

1-METHYLCYCLOPROPENE TREATMENT EFFICACY IN PREVENTING  
ETHYLENE PERCEPTION AND RIPENING IN INTACT AND FRESH-CUT  
'GALIA' MELON AND 'SUNRISE SOLO' PAPAYA FRUITS

By

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The objectives of this study were to determine the physiological responses of intact and fresh-cut melon (*Cucumis melo* L. var. *reticulatus* L. Naud. cv. Galia) and papaya (*Carica papaya*, L. var. Sunrise Solo) fruits in which ethylene perception was blocked through application of 1-methylcyclopropene (1-MCP). The whole fruits were treated with 1-MCP (melon, 1 or 1.5  $\mu\text{L L}^{-1}$ ; papaya, 2.5 or 9  $\mu\text{L L}^{-1}$ ) for 24 h at 20 °C and stored at 20 °C or processed and stored at 5 °C.

Inhibition of ethylene perception via application of 1-MCP delayed the onset of the respiratory and ethylene climacterics and reduced maximum respiration and ethylene production rates of 'Galia' fruit ripened at 20 °C. Softening of intact and fresh-cut 'Galia' melon was significantly reduced by 1-MCP, regardless of application time (ripening stage). Yellowing of the fruit surface during ripening was strongly and somewhat irreversibly delayed in 1-MCP-treated 'Galia'.

Papaya fruit ('Sunrise Solo') treated with 1-MCP exhibited delayed initiation of the respiratory and ethylene climacterics and suppressed ethylene production during ripening. Softening was significantly delayed in fresh-cut and intact fruit. Papaya treated with 1-MCP retained higher levels of titratable acidity compared with non-1-MCP-treated fruit throughout ripening. The color change of the fruit surface from green to yellow was significantly but temporarily inhibited in 1-MCP-treated fruit.

Cell wall modification was studied in intact and fresh-cut ripe papaya fruit stored for 10 days at 5 °C. Water-soluble polyuronides represented the major pectic fraction followed by the CDTA (1,2 cyclohexylenedinitrilotetraacetic acid)- and Na<sub>2</sub>CO<sub>3</sub>-soluble fractions irrespective of 1-MCP. Both hemicellulosic and pectic polysaccharides in intact and fresh-cut fruit showed some changes but this trend was slightly or not affected by 1-MCP. Neutral sugars from pectins and hemicelluloses including galactose, glucose and xylose decreased in both intact and fresh-cut fruit regardless of 1-MCP. Generally, either intact fruit or fresh-cut fruit pre-treated with 1-MCP exhibited little or no significant changes compared with fruit not treated with 1-MCP.

The studies presented herein have shown that 1-MCP has potential for extending the useful storage life of intact and fresh-cut melon and papaya fruits by delaying ethylene inducible ripening process.

## CHAPTER 1 INTRODUCTION

'Galia' (*Cucumis melo* L. var. *reticulatus* L. Naud. cv. Galia) fruit has excellent flavor and aroma characteristics; however, storage life is limited to 2-3 weeks even at low temperatures. The storage life of papaya (*Carica papaya* L.) fruit is also short due to its inherently high respiration rate, delicate skin, and high water content. Papaya fruit can be harvested at the mature green stage (10% to 20% yellow skin), however, and maintained at 10 to 12 °C for periods of up to 2 weeks. Approaches restricting ethylene synthesis, such as controlled atmosphere, have proven that storage life of melon and papaya fruits can be extended. Another, a more facile approach to extending the storage life and quality of harvested melon and papaya has been through the application of 1-methylcyclopropene (1-MCP), a potent anti-ethylene compound. 1-MCP is very volatile and antagonism ethylene effectively in the range of parts per billion (ppb) to parts per million (ppm). Treated fruit do not contain residue of 1-MCP. 1-MCP has been reported to delay or reduce ethylene-inducible effects on a variety of fruits. The investigation of the efficacy of 1-MCP to maintain quality of both the melon and papaya fruits will provide information value to designing and exploring new postharvest applications that contribute to reduce postharvest losses during market preparation, storage, transport, or at the wholesale, retail, or consumer level.

Fresh-cut produce is one of the new food-processing methods that is increasing in popularity. Featuring fresh-like quality in a ready to use package, fresh-cut produce has become very popular. Fresh-cut produce, however, can easily become unacceptable in a

few days because its tissues no longer retain their protective epidermal and cuticular layers, and become ruptured and damaged. Wounding associated with the preparation fresh-cut produce is responsible for rapid losses in appearance, aroma, flavor, firmness/texture, and resistance to microbial degradation. The knowledge of fresh-cut processing on either a theoretical or practical basis is quite limited since many fruits and vegetables have not been explored as fresh-cut commodities. Tropical fruits are high-priority commodities to be explored as fresh-cut produce due to their perishable characteristics and sensitivity low temperatures. For example, papaya is very susceptible to mechanical damage, pest attacks and diseases, has a storage life of less than one week under ambient tropical conditions.

Melon (*Cucumis melo* L. var. *inodorus* and *reticulatus* L. Naud.) is one of the most popular fresh-cut products; however, information about its behavior and characteristics after fresh-cut processing is limited to observations on discoloration, off-odor development, and water soaking. The behavior of fresh-cut fruits generally parallels that of wounded tissues and is affected by type of tissue, stage of maturity, extent of wounding, temperature, oxygen, ethylene, carbon dioxide, water vapor pressure, and microorganisms. The immediate response to wounding is cell rupture and loss of tissue integrity due to water loss and mix of sequestered enzymes and substrates, followed by physiological and biochemical changes including accelerated ethylene production. Ethylene, a stress-related hormone, may influence the events leading to texture loss and deterioration in fresh-cut fruits. Therefore, inhibition of ethylene synthesis or action may enhance the storage life of fresh-cut produce and also help to understand the



physiological changes that distinguish the behavior of fresh-cut fruit as compared with these events as they occur in ripening, intact fruit.

The primary objectives of the work reported herein were to examine the potential use of ethylene-perception inhibition for control of ripening in pre-ripe and ripe melon and papaya fruits, and to evaluate the effects of ethylene action on the postharvest qualities of either intact or fresh-cut melon and papaya fruits by measuring several physiological and biochemical properties. Ethylene action was manipulated through use of 1-methylcyclopropene, a strong and persistent inhibitor of ethylene action.

## CHAPTER 2 LITERATURE REVIEW

### Melon

#### Introduction

The melon (*Cucumis melo* L. var. *inodorus* and *reticulatus* L. Naud. ) plant, a member of the *Cucurbitaceae*, is thought to have originated from the tropical regions of Africa and the Middle East (Seymour and McGlasson, 1993). 'Galia' (*Cucumis melo* L. var. *reticulatus* L. Naud. cv. Galia) was bred on the basis of the green-flesh qualities of 'Ha'Ogen', introduced from Hungary to Israel, at the Newe Ya'ar research center in the mid-60's (Karchi, 2000). The melon plant is generally monoecious but occasionally andromonoecious (McGlasson and Pratt, 1963). *Cucumis melo* specie are classified into two main groups including *reticulatus*, the netted or muskmelon fruit types, and *inodorus*, the smooth-skinned or honeydew fruit types (Seymour and McGlasson, 1993). Melon fruit is categorized as an inferior berry whose edible flesh is derived from the placentae or mesocarp (Seymour and McGlasson, 1993). Melon fruits show high variation in flesh color (from green to orange), skin color (from white or green to orange or gray), skin texture (from smooth to netted), and size (Seymour and McGlasson, 1993). Some common melon types marketed in the United States are Western U.S., Eastern U.S., Charentais, LSL, Galia, Ananas, Honeydew, and Casaba (Zheng and Wolf, 2000).

#### Harvest Maturity

The abscission properties are the most useful criteria for estimating harvest maturity in muskmelon types whereas the abscission layer does not develop in

honeydew-type melons until they are over-ripe (Pratt et al., 1977). Therefore, external color (green to white), peel texture (hairy to smooth), aroma, fruit density (low to high) and soluble solids are also used to verify harvest maturity of melon fruits (Portela and Cantwell, 1998). Melon fruit should have a minimum of 10% soluble solids concentration before harvest (Bianco and Pratt, 1977). Skin color of 'Galia' fruit can be used as a maturation index. Fallik et al. (2001) categorized 6 maturity indices for 'Galia' based on skin color: (1) dark green, (2) green (3) light yellow with green, (4) light yellow, (5) yellow, (6) dark yellow to orange).

### **Ripening Process**

**Ethylene.** Muskmelon fruit types abscise at or near the climacteric (Altman and Corey, 1987) whereas honeydew types abscise after completion of the respiratory climacteric (Pratt et al., 1977). Exogenous ethylene treatment induces ripening in netted melons depending on maturity, temperature, treatment duration, and ethylene concentration (Seymour and McGlasson, 1993). Exogenous ethylene may be applied to non-netted types to achieve uniform ripening of fruit that have sufficient soluble solids content (Seymour and McGlasson, 1993). Since melons have no polymeric carbohydrate reserves such as starch, postharvest ethylene treatments do not enhance soluble solids content in harvested fruit of any maturity class (Bianco and Pratt, 1977). Orange-fleshed melon fruit (mostly netted types) produce higher ethylene levels than green- or white-flesh types (Zheng and Wolf, 2000). Melon fruit with a netted rind have higher ethylene production than do smooth types (Zheng and Wolf, 2000).

**Carbohydrates.** Both in netted and honeydew type melons, sugar accumulation reaches its maximum after full maturity (Seymour and McGlasson, 1993). Soluble solids

in melon fruits may accumulate to values as high as 17% (Bianco and Pratt, 1977), with sucrose and fructose comprising the most prevalent sugars (Hubbard et al., 1990).

**Structural polysaccharides.** An increase in soluble pectin, a decrease in pectin size, loss of galactosyl residues, and changes in size of hemicelluloses represent the most evident features of cell wall changes during ripening of melon fruits (Gross and Sams, 1984; McCollum et al., 1989). Galactosidases, pectin esterase, and cellulase are thought to be responsible for cell wall degradation (Gross and Sams, 1984; Lester and Dunlap, 1985; McCollum et al., 1989).

**Organic acids.** Citric and malic acids are the major organic acids in most melon fruits (Leach et al., 1989; Flores et al., 2001). Artes et al. (1999) reported that titratable acidity (% citric acid equivalents) ranged from approximately 0.50 ('Galia') to 0.14 ('Tendral') in 4 different varieties of muskmelons.

**Pigments.** In orange-fleshed muskmelons, the following pigments have been found:  $\beta$ -carotene (84.7 %),  $\delta$ -carotene (6.8 %),  $\alpha$ -carotene (1.2 %), phytoene (1.5 %), lutein (1 %), violaxanthin (0.9 %), and traces of other carotenoids (Seymour and McGlasson, 1993). During muskmelon ripening, pigment accumulation initiates in the placenta, progressing outward through the mesocarp (Reid et al., 1970). In green-fleshed types such as 'Galia', however, the carotenoid content in exocarp and mesocarp does not change significantly during ripening (Flugel and Gross, 1982).

**Volatiles.** The volatile ester profiles of ripe muskmelon and honeydew type melons are very similar except for ethyl butyrate, which is more abundant in muskmelons (Yabumoto et al., 1978). The following volatiles have been reported to be representative of muskmelons: ethyl-2-methyl butyrate, ethyl butyrate, hexanoate, hexyl acetate, 3-

methyl butyl acetate, benzyl acetate, (Z)-6-nonenyl acetate, (E)-6-nonenol, (Z,Z)-3, 6-nonadienol and (Z)-6-nonenal (Yabumoto et al., 1978; Wyllie and Leach, 1990). Butyl acetate (5), 2-methyl-butyl acetate (6), and hexyl acetate (9) are the most abundant volatiles in 'Galia' type melons (Fallik et al., 2001).

### **Postharvest Storage of Melon Fruit**

Melon fruit are typically stored at 7 to 10 °C whereas storage below 7 °C may cause chilling injury, especially for honeydew types (Lipton and Aharoni, 1979; Hardenburg et al., 1986). Controlled atmosphere conditions of 3% O<sub>2</sub> and 10% CO<sub>2</sub> at 7 °C, and 2% O<sub>2</sub> and 10% CO<sub>2</sub> at 3 °C have been shown to delay ripening and extend storage life of honeydew melon fruit (Hardenburg et al., 1986). Portela and Cantwell (1998) reported that fresh-cut honeydew and muskmelon fruit stored at 5 °C for 12 days maintained their appearance and color but exhibited a 50% decline in firmness. O'Connor-Shaw et al. (1994) reported that the storage life of fresh-cut honeydew-type melons was limited to 14 days while the storage life of fresh-cut muskmelon types was 4 days at 4 °C.

### **Postharvest Diseases**

Sour rot (*Galactomyces geotrichum* (Butl. & Peter) Redh. & Mall.; *Geotrichum candidum* Lk.), Rhizopus rot (*Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill.), Fusarium rot (*Fusarium* spp.), Trichothecium rot (*Trichothecium roeum* (Pers.:Fr.) Link), Botrytis rot (*Botryotinia fuckelina* (de Barry) Whetzel; *Botrytis cinerea* Pers.:Fr.), Lasiodiplodia rot (*Botryosphaeria rhodina* (Cooke) Arx; *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl.), and anthracnose (*Glomerella lagenarium* F. Stevens.; *Colletotrichum orbiculare* (Berk. & Mont.) Arx; *Gloeosporium orbiculare* Berk. & Mont; *Colletotrichum lagenarium* (Pass.) Ellis & Halst; *Gloeosporium lagenarium* (Pass.) Sacc.) are the most common postharvest diseases in melon fruit (Sommer et al., 1992). Netted types are very

susceptible to fungal diseases since organisms may locate easily in the skin net and enter the fruit through mechanical breaks or abrasions (Seymour and McGlasson, 1993).

Wounds and fresh stem-scars are likely sources of ingress for sour rot (*Galactomyces geotrichum* (Butl. & Peter) Redh. & Mall.; *Geotrichum candidum* Lk.; Sommer et al., 1992). The fungus is easily spread by wind, rain, and vinegar flies (*Drosophila* spp.; Sommer et al., 1992). Rhizopus (*Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill.) colonizes fruits via mechanical injuries and stem scars very rapidly at high temperatures (Sommer et al., 1992). Fusarium (*Fusarium* spp.) frequently originates from soil and becomes active when fruit ripen. Disease progression of Fusarium is typically rather slow and fruit losses do not become excessive (Sommer et al., 1992). Spores of anthracnose (*Colletotrichum orbiculare* (Berk. & Mont.) Arx; *Gloeosporium orbiculare* Berk. & Mont; *Colletotrichum lagenarium* (Pass.) Ellis & Halst; *Gloeosporium lagenarium* (Pass.) Sacc.) are distributed by water, wind, insects, or handling. As fruit mature and ripen, latent anthracnose becomes active and causes sunken and black lesions (Sommer et al., 1992)

### **Postharvest Disease Control**

Maintaining good physiological condition of the melon plant during growth and proper handling of fruit during harvesting, transportation and storage are essential in preventing or controlling postharvest deterioration and decay (Qi et al., 1988; Teitel et al., 1989; Aharoni et al., 1993; Seymour and McGlasson, 1993; O'Connor-Shaw et al., 1996; Fallik et al., 2000). Immersing fully ripe 'Galia' fruit in hot water (52 °C) for 2 min may provide antifungal protection for a limited period (Teitel et al., 1989). A combined treatment of a hot water rinse and brushing can improve the general appearance and maintain quality of melon fruit as well as also reduce postharvest decay. According to

Ayhan et al. (1998), 200 ppm free chlorine performed well in reducing microbial growth in intact and fresh-cut muskmelon fruit. Honeydew melons (intact or fresh-cut) may also be dipped in 150-ppm chlorinated water for 5 min to control decay development (Qi et al., 1988).

## Papaya

### Introduction

Papaya (*Carica papaya* L.) is an herbaceous plant and a member of the family *Caricaceae*. The plant is limited to the region within a latitudinal range of 32°N and 32°S (Morton, 1987). The plant may have female, male or hermaphroditic flowers (Nakasone, 1986; Morton, 1987). The flower type determines the final size and shape of the fruit. Fruit from bisexual flowers are usually pyriform in shape with a small seed cavity and thick wall of firm flesh (mesocarp). On the other hand, fruit from female plants are nearly round or oval, and of relatively thin flesh (Morton, 1987). Fruit shape is usually spherical to oblong and fruit are generally composed of five longitudinal carpels united around a large central cavity wherein seeds are attached to placental tissue by 0.5-10 cm stalks (Morton, 1987). Fruit with less than five carpels are long and cylindrical, resembling cucumber fruit in shape (Nakasone, 1986). Papaya fruit range from 15 to 50 cm in length with a diameter of 10 to 20 cm (Morton, 1987), and fruit weight ranges from 30 g to 9 kg, depending on the cultivars (Nakasone, 1986). The peel is thin, usually smooth and green when immature but fairly tough, and yellow to orange when ripe. A slight injury can induce milky latex containing the proteolytic enzyme, papain (EC 3.4.22.2), to exude (Sankat and Maharaj, 1997). Flesh (mesocarp) thickness varies from 1.5 to 5 cm (Nakasone, 1986; Sankat and Maharaj, 1997). During ripening, the flesh becomes aromatic, yellow to orange or reddish-yellow, juicy, sweetish, and melon-like in flavor

(Morton, 1987; Sankat and Maharaj, 1997). Seeds are generally dark gray or black, covered with a transparent gelatinous aril, and have high oil and protein contents (Sankat and Maharaj, 1997).

Some of the most cultivated varieties of papaya include 'Solo', 'Sunrise Solo', 'Maradol', 'Waimanalo', 'Higgins', 'Wilder', 'Hartus Gold', 'Bettina', 'Peterson', 'Singapore Pink', and 'Cariflora' (Morton, 1987).

### **Uses of Papaya Fruit**

Ripe papaya fruit are generally consumed fresh; however, processed papaya fruit products, such as nectar and juice, are competing against fresh papaya fruit. Fruit may also be added to ice creams, sauces, used in cooked desserts, or pickled or preserved as jam (Nakasone, 1986). Green papaya can be boiled and served as a vegetable and canned in sugar syrup (Morton, 1987). Papaya fruit is a good source of vitamins A and B and an excellent source of vitamin C (Sankat and Maharaj, 1987). Carotene, thiamine, riboflavin, niacin, tryptophan, methionine and lysine are usual constituents of papaya fruit (Kimura et al., 1991). Papaya latex contains two proteolytic enzymes, papain and chymopapain (Paull, 1993). Papain is used to tenderize meat, to clarify beer, and to treat wool and silk before dyeing. Moreover, papain is used in toothpastes, cosmetics and detergents, as well as in digestion aids (Morton, 1987), and has been used to treat ulcers and to reduce swelling and fever (Morton, 1987). In India, latex from papaya fruit or seed is applied to the uterus as an irritant to induce abortion (Morton, 1987). Young leaves are cooked and consumed like spinach in India (Nakasone, 1986).

### **Papaya Harvest Maturity**

Papaya development from pollination to full ripeness requires approximately 5.5 (Hawaii) to 10.5 (Africa) months (Nakasone, 1986). Color break, sugar composition



(decline in sucrose, increase in glucose and fructose), and soluble solids concentration are the most useful maturity indices (Nakasone, 1986; Paull, 1993; Sankat and Maharaj, 1997). Nondestructive methods including reflectance, delayed light emission, and body transmission spectroscopy have been used to measure papaya maturity (Sankat and Maharaj, 1997). For local markets, fruit may be harvested when the skin color reaches 80% of yellow. Otherwise, fruit destined for storage or long-distance transportation are picked at the mature-green stage. The fruit must be handled properly in order to avoid injuries causing leakage of latex, which stains the fruit and reduces consumer acceptance (Morton, 1987). The latex from the peel may irritate the skin of fruit handlers; therefore, protective measures should be taken during prolonged physical contact with papaya (Morton, 1987).

### **Papaya Ripening**

The optimum temperature for papaya ripening is between 22.5 and 27.5°C (Paull, 1993). Papaya is a climacteric fruit, and the increase in ethylene production parallels respiration rate, reaching a maximum 1-2 days after (Wills and Widjanarko, 1995) or simultaneously with (Paull, 1993) the respiratory maximum. Respiration and ethylene production of mature green papaya 'Solo' fruit are below 5 mL kg<sup>-1</sup> h<sup>-1</sup> and 1 µL kg<sup>-1</sup> h<sup>-1</sup>, respectively; however, respiration and ethylene production increase to approximately 45 mL kg<sup>-1</sup> h<sup>-1</sup> and 7 µL kg<sup>-1</sup> h<sup>-1</sup>, respectively, during ripening at 22 °C (Paull and Chen, 1983).

