolics exhibited an ethylene-independent regulation when ethylene was inhibited (Defilippi et al., 2004). It is concluded that 1-MCP did not affect the final levels of total soluble phenolics in guava, where probably polyphenolic regulation was not affected by inhibition of ethylene. The inhibition of ethylene itself, even at a relatively high concentration of 1-MCP, was not enough to affect polyphenolic biosynthetic pathways.

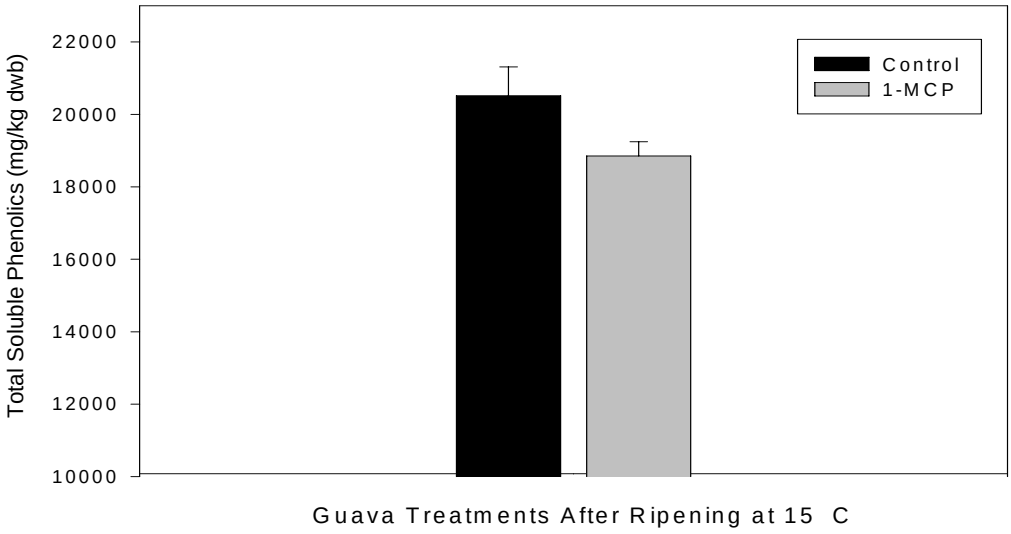


Figure 4-5. Effect of 1-MCP (1000 nL/L, 10°C, 24 h) on total soluble phenolics (mg/kg DW) in guava. Error bars represent the standard error of the mean, n = 23.

4.3.2.3 Antioxidant capacity

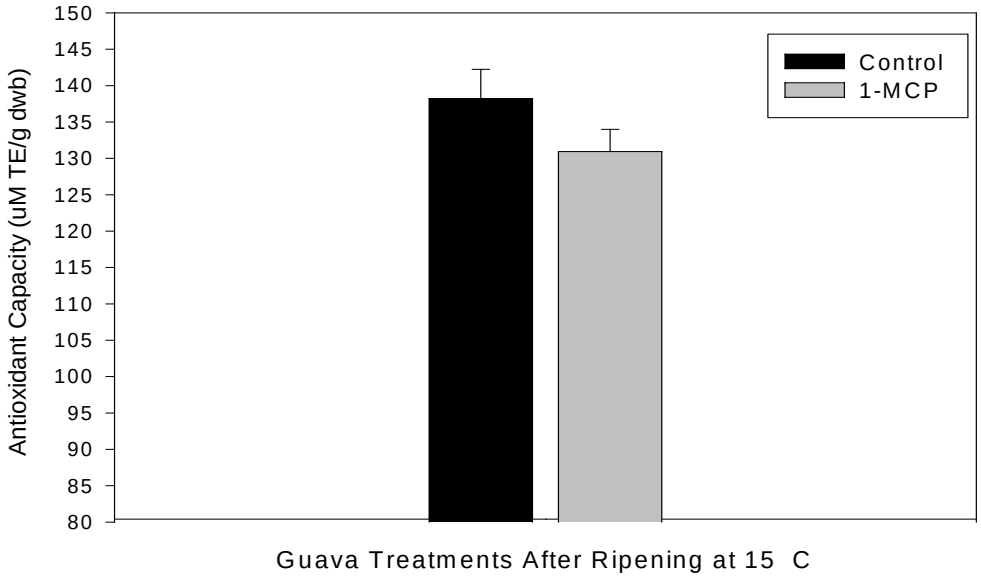


Figure 4-6. Effect of 1-MCP treatment (1000 nL/L, 10°C, 24 h) on guava antioxidant capacity (μM Trolox equivalents/g DW). Error bars represent the standard error of the mean, n = 23.

Antioxidant capacity of ripe guavas was unaffected by 1-MCP treatment (Figure 4-6). This observation supports results for TSP. Since antioxidant capacity in guava was not

affected by 1-MCP this might explain a degree of ethylene independence in the regulation of most antioxidant phytochemicals during climacteric ripening.

4.3.2.3 Ascorbic acid

1-MCP treated fruit presented significantly higher levels of ascorbic acid as compared to Control fruit (Figure 4-7). Differences in ascorbic acid might be attributed to fruit variability than the 1-MCP treatment itself. Basseto and partners (2005) reported no differences between 1-MCP and control fruit, determined by a titration method. The use of a more precise analytical method resulted in a more accurate quantification of ascorbic acid. However, it resulted in a high variability between samples. A variability can be attributed to the assay for running ascorbic acid, however, careful care was taken when making composites of five fruits and extracting guava juice and the standard for HPLC had a low standard error (1.7%). It has been reported a high variability in ascorbic acid levels in guava, even within the same variety (reviewed by Mitra, 1997). Additionally, the fact that antioxidant capacity and TSP were not influenced by 1-MCP is a good indicator that ascorbic acid probably was not affected.

Apart from being an essential nutrient for humans, ascorbic acid within the plant has numerous roles mainly related to three biological activities: as an antioxidant, as a donor/acceptor in electron transport at the plasma membrane or chloroplasts, and as an enzyme co-substrate (Davey et al., 2000). Although the biosynthetic pathway for ascorbic acid has not been completely elucidated, a pathway via hexose sugars (GDP-mannose, GDP-L galactose, L-galactose, galacto-1,4-lactone) has been proposed recently (Davey et al., 2000; Smirnoff and Wheeler, 2000; Barata-Soares, 2004). The relationship existent between ascorbic acid and ethylene lies within ethylene's biosynthetic pathway, where ACC oxidase (enzyme responsible for the last step in ethylene biosynthesis), uses

ascorbic acid as a co-substrate, apparently oxidizing it to dehydroascorbate (Davey et al., 2000; Smith et al., 2000). Ethylene levels produced by most fruits are very low (ppb range), therefore its normal production or subsequent inhibition might be to low to result in detectable changes in overall ascorbic acid levels (personal communication, Huber, 2005), specially in guavas, which contain a large pool of ascorbic acid as compared to most fruits. Additionally other mechanisms such as heat, light or wounding tend to affect ascorbic acid to a greater extent.