**Soluble carbohydrates.** The principal soluble carbohydrates in papaya fruit are sucrose, glucose and fructose, with sucrose being the predominant sugar at the full ripe stage (Chan, 1979; Selvaraj and Pal, 1982). Invertase (EC 3.2.2.26) activity increases

during ripening, presumably causing the conversion of sucrose to fructose and glucose (Selvaraj and Pal, 1982). Papaya fruit contains low levels of starch (Selvaraj et al., 1982).

**Structural polysaccharides and textural changes.** Softening of papaya fruit is associated with a dramatic increase in the solubility of cell wall pectins (Paull, 1993; Lazan et al., 1995). Pectin depolymerization is also observed, occurring first in the inner mesocarp tissues (Sankat and Maharaj, 1997). Cellulase (EC 3.2.1.5), pectin methyl esterase (EC 3.1.111), xylanase (EC 3.2.1.8), polygalacturonase (EC 3.2.1.15; PG; highest in inner mesocarp) and  $\beta$ -galactosidase (EC 3.2.1.23; highest in outer mesocarp) activities have been reported to increase during papaya ripening (Paull and Chen, 1983; Ali et al., 1999).

**Organic acids.** Citrate and malate are the predominant organic acids in papaya, but tartaric, fumaric and succinic acids have also been noted (Selvaraj et al., 1982). The concentration of total and nonvolatile acids decreases during fruit development, reaching a minimum  $1.54 \text{ mEq } 100\text{g}^{-1}$  (fresh weight) with a pH in the range from 5.0-5.5 at the full-ripe stage (Paull, 1993). Ascorbic acid increases nearly 4-fold, reaching levels of  $5.5\text{-mg}\cdot 100^{-1}$  (fresh weight) during ripening (Paull, 1993). Compared with other fruit, total titratable acidity remains low during ripening, which may contribute to the sweet taste of papaya (Selvaraj et al., 1982). Non-volatile organic acids comprise 75% to 92% of total acidity (Selvaraj et al., 1982).

**Pigments.** Total carotenoid content of mesocarp increases up to 14-fold during papaya ripening, with levels ranging from  $0.28 \text{ mg } 100^{-1}$  dry pulp at the mature-green stage to nearly  $4 \text{ mg } 100^{-1}$  dry pulp when full ripe (Selvaraj et al., 1982).  $\beta$ -carotene (62%) is the predominant carotenoid in yellow-flesh cultivars whereas lycopene is the

major carotenoid in red-fleshed cultivars (Selvaraj et al, 1982). Lycopene constitutes 61% of the total carotenoid content of the red-fleshed 'Solo' papaya (Kimura et al., 1991).  $\beta$ -cryptoxanthin,  $\beta$ -zeacarotene, and cryptoflavin are found in minor quantities in papaya fruit (Kimura et al., 1991).

**Proteins and amino acids.** Several proteases, papain, chymopapain A and B (EC 3.4.22.6), and papaya peptidase (EC 3.4.22.30), are found in papaya latex (Paull, 1993). Total protease activities in papaya mesocarp tissue decline during ripening (Paull, 1993). At least 13 free amino acids have been identified in papaya fruit (Selvaraj et al., 1982; Morton, 1987).

**Volatiles.** At least 199 volatiles have been identified in papaya fruit, with linalool being the most abundant (MacLeod and Pieris, 1983; Flath et al., 1990). Volatiles in the cultivar 'Solo' are comprised of up to 94% linalool followed, in declining abundance, by benzyl isothiocyanate, methyl butanoate and methyl benzoate (Flath et al., 1990). Only one volatile, methyl benzoate, is described as having papaya qualities (Paull, 1993).

### **Postharvest Handling and Storage of Papaya**

Papaya fruit have a maximum storage life of 7 days under ambient tropical conditions (30 °C); temperatures above 32.5 °C cause abnormal ripening (An and Paull, 1990). Storage between 12 to 16 °C appears to represent the most compatible temperature range for storage. Storage below 10 to 12 °C may cause chilling injury, depending upon the maturity stage (Chen and Paull, 1986). 'Solo' fruit stored at 25 °C and 30 °C had higher total carotene and ascorbic acid, lower benzyl isothiocyanate (bitterness compound), more intense yellow peel color, and more acceptable eating attributes compared with fruit held at 20 °C (Wills and Widjanarko, 1995). This may have been the result, however, of more advanced ripening of the fruit held at the higher temperature.

Maharaj and Sankat (1990) reported that the best atmospheric conditions for maintaining acceptability and market quality of papaya fruit during storage were 1.5 to 2% O<sub>2</sub> and 5% CO<sub>2</sub> at 26 °C. Plastic film wraps are more effective than waxes and other coatings in reducing water loss (Maharaj and Sankat, 1990).

### **Chilling Injury**

Chilling injury is a major physiological disorder induced by low non-freezing temperatures (Chen and Paull, 1986; Chan, 1988). Chen and Paull (1986) reported that mature-green 'Solo' papaya stored at 7.5 °C showed chilling injury symptoms after 20 days. Chilling injury symptoms of papaya fruit include epidermal discoloration of the mesocarp, development of hard areas in the flesh and around vascular bundles, enhanced mesocarp water soaking and electrolyte leakage, increased ethylene production, and increased susceptibility to decay (Chen and Paull, 1986).

### **Postharvest Pathology**

Postharvest diseases are very important in reducing market quality of papaya fruit and they are primarily responsible for the losses that occur during shipment. In Hawaii, postharvest losses of papaya fruit due to diseases extended up to 93% before 1987 depending on postharvest handling and packing procedures (Alvarez and Nishijima, 1987). Diseases are of three general types: fruit surface rots, stem-end rots, and internal infections (Alvarez and Nishijima, 1987).

**Fruit surface rots.** Anthracnose (*Glomerella cingulata* (Stonem.) Spauld. & Schr.; *Colletotrichum gloeosporioides* (Penz.) Arx), chocolate spot (*Colletotrichum gloeosporioides* spp.), dry rot (*Mycosphaerella* spp.), wet rot (*Phomopsis caricae-papayae* Petr. & Cif.), Alternaria fruit spot (*Alternaria alternata* (Fr.) Keissler), Fusarium rot (*Fusarium solanifer* Snyder & Hans.) and Guignardia spot (*Guignardia* spp.) are the

most common fruit surface pathogens found in papaya fruit (Alvarez and Nishijima, 1987; Sommer et al., 1992). Anthracnose is the major postharvest disease of papaya, and the symptoms of anthracnose (causing initially tiny, brown, superficial, water-soaked lesions that may enlarge to 2.5 cm or more in diameter) are most prominent at the full-ripe stage (Alvarez and Nishijima, 1987). Chocolate spot is a surface disease and causes reddish brown lesions on the skin (Sommer et al., 1992). As fruit ripen, lesions of chocolate spot become sunken, displaying water-soaked margins (Nakasone, 1986; Sommer et al., 1992). Wet rot (*Phomopsis caricae-papayae* Petr. & Cif.) generates soft and translucent areas on the fruit surface (Alvarez and Nishijima, 1987). Circular or oval black lesions are the symptoms of *Alternaria* fruit spot (Alvarez and Nishijima, 1987). Infections by *Fusarium solanifer* produce small dry lesions with water soaked areas (Alvarez and Nishijima, 1987). Guignardia spot, evident as greenish-black lesions, is often seen when papaya are pretreated in water at 42 °C for at least 40 minutes (Alvarez and Nishijima, 1987).

**Stem end rots.** *Lasiodiplodia* rot (*Botryosphaeria rhodina* (Cooke) Arx; *Lasiodiplodia theobromae* (Pat.) Griffin & Maulb.), *Phytophthora* rot (*Phytophthora nicotianae* Breda de Haan var. *parasitica* (Dast.) Waterh.), and *Rhizopus* (*Rhizopus stolonifer* (Her. Ex Fr.) Lind.) are among the most widespread stem end rots reported in papaya fruit (Alvarez and Nishijima, 1987; Sommer et al., 1992). *Lasiodiplodia* rot usually occurs at injuries to fruit skin or near fruit peduncle (Sommer et al., 1992). *Rhizopus* fungus invades through wounds and colonizes the entire fruit rapidly, often spreading to other fruits (Alvarez and Nishijima, 1987).

**Internal fruit infections.** Purple-stain (*Erwinia herbicola* (Loehnis) dye) and internal yellowing (*Enterobacter cloacae* (Jordan) Hormaeche & Edwards) are the two most reported internal diseases in papaya fruit (Alvarez and Nishijima, 1987). The tissue invaded by *Erwinia herbicola* becomes translucent and later rots, resulting in extensive off-odors (Alvarez and Nishijima, 1987). Fruit flesh infected by *Enterobacter cloacae* is translucent with a bright yellow to lime-green discoloration (Alvarez and Nishijima, 1987).

**Postharvest disease control.** Since many infections affecting papaya during postharvest handling become established in the field, postharvest control measures begin with choosing resistant varieties and implementing good cultural practices during fruit growth (Nakasone, 1986). After harvest, proper temperature measurement during transportation and marketing, the use of vapor/hot water treatments, and dipping in aurefungin and carnauba waxing are some of the control measures effective in controlling disease progression (Nakasone, 1986; Alvarez and Nashijma, 1987; Sommer et al., 1992; Sankat and Maharaj, 1997). Hot water treatment at 43 °C to 49 °C for 20 minutes has been reported to prevent/control postharvest decays (Akamine and Arisumi, 1953).

## **1-Methylcyclopropene**

### **Introduction**

The promotion of plant senescence by ethylene can be inhibited by a number of cyclic olefins including cyclopropene, 1-methylcyclopropene (1-MCP), 3-methylcyclopropene, 1,3-dimethylcyclopropene, 3,3-dimethylcyclopropene, 1,3,3-trimethylcyclopropene, 3-methyl-3-vinylcyclopropene and 3-methyl-3-ethynylcyclopropene (Sisler et al., 2001). The compounds evidently compete with ethylene at the site of ethylene receptors, blocking tissue responsiveness to the growth

regulator (Sisler and Serek, 1997; Sisler et al., 2001). 1-MCP has been used as a tool to investigate ethylene action and tissue responses to ethylene during fruit ripening and flower senescence since it is effective in the ppb range, odorless, stable (non-explosive), and non-toxic (Sisler and Serek, 1997; Sisler and Serek, 1999). Application of 1-MCP delays ripening of climacteric fruits and flower senescence, presumably via its blocking effect on the ethylene signal transduction pathway. 1-MCP as commercial powder (active ingredient 0.14% 1-MCP) from Agrofresh, Philadelphia, PA., has been approved for use apple fruit.

#### **Treatment Procedures for 1-MCP**

1-MCP can be applied to plant tissues as a gas. The concentration required to inhibit ethylene action decreases as the exposure period increases (Serek et al., 1995a). Actively growing vegetative tissues and abscission layers, some of which involve mitotic activity, may need higher 1-MCP concentrations (Sisler and Serek, 1997). Sisler et al. (1997) reported that at higher temperatures less 1-MCP is required. Very low quantities of 1-MCP (20 nL L<sup>-1</sup>) were effective in extending the storage life of cut flowers including *Rosa hybrida*, *Begonia*, and *Kalanchoe* by preventing bud and flower abscission, leaf abscission, and flower senescence (Serek et al., 1994). The duration of the prophylactic period varies from plant to plant. Some cut flowers, banana and tomato fruits remain insensitive to ethylene almost for 12 days at 24 °C (Sisler and Serek, 1997).

#### **1-MCP and Ethylene**

Sisler et al. (1997) proposed that 1-MCP binds to a metal in the ethylene receptor and would thus compete with the ethylene receptor, maintaining the active form of the receptors until ethylene concentration becomes adequate, new receptors are synthesized, or released from the receptor sites (Sisler and Serek, 1997). However, reports have

indicated that 1-MCP may bind to other receptors showing homology to ethylene receptors and/or it may not permanently attach to the ethylene receptors (based upon the continuous presence of 1-MCP that further improved storage life of pak choy and broccoli compared to daily application of 1-MCP) (Abble et al., 2002). Furthermore, Jiang et al. (1999b) reported that the  $K_m$  (substrate concentration at half the maximum velocity) for 1-MCP ( $17 \text{ nL L}^{-1}$ ) was lower than that for ethylene ( $96 \text{ nL L}^{-1}$ ) for control of banana softening, suggesting that 1-MCP has a stronger affinity than ethylene for the ethylene-binding sites.

A consistently observed effect of 1-MCP treatment with climacteric fruit is the dramatically reduced level of ethylene production. 1-MCP treatment of tomato fruit decreased transcript abundance for the enzymes 1-aminocyclopropene-1-carboxylate oxidase (ACO) (EC 4.2.1.3) and ACC synthase (ACS) (EC 4.4.14); however, ACC content did not change (Nakatsuka et al., 1997). Peach fruit exhibited suppressed ethylene production, ACO activity, and accumulation of *PP-ACS1* mRNA in response to 1-MCP treatment (Mathooko et al., 2001). 1-MCP inhibited the ethylene-induced triple response in arabidopsis seedlings (Hall et al., 2000). ETR1 and ERS1 (ethylene response sensors) showed nearly identical sensitivity to 1-MCP in arabidopsis, suggesting the ethylene-binding sites of ETR1 and ERS1 have similar affinities for ethylene (Hall et al., 2000). Accumulation of ACO mRNA during storage of 'Flavortop' nectarine was inhibited by 1-MCP, and this inhibition persisted during post-storage ripening (Dong et al., 2001).

In a few reports, fruits including grapefruit (Mullins et al., 2000) and strawberry (Tian et al., 2001) were noted to show increased ethylene production in response to 1-



MCP treatment. However, both grapefruit and strawberry are non-climacteric fruits in which the triggering end regulation of the ripening process as a whole does not require ethylene unlike climacteric fruits. Furthermore, pre-treatments of citrus leaves and leaf explants with 1-MCP induced ethylene production upon transfer of the leaves to air (Zhong et al., 2001). The reason for higher ethylene production could be stress-related ethylene production, regulation of ethylene production, and/or excessive ethylene production due to loss of ethylene feedback control mechanisms (Mullins et al., 2000).

### **1-MCP and Fruit Softening**

One of the most commonly reported effects of treating fruit with 1-MCP is the dramatic decline in the rate of softening, presumably a consequence of reduced accumulation of specific, ethylene-induced cell wall enzymes (Huber et al., 2003). The accumulation of polygalacturonase and cellulose (EC 3.2.1.5) was significantly delayed and suppressed in 1-MCP-treated avocado fruit (Feng et al., 2000; Jeong et al., 2002). However, eventually 1-MCP-treated avocado fruit softened eventually as high as non-treated fruit, which indicates that PG and cellulose are not essential for softening of avocado fruit (Feng et al., 2000; Jeong et al., 2002). The solubilization and degradation of polyuronides of avocado fruit was significantly reduced and delayed by 1-MCP application as well (Jeong et al., 2003). The mRNA abundance of PG and pectinesterase (EC 3.1.1.11) during storage of 1-MCP-treated 'Flavortop' nectarine was reduced, and inhibition of PG expression persisted during post-storage ripening (Dong et al., 2001). In contrast to the general inhibition of accumulation of cell wall enzymes in response to 1-MCP treatment, the accumulation of endoglucanase (EC 3.2.1.4) and its transcript in 'Flavortop' nectarine were enhanced by 1-MCP and inhibited by ethylene at all stages ripening (Dong et al., 2001). The nectarine fruit with 1-MCP showed severe flesh

woolliness (soft dry texture) and reddening, and lower juice compared to ethylene-treated fruit, and the authors proposed that these disorders may be enhanced by the high level of expression of endoglucanase (Dong et al., 2001). 1-MCP treatment decreased the mRNA abundance of expansin 1 in mature green or ripe tomato fruit (Hoeberichts et al., 2002). Since it is believed that expansin is stimulated by ethylene, expansin may contribute to tomato fruit softening at early stage of ripening (Hoeberichts et al., 2002).

### **The Influence of Suppressed Ethylene Perception on Ripening Physiology and Biochemistry**

Table 2-1 summarizes from the literature some of the physiological and biochemical responses (PBR) of fruits in which ethylene perception is suppressed by 1-MCP. PBR are divided into three groups (columns) as reduced or delayed, increased and unaltered. Some of the PBR of a crop are listed in three columns because of the different sources possibly caused by cultivar and ripeness stage differences.

Table 2-1. 1-MCP-induced effects on ripening fruits

Fruit	Reduced or delayed PBR	Increased PBR	Unaltered PBR	References
Apple ( <i>Malus sylvestris</i> L.)	Ethylene production, respiration, softening, color change, loss of titratable acidity and water loss, decay, aroma production, coreflush, and scald	Respiration, soluble solids, and internal injury	Respiration, soluble solids, and loss of titratable acidity	(Fan and Mattheis, 1999; Fan et al., 1999; Watkins et al., 2002; Jiang and Joyce, 2002; Saftner et al., 2003)
Apple (fresh-cut) ( <i>Malus sylvestris</i> L.)	Ethylene production, respiration, softening, and color change.			(Jiang and Joyce, 2002)
Apricot ( <i>Prunus armeniaca</i> L.)	Ethylene production, respiration, softening, color change, titratable acidity, decay, and aroma production	Days to ripen		(Fan et al., 2000; Dong et al., 2002)

Table 2-1. Continued

Fruit	Reduced or delayed PBR	Increased PBR	Unaltered PBR	References
Banana ( <i>Musa</i> sp. AAA group)	Ethylene production, softening, peel color change, chlorophyll loss, and aroma production	Ethylene production and uneven skin color development		(Sisler et al., 1996; Golding et al., 1998; Jiang et al., 1999a; Jiang et al., 1999b)
Grapefruit ( <i>Citrus paradisi</i> )	Degreening	Ethylene production	Decay	(Mullins et al., 2000)
Mango ( <i>Mangifera indica</i> L.)	Softening, and color change	Days to ripen	Soluble solids, titratable acidity, weight loss, and rots	(Jiang and Joyce, 2000; Hofman et al., 2001)
Nectarine ( <i>Prunus persica</i> Lindl.)	Ethylene production, and softening	Woolliness and discoloration	Respiration	(Dong et al., 2001)

Table 2-1. Continued

Fruit	Reduced or delayed PBR	Increased PBR	Unaltered PBR	References
Papaya ( <i>Carica papaya</i> L.)	Ethylene production, respiration, softening, and color change.	Days to ripen, soluble solids, rots, anthracnose, and skin blemishes.	Soluble solids	(Hofman et al., 2001; Jacomino et al., 2002)
Pear ( <i>Pyrus communis</i> L.)	Ethylene production, softening, and water loss			(Lelievre et al., 1997; Baritell et al., 2001)
Peach ( <i>Prunus persica</i> L.)	Ethylene production, ACS and ACO activities, respiration, softening, and loss of titratable acidity.	Mesocarp browning		(Mathooko et al., 2001; Fan et al., 2002)
Pineapple ( <i>Ananas comosus</i> Merr.)	Ethylene production, color change, loss of soluble solids, and chilling injury.			(Selvarajah et al., 2001)

Table 2-1. Continued

Fruit	Reduced or delayed PBR	Increased PBR	Unaltered PBR	References
Plum	Ethylene production, respiration,	Browning	Color change	(Abdi et al., 1998; Dong et al., 2002)
<i>(Prunus salicina</i>	softening, color change, loss of			
<i>L.</i> ; <i>Prunus x</i>	titratable acidity, aroma			
<i>domestica L)</i>	production, browning, and decay			
Strawberry	Ethylene production, softening,	Ethylene	Respiration, and	(Ku et al., 1999; Tian et
<i>(Fragaria x</i>	color change, decay, phenolics,	production, and	decay	al., 2000; Jiang et al.,
<i>ananassa Duch)</i>	and phenylalanine ammonia-lyase	decay		2001)
	(PAL)			
Tomato	Ethylene production, ACO and		Soluble solids	(Nakatsuka et al., 1997;
<i>(Lycopersicon</i>	ACS, color development, phytoene			Wills and Ku, 2002;
<i>esculentum L.)</i>	synthase, expansin, respiration,			Hoerberichts et al., 2002;
	loss of titratable acidity, softening			Mostofi et al., 2003)

## **Physiology of Fresh-cut Produce**

### **Introduction**

The processing or preparation of lightly processed (fresh-cut) fruits and vegetables can be defined as washing, sorting, trimming, peeling, skinning or chopping of horticultural commodities in a manner that does not reduce fresh-like quality (Rolle and Chism, 1987; O'Conner-Shaw et al., 1994). The preparation can result stress similar to that found in wounded tissues (Brecht, 1995). Excessive water loss, synthesis of secondary compounds, higher ethylene production and respiration, softening, browning and degreening are examples of such behaviors shown by fresh-cut produce (Rolle and Chism, 1987; Miller, 1992). These behaviors plus temperature, relative humidity and atmospheric composition can greatly influence quality maintenance of fresh-cut produce (King and Bolin, 1989).

### **Consequences of Processing**

The physiology of fresh-cut produce is similar to that of wounded tissues (Brecht, 1995). The process necessary for fresh-cut produce (fresh-cut processing) requires abrasion, peeling, slicing, chopping and shredding (O'Conner-Shaw et al., 1994). Each of the previous steps can generate stress conditions in living tissues. Since fruits and vegetables remain viable after the fresh-cut processing, their behavior is generally comparable to plants exposed to stress conditions in nature such as wind damage. This behavior includes enhanced ethylene and respiration rates, wound-healing processes (synthesis of secondary compounds, suberization and lignification), biochemical changes (membrane changes, browning and degreening) and physical changes (softening and water loss; Rolle and Chism, 1987; Miller, 1992; Brecht, 1995).

**Ethylene production and respiration.** Wounding caused by fresh-cut processing may accelerate ethylene production and respiration (Rolle and Chism, 1987). In climacteric fruits, wounding causes more ethylene production in the preclimacteric and climacteric periods than in the postclimacteric period (Brecht, 1995). Respiration of fresh-cut produce generally rises with temperature depending on the severity of damage during processing. Higher ethylene production due to wounding may result in an increase in respiration rate as well (Rolle and Chism, 1987). Additionally, starch break down and oxidation of fatty acids may contribute to this respiratory inclination (Miller, 1992).

**Secondary metabolism.** Wounding causes tissues to synthesize secondary compounds, which are mostly related to wound-healing and defense mechanism processes. These secondary compounds may affect aroma, appearance, nutritive value, and safety of fresh-cut produce (Brecht, 1995). Some of these secondary compounds are phenolics, flavonoids, terpenoids (Sakai and Nakagawa, 1988), alkaloids, glucosinolates, and long-chain fatty acids and alcohols (Miller, 1992). Wounding increases the activities and transcripts of the following enzymes associated with the secondary compounds: PAL (EC 4.1.3.5) (Fritzemeier et al., 1987; Liang et al., 1989), 4-coumarate:CoA ligase (EC 6.2.1.13) (Fritzemeier et al., 1987), chalcone synthase, 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (EC 4.2.3.4) (Miller, 1992), peroxidases (EC 1.11.1.6) (Bostock et al., 1987; Miller and Thomas, 1989), and stilbene synthase (EC 2.3.1.95) (Vornam et al., 1988).

Structural changes of the tissue surface may occur as a consequence of the wound-healing process. Desiccation of the wounded surface is the first observable change of fresh-cut produce (Varoquax and Wiley, 1994). Desiccation is followed by suberin and



lignin production and deposition in cell walls, and is possibly preceded by periderm occurrence beneath the suberin layer in many tissues for example potato tuber, bean pod, and cucumber pericarp (Burton, 1982).

**Browning and degreening.** Browning results from enzymatic oxidation of phenols and polyphenols (Ahvenainen, 1996). Polyphenol oxidase (EC 1.14.18.1), PAL, tyrosine ammonia lyase (EC 4.1.99.2), cinnamic acid -4-hydroxylase (EC 1.14.13.11), lipoxygenase (EC 1.13.11.13) and catechol oxidase (EC 1.1.3.14) are the enzymes that likely cause enzymatic browning (Ahvenainen, 1996).

Increased ethylene production and loss of membrane integrity may start rapid chlorophyll degradation due to induction of chlorophyll-degrading enzymes (Varquaux and Wiley, 1994). The enzymes responsible for the chlorophyll degradation are chlorophyll oxidase, chlorophyllase (EC 3.1.1.15), lipolytic acid hydrolase, and other peroxidases (EC 1.11.1.6) (Varquaux and Wiley, 1994).

**Membrane changes.** Wounding causes cellular disruption, leading to compartmentation of enzymes and substrates (Rolle and Chism, 1987). Wounding enhances the activities of lipid acyl hydrolyse (act like phospholipase D), polyphenol oxidase (Ikediobi et al., 1989) and lipoxygenase (Lulai, 1988; Ikediobi et al., 1989). These enhanced enzyme activities may result in increases in free fatty acids and free radicals that are toxic to many cellular processes and capable of causing organelle inactivating proteins and lysis (Brecht, 1995). Additionally, excessive ethylene production enhances permeability of membranes and reduces phospholipid biosynthesis (Watada et al., 1990). For example, in fresh-cut carrot, total phospholipids and phosphatidic acid increased but phosphatidylcholine decreased (Picchioni et al., 1994).

Picchioni et al. (1994) also found that rough endoplasmic reticulum numbers increase in fresh-cut carrot, which may be correlated to lipid synthesis and the enzyme induction process.

**Textural and cell wall changes.** Firmness loss is immediate and faster in wounded tissues due to cell rupture and loss of tissue integrity (Miller, 1992). Cell wall enzyme activity may be accelerated by wounding (Miller, 1992; Karakurt and Huber, 2002), which may contribute extensive softening in fresh-cut tissues. To illustrate, Karakurt and Huber (2002) found that PG and  $\alpha$ - (EC 3.21.22) and  $\beta$ -galactosidase activity (EC 3.2.1.23) was higher in fresh-cut papaya fruit compared to intact fruit at 5 °C. The enhanced cell wall enzyme activity, thus, causes depolymerization of pectic and hemicellulosic polyuronides, which may result in further textural changes in fresh-cut tissues.

**Dehydration.** Fresh-cut processing causes interior tissues to be exposed to air and to increase in evaporation rate which results in water loss. Dehydration at the cut surface is sometimes obligatory to control microbial growth; however, it may provoke undesirable visual appearances such as color fading in carrot skin (Watada et al., 1996).

### **External and Internal Factors Contributing to Quality of Fresh-cut Fruits and Vegetables**

Wounded tissues rapidly deteriorate and senesce. Hence, minimizing the negative consequences of wounding is a crucial step that affects storage life and maintenance of interior and exterior qualities of fresh-cut produce. These qualities are greatly affected by morphological, physiological, environmental, pathological and practical factors.

**Raw product.** Since fresh-cut fruits and vegetables are already at the table-ripe edible stage, they should have excellent interior and exterior qualities. Fresh-cut produce

should also have superior characteristics such as slower ripening rate, good texture and flavor qualities and less sensitivity to chilling injury and microorganisms than their counterparts because they are more perishable compare to intact produce (Watada et al., 1996).

**Temperature.** Fresh-cut fruits and vegetables should be held at lower temperatures to slow down metabolic activity. Lower temperatures are also necessary to control microbial growth. However, most of tropical and some subtropical commodities are chill sensitive; therefore, storage at a lower temperature may lead to chilling injuries. On the other hand, keeping fresh-cut produce at lower temperatures suppresses the development of chilling injury symptoms for a limited period (Watada and Qi, 1999).

**Relative humidity.** Relative humidity of the atmosphere of fresh-cut produce should be higher to reduce extensive water loss (Schlimme, 1995). Edible or non-edible coating and proper packing may reduce water loss from fresh-cut produce (Watada et al., 1996; Schlimme, 1995). In many cases, water loss in fresh-cut produce results from epidermal membrane deterioration. Particularly, at higher temperatures where the water vapor deficit is large, the water loss hastens in fresh-cut produce (Watada et al., 1996).

**Controlled atmosphere and modified-atmosphere packing.** Reduced oxygen and elevated carbon dioxide levels are basic practices of controlled atmosphere condition. The response of fresh-cut produce stored in controlled atmosphere is different from that of intact produce; therefore, fresh-cut produce should be stored differently and separately. On the other hand, because of the short handling period, controlled atmosphere may not be economically applicable for fresh-cut produce (Watada et al, 1996). Gas compositions

in film-packed and edible-coated fresh-cut produce can be modified and this modification may extend storage life of fresh-cut produce (Watada et al., 1996; Schlimme, 1995).

**Packing and edible coating.** Fresh-cut produce may be packed or coated to reduce mechanical damage and water loss, to identify produce and to carry information to consumer (Schlimme, 1995). Nevertheless, packing may cause an increase in temperature that evokes higher respiration and ethylene production rate. Therefore, ethylene must be excluded or absorbed by ethylene absorbents such as charcoal and palladium chloride (Schlimme, 1995). Edible films reduce moisture loss, limit gas exchange, retard ethylene production and keep aroma inside (Ahvenainen, 1996). Lipids, resins, polysaccharides and proteins are the basic components of edible films (Baldwin et al., 1995). Some coatings may carry some additives that can prevent discoloration and microbial growth by serving as antioxidants and/or anti-microbial agents (Baldwin et al., 1995). Some of these additives are sucrose polyesters of fatty acids, sodium salts of carboxymethylcellulose, carrageenan and chitosan (Ahvenainen, 1996).

**Chemical application.** Chemical applications are mostly used for reducing decay and browning, and retaining firmness in fresh-cut produce. Chlorine is the standard sanitizing agent for fresh-cut produce in proper concentrations (100-300 ppm; pH, 7). Higher chlorine concentrations (> 500 ppm) may cause fresh-cut produce to discolor, equipment to corrode and aromatic hazardous chloramines to form (Hurst, 1995). Hong and Gross (1998) reported that sodium hypochlorite caused some physiological and biochemical alterations in fresh-cut tomato fruit (higher electrolyte leakage and ethylene production). Sulphating agents are the most common chemicals for inhibiting browning reactions. In addition to sulphites, ascorbic acid, sodium dehydroacetic acid, potassium

sorbate, citric acid, zinc, chloride and calcium chloride, resorcinol derivatives, and carbon dioxide and carbon monoxide are used as anti-browning agents (Ahvenainen, 1996).

**Microorganism.** Microorganisms readily grow on and in fresh-cut produce, and some of them may be detrimental to human being such as *Escherichia coli*. The following microorganisms have been found in fresh-cut produce: mesophilic bacteria, lactic acid bacteria, coliforms and fecal coliforms, yeast and molds, and pectinolytic microflora such as *Pseudomonas fluorescens* and *Xanthomonas maltophila* (Nguyen-the and Carlin, 1994). Mesophilic microflora is the largest population followed by lactic acid bacteria (Watada et al., 1996). Moreover, some food-borne microorganisms have been reported in fresh-cut produce that includes *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aeromonas hydrophila* (Nguyen-the and Carlin, 1994; Alfred, 1994), *Staphylococcus aureus* (Nguyen-the and Carlin, 1994), *Escherichia coli* (Nguyen-the and Carlin, 1994; Alfred, 1994), *Salmonella* spp. (Nguyen-the and Carlin, 1994), *Clostridium botulinum* (Alfred, 1994), *Bacillus cereus*, *Giardia lamblia* (Beuchat, 1995), *Shigella* spp., and *Plesiomonas shigelloides* (Alfred, 1994). The hepatitis A and Norwalk agent virus also have been reported in fresh-cut produce (Beuchat, 1995).

Microbial growth, particularly of human pathogens, may be inhibited by competing bacteria such as lactic acid bacteria or naturally existing antimicrobials released during fresh-cut processing (Luna-Guzman and Barrett, 2000). Low temperature is one of the most effective methods to control and prevent microbial growth (Nguyen-the and Carlin, 1994) while some of the organisms can survive in low temperatures such as *Listeria*, *Yersinia* and *Aeromonas* (Alfred, 1994). Washing fresh-cut produce with chlorine solution (up to 300 ppm) is another effective way to impede development of

microorganisms (Nguyen-the and Carlin, 1994). This process cannot completely eliminate all microorganisms because microorganisms can survive when they are inside tissues where disinfectants cannot penetrate (Watada et al., 1996). Washing fresh-cut produce in trisodium phosphate is one of the other effective ways to control microbiological growth (Beuchat, 1995). Ozone, a strong oxidant, is also used for its lethal activity upon microorganisms at microgram per milliliter concentrations (Beuchat, 1995). In addition to chemical solutions, organic antagonism, gamma irradiation, boiling in water and edible coatings containing biochemical agents are also used for the control of microbial growth (Watada et al., 1996; Nguyen-the and Carlin, 1994). Irradiation is a safe, effective and hazard-free antimicrobial method (Farkas, 1998). *Campylobacter*, *Yersinia*, *Vibrio* and *Escherichia coli* have low resistance to ionizing radiation (Farkas, 1998). Slow ripening and senescence induced by restricted ethylene action or synthesis may extend the storage life of fresh-cut produce: in a less ripe condition, the growth of most opportunistic microorganisms would be expected to be retarded since they tend to grow most rapidly on senescent tissues (Zagoray, 1999).

## **Cell Wall**

### **Introduction**

The plant cell wall is an important structure that determines cell shape, connects cells to each other, provides essential mechanical strength and ridges and acts as vital barrier against abiotic and biotic invaders such as insects and dusts. The chemical and physiological structure of the plant cell wall varies widely from plant group to plant group and from cell type to cell type. The plant cell wall is a dynamic structure that changes during the life cycle of a cell.

## Cell Wall Structure

The plant cell wall is constructed by a very complex but highly organized composite of many different polysaccharides, proteins and aromatic substances. The cell wall consists of three main divisions; the primary cell wall, the middle lamella and the secondary cell wall (Carpita and McCann, 2000). The primary cell wall, born in the cell plate during cell division, is capable of growth by expansion; the middle lamella forms the interface between adjacent cells; and secondary cell wall builds up upon the primary cell wall when the cells mature and are no longer growing (Goldwin, 1983; Brett and Waldron, 1996). The model of cell wall structure is thought to be by three coextensive networks: the cellulose-hemicellulose framework, the pectic matrix, and a network of structural proteins (Carpita and Gibeau, 1993). Cellulose, the most abundant plant polysaccharide, exists in the form of microfibrils that are an unbranched  $\beta$  (1-4) linked polymer of D-glucose strengthened by hydrogen bonds (Goodwin, 1983; Carpita and MacCann, 2000). Pectins are a mixture of heterogeneous and highly hydrated polysaccharides, rich in D-galacturonic acid. Pectins consist of 6 different polymers rhamnogalacturonan I, rhamnogalacturonan II, homogalacturonan, arabinan, galactan, and arabinogalactan I (Carpita and MacCann, 2000). Hemicelluloses are a class of polysaccharide, hydrogen-bonded to cellulose microfibrils (Carpita and MacCann, 2000). The two major hemicellulosic polymers are xyloglucans and glucomannans in flowering plants; the other polymers include xylans, mannans, galactomannans and arabinogalactan II, callose, and  $\beta$ 1,3 and  $\beta$ 1,4-glucans. Proteins such as extension and expansin, and phenolics such as lignin and ferulic acid are part of the plant cell structure (Reiter, 1994).

### **Cell Wall Loosening and Growth**

Cell growth is provided by expansion or elongation that creates an irreversible increase in cell volume. The cell wall must change its structure to expand or elongate: cell wall loosening is probably the primary event in this process followed by continued deposition of new materials. Cellulose microfibril orientation controls cell expansion or elongation and decides the plane of elongation (Carpita and MacCann, 2000). The multinet growth hypothesis explains displacement of the microfibrils during growth. New microfibrils deposited in strata on the inner surface of the cell wall in mostly transverse orientation replace older ones that are pushed towards the outer layers of the cell wall and reoriented in the direction of the cell elongation (Carpita and MacCann, 2000). The acid-growth hypothesis proposes that auxin causes lower pH conditions by pumping proton, which activates apoplast-localized growth-specific hydrolyses that cleave the load-bearing bonds that join cellulose microfibrils to other polysaccharides (Cosgrove, 2000). This cleavage produces a loosening in the cell wall as well as water uptake leading an increase in cell size (Cosgrove, 2000). Two kinds of enzymes xyloglucan endo-transglycosylase (EC 2.4.1.72) and expansins are thought to be involved in cell wall loosening.

### **Fruit Ripening and the Cell Wall**

The textural changes during fruit ripening are thought to be related to alterations in cell wall structure (Huber, 1983; Tucker and Grierson, 1987). The changes are mostly correlated with structure and composition of pectic components (Seymour et al., 1987). Solubilization and depolymerization of pectins (Fischer and Bennett, 1991) and hemicelluloses (Lashbrook et al., 1997) during ripening are frequently related to cell wall loosening and disintegration. Cell wall modifications have been extensively studied in



tomato fruit, and early reports indicated that pectin degradation by PG represented the model of fruit softening; however, PG-antisense tomato fruit revealed that pectin degradation is not essential for fruit ripening (Smith et al., 1988; Giavannoni, 1990). The other major changes during ripening occur in hemicellulose content. Xyloglucan, the chief hemicellulose in dicotyledonous plants, undergoes depolymerization in most fruits, including tomato (Sakurai and Nevins, 1993). Besides the depolymerization of both pectic and hemicellulosic polyuronides, there is a loss of neutral sugar from neutral pectins, primarily galactose and arabinose (Tucker, 1993).

### **The Cell Wall and Pathogen Attacks**

The plant cell wall fortifies cells against attacks from microorganisms and even other plants. Callose and lignin are thought to act as a physical barrier, blocking fungal penetration into plant cells (Hammond-Kosack and Jones, 2000). Hydroxyproline-rich glycoproteins contribute to defense against fungal attacks by cross-linking to the cell wall matrix and initiating additional lignin formation. PG-inhibiting proteins restrain PG activity, originated from pathogens, which is also a part of defense mechanisms (Hammond-Kosack and Jones, 2000). Oligosaccharides derived from the cell walls of fungi and plants, including  $\beta$ -glucans, chitin, chitosan, and pectin, are inducers of the synthesis of a wide spectrum of defensive chemicals in plant tissues (Ryan, 1988). The oligosaccharides are generated at infection or wound sites and may be early signals to activate genes whose products, such as antibiotic phytoalexins, extensins, proteinase inhibitors, pathogenesis-related proteins and lignin, enhance the plant's defense system against pathogens and herbivores (Ryan, 1988).

CHAPTER 3  
DELAYING ETHYLENE-INDUCIBLE RIPENING PROCESS 1-  
METHYLCYCLOPROPENE IN 'GALIA' MELON FRUIT

**Introduction**

Ethylene has been known to regulate fruit ripening and softening in climacteric fruits (Lelievre et al., 1997). Accidental exposure to ethylene or natural ethylene production can reduce the postharvest life of climacteric fruits by accelerating ripening and senescence (Reid, 1985). The ethylene inducible effect, however, can be delayed/prevented by some ethylene antagonists or ethylene action inhibitors including silver thiosulphate (STS), 2-5 norbornadiene, diazocyclopentadiene, and 1-methylcyclopropene (1-MCP) (Sisler and Serek, 1997; Sisler and Serek, 1999). Commercial use of STS in cut flowers is being considered in some countries due to Ag<sup>+</sup> heavy metal in STS complex (Sisler and Serek, 1997). 1-MCP, therefore, seems to be the most practical ethylene action inhibitor due to its stability, activity in low concentration, and non-toxic and odorless properties (Sisler and Serek, 1997; Sisler and Serek, 1999). The ability of 1-MCP to inhibit ethylene action in apple fruit resulted in promising commercial development (Safner et al., 2003). Furthermore, studies with 1-MCP confirmed that post-storage life and quality of tomato fruit at early and advanced stage of ripening can be improved by 1-MCP (5, 10, 20 and 100  $\mu\text{L L}^{-1}$  for 24 h at 20 °C, Wills and Ku, 2002; 50 to 150  $\text{nL L}^{-1}$  1-MCP for 20 h at 20 °C, Hoeberichts et al., 2002).

'Galia' fruit (*Cucumis melo* var. *reticulatus* L. Naud. cv. Galia) is a climacteric fruit in which ripening is achieved by the help of ethylene, and ethylene and respiratory

climacterics (Seymour and McGlasson, 1993). The fruit has excellent flavor and aroma characteristics; however, storage life of 'Galia' fruit, harvested an early stage of ripening (green peel color), is limited to 2-3 weeks even at low temperatures (Aharoni et al., 1993; Fallik et al., 2001). Restriction of ethylene synthesis has proved that storage life of melon fruit can be extended. For example, 'Galia' fruit at an early stage of ripening held in controlled atmosphere of 10% CO<sub>2</sub> and 10% O<sub>2</sub> with an ethylene absorbent, potassium permanganate, for 14 days at 6 °C plus an additional 6 days at 20 °C had higher quality (reduced fruit softening and decay) than control fruit stored in controlled atmosphere only (Aharoni et al., 1993).

The present study was performed to characterize the physiological responses of 'Galia' melon fruit to 1-MCP treatment and determine whether 1-MCP treatment could be effective as a postharvest application for the extension of the storage period or storage life of pre-ripe or ripe 'Galia' fruit.