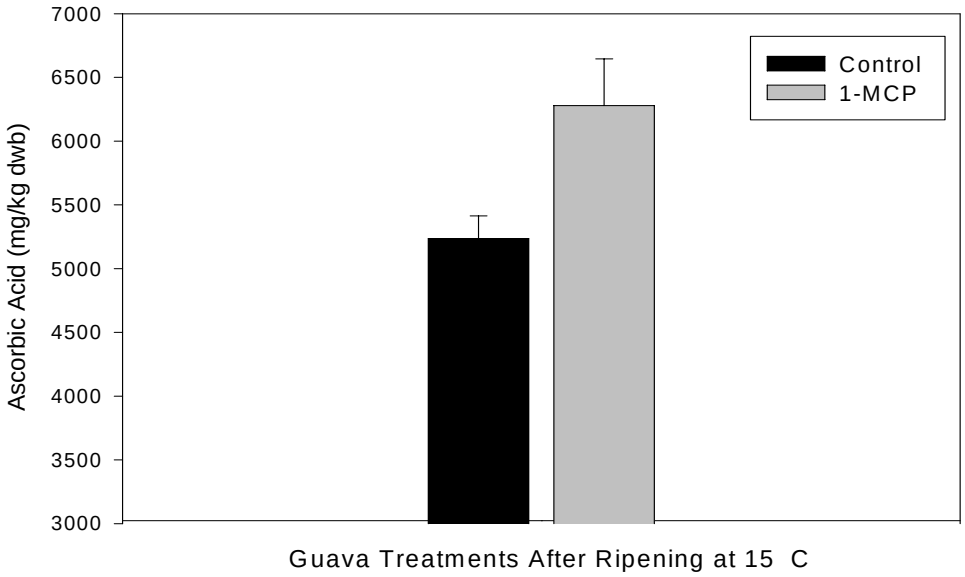


Figure 4-7. Guava ascorbic acid content (mg/kg DW) as affected by 1-MCP (1000 nL/L, 10°C, 24 h). Error bars represent the standard error of the mean, n = 23.

4.3.2.4 Lycopene

Results from lycopene analysis presented significantly higher content in 1-MCP fruits as opposed to control fruits (Figure 4-8). Equal or even higher lycopene values in 1-MCP fruit as compared to control indicates that although there was an alteration on ethylene production and other ripening processes were affected, lycopene accumulation resulted in guava during its ripening. This might suggest that lycopene accumulation pathways in guava may not be directly related to ethylene pathways, although they might

affect the rate of lycopene accumulation during ripening. Comparably, Mostofi and partners (2003) reported final lycopene values in 1-MCP treated tomatoes as insignificantly different from control, however, the treatment delayed the onset of lycopene accumulation during storage at 15 °C. In another study on fresh cut tomatoes using other ethylene inhibitors and exogenous ethylene, although ethylene inhibition slowed down the rate of lycopene accumulation probably due to other processes slowing down, it was concluded that ethylene production is not essential for lycopene biosynthesis in tomato fruit (Edwards et al., 1983).

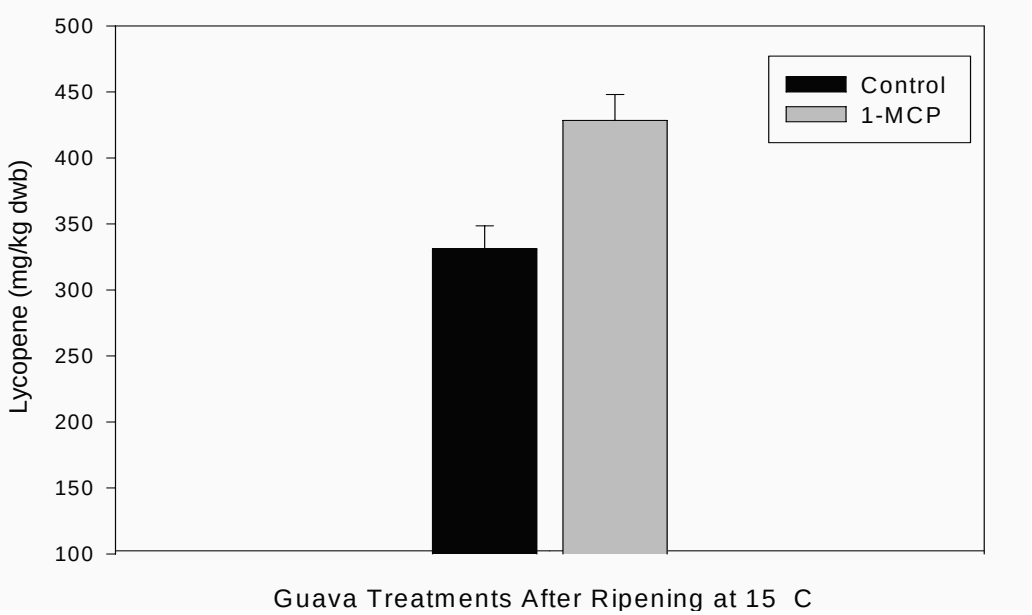


Figure 4-8. Effect of 1-MCP (1000 nL/L ,10°C, 24 h) on guava lycopene content (mg/kg DW). Error bars represent the standard error of the mean, n = 23.

It is known from studies on tomatoes that lycopene accumulation is mainly in the last period of the ripening process (Giovanelli et al., 2004). Although ethylene plays an important role in accumulation of tomato carotenoids during ripening, since it regulates phytoene synthase, the lycopene-producing enzyme, it has been discussed that it might not be the key regulator for lycopene accumulation in particular. Work conducted on

tomatoes by Alba et al. (2002) demonstrated that lycopene accumulation in pericarp tissue during tomato ripening is mainly regulated by phytochromes or chromoproteins, and this can be independent of ethylene biosynthesis. The regulation of carotenoid biosynthesis genes in particular has also been proposed as primary mechanism that controls lycopene accumulation in tomato fruits (Ronen et al., 1999).

Lycopene is the major carotenoid in guava and its content is comparable or sometimes higher than tomato. Unfortunately the lack of lycopene in the skin hides its presence in the pulp, making fruit selection based on lycopene an impossible selling point in the market. Considering new evidences for correlation between lycopene consumption and reduced rates of prostate cancer (Giovannuci et al., 1995; Rao and Agarwal, 1999), it is of particular importance preserving or even enhancing its content during postharvest operations. The opportunity of marketing guava as an excellent source of lycopene is present.

4.3.2.5 Polyphenolics by HPLC

Polyphenolics by HPLC were identified by retention time, spectral properties, and comparison to authentic standards. Based on the work done on the HW treatment study, approximately 16 peaks were selected from the guava chromatogram. These peaks were divided into 4 groups based on spectral properties and retention time: gallic acid, procyanidins, characteristic phenolics, and an ellagic acid derivative. Gallic acid and ellagic acid derivative were two individual identifiable compounds. Characteristic phenolics group contained characteristic guava compounds which are unknown, with spectral properties similar to the ones described for the HW treatment study. The polyphenolic profile of guavas from the 1-MCP study was similar to the HW treatment study.

1-MCP did not have an influence on the levels of procyanidins, ellagic acid derivative and other characteristic polyphenolics of ripe guava (Figures 4-9 and 4-10). The observed results confirm results observed for antioxidant capacity and TSP, which help explain an ethylene-independent synthesis guava polyphenolic compounds when ethylene is inhibited. In the case of gallic acid (Figure 4-11), however, 1-MCP treated fruit exhibited higher content, which might be attributed more to variability in content between samples, specially within the control group. However, due to gallic acid's abundance in a wide variety of commodities and its still not completely known synthesis mechanism, the possibility of an interaction with ethylene inhibition should also be considered.

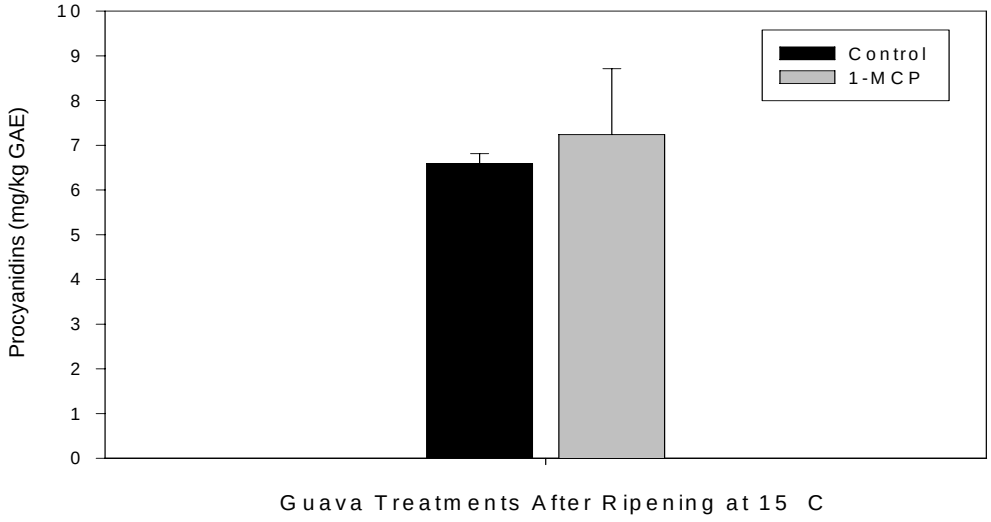
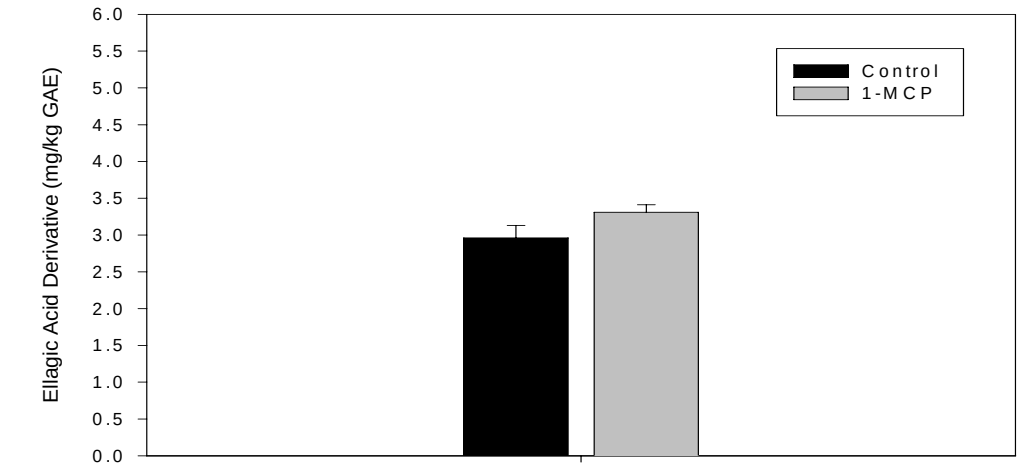
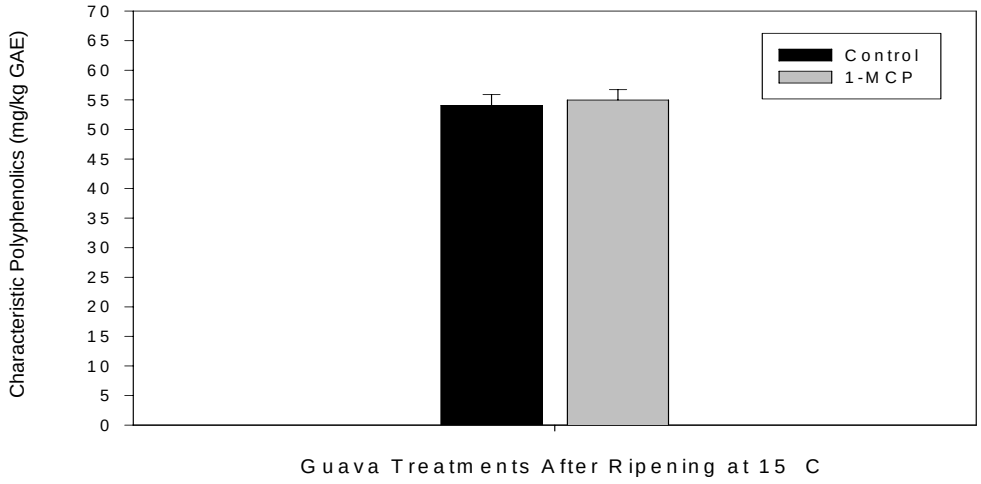


Figure 4-9. Guava procyanidin content (mg/kg GAE) as affected by 1-MCP. Error bars represent the standard error of the mean, n = 23.



A



B

Figure 4-10. Guava ellagic acid derivative content (A) and characteristic polyphenolics content (B) (mg/kg GAE) as affected by 1-MCP. Error bars represent the standard error of the mean, n = 23.