## **Materials and Methods**

### **Plant Material**

'Galia' plants were grown according the growing techniques and production practices established by Shaw et al. (2001) in Greenhouse Facilities at the University of Florida Horticultural Farm near Gainesville, FL in spring 2001. Temperatures were recorded every 15 min at various locations in the greenhouse using thermocouples and a datalogger (CR-10 Campbell Scientific, Inc., N. Logan, UT). No additional heating or cooling units installed in the greenhouse. Fruit were harvested at two stages of maturity, green (GRN, early stage of ripening) and yellow (YLW, advanced stage of ripening) according a color chart (1, dark green; 2, green; 3, light yellow with green; 4, light yellow; 5, yellow; 6, dark yellow to orange) reported by Fallik et al. (2001). The

harvested fruit were transferred to the Postharvest Horticulture Laboratory of Gainesville. The fruit were then selected on the basis of uniformity of size and freedom from defects; afterwards, the fruit were gently brushed, washed with tap water (23 °C), dipped into 200  $\mu\text{L L}^{-1}$  chlorinated water for 1 min and air-dried.

### **1-MCP Application**

A commercial powder formulation provided by Agrofresh (active ingredients 0.14%) Philadelphia, Penn. was used to generate 1-MCP. 1-MCP was released from three g of the powder to the vapor phase by adding 50 mL deionized water, generating a 7.5  $\text{mL L}^{-1}$  concentrated stock in a 136-mL sealed vial. 1-MCP concentration (1 mL) in the headspace of the vial was measured using a gas chromatograph (GC) (Hewlett Packard 5890 II GC; Avondale, PA) furnished with a 80-100 mesh Chromosorb PAW stainless steel column (1.8 m x 3.18 mm i.d.; Supelco, Bellefonte, PA). Injector, oven, and detector (FID) temperatures were set at 150, 150 and 200 °C, respectively. Isobutylene gas, which has a FID response similar to that of 1-MCP (Jiang et al., 1999) was used as a standard. Approximately a 10-mL sample of vial headspace gas was injected into a 179-L metal chamber containing a 50-L void space, yielding a final 1-MCP concentration of 1.5  $\mu\text{L L}^{-1}$  and held for 24 h at 20 °C. The treatment containers were vented for 5 minutes, resealed, and reinjected with fresh 1-MCP at 6-h intervals. Control fruit were kept under identical condition.

1-MCP concentration and efficacy was investigated in a preliminary experiment in which GRN fruit were treated with air (control), 0.09, 0.9 and 9  $\mu\text{L L}^{-1}$  1-MCP for 24 h at 20 °C and stored at 15 °C.

### **Respiration and Ethylene Production**

Air-(control) and 1-MCP-treated fruit were placed in airtight plastic containers (1 fruit per container) (3.6 L) and sealed for 1 h at 20 °C. Respiration and ethylene production from each the treatment (5 replications) was determined by measuring the CO<sub>2</sub> and C<sub>2</sub>H<sub>4</sub> concentration in the headspace of the containers. For CO<sub>2</sub>, 0.5-mL headspace gas sample was injected to a GC (Gow-Mac, Bridge Water, NJ) equipped with thermal conductivity detector, and for C<sub>2</sub>H<sub>4</sub>, 1-mL headspace gas sample was infused into the Hewlett-Packard-5890 GC fitted with a flame ionization detector.

#### **Firmness Assessment**

Mesocarp fruit firmness was measured on opposite sides of (two equidistant points on the equatorial region) pared fruit using an Instron Universal Testing Instrument (Model 441-C8009, Canton, MA). The probe (convex, 11 mm diameter) located at zero force and contacted with the pared fruit surface was driven to a depth of 10 mm with a crosshead speed of 50 mm min<sup>-1</sup>. Firmness data was expressed as the maximum force Newton (N) acquired during penetration. All tests were conducted with fruit pulp temperature of 20 °C.

#### **Electrolyte Leakage Assessment**

Five mesocarp cylinders were removed the equatorial position of a fruit with an 8-mm diameter cork borer. From each cylinder, 1 disk (8 x 8 x 8 mm<sup>3</sup>) was excised by the same cork borer, yielding to a total of 5 disks per fruit. The disks were briefly rinsed with deionized water and blotted dry on a slightly moistened Whatman filter paper. The disks (five per fruit) were then incubated in 15 mL of 500 mM mannitol for 6 h in a capped polypropylene tube. Conductivity was measured with a conductivity bridge (YSI-31A, Yellow Springs, OH) furnished with a conductivity cell (3403, Yellow Springs, OH) immediately after addition of the bathing solution to the disks and the end of the

incubation period. The aliquot removed from the bathing solution for the conductivity measurement was added back to the bathing solution. The disks and bathing solution were then stored at -20 °C for at least 24 h, thawed, boiled in water for 30 min, cooled to room temperature, and conductivity measurement was measured once more. The electrolyte leakage was expressed as percentage of the conductivity of total tissue electrolytes.

### **Soluble Solids Concentration, pH and Titratable Acidity Determination**

Soluble solids concentration (SSC), titratable acidity (TA), and pH were quantified using a digital refractometer (Abbe Mark-10480, Buffalo, NY), a Fisher-395 dispenser (Fisher 395, Pittsburg, OH) equipped with an electrometer (Fisher 380, Pittsburgh, PA), and a digital pH meter (Corning, NJ), respectively. Mesocarp tissue (80 g) was macerated with a mortar and pestle and centrifuged at 27,200 RFC for 10 min at 21 °C. Fruit juice collected from the macerated/centrifuged tissue was used for SSC and pH measurement. For TA, 6-g fruit juice was titrated with 0.1 N NaOH to an end point of pH 8.2, and TA was expressed as percentage of malic acid using the volume of mL NaOH recorded from the dispenser.

### **Statistical and Informal Taste Analyses**

General linear model program of SAS (SAS institute, Carey, NC) and Duncan's Multiple Range Test were performed for Completely Randomized Designs. Informal taste analyses to determine the edible stage on fruit surface and flesh appearance, odor, flavor and texture quality were performed by untrained personnel of the postharvest research group of University of Florida.

## Results

### 1-MCP Concentration and Efficacy

The firmness of GRN control fruit decreased from 66.7 to 6.3 N while fruit treated with  $0.09 \mu\text{L L}^{-1}$  1-MCP only softened from 67.8 to 11.3 N, fruit treated with  $0.9 \mu\text{L L}^{-1}$  1-MCP from 67.1 to 17 N and fruit treated with  $9 \mu\text{L L}^{-1}$  1-MCP 70.3 to 18.1 N over a 21-day period at  $15^\circ\text{C}$  (Figure 3-1). The decrease in firmness from day 1 to 21 was over 10-fold in the control whereas approximately 6-fold in  $0.09 \mu\text{L L}^{-1}$ -1-MCP-treated fruit and 4-fold in both  $0.9 \mu\text{L L}^{-1}$ -1-MCP- and  $9 \mu\text{L L}^{-1}$  1-MCP-treated fruit. The firmness level of fruit treated with  $9 \mu\text{L L}^{-1}$  1-MCP did not result in a significant difference in firmness relative to the fruit treated with  $0.9 \mu\text{L L}^{-1}$  1-MCP from on days 9 through 21, indicating that the saturation level of 1-MCP is between  $0.9$  to  $9 \mu\text{L L}^{-1}$  1-MCP. Therefore, with the evaluation of previous 1-MCP-related publications, the present studies were performed with  $1.5 \mu\text{L L}^{-1}$  1-MCP.

### Respiration and Ethylene Production

Except for the climacteric rise, respiration of both control and 1-MCP-treated fruit decreased during storage (Figure 3-2A). However, GRN control fruit reached its respiratory climacteric peak at day 6 ( $9.4 \text{ mL kg}^{-1} \text{ h}^{-1}$ ) as equal with 1-MCP-treated fruit at day 15 ( $6 \text{ mL kg}^{-1} \text{ h}^{-1}$ ), resulting in an 11-day delay in the climacteric respiratory peak and a 36% reduction of the magnitude of respiratory climacteric peak. Ethylene production from GRN control fruit increased rapidly, reached a peak at day 3 ( $7.8 \mu\text{L kg}^{-1} \text{ h}^{-1}$ ), and then decreased while ethylene climacteric of GRN 1-MCP-treated fruit started to peak at day 3 and reached its maximum at 9 days ( $2.7 \mu\text{L.kg}^{-1} \text{ h}^{-1}$ ), leading to a 6-day delay and 65% reduction in the magnitude of climacteric ethylene peak (Figure 3-2A). The ethylene production by GRN 1-MCP-treated fruit was statistically lower relative to

GRN control on days 1 through 5 when ethylene climacteric of GRN control fruit occurred.

Respiration of YLW control and 1-MCP-treated fruit gradually decreased during storage, with no statistical differences between the two treatments (Figure 3-2B).

Ethylene production in both YLW control and 1-MCP-treated fruit also declined during storage. The ethylene production of YLW 1-MCP-treated fruit showed a 56% decrease from the first day of storage ( $5.4 \mu\text{L kg}^{-1} \text{L}^{-1}$  at day 1) to the last day ( $2.4 \mu\text{L kg}^{-1} \text{L}^{-1}$  at day 11) whereas YLW control fruit nearly a 90% decrease from day 1 ( $3.8 \mu\text{L kg}^{-1} \text{L}^{-1}$ ) to day 9 ( $0.4 \mu\text{L kg}^{-1} \text{L}^{-1}$ ), resulting a difference between treatments after day 3. YLW fruit treated with 1-MCP produced higher ethylene after day 3 while the ethylene rate was continued to decrease in YLW control fruit.

### **Firmness**

Firmness of either GRN control or 1-MCP-treated fruit declined during storage as shown in Figure 3-3A. GRN control fruit soften very quickly within first 5 days, losing 66% of their original firmness, while GRN 1-MCP-treated lost only 46%. At the last day of storage of GRN control (day 13), GRN control maintained only 6% of their initial value while GRN 1-MCP-treated fruit 20%, from then on, 1-MCP-treated fruit remained relatively firm and preserved 10% their initial firmness at the end of their storage (day 21).

Firmness of YLW control fruit and 1-MCP-treated fruit was not significantly different from each other during the first 2 days of storage (Figure 3-3B). After 2 days of storage, firmness of the YLW control fruit sharply decreased but not that of the YLW 1-MCP-treated fruit. Softening of both treatments remained unchanged from day 5 to 9, from then on, that of YLW 1-MCP-treated showed a sharper decline. Within 5 days,



YLW control fruit softened from 16.5 to 4.9 N (a 70% loss) while YLW 1-MCP-treated fruit from 18 to 12.8 N (a 29% loss). At the end of the storage life (YLW control, day 9; YLW 1-MCP, day 11), YLW control maintained only 30% of their initial firmness while YLW 1-MCP-treated fruit 43%.

### **Electrolyte Leakage**

A continuous increase in electrolytes released from mesocarp tissue of either GRN control or GRN 1-MCP-treated fruit was observed until day 13. The treatments peaked their maxima of 35.8% (control) and 28.5% (1-MCP) at day 13 (Figure 3-4A). GRN control displayed statistically higher leakage rates than GRN 1-MCP-treated fruit after day 3. Electrolyte efflux of GRN 1-MCP-treated fruit slightly decreased from day 11 to 21. YLW control fruit showed an increasing electrolyte leakage through day 7; afterwards, showing a minimal decrease (Figure 3-4B). Electrolytes of YLW 1-MCP-treated fruit slightly increased through day 11 as well (Figure 3-4B). The maxima of electrolyte efflux of YLW control fruit was 36.7% at day 7 whereas in YLW 1-MCP-treated fruit 27.9% at day 11, resulting a significant difference between the two treatments after day 5.

### **Soluble Solids Concentration, pH and Titratable Acidity**

Soluble solids of either GRN control or GRN 1-MCP-treated fruit showed very little change, with no differences between treatments, and averaged from 8.1% to 8.9% as shown in Figure 5A. SSC in YLW control fruit slightly decreased during storage whilst in YLW 1-MCP-treated fruit somewhat increased but magnitude of change and differences were unremarkable, and the soluble solids ranged from 10 and 11% (Figure 3-5B).

TA of GRN control increased a little until day 5, after that point, moderately decreased; however, TA of GRN 1-MCP-treated fruit remained unchanged starting on days 5 through the end of storage (Figure 3-6A). Neither differences nor changes of TA of GRN control and 1-MCP-treated fruit were noted during storage. TA of either YLW control or 1-MCP-treated fruit slightly increased during storage though a minimal decrease was observed at the end (Figure 3-6B). YLW 1-MCP-treated fruit had significantly higher TA than the control on days 7 through 9.

The pH of GRN control slightly decreased until day 7, and then, increased; however, in 1-MCP-treated fruit did not show a unique pattern as illustrated in Figure 3-7A. In either YLW control fruit or 1-MCP-treated fruit, pH very slightly decreased while a small peak was noted at the end (Figure 3-7A). The magnitude of changes and differences in pH of either GRN or YLW fruit treated with and without 1-MCP was unremarkably low.

### **Informal Quality Analysis**

The color change of fruit surface from green to yellow was deferred in GRN 1-MCP-treated fruit (Figure 3-8). The color change of fruit skin from green to greenish-yellow was also deferred in YLW 1-MCP-treated fruit (Figure 3-9). The edible stage (determined by the informal quality analysis with the help of firmness and color evaluation data) lasted on days 5 through 9 for GRN control and on days 13 through 19 for 1-MCP-treated fruit, leading a 4-day delay in edibility and a 40% extension of edible stage. YLW control fruit persisted their edibility through day 5 whereas YLW 1-MCP-treated fruit through day 9, representing almost a two-fold extension (80%). Fruit exhibited  $< 4$  N firmness were not edible. Neither YLW nor GRN fruit treated with and without 1-MCP did show significant external and internal decay occurrences.

### Discussion

Treatment with  $0.9 \mu\text{L L}^{-1}$  1-MCP significantly improved firmness retention of GRN 'Galia' fruit relative to 0 and  $0.09 \mu\text{L L}^{-1}$  1-MCP concentration at  $15^\circ\text{C}$ . Increasing 1-MCP concentration from  $0.9$  to  $9 \mu\text{L L}^{-1}$  did not confer additional benefit upon firmness of GRN 'Galia' fruit. In a previous study, charentais melon fruit exposed to  $1 \mu\text{L L}^{-1}$  1-MCP (for 24 h at  $22^\circ\text{C}$ ) stored at  $2^\circ\text{C}$  for 16 days and rewarmed for additional 5 days at  $22^\circ\text{C}$  became insensitive to low-temperature damage (estimated by visually rating the extend of the fruit surface pitting and browning) compared to non-1-MCP-treated fruit (Ben-Amor et al., 1999). Thereby, we propose that the commercial 1-MCP concentration for 'Galia' melon fruit would be approximately 1 to  $1.5 \mu\text{L L}^{-1}$  for 24 h at  $20^\circ\text{C}$ .

'Galia' fruit is characterized by a classic climacteric ethylene and respiration pattern (Figures 3-2A and 2B). The maximum ethylene production rate of 'Galia' fruit was below  $10 \mu\text{L kg}^{-1} \text{h}^{-1}$  during ripening, a comparable result noted by Zheng and Wolf (2002) at  $24^\circ\text{C}$ . Respiration of 'Galia' fruit ranged from 6 to  $13 \text{ mL kg}^{-1} \text{h}^{-1}$  and declined during ripening excluding climacterics. Our results indicate 'Galia' fruit is an inferior ethylene producers; the ethylene climacteric occurs earlier than the respiratory climacteric during ripening.

1-MCP suppressed both ethylene production and ethylene climacterics in GRN 'Galia' fruit, indicating that 1-MCP efficiently binds the ethylene receptors, thereby, limiting the positive feedback regulation of ethylene production in 'Galia' fruit during ripening. 1-MCP delayed both ethylene and respiratory climacteric rise of 'Galia' fruit by 6 and 11 days, respectively. Similarly, preclimacteric Charentais muskmelon melon fruit exposed  $1 \mu\text{L L}^{-1}$  for 24 h at  $14^\circ\text{C}$  exhibited a delay in ethylene climacteric peak (4 days)

relative to non-1-MCP-treated fruit stored and measured at 14 °C (Chatenet et al., 2000). YLW 'Galia' control fruit showed a declining ethylene rate compared with 1-MCP-treated fruit, resulting a higher ethylene production in YLW 1-MCP-treated fruit. A possible explanation of this observation is: blocking ethylene binding sites of YLW 'Galia' fruit by 1-MCP may cause an interference between ethylene and the ethylene control mechanism, consequently, ethylene fails to perceive the quantity of ethylene production, and is being continued to synthesized (Mullins et al., 2000; Zhong et al., 2001).

1-MCP delayed the respiratory climacteric and suppressed its magnitude in GRN 'Galia' fruit, proving that ripening of melon fruit is strongly regulated by ethylene (Flores et al., 2001). The respiration of YLW 1-MCP-treated fruit, however, was not affected by 1-MCP, which implies respiration might not be directly related to senescence or over-ripening in melon fruit (Saltveit, 1993; Bower et al., 2002).

Softening of GRN 'Galia' fruit strongly deferred by 1-MCP, consisting with the fact that most fruits exposed to 1-MCP at the early stage of ripening showed firmness retention relative to non-1-MCP-treated fruit (Fan et al., 1999, Jiang et al., 1999; Jeong et al., 2002; Wills and Ku, 2002). 1-MCP delayed loss of firmness in YLW 'Galia' fruit (at the advanced stage of ripening) as well. Apple ( $0.7 \mu\text{L L}^{-1}$  1-MCP for 16 h at 20 °C; Mir et al., 2001 or  $10 \mu\text{L L}^{-1}$  1-MCP for 6 h at 20 °C; Jiang and Joyce, 2002) and tomato (treated with  $50-150 \text{ nL L}^{-1}$  1-MCP for 20 h at 20 °C; Hoerberichts et al., 2002) fruit at advanced stage of ripening treated with 1-MCP also showed delayed softening. Thus, the softening process in melon fruit even at the advanced stage of ripening is regulated by ethylene (Lelievre et al., 1997; Flores et al., 2001).

Electrolyte efflux, a measurable symptom of membrane damage (Marongoni et al., 1996), of both GRN and YLW 'Galia' fruit treated with and without 1-MCP increased during storage. The increase in electrolytes during ripening has been previously reported for muskmelon type melon fruit (Lester and Stein, 1996; Lacan and Baccou, 1996). The increase in leakage of both GRN and YLW 'Galia' fruit was repressed by 1-MCP, showing that membrane deterioration during melon fruit ripening is regulated by ethylene. Ethylene has been reported to stimulate the activities of free-radical-producing enzymes that contribute membrane deterioration (Paliyath and Droillard, 1992). To date only one study has been reported for the effects of 1-MCP upon electrolyte efflux: petunia flower corollas treated with  $150 \text{ nL L}^{-1}$  1-MCP for 6 h at  $22 \text{ }^\circ\text{C}$  after  $12 \text{ } \mu\text{L L}^{-1}$   $\text{C}_2\text{H}_4$  application displayed lower leakage rates compared to the corollas treated with ethylene; however, direct application of 1-MCP (no pre-ethylene treatment) did not affected electrolyte leakage (Serek et al., 1995b).

Soluble solids concentration in either GRN or YLW 'Galia' fruit was not significantly affected by 1-MCP since muskmelon fruit types have little or no starch reserve (Seymour and McGlasson, 1993). The effects of 1-MCP upon TA and pH were minimal. 1-MCP caused slightly higher TA in YLW fruit while did not have a significant effect on GRN fruit. Higher TA due to 1-MCP application has been noted for tomato fruit at an advanced stage of ripening ( $5$  to  $100 \text{ } \mu\text{L L}^{-1}$  1-MCP for 2 h at  $20 \text{ }^\circ\text{C}$ ; Wills and Ku, 2002). 1-MCP had no influence upon pH of either GRN or YLW 'Galia' fruit. The color change of GRN 'Galia' fruit surface from green to yellow in YLW fruit were deferred by 1-MCP, which confirms that loss of chlorophylls and increase in carotenoids are ethylene-dependent process in melon fruit (Flores et al., 2001). The inhibitory effect of 1-

MCP upon color change or development has been reported for most climacteric fruits at an early stage of ripening (Golding et al., 1998; Jiang and Joyce, 2000; Jeong et al., 2002). Fruits treated at the advanced stage of ripening responded to 1-MCP by deferring color change/development as well such as 'Golden Delicious' apple at 4 °C (1 10  $\mu\text{L L}^{-1}$  1-MCP for 6 h at 20 °C; Jiang and Joyce, 2002) and tomato at 20 °C (50 to 150  $\text{nL L}^{-1}$  1-MCP for 2 h at 20 °C; Hoeberichts et al., 2002).