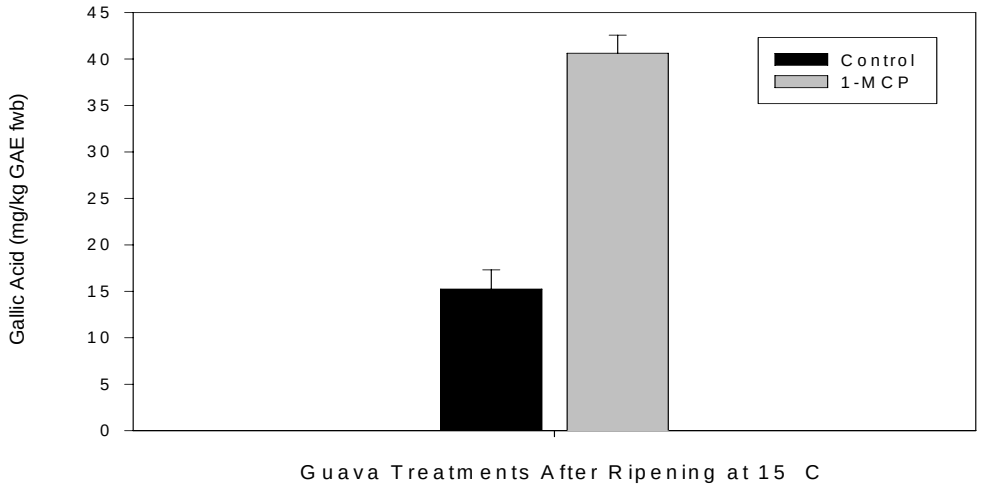


Figure 4-11. Guava gallic acid content (mg/kg GAE) as affected by 1-MCP. Error bars represent the standard error of the mean, n = 23.

4.3.3 1-MCP Treatment to Boxed Guavas

The present study evaluated the effects of 1-MCP on guava quality and phytochemical content when application was conducted on guavas arranged inside their boxes, in a simulated environment prior to shipment. Guavas treated in boxes presented similar aesthetic characteristics during storage as loose fruit from the previous experiment. On Day 0, during 1-MCP application, guavas within the boxes presented no differences among themselves. From Days 5 to 8 in storage the first identifiable differences were detected, where 1-MCP presented a better retention of green coloration and firmness, as compared to control fruit. Similarly to the main study, Day 9 was characterized by the first collection of ripe guavas, which accounted for 14% of the control group; while most 1-MCP guavas maintained their firmness and green coloration. The first collection of ripe 1-MCP guavas (17% of the 1-MCP group) was conducted on Day 12, when already more than 50% of the control fruit had already ripened and been procured. Similar to the main experiment, collection of the last group of control guavas was conducted on Day 15, while collection of 1-MCP ripe guavas continued until Day

26. Results from aesthetic quality evaluations indicated an effect due to 1-MCP treatment in color retention, where 1-MCP treated fruits presented a delay in skin coloration development for at least 5 days, comparably to the main study. Chemical analysis performed resulted in no significant differences in moisture content (86.1 %), total soluble phenolics (19,100 mg/kg DW), antioxidant capacity (121 µM Trolox equivalents/g DW), ascorbic acid (7,090 mg/kg DW), lycopene (853 mg/kg DW), soluble solids (8.13 °Brix) and pH (4.10).

Firmness measured over time, however, presented no significant differences during storage (Figure 4-12), as opposed to the main experiment. However, as observed by the large error rates, this statistical lack of effect might be due to variation in samples. A clear distinction between treatments can be observed, especially after Day 9, where 1-MCP fruit presents higher firmness values. Probably, a larger number of replications

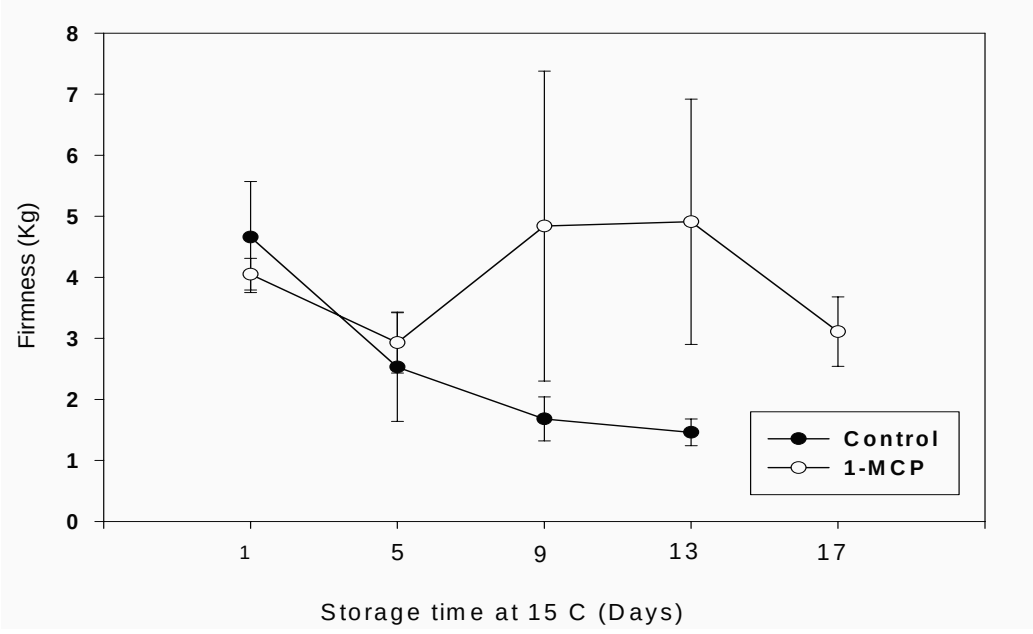


Figure 4-12. Firmness (kg) of boxed guavas treated with 1-MCP (1000 nL/L ,10°C, 24 h) during storage at 25 °C. Error bars represent the standard error of the mean, n=3.

might have demonstrated a clearer effect. Titratable acidity (Figure 4-13) and soluble solids (Figure 4-14) were unaffected by 1-MCP during storage, comparable to the main experiment. The nature of the packaging material used is an important factor to consider. It seems that the 1-MCP gas was able to penetrate to the walls of the cardboard box, diffusing itself among the fruit, delaying skin yellowing and retaining firmness, as described by aesthetic quality evaluations. Probably, if the boxes had more perforations, a better penetration and diffusion of the gas would have happened. In a work conducted in plums packaged similarly inside perforated boxes, 1-MCP proved better effectiveness than plums treated in bulk (Valero et al., 2004). Therefore, the possibility of applying 1-MCP on packaged guavas has a potential of being explored further for commercial applications, especially due to the nature of the fruit, since an easier way to handle any postharvest process might be beneficial.

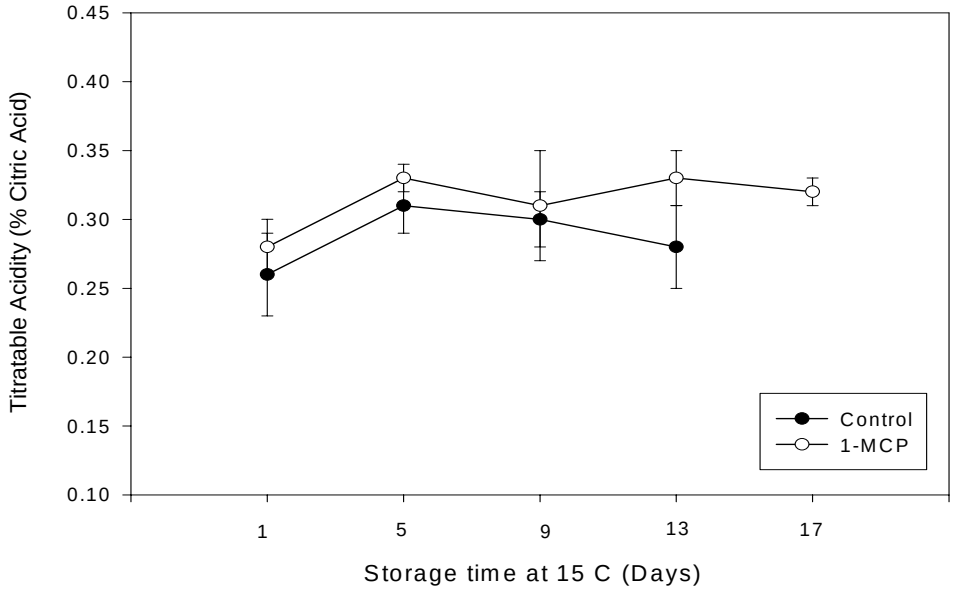


Figure 4-13. Titratable acidity (% citric acid) of boxed guavas treated with 1-MCP during storage at 15 °C. Error bars represent the standard error of the mean, n = 3.

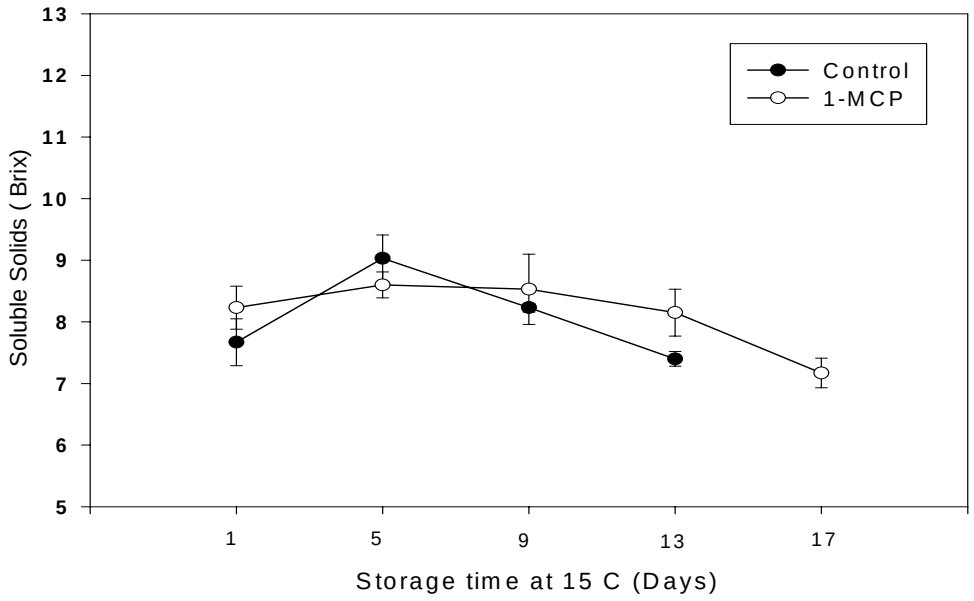


Figure 4-14. Effect of 1-MCP treatment (1000 nL/L at10°C, 24 h) on boxed guavas soluble solids (°Brix) during storage at 15 °C. Error bars represent the standard error of the mean, n = 3.

4.4 Conclusions

Extension of shelf-life in guavas is of extreme value due the highly perishable nature of these exotics. A 1-MCP treatment (1000 nL/L for 24 h at 10 °C) applied to pink fleshed guavas was effective in extending their shelf life and retaining quality characteristics, without detrimental effects to their phytochemicals. Guava shelf life was extended for at least five days during storage at 15 °C, with a delay in skin yellowing and retention of firmness. Quality parameters such as titratable acidity, soluble solids, and pH were maintained during storage, and unaffected by a 1-MCP treatment. Additionally, there was no significant effect of 1-MCP in total soluble phenolics and antioxidant capacity. Ascorbic acid and lycopene presented significantly higher values in 1-MCP treated fruit; however these differences were not attributed to a treatment effect, but rather to fruit variability and certain independence of ethylene in their biosynthetic

pathways. Procyanidin compounds, total polyphenolics, and an ellagic acid derivative were not affected by a 1-MCP treatment, which supports results for antioxidant activity and TSP. An ethylene inhibition in guava, even at a relatively high concentration, resulted in insignificant effect in most of its phytochemicals. Results from the boxed guavas study indicate a potential for applying 1-MCP on boxed guavas, and further investigation in a near future would be beneficial for packers. Literature presents mixed results on the effects of 1-MCP on physicochemical properties of various commodities, being species specific and very dependent on application conditions. Results of this study conclude that 1-MCP can be applied to guava successfully, without negative impacts on its aesthetic quality, phytochemicals, and antioxidant properties. Further research is needed to determine optimal application conditions.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Guava marketability as a fresh fruit is somewhat limited due to lack of an established quarantine treatment and its highly perishable nature. To provide an overview, these studies consisted of application of two postharvest treatments, a hot water immersion technique as a quarantine treatment and a 1-methylcyclopropene (1-MCP) application to extend guava shelf-life. The effects evaluated not only included overall quality parameters, but guava phytochemicals (polyphenolics, ascorbic acid, carotenoids, and lycopene) and antioxidant properties. A hot water immersion treatment at 46 °C for up to 30 min may be applied to guavas at three ripening stages without affecting their quality and phytochemical content. Stage I fruits treated longer than 30 min experienced an increase in certain polyphenolic compounds and a decrease in lycopene content. This was a response to heat stress, where biosynthesis of certain polyphenolic compounds was enhanced and lycopene biosynthesis might have been reversibly inhibited affecting its final concentrations. Other differences reported were mainly attributed to ripening stage than the HW treatment itself. 1-MCP application (1000 nL/L for 24 h at 10 °C) successfully extended the shelf life of guavas for at least 5 days during storage at 15 °C, presenting positive effects which included skin yellowing delay and retention of firmness. Although shelf-life was extended, 1-MCP insignificantly affected quality parameters (titratable acidity, soluble solids, and pH) and phytochemical content. It was observed a higher ascorbic acid and lycopene content in 1-MCP treated fruits, which was not directly related to a 1-MCP effect. It was concluded that the

biosynthesis pathways for most antioxidant compounds in guava are independent from inhibition of ethylene action. A HW immersion treatment and a 1-MCP treatment may be applied successfully to guavas, maintaining their quality attributes and especially not affecting detrimentally phytochemical compounds.

LIST OF REFERENCES

Abu-Goukh, A.; Bashir, H. A. Changes in pectic enzymes and cellulose activity during guava fruit ripening. *Food Chem*. **2003**, 83, 213-218.