1-MCP significantly extended the edible stage of both GRN and YLW 'Galia' fruit by 40 and 80%, respectively. One of the affirmative effects of 1-MCP is the extended storage life for most fruits treated at the early stage of ripening (Hofman et al., 2001). Recently, apple (Mir et al. 2001; Pre-Aymard et al., 2002; Jiang and Joyce, 2002) and tomato (Wills and Ku, 2002; Hoeberichts et al., 2002) fruit at an advanced stage of ripening has been reported to respond to 1-MCP by improving their shelf life. Thus, over-ripening or senescence in climacteric fruits can be delayed by 1-MCP. Coriander leaf senescence, as assessed by chlorophyll and protein loss, was significantly delayed by 1-MCP (Jiang et al., 2002). Tucker and Brady (1987) and Smith et al. (1989) earlier reported that silver thiosulphate arrested tomato ripening once initiated. Thereby, climacteric fruit ripening from the early to the advanced stage of ripening necessitates ethylene.

In summary, 1-MCP extended storage and storage life of 'Galia' fruit at different stages of maturity. Therefore, the use of 1-MCP seems to be a novel postharvest application that has commercial potential for melon shippers, retailers and even consumers. The affirmative effects of 1-MCP upon ripe fruit would benefit for fresh-cut fruit industry as well. Our results demonstrate 'Galia' fruit was strongly benefited from 1-

MCP application; however, 'Galia' fruit does not represent all types of melon fruit. Thus, future studies are needed involving different melon types and cultivars.

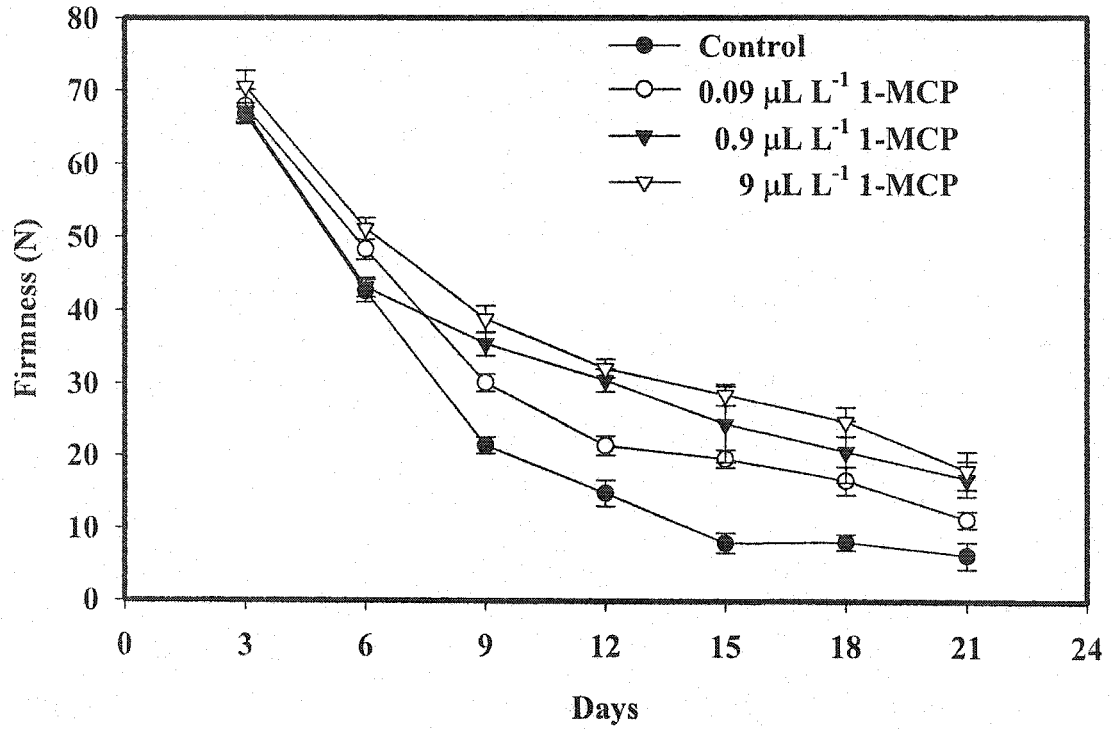


Figure 3-1. Unpared fruit firmness of green 'Galia' fruit treated with air (control), 0.09, 0.9 µL L<sup>-1</sup> and 9 µL L<sup>-1</sup> 1-MCP during storage at 15 °C. Vertical bars are standard deviations of means.



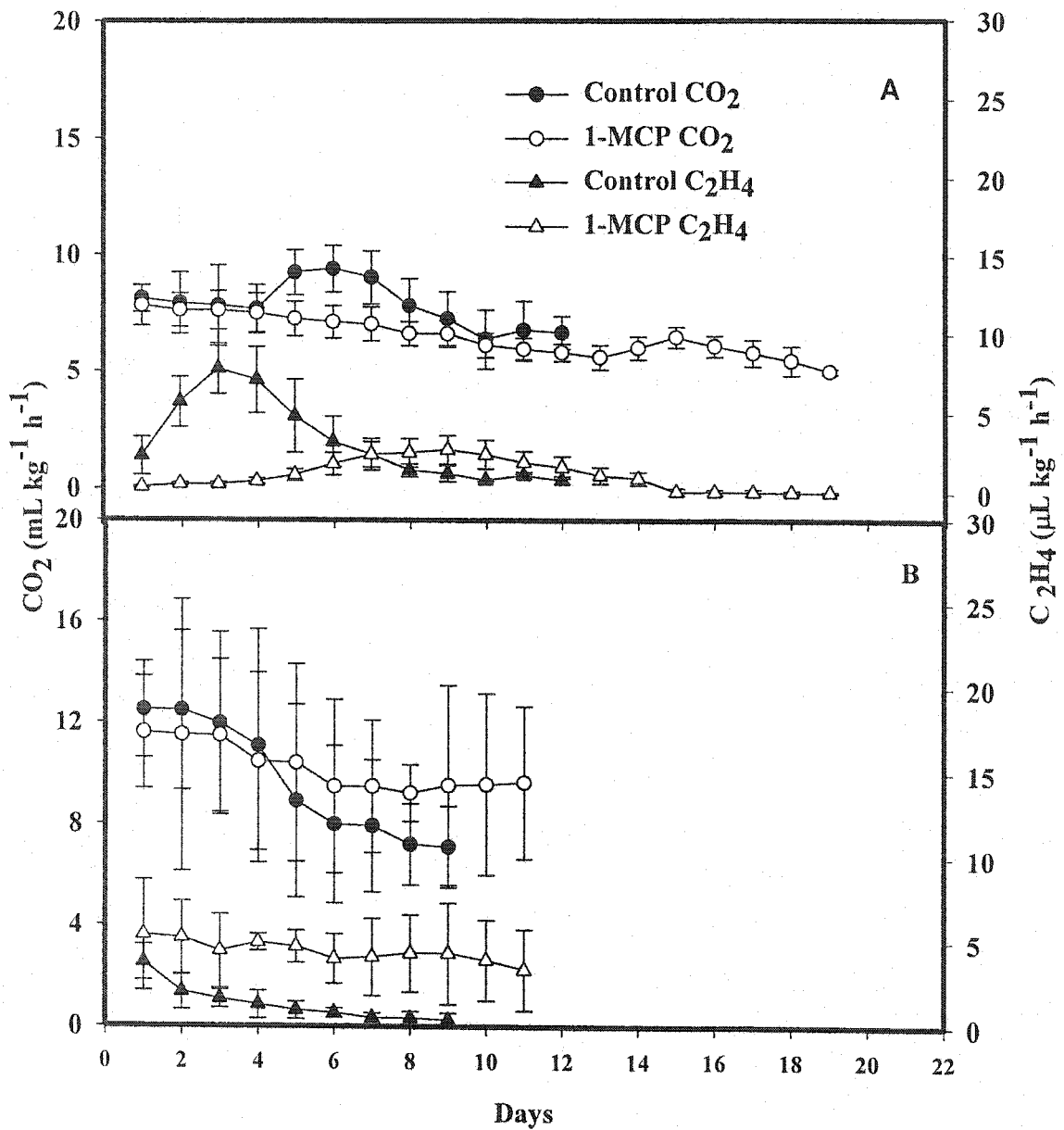


Figure 3-2. Respiration and ethylene production of green (A) and yellow (B) 'Galia' melon fruit treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP and air (control) during storage at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

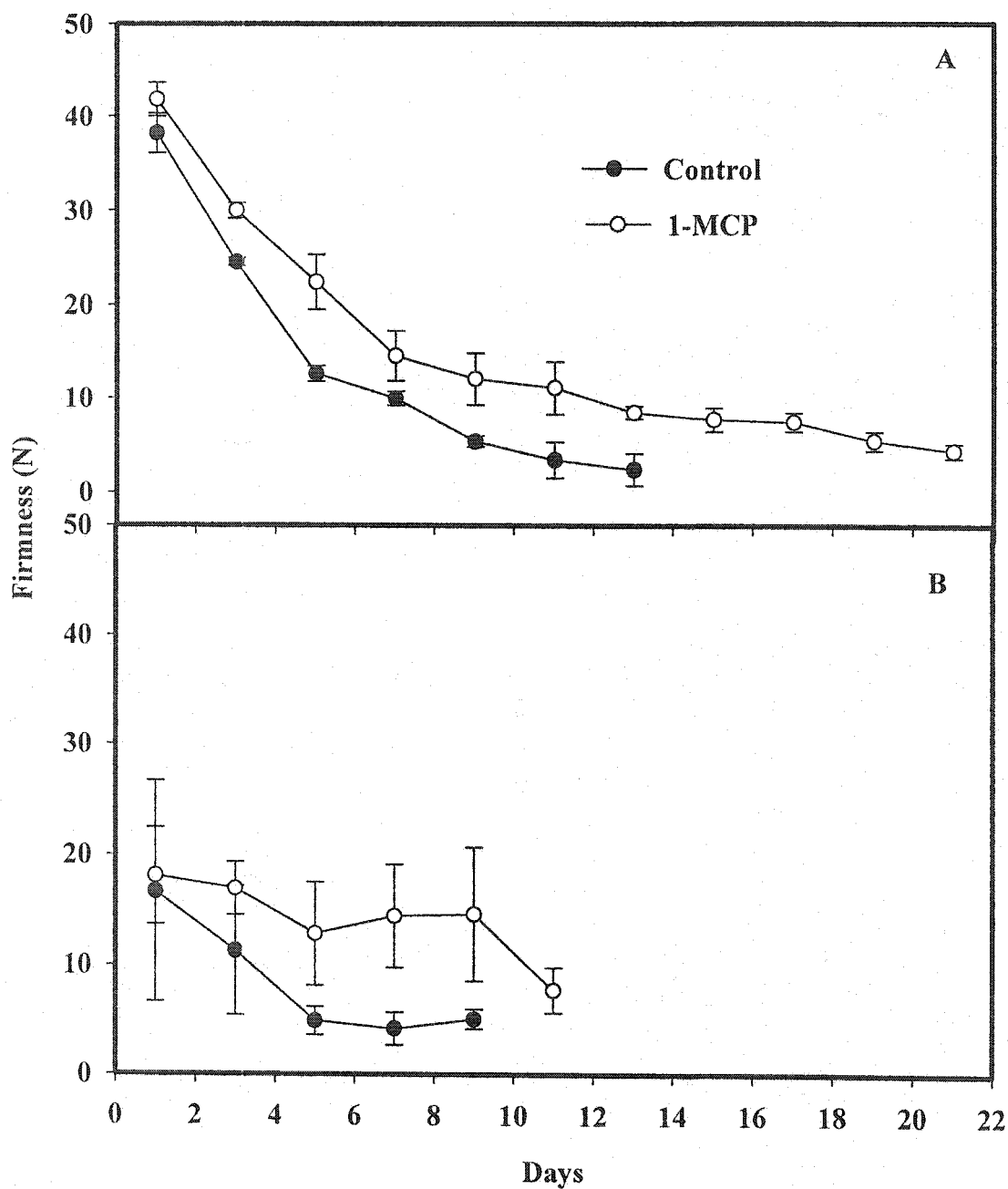


Figure 3-3. Mesocarp firmness of green (A) and yellow (B) 'Galia' melon fruit treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP and air (control) during storage at 20 °C. Vertical bars represent standard deviation of the means (n = 5).

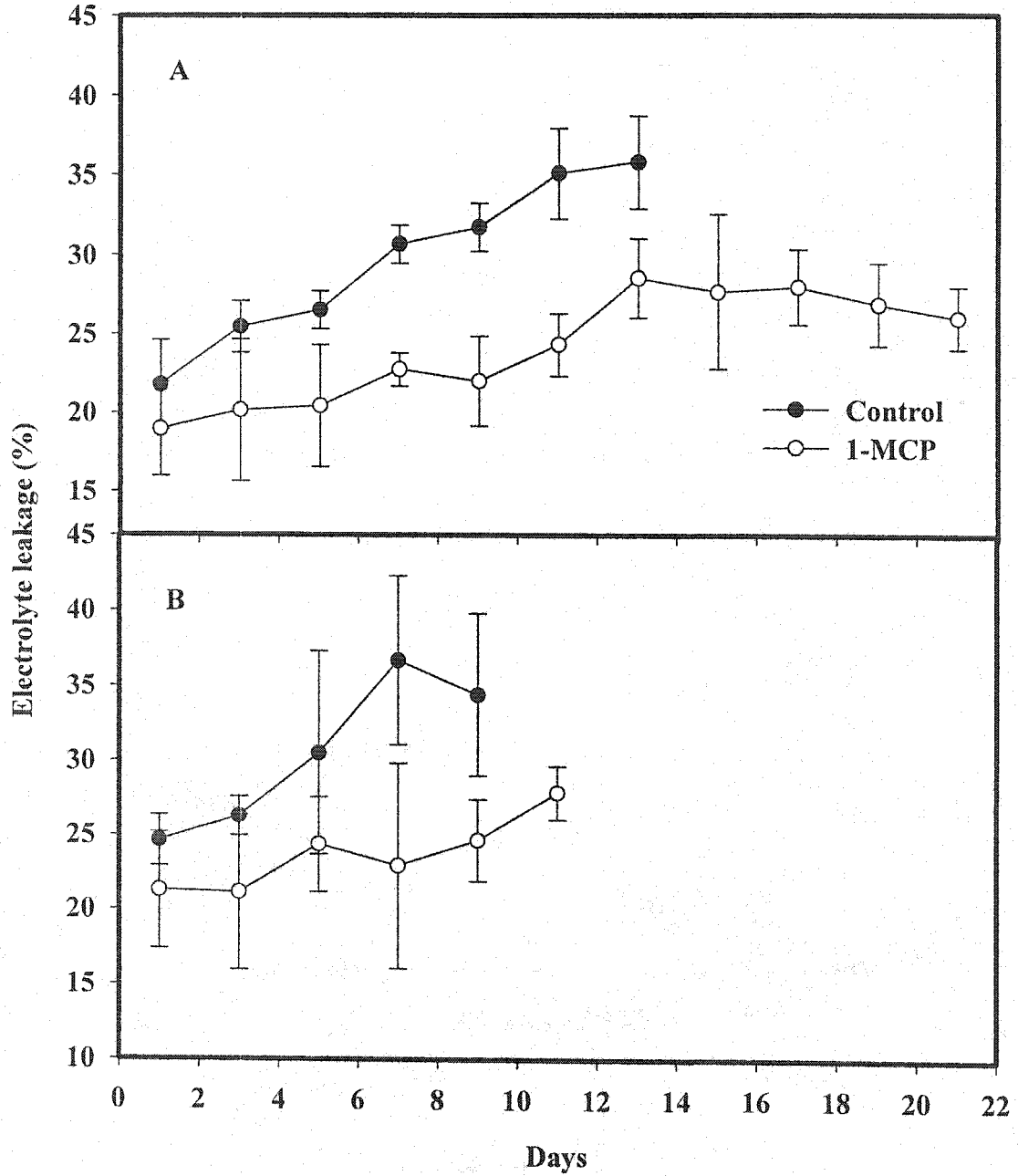


Figure 3-4. Electrolyte leakage of green (A) and yellow (B) 'Galia' melon fruit treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP and air (control) during storage at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

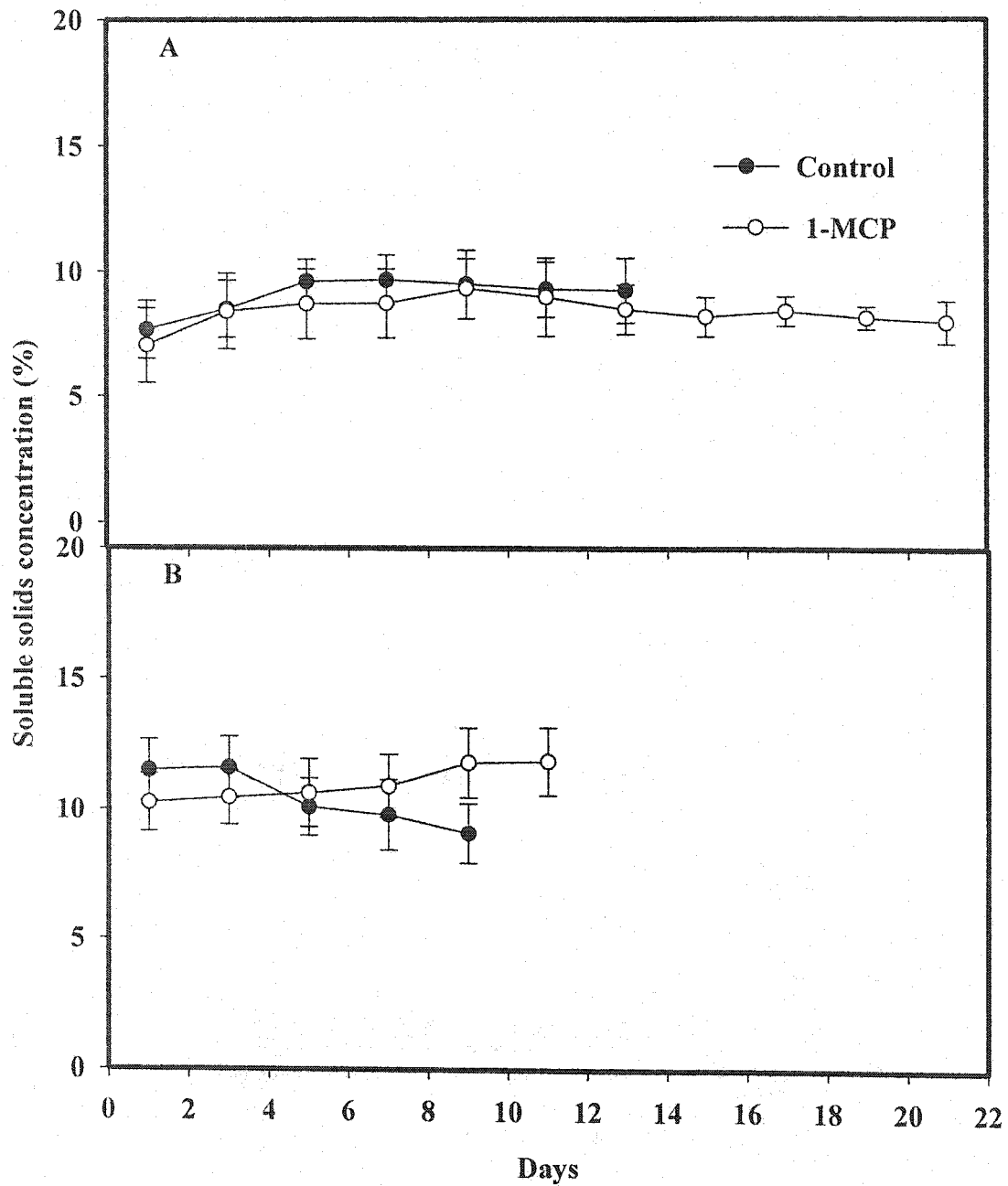


Figure 3-5. Soluble solids concentration of green (A) and yellow (B) 'Galia' melon fruit treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP and air (control) during storage at 20 °C. Vertical bars represent standard deviation of the means (n = 5).

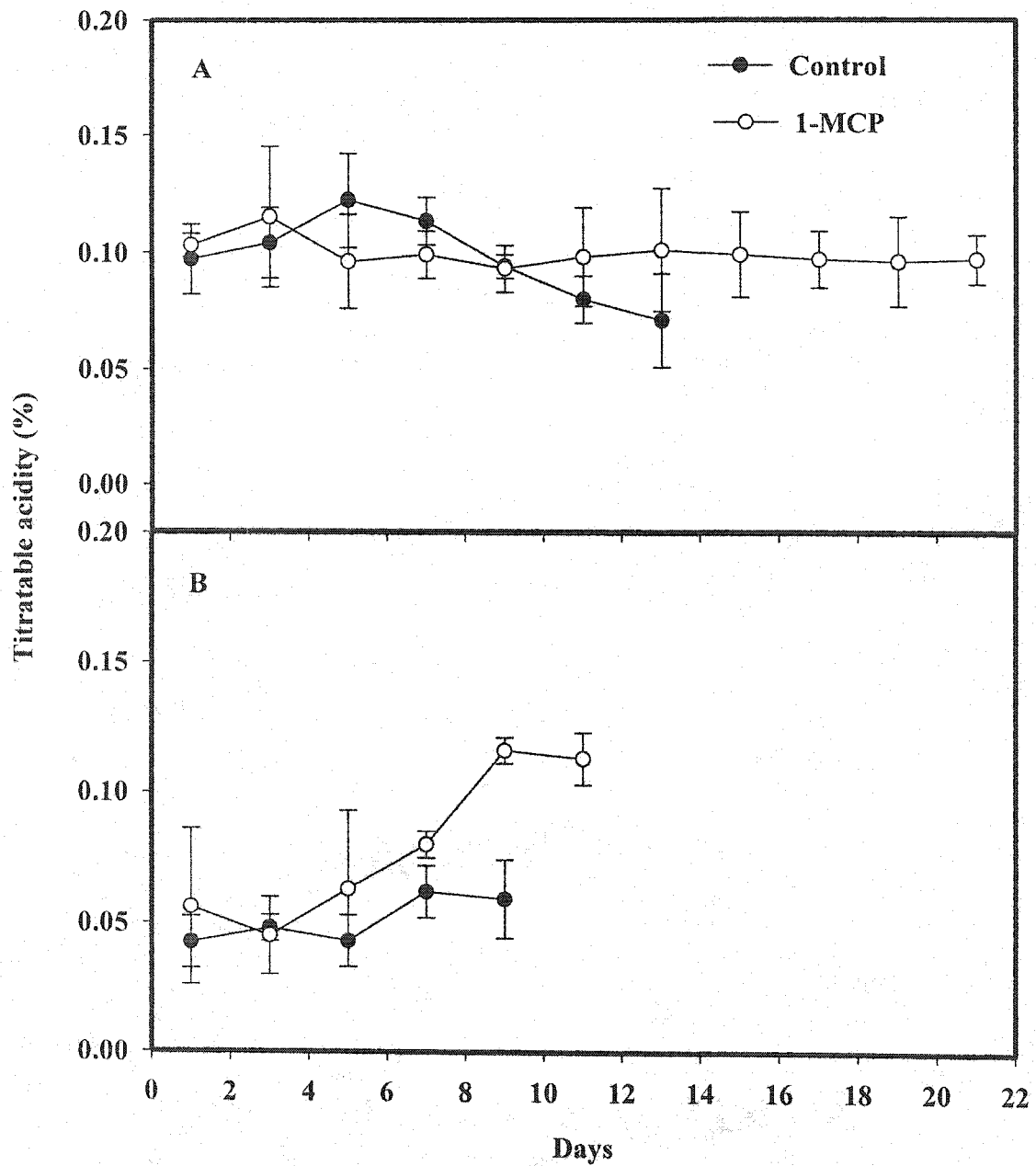


Figure 3-6. Titratable acidity of green (A) and yellow (B) 'Galia' melon fruit treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP and air (control) during storage at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

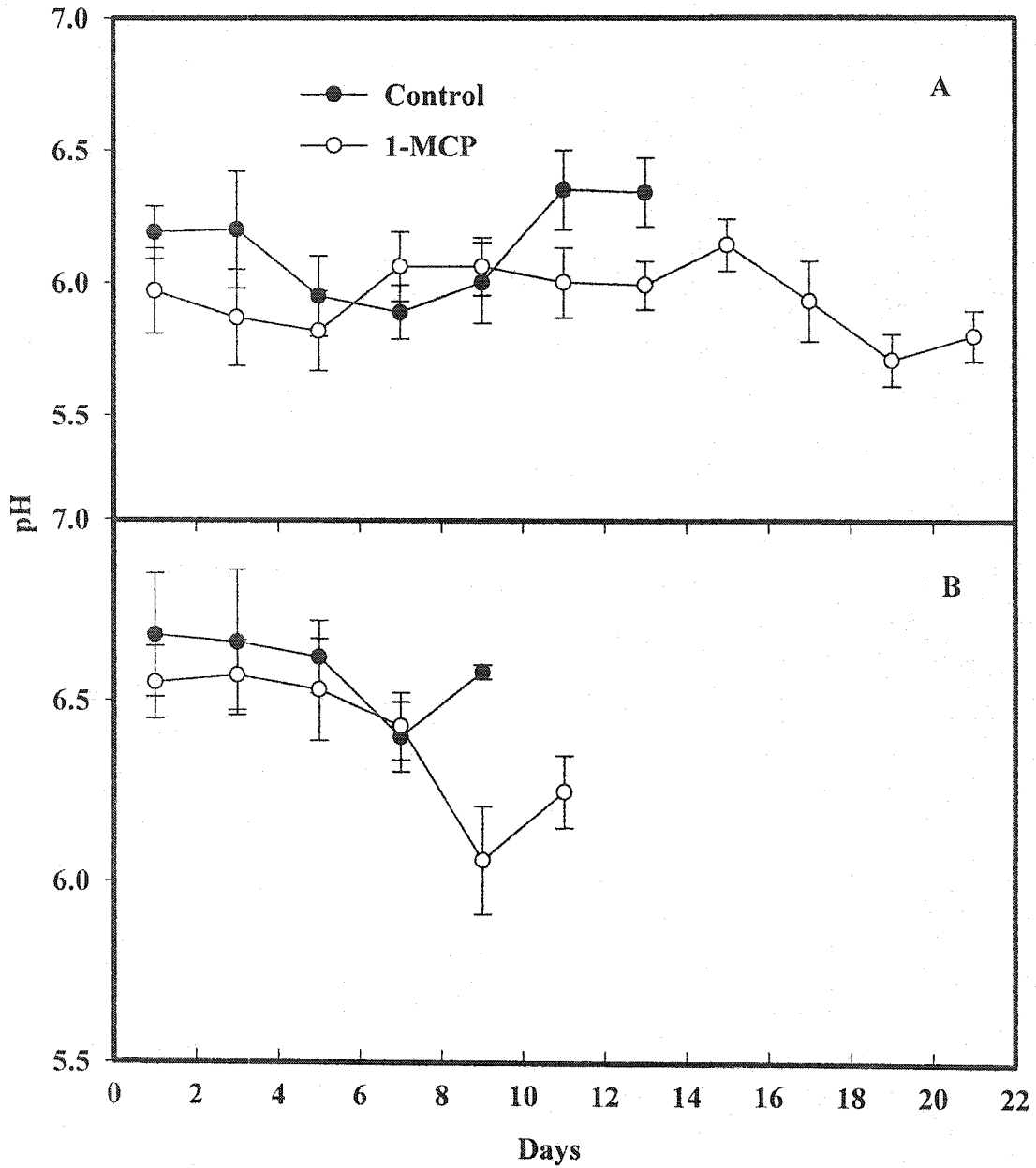


Figure 3-7. The pH of green (A) and yellow (B) 'Galia' melon fruit treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP and air (control) during storage at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

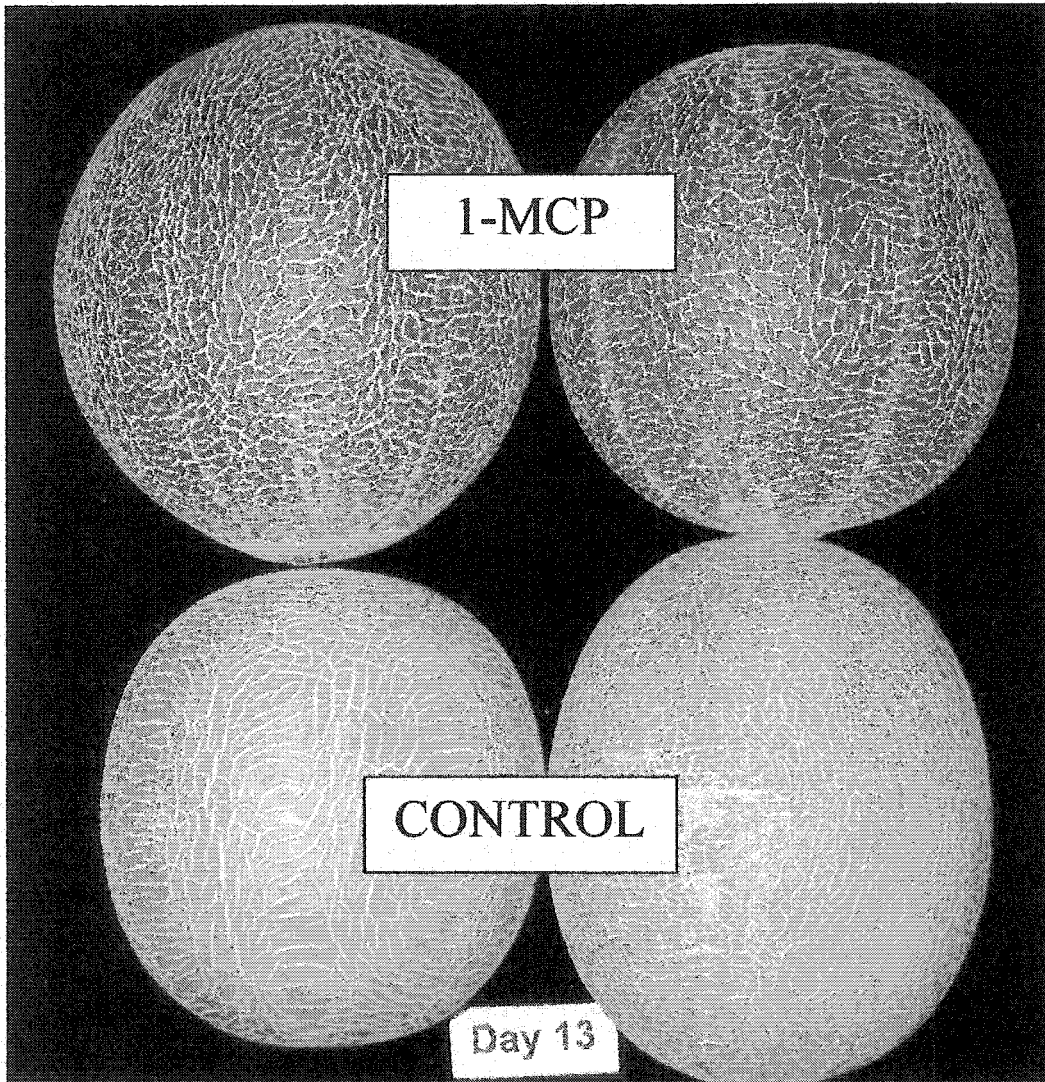


Figure 3-8. 'Galia' fruit harvested at the pre-ripe stage (green surface) were treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP or air (control) and then stored for 13 days at  $20^\circ\text{C}$ .

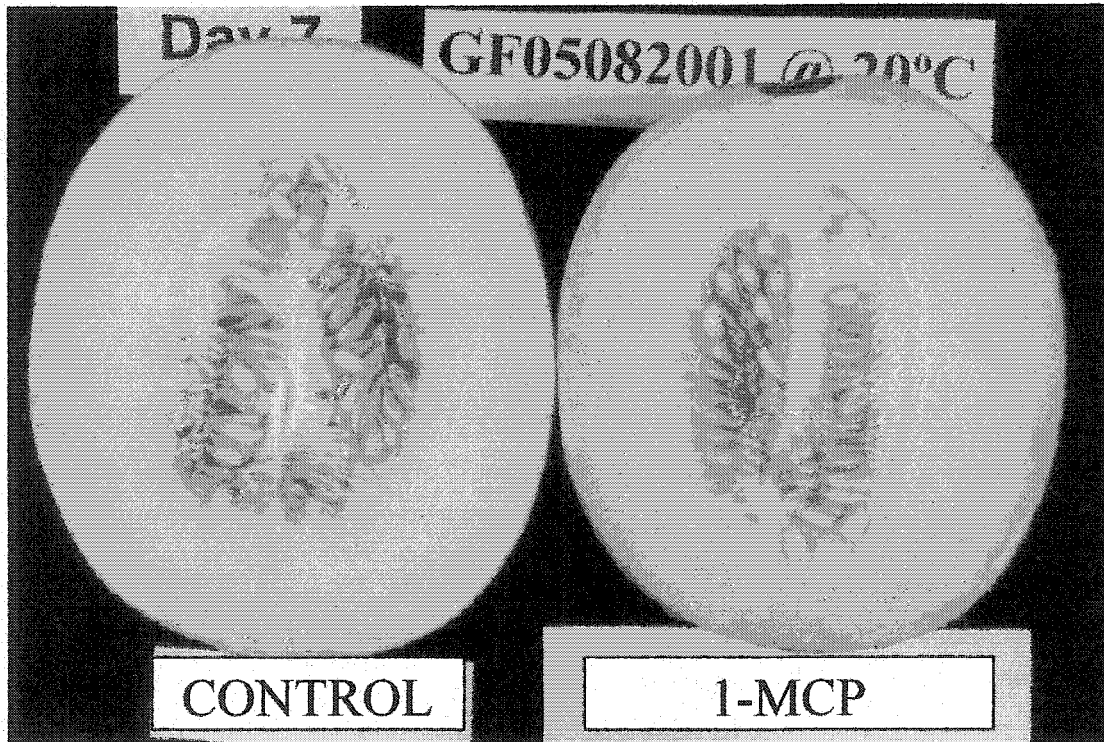


Figure 3-9. 'Galia' fruit harvested at the ripe stage (yellow surface) were treated with  $1.5 \mu\text{L L}^{-1}$  1-MCP or air (control) and then stored for 7 days at 20 °C.



CHAPTER 4  
PHYSIOLOGICAL CHANGES IN FRESH-CUT AND INTACT 'GALIA' MELON  
FRUIT WITH TREATED 1-METHYLCYCLOPROPENE

**Introduction**

The increase in consumer demand for fresh-cut produce has prompted increased research interest in devising and implementing methods for improving and prolonging the quality of these highly perishable products. Fresh-cut processing involves several steps including peeling, and cutting, shredding, etc. The physical injury attendant to fruit processing initiates a series of events such as increased respiration and ethylene production, stimulated phenol metabolism, and increased enzyme activities (Rolle and Chism, 1987; King and Bolin, 1989). The secondary events resulting from wounding contribute to the challenge for improving the keeping quality of fresh-cut produce. The storage life of fresh-cut commodities can also be compromised by the proliferation of microorganisms including mesophilic microflora, lactic acid bacteria, coliforms and fecal coliforms, yeasts and other fungi, and pectinolytic microflora (Nguyen-the and Carline, 1994). Low temperature has been used to preserve quality and extend storage life of fresh-cut produce. Although cold storage retards many biological processes in fresh-cut produce, events leading to tissue softening and deterioration continue at low temperature especially for fresh-cut fruits. Fresh-cut melon, one of the most popular fresh-cut fruit (International Fresh-cut Produce Association, 2003), is not an exception for these fresh-cut fruits displaying rapid tissue softening and deterioration (Lamikanra et al., 2000). Postharvest applications such as dipping fruit slices/cubes in dilute hypochlorite 50  $\mu$ L

L<sup>-1</sup> total available chlorine (pH 6) (Ayhan et al., 1997), in 2.5% calcium chloride solution (Luna-Guzman and Barrett, 2000), or storing in controlled atmosphere of 2% O<sub>2</sub> + 10% CO<sub>2</sub> at 5 °C and 4% O<sub>2</sub> + 10% CO<sub>2</sub> at 10 °C (Qi et al., 1998) have been reported to improve and extend storage life of fresh-cut melons. To date, no studies have been reported upon fresh-cut 'Galia' melon fruit while other melon types especially muskmelon and honeydew fruit have been often studied as fresh-cut produce. It has been reported that storage life of fresh-cut honeydew melon were limited to 11 days at 4 °C whereas storage life of fresh-cut muskmelon 4 to 6 days at 4 or 5 °C (O'Connor-Shaw et al., 1994; Qi et al., 1998).

'Galia' melon is a climacteric fruit (Seymour and McGlasson, 1993); thus, ripening process is ethylene-mediated. The ethylene-mediated effects on climacteric fruit can be significantly delayed by the use of ethylene binding inhibitors. One of these, 1-methylcyclopropene (1-MCP; Sisler and Serek, 1997) has been shown to extend the storage life and period of optimum quality of apple (Jiang and Joyce, 2002) and tomato (Wills and Ku, 2002; Ku et al., 2002) fruits at advanced stages of ripening. Exposure of fresh-cut postclimacteric apple fruit before or after cut to 1-MCP (1 or 10 µL L<sup>-1</sup> for 6 h at 20 °C) improved their storage life by reducing softening and color change of epidermal tissue (loss of green color) at 4 °C (Jiang and Joyce, 2002). Thus, we proposed that fresh-cut 'Galia' fruit should benefit from 1-MCP application as well. The objectives of this study were to investigate responses of fresh-cut ripe (derived from postclimacteric intact fruit subject to treatments) versus intact ripe 'Galia' fruit to 1-MCP.

## Materials and Methods

### Plant Material

'Galia' plants were grown according the growing techniques and production practices established by Shaw et al. (2001) in Greenhouse Facilities at the University of Florida Horticultural Farm near Gainesville, FL in spring 2002. Temperatures were recorded every 15 min at various locations in the greenhouse using thermocouples and a datalogger (CR-10 Campbell Scientific, Inc., N. Logan, UT). No additional heating or cooling units installed in the greenhouse. 'Galia' fruit were harvested at three-quarter to full-slip stage and transferred to the postharvest facilities at the University of Florida in Gainesville. The fruit were selected for uniform size (approximately 1200 to 1300 g), external color (yellow) and netting development. Ethylene production and respiration rate for the fruit at the time of harvest was approximately  $2 \mu\text{L kg}^{-1} \text{h}^{-1}$  and  $10 \text{ mL kg}^{-1} \text{h}^{-1}$  at  $20 \text{ }^\circ\text{C}$ , respectively, and soluble solids concentrations were about 11 to 12%. The fruit were gently washed with tap water, immersed in  $200\text{-}\mu\text{L L}^{-1}$  chlorinated water for 1 min ( $23 \text{ }^\circ\text{C}$ ), and air-dried before transferring to  $20 \text{ }^\circ\text{C}$  for 1-MCP application.

### 1-MCP Quantification and Treatment

The source of 1-MCP was Agrofresh commercial powder (active ingredient 0.14% 1-MCP) from Agrofresh, Philadelphia, PA. Three g powder were dissolved in 50-mL deionized water of a 136-mL vial; afterwards, the vial was sealed with a septum and incubated on oscillating shaker for 2 h at room temperature. 1-MCP concentration in the vial headspace was measured using a gas chromatograph (Hewlett Packard-5890, Avondale, PA) equipped with a 80-100 mesh Chromosorb PAW stainless steel column ( $1.8 \text{ m} \times 3.18 \text{ i.d.}$ ; Supelco, Bellefonte, PA) at an injector, oven and detector (FID) temperature of 150, 150 and  $200 \text{ }^\circ\text{C}$ , respectively. Isobutylene gas was used as standard to

calculate 1-MCP concentration (Jiang et al., 1999). Approximately 7.5 mL L<sup>-1</sup> 1-MCP stock in the headspace of the vial was generated from the 3-g powder. Vial-headspace gas sample (7.5 mL) was injected into a 174-L metal chamber having a 56.5-L void volume, yielding a final 1-MCP concentration of 1 µL L<sup>-1</sup>, and maintained for total exposure period of 24 h at 20 °C. The metal chamber was vented for 5 min at 6-h intervals and reinjected with fresh 1-MCP avoid CO<sub>2</sub> accumulation. Control fruit was kept under similar condition.

### **Preparation of Fresh-cut 'Galia', and Treatment Design**

The fruit were transferred from 20 °C to a 5 °C facility that had been sanitized using 200 µL L<sup>-1</sup>-chlorinated water prior to use. After a 1-h period to allow temperature equilibration, the blossom and pedicle ends of the fruit were removed, and the fruit were longitudinally (from the pedicel and to the stem end) cut into 2.5 cm slices using a plastic Bread Slicer (Cuope-Pain). The slices were peeled and cut into cubes (2.5 x 2.5 x 2.5 cm<sup>3</sup>, 15 to 16 g) using a double bladed knife. The cubes were then flushed with a sterile isotonic mannitol solution (500 mM) using a squeeze bottle, and placed in non-airtight plastic containers (1.7 L, FridgeSmart) that has built-in grid on the bottom lifts (9 cubes/container). A total of 60 containers (30 each for 1-MCP and control fresh-cut tissue) were used in this experiment, and 10 of these (5 each of each treatment) were removed at 2-day intervals for quality evaluation. Additionally, 80 intact fruit (40 each of control and 1-MCP-treated fruit) were stored along with the fresh-cut tissue at 5 °C. The 4 treatments included, fresh-cut tissue derived from intact ripe fruit pre-treated with air (FC-CNT: fresh-cut control), fresh-cut tissue derived from intact ripe fruit pre-treated with 1-MCP (FC-MCP: fresh-cut 1-MCP), intact ripe fruit pre-treated with air (IF-CNT:

intact fruit control), and intact ripe fruit pre-treated with 1-MCP (IF-MCP: intact fruit 1-MCP).