Abushita, A.A,; Daood, H.G.; Biacs, P.A. Changes in carotenoids and antioxidant vitamins in tomatoes as a function of varietal and technological factors. *J. Agric. Food Chem.* **2000**, 48, 2075-2081.

Akamine, E.; Goo, T. Respiration and ethylene production in fruits of species and cultivars of *Psidium* and species of *Eugenia*. *J. Amer. Soc. Hort. Sci.* **1979**, 104, 632-635.

Alba, R.; Cordonnier-Pratt, M.; Pratt, L. Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. *Plant Physiol.* **2000**, 123, 363-370.

Association of Analytical Communities. *Official methods of analysis of AOAC International,* 17th Ed.; Horwitz: Gaithersburg, MD, 2000.

Barata-Soares, A.D.; Gomez, M.; De Mesquita, C.; Lajolo, F. Ascorbic acid biosynthesis: A precursor study on plants. *Brasilian J. Plant Physiol.* **2004**, 3, 147-154.

Bashir, H. A.; Abu-Goukh, A. Compositional changes during guava fruit ripening. *Food Chem*. **2002**, 80, 557-563.

Basseto, E.; Jacomino, A.P.; Pinheiro, A.L.; Kluge, R.A. Delay of ripening of 'Pedro Sato' guava with 1-Methylcyclopropene. *Postharvest Biol. Technol.* **2005**, 35, 303-308.

Blankenship, S.; Dole, J. 1-Methylcyclopropene: A Review. *Postharvest Biol. Technol*. **2003**, 28, 1-25.

Boyle, F.; Seagrave, H.; Sakata, S.; Sherman, D. *Commercial guava processing in Hawaii,* Bulletin.University of Hawaii: Honolulu, Hawaii, 1957.

Brasil, I.; Arraes Maia, G.; Wilane de Figueiredo, R. Physical-chemical changes during extraction and clarification of guava juice. *Food Chem*. **1995**, 54; 383-386.

Brecht,J.; Saltveit, M.; Talcott, S.; Schneider, K.; Felkey, K.; Bartz, J. Fresh-cut vegetables and fruits. In: Janick (ed.). *Horticultural reviews.* Vol. 30; John Wiley and Sons: Hoboken, New Jersey, 2004.

Brummel, D.; Harpster, M. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Mol. Biol.* **2001**, 77, 311-340.

Bulk, R.; Babiker, E.; Tinay, A. Changes in chemical composition of guava fruits during development and ripening. *Food Chem.* **1997**, 59, 395-399.

Campos-Vargas, R.; Saltveit, M.E. Involvement of putative chemical wound signals in the induction of phenolics metabolism in wounded lettuce. *Physiol. Plant.* **2002,** 114, 73-84.

Cheng,T.; Floros, J.; Shewfelt, R.; Chang, C. The effect of high temperature stress on ripening of tomatoes (*Lycopersicum esculentum*). *J. Plant Physiol.* **1988**, 132, 459-464.

Ching, L.; Mohamed, S. Alpha-tocopherol content in 62 edible tropical plants. *J. Agric. Food Chem*. **2001**, 49, 3101-2105.

Cisneros-Zevallos, L. The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. *J. Food Sci*. **2003**, 68, 1560-1565.

Civello, P.; Martinez, G.; Chaves, A.; Anon, M. Heat treatments delay ripening and postharvest decay of strawberry fruit. *J. Agric. Food Chem.* **1997**, 45, 4589-4594.

Cook, N.; Samman, S. Flavonoids: Chemistry, metabolism, cardioprotective and dietary sources. *Nutr. Biochem.* **1996**, *7*, 66-76.

Davey, M.; Montagu, M.; Inze, D.; Sanmartin, M.; Kanellis, A.; Smirnoff, N.; Benzie, I.; Strain, J.; Favell, D.; Fletcher, J. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci. Food Agric.* **2000,** 80, 825-860.

DeEll, J.; Murr, D.; Porteous, M.; Rupasinghe, H. Influence of temperature and duration of 1-methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biol. Technol.* **2002**, 24, 349-353.

De Bruyne, T.; Pieters, L.; Deelstra, H., Vlietinck, Condensed vegetable tannins: Biodiversity in structure and biological activities. *Biochemical Systematics and Ecology.* **1999**, 27, 445-449

Defilippi, B.G.; Dandekar, A.M.; Kader, A.A. Impact of suppresion of ethylene action or biosynthesis on flavor metabolites in apple. *J. Agric. Food Chem.* **2004**, 52, 5694-5701.

Dewanto, V.; Wu, X.; Adom, K.K.; Liu, R.H. Thermal processing enhances the nutritional Value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, 50, 3010-3014.

Dixon, R.; Paiva, N. Stress-induced phenylpropanoid metabolism. *Plant Cell.* **1995.** 7, 1085-1097.

Dong, L.; Lurie, S.; Zhou, H. Effect of 1-methylcyclopropene on ripening of 'Canino' apricots and 'Royal Zee' plums. *Postharvest Biol. Technol.* **2002,** 24, 135-145.

Edmundo, M.P.; Elia, N.; Edmundo, M.S. Antioxidant capacity of guava fruit and jicama roots under chilling injury conditions. Annual IFT Meeting and Expo.2002.

Edwards, J.I.; Saltveit, M.E.; Henderson, W.R. Inhibition of lycopene synthesis in tomato pericarp tissue by inhibitors of ethylene biosynthesis and reversal with applied ethylene. *J. Amer. Soc. Hort. Sci.* **1983**, 108, 512-514.

El-Zoghbi, M. Biochemical changes in some tropical fruits during ripening. *Food Chem*. **1994**, 49, 33-37.

Environmental Protection Agency [EPA]. Biopesticide Federal Register Notices by Active Ingredient. 1-MCP. 2003. Available online: www.epa.gov Last accessed: April, 2005.

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

Fan, X.; Blankenship, S.; Mattheis, J. 1-Methylcyclopropene inhibits apple ripening. *J. Am. Soc. Hort. Sci.* **1999**, 124, 690-695.

Fennema, O.R. *Food Chemistry*, 3rd Ed.; Marcel Dekker: New York, NY, 1996.

Food and Agriculture Organization [FAO]. Commodity Market Review 2003-2004. Available online: http://www.fao.org/documents. Last accessed: August, 2004.

Giovanelli, G.; Lavelli, V.; Peri, C.; Simona, Nobili. Variation in antioxidant components of tomato during vine and post-harvest ripening. *J. Sci. Food Agric.* **1999**, 79, 1583-1588.

Goldstein, J.; Swain, T. Changes in tannins in ripening fruits. *Phytochemistry.* **1963,** 2, 371-383.

Gorinstein, S.; Zemser, M.; Haruenkit, R.; Chuthakorn, R.; Grauer, F.; Martin-Belloso, O.; Trakhtenberg, S. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *J. Nutr. Biochem*. **1999**, 10, 367-371.

Gould, W.; Sharp, J. Hot-water immersion quarantine treatment for guavas infested with Caribbean fruit fly (diptera: tephritidae). *J. of Economic Entomology*. **1992**, 85, 1235-1239.

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

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

Ho, C.; Lee, C.; Huang M. *Phenolic compounds in food and their effects on health I.*; American Chemical Society: Washington, DC, 1992.

Hofman, P.; Jobin-De cor, M.; Meiburg, G.; Macnish, A.; Joyce, D. Ripening and quality responses of avocado, custard apple, mango and papaya fruit to 1-methylclopropene. *Aust. J. Exp. Agric.* **2001**, 41, 567-572.

Huber, D.J. The role of cell wall hydrolases in fruit softening. *Hort. Rev.***1983,** 5,169-219.

Itoo, S.; Matsuo, T.; Ibushi, Y.; Tamari, N. Seasonal changes in the levels of polyphenols in guava fruit and leaves and some of their properties. *J. Japan. Soc. Hort. Sci.* **1987**, 56, 107-113.

Jacobi, K.; Giles, J. Quality of Kensington mango (*Mangifera indica* Linn.) fruit following combined vapour heat dissinfestation and hot water disease control treatments. *Postharvest Biol. Technol.* **1997**, 12, 285-292.

Jacobi, K.; MacRae, E.; Hetherington, S. Postharvest heat disinfestation treatments of mango fruit. *Scientia Horticulturae.* **2001,** 89, 171-193.

Janick, J. *Horticultural Reviews: Volume 30*; John Wiley and Sons: Hoboken, HJ, 2004.

Jeong, J.; Huber, D.J.; Sargent, S. Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (*Persea americana*) fruit. *Postharvest Biol. Technol.* **2002**, 25, 241-256.

Jiang, Y.; Joyce, D.; Terry, L. 1-methylcyclopropene treatment affects strawberry fruit decay. *Postharvest Biol. Technol.* **2001,** 23, 227-232.

Jiménez-Escrig, A.; Rincón, M.; Pulido, R.; Saura-Calixto, F. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *J. Agric. Food Chem*. **2001**, 49, 5489-5493.

JMP; SAS Institute, Inc., SAS Campus Drive, Cary, NC, 1996.

Ke, D.; Salveit, M.E. Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. *Plant Physiol.* **1988**, 1136-1140.

Kipe, S. The World Fresh Fruit Market. USDA Foreign Agricultural Service. Horticultural and Tropical Products Division. 2004. Available online: www.fas.usda.gov/htp/Presentations/2004 Last accessed: August, 2004.

Ko, W.; Kunimoto, R. Guava fruit firm rot induced by bruising. *Hort. Science*. **1980**, 15, 722-723.

Kondo,S.; Kittikorn, M.; Kanlayanarat, S. Preharvest antioxidant activities of tropical fruits and the effect of low temperature strage on antioxidants and jasmonates. *Postharvest Biol. Technol.* **2005**, In Press.

Lee, J.H.; Johnson, J.V.; Talcott, S.T. Identification of ellagic acid conjugates and other polyphenolics in mature muscadine grapes by HPLC-ESI-MS. **2005**, ASAP Article.

Leonardi, C.; Ambrosino, P.; Esposito, F.; Fogliano,V. Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *J. Agric. Food Chem.* **2000,** 48, 4723-4727.

Leong, L.; Shui, G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem.* **2002**, 76, 69-75.

Lin, C.H.; Chen, B.H. Determination of carotenoids in tomato juice by liquid chromatography. *J.of Chromatography*. **2003**, 1012, 103-109.

Liguori, G.; Weksler, A.; Zutahi, Y.; Lurie, S.; Kosto, I. Effect of 1-MCP on melting flesh peaches and nectarines. *Postharvest Biol. Technol*. **2004**, 31, 263-268.

Lohani, S.; Trivedi, P.; Nath, P. Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA, and IAA. *Postharvest Biol. Technol*. **2004**, 31, 119-126.

Lurie, S. Postharvest heat treatments. *Postharvest Biol. Technol.* **1998**, 14, 257-269.

Lurie, S.; Handros, A.; Fallek, E.; Shapira, R. Reversible inhibition of tomato gene expression at high temperature. *Plant Physiol*. **1996**, 110, 1207-1214.

Lurie, S.; Klein, J. Acquisition of low-temperature tolerance in tomatoes by exposure to high-temperature stress. *J. Amer. Soc. Hort. Sci.* **1991**, 116, 1007-1012.

Malo, S.E; Campbell,C.W. 1994.The Guava. University of Florida-IFAS extension. Bulletin. Available online: http://edis.ifas.ufl.edu/MG045. Last accessed: August, 2004.

Marcelin, O.; Williams, P.; Brillouet, J. Isolation and characterization of the two main cell-wall types from guava (*Psidium guajava* L.) pulp. *Carbohydr. Res.* **1993**, 240, 233-243.

Martinez-Valverde, I.; Periago, M.; Provan, G.; Chesson, A. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *J. Sci. Food Agric.* **2002**, 82, 323-330.

Mercadante, A.; Steck, A.; Pfander, H. Carotenoids from Guava (*Psidium guajava L.*): Isolation and Structure Elucidation. *J. Agric. Food Chem*. **1999**, 47, 145-151.

Mercado-Silva, E.; Benito-Bautista, P.; Garcia-Velasco, M. Fruit development, harvest index and ripening changes of guavas produced in central Mexico. *Postharvest Biol. Technol.* **1998**, 13, 143-150.

Miean, K.H.; Mohamed, S. Flavonoid (myricetin, quercetin, keampferol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem*. **2001**, 49, 3106-2112.

Mir, N.; Curell, E; Khan, N.; Whitaker, M.; Beaudry, R. Harvest maturity, storage temperature, and 1-MCP application frequency alters firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *J. Am. Soc. Hort. Sci.* **2001**, 126, 618-624.

Misra, K.; Seshadri, T. Chemical components of the fruits of *Psidium guajava*. *Phytochemistry.* **1967**, 7, 641-645.

Mitra, S. *Postharvest physiology and storage of tropical and subtropical fruits,* CAB International: New York, New York, 1997.

Morton, J.F. *The Guava. Fruits of warm climates*, Media Incorporated: Greensboro, NC, 1987.

Mostofi, Y.; Toivonen, P.; Lessani, H.; Babalar, M.; Lu, C. Effects of 1-methylcyclopropene on ripening of greenhouse tomatoes at three storage temperatures. *Postharvest Biol. Technol.* **2003**, 27, 285-292.

Mowlah, G.; Itoo, S. Quantitative changes in guava polyphenols and the polyphenoloxidase (PPO) at different stages of maturation, ripening, and storage. *Nippon Kogyo Gakkaishi.* **1982**, 29, 413-417.

Mueller-Harvey, I. Analysis of hydrolysable tannins. *Anim. Feed Sci. Technol.* **2001**, 91, 3-20.