### **Ethylene Analysis**

Ethylene production was measured at room temperature every other day enclosing fresh-cut and intact fruit in plastic containers (0.9 L and 3.6 L, respectively) allowing ethylene to accumulate for 2 h at 2-day interval at 5 °C. Nine cubes per fruit for FC-CNT or FC-MCP and 1 fruit for IF-CNT or IF-MCP were placed in the airtight containers prior to sampling. A 1-mL headspace sample was withdrawn by a hypodermic syringe through a rubber septum, ethylene production was measured using a GC (Hewlett Packard 5890 II, Avondale, PA) equipped with a flame ionization detector. The carrier gas (Nitrogen) was 30 mL min<sup>-1</sup>. Oven, injector and detector temperature was 70, 200 and 250 °C, respectively.

### **Firmness Assessment**

Mesocarp firmness of a fruit cube was measured using an Instron Universal Testing Instrument (Model 4411, Canton, MA) equipped with a 5-kg load cell and an 8-mm convex probe at 20 °C. During firmness measurement, intact fruit or fresh-cut fruit containers were kept coolers. Intact fruit prior to firmness measurements were diced into cubes using the procedures described above for fresh-cut processing. The probe was positioned at zero force contact with a fruit cube surface, and driven to a depth of 10 mm at a crosshead speed of 50 mm min<sup>-1</sup>. Firmness data are reported as the maximum force (Newton) recorded during penetration.

### **Electrolyte Leakage**

Mesocarp disks (5 discs per cube or fruit), in 8 mm diameter and 8 mm thickness, were removed from centermost part of either fresh-cut tissue or intact fruit with an 8-mm

diameter cork borer, rinsed with deionized water, blotted dry, and incubated in 15 mL of 500 mM mannitol for 1 h in capped polypropylene tubes. Incubations were conducted at room temperature, on an oscillating shaker set at 1.4 cycle sec<sup>-1</sup>. The conductivity of the bathing solution was measured at the end of the 1-hour incubation using a conductivity bridge (YSI-31A, Yellow Springs, OH) equipped with a conductivity cell (Model 3403, Yellow Springs, OH). The aliquot removed for the conductivity measurement was added back to the bathing solution. The disks and bathing solution were then stored -20 °C for at least 24 h, thawed, and heated in a boiling water bath for 30 min, cooled to room temperature, and conductivity again measured. Electrolyte leakage was expressed as a percent of total conductivity estimated from the frozen/heated samples.

### **Pectin Efflux**

Five mesocarp cylinders were removed from the mid section of fresh-cut tissue or an equatorial section of an intact fruit using a 15-mm diameter cork borer. The cylinders were then sliced into disks perpendicularly from the long axis of a cylinder using a double bladed razor in which a razor was mounted from the other razor by 10 mm. Each disk, 15 mm diameter and 10 mm thick, weighed approximately 5 g. The disks (5 per replication) were briefly rinsed with distilled water, blotted dry, and incubated in 10 mL of 500 mM sucrose on an oscillating shaker for 6 h at room temperature. Afterwards, the bathing solution was filtered through a Whatman GF/C filter, and 40 mL of 95% ethanol was added to the filtrate. The filtrates were centrifuged at 2,000 g for 20 min at 4 °C and the supernatant was discarded. The pellet was washed with 40 mL of 80% ethanol (2 times) and dissolved in 5 mL distilled water, and total uronic acid was determined by the *m*-phenylphenol method (Blumenkrantz and Asboe-Hansen, 1973).

### **Quality Evaluation**

Fruit surface and flesh color were measured using a chromameter (Minolta-CR-200, Japan). The results were presented as lightness ( $L^*$ , representing the lightness or grey scale), hue angle (the dimension of color that specifies a position on a color wheel of 360°, with 0°, 90°, 180° and 270° representing the hues red, yellow, green and blue, respectively) and chroma (distinguishing the difference from a grey shade to a pure hue) values. Water soaking on the flesh was expressed as the percentage of areas of a cube with a 5% interval (total 5 cubes for each treatment) using only the top face. Five intact fruit or five fresh-cut cubes from each treatment were evaluated for mesocarp water soaking. Informal descriptive analysis was used to profile the quality of either fresh-cut cubes and intact fruit flesh by untrained personnel, evaluating appearance, odor, texture and flavor (O'Connor-Shaw et al., 1994) according to the following hedonic chart: 1, poor; 2, poor-good; 3, fair; 4, good-excellent; and 5, excellent. The informal descriptive analyses were done immediately after removing fresh-cut or intact fruit from the 5-°C cold room at days 0, 2, 4, 6, 8 and 10. The samples were tested under white light and at the room temperature, 23 °C, with testing at least 3 samples for each treatment.

### **Microbial Counts**

Fruit tissue (5 g) was removed with a flame-sterilized cork borer (21.5 mm diameter) and knife from innermost part of a fruit or with a flame-sterilized knife from a fruit cube (approximately 1/3 of a cube) on sterilized aluminum foil in an air-circulated fume. The fruit tissue then was incubated in a 45-mL sterile phosphate buffered solution (PBS), pH 7. The bathing solution and fruit tissue were vortexed at high speed using a vortex (Fisher-Genie 2, Scientific Industries Inc., Bohemia, NY) for 1 min. Afterward, a series of dilutions were prepared using sterile PBS as needed. Total aerobic,

Enterobacteriaceae, yeasts and other fungi, total coliforms, and lactic acid bacteria counts were made using 1 mL inoculum of the bathing solution. The plates and incubation conditions for each count were: total aerobic count, 3M Petrifilm aerobic count plate (3M Microbiology Products, St. Paul, MN), 3 days at 30 °C; Enterobacteriaceae, 3M Petrifilm Enterobacteriaceae count plate, 1 day at 30 °C; yeast and other fungi, 3M Petrifilm yeast and mold count plate, 5 days at 25 °C; total coliforms, 3M Petrifilm coliform count plate, 1 day at 30 °C; and lactic acid bacteria, 3M Petrifilm aerobic count plate anaerobic incubation for 2 days at 30 °C in a 1.9-L airtight plastic container with an anaerobic system envelope (Gas Pak, Becton and Dickinson Co., Cockeysville, MD). The plates were prepared in an air-circulated hood after 0 (immediately after dicing), 5 and 10 days at room temperature, and microbial counts were reported as colony forming units per gram of tissue (CFU g<sup>-1</sup>).

#### **Experimental Design and Statistics**

The experiments were conducted in a randomized complete-block design, using 3 to 5 replications per treatment. Statistical procedures were performed using the PC-SAS software package. Differences between means were determined using the Duncan Mean Comparison Test.

### **Results and Discussion**

#### **Ethylene Production**

Ethylene production of fresh-cut cubes of ripe 'Galia' fruit stored at 5 °C (FC-CNT, 10.4 µL kg<sup>-1</sup> h<sup>-1</sup>; FC-MCP, 8.5 µL kg<sup>-1</sup> h<sup>-1</sup>) was at least 4-fold higher than that of intact fruit (IF-CNT, 1.9 µL kg<sup>-1</sup> h<sup>-1</sup>; IF-MCP, 1.6 µL kg<sup>-1</sup> h<sup>-1</sup>) at day 1 (Figure 4-1).

Ethylene production of both fresh-cut fruit and intact fruit declined during storage; however, intact fruit displayed statistically lower ethylene production and declining rates



relative to fresh-cut fruit on days 1 through 7. Ethylene production declined approximately 5-fold in FC-CNT (from 10.3 to 2.1  $\mu\text{L kg}^{-1} \text{h}^{-1}$ ) and 4-fold in FC-MCP (from 8.5 to 1.9  $\mu\text{L kg}^{-1} \text{h}^{-1}$ ) over a 9-day period. Ethylene production in intact fruit declined approximately 2 to 3 folds in both IF-CNT (from 1.9 to 0.8  $\text{kg}^{-1} \text{h}^{-1}$ ) and IF-MCP (from 1.6 to 0.6  $\text{kg}^{-1} \text{h}^{-1}$ ). Generally, ethylene production of intact ripe 'Galia' fruit was under 2  $\mu\text{L kg}^{-1} \text{h}^{-1}$  at 5 °C, which is similar to the case for ethylene production rate of intact ripe or postclimacteric muskmelon fruit held at 5 °C (1 to 3  $\mu\text{L kg}^{-1} \text{h}^{-1}$ ; Luna-Guzman et al., 1999). These authors, however, reported slightly higher ethylene production rates in intact ripe muskmelon fruit (approximately from 1 to 3  $\mu\text{L kg}^{-1} \text{h}^{-1}$ ) compared with fresh-cut ripe muskmelon fruit (approximately from 0.5 to 2  $\mu\text{L kg}^{-1} \text{h}^{-1}$ ) during 12 days of storage. Fresh-cut 'Galia' fruit in this study had higher ethylene production rates than intact 'Galia' fruit, likely due to both the wound response, stress-related ethylene production in wounded cells (Rolle and Chism, 1987), and increased surface area exposed to the atmosphere after dicing, facilitating oxygen diffusion to interior cells (Zagory et al., 1995). The decrease in ethylene production in intact and fresh-cut ripe 'Galia' fruit during storage is in agreement with the findings of Luna-Guzman et al. (1999), who reported that intact or fresh-cut ripe muskmelon fruit stored at 5 °C exhibited declining ethylene production rates during the first 7 days of storage.

In the present study, the effect of 1-MCP on ethylene production was insignificant though some small differences were noted at day 1 between IF-CNT and IF-MCP. However, fresh-cut postclimacteric 'Golden Delicious' apple fruit treated with 1-MCP (1 or 10  $\mu\text{L L}^{-1}$  for 6 h at 20 °C) before or after cutting had lower ethylene production relative to non-1-MCP-treated fruit (fruit sample sealed in glass jars at 20 °C) after 5 or

10 days of storage (Jiang and Joyce, 2002). The low ethylene production of fresh-cut and intact 'Galia' fruit at 5 °C may be due to the low storage temperatures employed and/or the inherently low ethylene production of 'Galia' fruit relative to other muskmelon types such as both preclimacteric 'Giant Perfection' and 'Iroquois' that, have ethylene rate of over 80  $\mu\text{L kg}^{-1} \text{h}^{-1}$  (Zheng and Wolf, 2000). In contrast, the ethylene production rate of intact ripe 'Galia' fruit is under 6  $\mu\text{L kg}^{-1} \text{h}^{-1}$  during ripening at 20 °C (Chapter 3).

### **Firmness Assessment**

As shown in Figure 4-2, both fresh-cut and intact ripe 'Galia' fruit regardless of 1-MCP softened moderately during storage. IF-CNT and FC-CNT lost about 31% and 22% of their original firmness, respectively, after 10 days while IF-MCP and FC-MCP softened 16% and 11%, respectively. At day 10, IF-MCP (10.6 N) had 22% higher firmness compared to IF-CNT (8.7 N), and FC-MCP (11.6 N) had 32% higher firmness than FC-CNT (8.5 N). Firmness loss in either fresh-cut tissue derived from ripe 1-MCP-treated fruit, or in intact ripe fruit treated with 1-MCP was significantly lower compared with either fresh-cut ripe control or intact ripe control fruit during storage, proving that melon fruit softening is an ethylene-dependent process (Flores et al., 2001). Recent studies have shown that 1-MCP can delay softening of climacteric fruits when applied at advanced stages of ripening. The deferred softening has been shown for apple (1 or 10  $\mu\text{L L}^{-1}$  1-MCP for 6 h at 20 °C; Jiang and Joyce, 2002) and tomato (50 to 150  $\text{nL L}^{-1}$ , for 24 h at 20 °C; Hoeberichts et al., 2002). Additionally, fresh-cut postclimacteric 'Golden Delicious' apple fruit treated with 1-MCP (1 and 10  $\mu\text{L L}^{-1}$  for 6 h at 20 °C) before or after cutting showed significant firmness retention relative to fresh-cut control fruit after 5 or 10 days at 4 °C (Jiang and Joyce, 2002).

### **Electrolyte Leakage**

Electrolyte leakage, a estimate of membrane permeability and integrity (Marangoni et al., 1996), slightly increased in both intact ripe and fresh-cut ripe 'Galia' fruit during storage (Figure 4-3). The increase in leakage of both IF-CNT (from 13.1% to 14.3%) and IF-MCP (from 12.3% to 14.1%) was below 15% on days 0 through 10 while the increase FC-CNT (from 14.9 to 20.7) was 38% and in FC-MCP (from 14.24 to 18) was 26%. 1-MCP did not suppress the leakage increase in either intact or fresh-cut fruit. However, intact ripe 'Galia' fruit treated 1-MCP ( $1.5 \mu\text{L L}^{-1}$ ) displayed slightly lower leakage during a limited time (on days 7 through 9) compared with intact ripe control at 20 °C (chapter 3). Fresh-cut ripe 'Galia' fruit showed slightly higher leakage compared with intact ripe fruit during storage at 5 °C, which supports the fact membrane permeability increases in response to wounding (Portela and Cantwell, 2001). Membrane permeability is a common feature of senescing organs and ripening fruit (Lester and Stein, 1993; Flores et al., 2001). Increased membrane permeability results in a loss of cellular components and accumulation of liquid in intercellular spaces (Saltveit, 1997). Similar to fresh-cut ripe 'Galia' fruit, increased leakage (measured at 22 °C) for fresh-cut climacteric/postclimacteric muskmelon fruit (approximately a 15% increase) was reported by Portela and Cantwell (2001) on days 0 through 12 stored at 5 °C. Portela and Cantwell (2001) further noted that fresh-cut muskmelon discs prepared by a blunt cork borer caused slightly higher leakage (22%) than fresh-cut fruit prepared by a sharp borer since the blunt borer resulted in more severe damage in tissues relative to the sharp borer.

### **Pectin Efflux**

Pectin efflux values from mesocarp disks of both FC-CNT ( $18.8 \text{ g kg}^{-1}$  fresh weight) and FC-MCP ( $19.1 \text{ g kg}^{-1}$  f.w.) were 2-fold higher than those of IF-CNT ( $9.1 \text{ g}$

kg<sup>-1</sup> f.w.) or IF-MCP (9.3 g kg<sup>-1</sup> f.w.), respectively, at day 2, and the efflux remained over 2 folds throughout the remainder of storage (Figure 4-4). Pectin efflux from mesocarp disks of FC-CNT increased by 25% and that of FC-MCP by 22% on days 2 through 4; afterwards, the efflux decreased by 15% in FC-CNT and by 11% in FC-MCP through day 10 compare to the values at day 2. However, efflux from mesocarp disks of both IF-CNT and IF-MCP did not change during storage, with having the approximate average efflux of 9.3 g kg<sup>-1</sup> f.w. Pectin efflux from mesocarp disks was unaffected by prior 1-MCP treatment in either fresh-cut or intact ripe 'Galia' fruit; however, pectin efflux increased significantly in fresh-cut ripe fruit relative to intact ripe fruit, indicating that pectin degradation may be affected by wounding. The insignificance of 1-MCP upon pectin efflux was reported for pericarp disks of ripe tomato fruit treated with 1-MCP (4.8 μL L<sup>-1</sup> for 24 at 18 °C) and stored for 2 to 3 weeks at 5 °C (Almeida, 1999). The wounding effect upon pectin degradation was noted for fresh-cut ripe papaya fruit by Karakurt and Huber (2003), who reported that the levels of water and CDTA-soluble polyuronides increased significantly within 24 h after cutting and remained higher (over 50%) compared to intact ripe fruit during 8 days of storage at 5 °C. Higher uronic acid efflux in fresh-cut 'Galia' fruit may be the result of increased activities of cell wall enzymes including polygalacturonase and β-galactosidase. (Miller et al., 1987; Karakurt and Huber, 2003). Recently, Moctezuma et al. (2003) reported the TBG4 (tomato β-galactosidase) gene was up-regulated by ethylene while the TBG6 gene was down-regulated by ethylene in ripe tomato fruit. Additionally, 1-MCP (1 μL L<sup>-1</sup> for 12 h at 20 °C) has been shown to decrease the activity of pectinmethylesterase, α-mannosidase and β-glucosidase in ripe 'SanCastrese' apricot fruit at 20 °C (Botondi et al., 2003).

## Quality Evaluation

No changes in color parameters of hue angle and L\* (lightness) of the skin of both IF-CNT and IF-MCP were noted during storage (Figure 4-5A and 5B), indicating that the original skin color of intact fruit regardless of 1-MCP remained unchanged. However, the skin color intensity became duller during storage, as evaluated by the development of chroma value (Figure 4-5C). Flesh color and intensity/purity of either FC-CNT or FC-MCP remained unchanged during storage, as estimated by L\*, hue angle and chroma (Figure 4-5D, 5E and 5F). Flugel and Gross (1982) observed relatively low levels of chlorophyll and carotenoids in the flesh of 'Galia' fruit with a gradual decrease in both types of pigment during ripening, which might explain the insignificant color change and insignificant variation among treatments noted here.

Water soaking in both FC-CNT and FC-MCP 'Galia' fruit increased with storage while the flesh of either IF-CNT or IF-MCP showed no sign of the disorder (Figure 4-6A). The extent of water soaked areas in FC-CNT was 44% after 10 days while that of FC-MCP was only 15%, leading a significant difference between these treatments from day 8 to 10. All 4 treatments had excellent quality at day 0, as assessed by sensory evaluation (Figure 4-6B). The score for sensory of IF-CNT or IF-MCP never dropped below 5 (excellent) during storage while FC-CNT scored 2.8 (poor-good to fair) at day 10 (Figure 4-8). On days 4 through 8 the sensory scores for FC-CNT fruit were below 4 (fair to good-excellent) whereas that of FC-MCP fruit were over 4 (good-excellent to excellent), which indicates the limit of acceptability, the average keeping quality, of fresh-cut ripe 'Galia' fruit was 4 days (Figure 4-7) at 5 °C while that of fresh-cut ripe fruit derived from intact ripe 1-MCP-treated fruit was 8 days at 5 °C.

Changes in either mesocarp or fruit skin color of 'Galia' melon fruit is due a gradual decrease in both chlorophyll and carotenoid content during ripening (Flugel and Gross, 1982). The color data 'Galia' fruit during storage showed no color changes (based on hue angle) in either fruit skin or mesocarp were recorded regardless of treatment, which may indicate ripening progress was deferred by the low temperature, 5 °C. Water soaking of fresh-cut 'Galia' fruit increased with storage time and water soaking of fresh-cut fruit was significantly higher than those of intact 'Galia' fruit on days 6 through 10. 1-MCP did not have a significant effect on water soaking of fresh-cut 'Galia' fruit during most of the storage period but significantly suppressed the increase in water soaking noted during the period from 8 through 10 days. In contrast, 1-MCP (1  $\mu\text{l L}^{-1}$  for 24 h) did not affect water soaking in Charentais melon flesh, treated at the preclimacteric stage, after 35 days of storage at 14 °C (Chatenet et al. 2000). The authors further reported that water soaking in Charentais melon mesocarp during the late stage of ripening was not an ethylene-inducible event based on the absence of expression of genes encoding 1-aminocyclopropane-1-carboxylic acid synthase and 1-aminocyclopropane-1-carboxylic acid oxidase. Water soaking in fresh-cut 'Galia' fruit, however, seems to be an ethylene-dependent process which may be related to the stage of ripeness and/or wounding. Chatenet et al. (2000) attributed the water soaking phenomenon to a depletion of cell wall calcium and Karakurt and Huber (2002) to ethylene-inducible changes in membrane permeability. Lipolytic enzymes including lipoxygenase and phospholipase D are like involved in wound-induced degradation of membrane lipids (Karakurt and Huber, 2003). Lipoxygenase can contribute to the membrane permeability by involving production of

reactive oxygen species, participating in peroxidative reactions (Huber et al., 2001), and inactivating protein synthesis (Karakurt and Huber, 2003)

### Microbial Counts

Total aerobic load in fresh-cut ripe 'Galia' fruit increased sharply during days 5 to 10 of storage at 5 C, with FC-CNT having higher counts ( $1.8 \times 10^3$  CFU g<sup>-1</sup>) than FC-MCP ( $1.5 \times 10^3$  CFU g<sup>-1</sup>) at day 10 (Table 4-1). Total aerobic population of intact ripe fruit were very low compared to fresh-cut fruit and showed a very slim increase through day 10 (Table 4-1). Total coliforms or other fungi in either fresh-cut or intact fruit were negligible throughout storage (Table 4-1). The Enterobacteriaceae population in FC-CNT and FC-MCP increased significantly during storage, and FC-CNT showed statistically higher counts ( $8.7 \times 10^1$  CFU g<sup>-1</sup>) than FC-MCP ( $5.2 \times 10$  CFU g<sup>-1</sup>) at day 10 (Table 4-1). Enterobacteriaceae were almost undetectable in intact fruit over the 10-day storage period (Table 4-1). There was also an increase in lactic acid bacteria of both fresh-cut and intact fruit; however, fruit with 1-MCP (IF-MCP) or derived 1-MCP-treated fruit (FC-MCP) displayed higher counts compared to fruit without 1-MCP at day 10 (IF-CNT and FC-CNT; Table 4-1). Yeasts accumulated significantly in fresh-cut ripe fruit on days 5 through 10 (FC-CNT,  $1.2 \times 10^3$  CFU g<sup>-1</sup>; FC-MCP,  $1.1 \times 10^3$  CFU g<sup>-1</sup>), with no variations between the two treatments while yeasts in intact fruit were imperceptible (Table 4-1).