Mullins, E.D.; McCollum, T.G.; McDonald, R.E. Consequences of ethylene metabolism of inactivating ethylene receptor sites in diseased non-climacteric fruit. *Postharvest Biol. Technol.* **2000**, 19, 155-164.

National Agriculture Statistics Service [NASS]. 2004. Guava output falls for the third straight year.Bulletin: Hawaii Guavas. Available online: www.**nass**.usda.gov. Last accessed: February, 2005.

Noogle, G. and Fritz, G. *Introductory plant physiology* 2nd Edition, Prentice Hall Inc.: Englewood Cliffs, New Jersey, 1983.

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

Padula, M.; Rodriguez-Amaya, D. Characterisation of the carotenoids and assesment of the vitamin A value of Brasilian guavas (Psidium guajava L). *Food Chem.* **1986**, 20, 11-19.

Paull, R. Response of tropical horticultural commodities to insect desinfestation treatments. *Hort. Sci.* **1994**, 29, 988-996.

Paull, R.; Chen, N. Heat treatment and fruit ripening. *Postharvest Biol. Technol.* **2000**, 21-37.

Rao, A.; Agarwal, S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. *Nutr. Res.* **1999**, 19, 305-323.

Regalado-Contreras, E.; Mercado-Silva, E. Effect of hot water treatment on the ascorbic acid and reduced glutathione levels in guava fruit during cold storage. Annual IFT Meeting and Expo. 1998.

Reyes, M.U.; Paull, R.E. Effect of storage temperature and ethylene treatment on guava (Psidium guajava L.) fruit ripening. *Postharvest Biol. Technol*. **1995**, 6, 357-365.

Rice-Evans, C.; Miller, N.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1995**, 20, 933-956.

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

Ronen, G.; Cohen, M.; Zamir, D.; Hirschberg, J. Regulation of carotenoid biosynthesis during tomato fruit development expression of the gene for lycopene epsilon-cyclase is down regulated during ripening and is elevated in the mutant Delta. *Plant Journal* **1999**, 17, 341-351.

Sahlin, E; Savage; G.P.; Lister, C.E. Investigation of the antioxidant properties of tomatoes after processing. *Journal of Food Composition and Analysis*. **2004**, 17, 635-647.

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

Salveit, M. Effect of 1-methylcyclopropene on phenylpropanoid metabolism, the accumulation of phenolics compounds, and browning of whole and fresh-cut ‘iceberg’ lettuce. *Postharvest Biol. Technol.* **2004**, 34, 75-80.

Selvarajah, S.; Bauchot, A.D.; and John, P. Internal browning in cold-stored pineapples is suppressed by a postharvest application of 1-methylcyclopropene. *Postharvest Biol. Technol.* **2001**, 23: 167–170

Seybold, C.; Frohlich, K.; Bitsch, R.; Otto, Konrad; Bohm, V. Changes in contents of carotenoids and vitamin E during tomato processing. *J. Agric. Food Chem*. **2004**, 52, 7005-7010.

Shahidi, F.; Wanasundara, P. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, 32, 67-103.

Sisler, E.C., Serek, M. Inhibitors of ethylene responses in plants at a receptor level. Recent developments. *Physiol. Plant.* **1997,** 100, 577-582.

Skerget, M.; Kotnik, P.; Hadolin, M.; Rizner, A.; Marjana, S.; Zeljko, K. Phenols, proanthocyanidins, flavones, and flavonols in some plant materials and their antioxidant activities. *Food Chem.* **2005**, 89, 191-198.

Smirnoff, N.; Wheeler, G. Ascorbic acid in plants: Biosynthesis and function. Critical Reviews in Plant Sciences, **2000**, 19, 267-290.

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

Takeoka, G. R.; Dao, L.; Flessa, S.; Gillespie, D. M.; Jewell, W. T.; Huebner, B.; Bertow,D.; Edeler, SE. Processing effects on lycopene content and antioxidant activity oftomatoes. *J. Agric. Food Chem.* **2001**, 49, 3713-3717.

Talcott, S.T., Howard, L, 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., Percival, S.S., Pittet-Moore, J. Celoria, C. Phytochemical composition and antioxidant stability of fortified yellow passion fruit (*Passiflora edulis*). *J. Agric. Food Chem*. **2003**, *48*, 4, 1315-1321

Thimann, K. *Senescence in plants*, CRC Press: Boca Raton, FL, 1980.

Thompson, K.; Marshall, M.; Sims, C.; Sargent, S.; Scott, J. Cultivar, maturity, and heat treatment on lycopen content in tomatoes. *J. Food Sci.* **2000**, 65, 791-793.

Tomas-Barberan, F.A.; Loaiza-Velarde, J.; Bonfanti, A.; Saltveit, M.E. Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. *J. Am. Soc. Hortic. Sci.* **1997**, 122, 399-404.

United States Department of Agriculture [USDA]. Available online: http://www.usda.gov. Last accessed: August, 2004.

United States Department of Agriculture-Animal and Plant Health Inspection Service [USDA-APHIS]. 2004. Available online: http://www.aphis.usda.gov. Last accessed: March, 2005.

Van de Berg, H.; Faulks, R.; Granado, H. F.; Hirschberg, J.; Olmedilla, B.; Sandmann,G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J. Sci. Food. Agric.* **2000**, 80, 880-912.

Valero, D.; Martinez-Romero, J.M.; Guillen, F.; Castillo, S.; Serrano, M. Could 1-MCP treatment effectiveness in plum be affected by packaging? *Postharvest Biol. Technol.* **2004**, 295-303.

VERIS Research Information Service. **2000.** Carotenoid fact book. VERIS, La Grange, IL.

Wilberg, V.; Rodriguez-Amaya, D. HPLC quantitation of major carotenoids of fresh and processed guava, mango, and papaya. *Lebensm-Wiss. U. Technol*. **1995,** 28, 478-480.

Wilson, C.; Shaw, P.; Campbell, C. Determination of organic acids and sugars in guava(*Psidium guajava* L.) cultivars by high-performance liquid chromatography. *J. Sci. Food Agric.* **1982**, 33, 777-780.

Xie, D.; Dixon, R. Proanthocyanidin biosynthesis-still more questions than answers? *Phytochemistry.* In Press.

Yadava, U. Guava production in Georgia under cold-protection structure. In: J. Janick (ed.) *Progress in new crops*; ASHS Press: Arlington, VA, 1996.

BIOGRAPHICAL SKETCH

Flor de Maria Nunez Rueda was born on January 4, 1981, and raised in Tegucigalpa, Honduras, Central America. After graduating from high school, she entered the Escuela Agricola Panamericana (University of Zamorano), earning her Bachelor of Science in agroindustry in December 2003. During spring 2003 she performed an internship with Dr. Steve Talcott at the Food Science and Human Nutrition Department at University of Florida and was offered an assistantship to pursue her graduate studies. She earned a Master of Science in food science and human nutrition at the University of Florida in August 2005.