The significant increase in microbe populations of fresh-cut 'Galia' arose after day 5, which is in agreement with the finding of Luna-Guzman and Barrett (2000) who noted a significant raise of total aerobic counts in non-1-MCP-treated fresh-cut ripe muskmelon fruit (sanitized with 50  $\mu$ L L<sup>-1</sup>-chlorinated water) after 4 days of storage at 5 °C. Luna-Guzman and Barrett (2000) recorded total aerobic counts ranging from approximately  $2 \times 10^2$  to  $9 \times 10^9$  CFU g<sup>-1</sup>, plated at days 4, 8 or 12. These values are

quite high compared to the values noted herein for fresh-cut ripe 'Galia' fruit. Higher total aerobic (from  $1.4 \times 10^4$  to  $8.8 \times 10^4$  CFU g<sup>-1</sup>), lactic acid bacterium (from  $1.4 \times 10^2$  to  $7.2 \times 10^2$  CFU g<sup>-1</sup>) and Enterobacterium (from  $7.8 \times 10^3$  to  $3.2 \times 10^4$  CFU g<sup>-1</sup>) counts were also documented in muskmelon fruit (undefined ripeness stage) stored 11 days at 4 °C by O'Connor-Shaw et al. (1994). However, Ayhan et al. (1998) reported a similar or slightly lower microbial count fresh-cut muskmelon fruit (undefined ripeness stage) ( $10 \times 10^1$  CFU cm<sup>2</sup> for total aerobic counts;  $1 \times 10^1$  CFU cm<sup>2</sup> for yeast; and  $1 \times 10^1$  CFU cm<sup>2</sup> for other fungi) washed with chlorinated water ( $50 \mu\text{L L}^{-1}$  total available chlorine) and sealed with modified atmosphere (95% nitrogen and 5% oxygen) stored 10 days at 2.2 °C. Low microbial population ( $1.5 \times 10^3$  to  $3 \times 10^3$  CFU g<sup>-1</sup> for total aerobic count and  $1 \times 10^2$  CFU g<sup>-1</sup> for yeast count) in pre-ripe/ripe non-1-MCP-treated fresh-cut muskmelon fruit stored for 6 days at 5 °C (not washed previously with chlorinated water) was also noted by Portela and Cantwell (2001). Portela and Cantwell (2001) attributed the low microbial count in the fresh-cut muskmelon to strict sanitation procedures during fresh-cut processing. 1-MCP slightly suppressed total aerobic and Enterobacterium count increase in fresh-cut 'Galia' fruit whereas lactic acid bacterium growth was promoted. Even so, the lactic acid bacterium population was very low in all treatments and at all times of evaluation, with thinking the maximum total limit for microbial growth for fresh-cut vegetables (is this value for vegetables of relevance to fruits?) is  $5 \times 10^7$  CFU g<sup>-1</sup> (Francis et al., 1999). The 'Solo' papaya variety treated with  $25 \mu\text{L L}^{-1}$  1-MCP at 20 °C for 14 h at mature green stage and stored at 20 °C for approximately for 20 days showed slightly higher symptoms of stem rots, body black rots, and anthracnose compared to untreated fruit lasted approximately 5 days (Hofman et al., 2001). Hofman et al. (2001)



attributed the slight increase in the symptoms of the diseases in 1-MCP-treated fruit to a reduction in antifungal concentrations due to extended storage life . Jiang et al. (2001) found that ripe 'Everest' strawberry fruit treated with 500 to 1000 nL L<sup>-1</sup> 1-MCP (24 h at 20 °C) displayed accelerated leak disease development at 20 °C compared to non-1-MCP-treated fruit; however, 1-MCP at 100 and 250 nL L<sup>-1</sup> delayed the onset of the decay. Tomato plant lines expose to 10 nL L<sup>-1</sup> 1-MCP (24h at 18 to 24 °C) resulted in a significant increase in *Botrytis cinerea* frequency in the cultivars 'Moneymaker' and 'Castlemart' but no increase in 'Pearson' (Diaz et al., 2002). Hence, the effects of 1-MCP upon microbial growth are complex and depend on the type of microorganisms, 1-MCP concentration, cultivars, and tissue type and development.

In summary, the storage life of fresh-cut melon fruit derived from intact ripe 'Galia' fruit treated with 1-MCP was extended by 4 days compared to fresh-cut fruit, derived from intact ripe non-1-MCP-treated fruit that lasted 4. Thus, 1-MCP can be considered a safe potential growth regulator or conditioner for fresh-cut melon fruit alternative to calcium chloride/lactate, ethylene absorbent or controlled atmosphere. Finally, the natural resistance of melon fruit to microbial growth can be supported by using sanitized equipments, containers, spaces and low temperature.

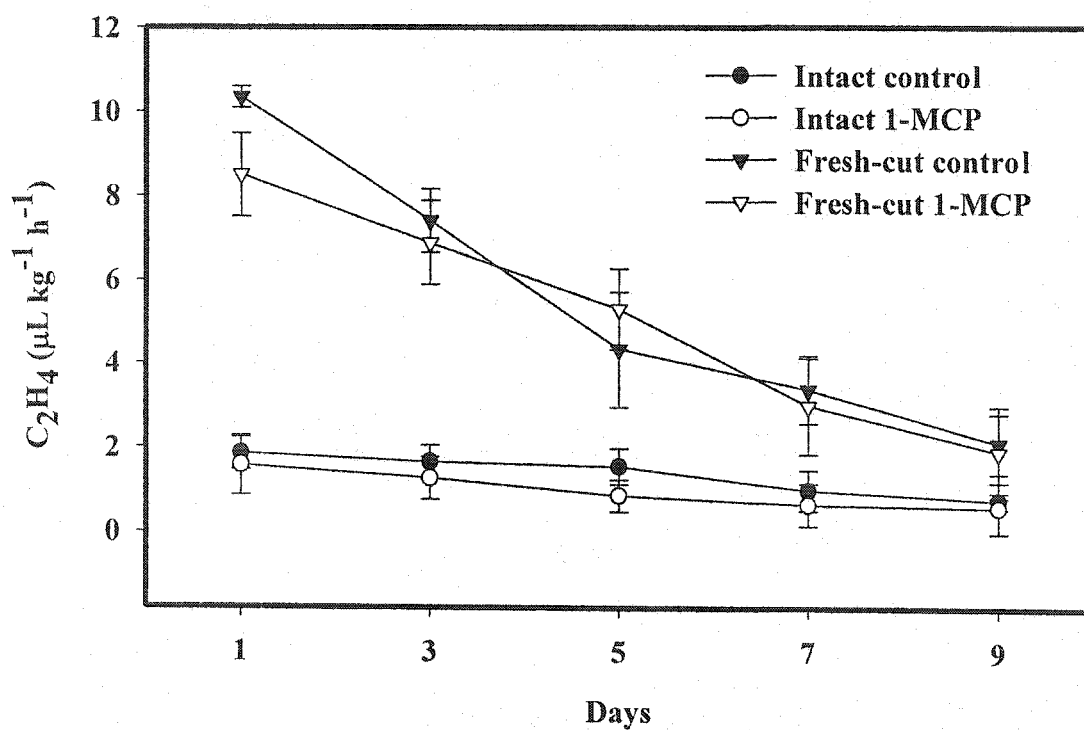


Figure 4-1. Ethylene production for intact ripe 'Galia' fruit with and without 1-MCP and fresh-cut fruit derived from intact ripe fruit treated with and without 1-MCP during storage at 5 °C. Vertical bars represent standard deviation of the means (n = 5).

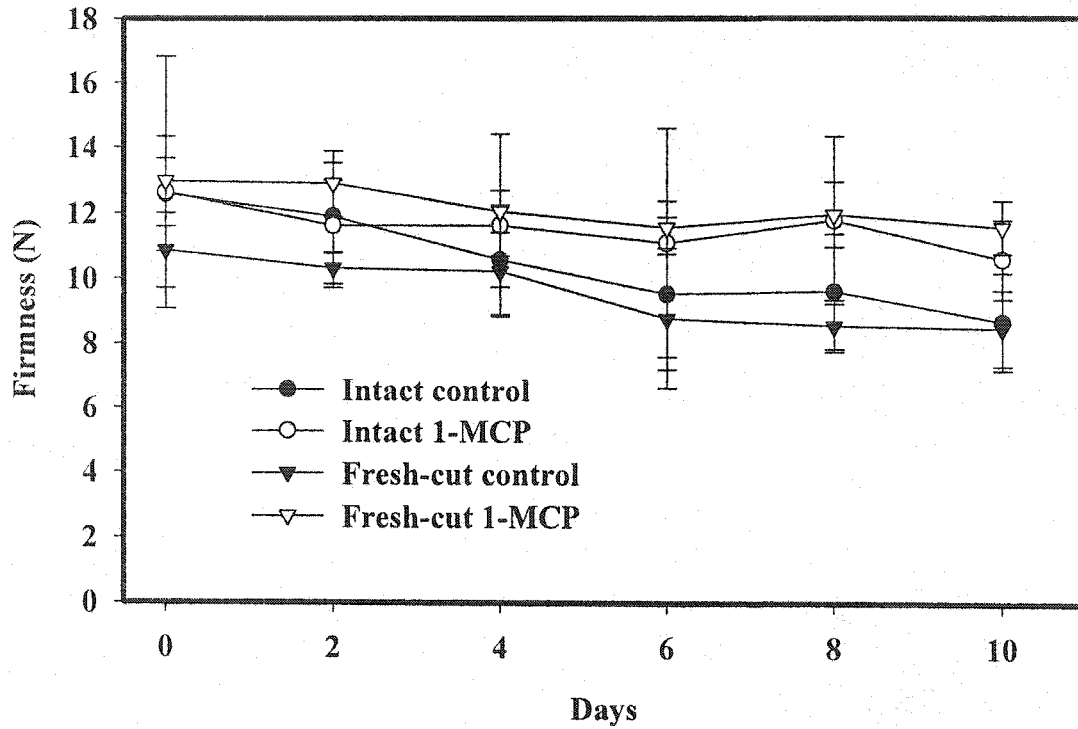


Figure 4-2. Mesocarp firmness for intact ripe 'Galia' fruit with and without 1-MCP and fresh-cut fruit derived from intact ripe fruit treated with and without 1-MCP during storage at 5 °C. Vertical bars represent standard deviation of the means (n = 5).

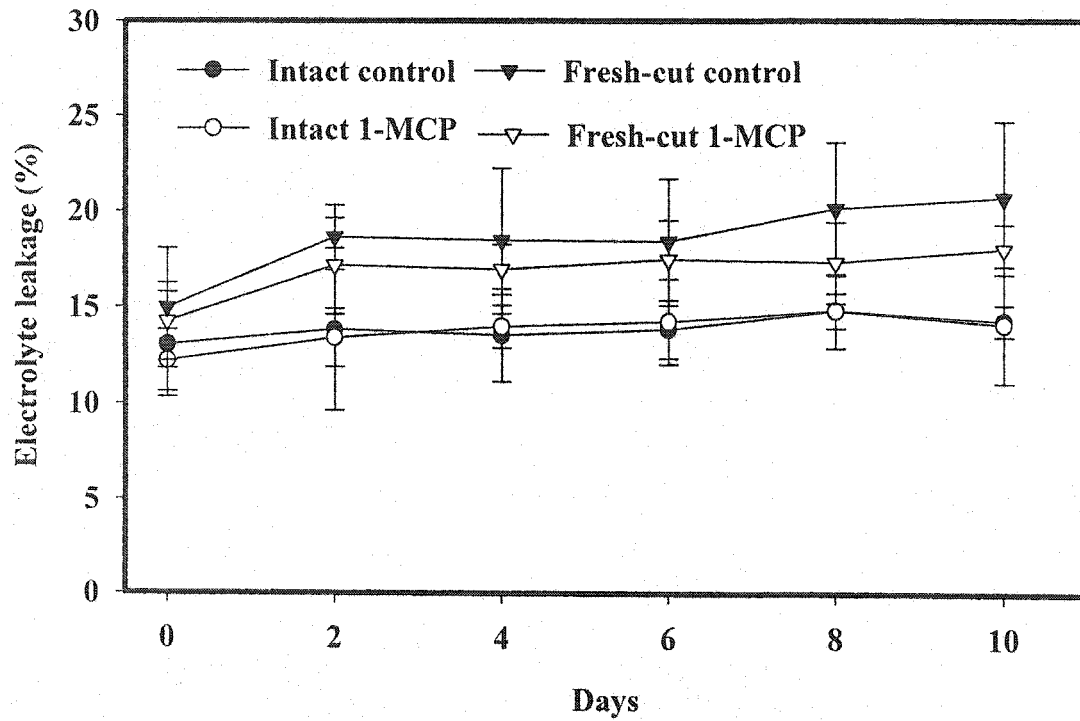


Figure 4-3. Electrolyte leakage from mesocarp tissues of intact ripe 'Galia' fruit with and without 1-MCP and fresh-cut fruit derived from intact ripe fruit treated with and without 1-MCP during storage at 5 °C. Vertical bars represent standard deviation of the means (n = 5).

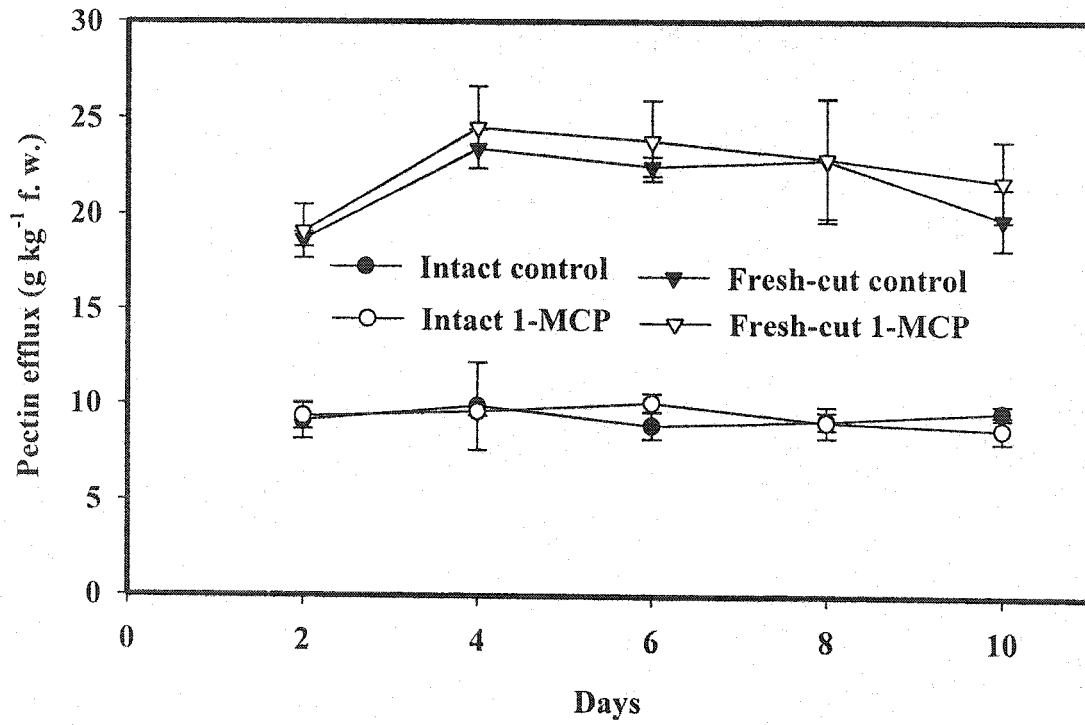


Figure 4-4. Pectin efflux from mesocarp tissues of intact ripe 'Galia' fruit with and without 1-MCP and fresh-cut fruit derived from intact ripe fruit treated with and without 1-MCP during storage at 5 °C. Vertical bars represent standard deviation of the means (n = 5).

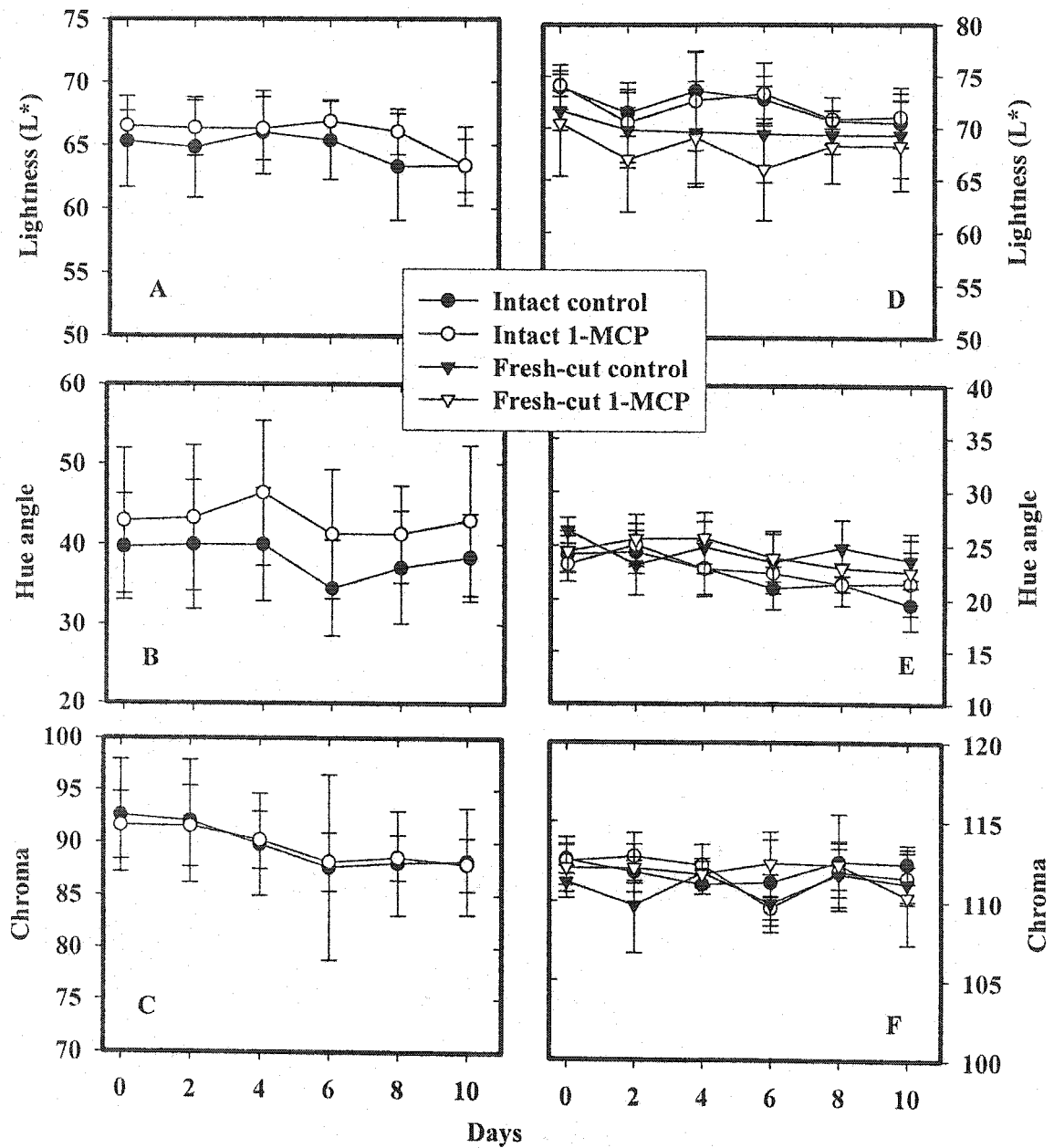


Figure 4-5. Color parameters, L\* lightness (A), hue angle (B) and chroma (C) for ripe 'Galia' fruit skin treated with and without 1-MCP ( $1 \mu\text{L L}^{-1}$ ) and the color parameters (D, E and F) for intact ripe fruit with and without 1-MCP and fresh-cut fruit derived from intact ripe fruit treated with and without 1-MCP during storage at  $5^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

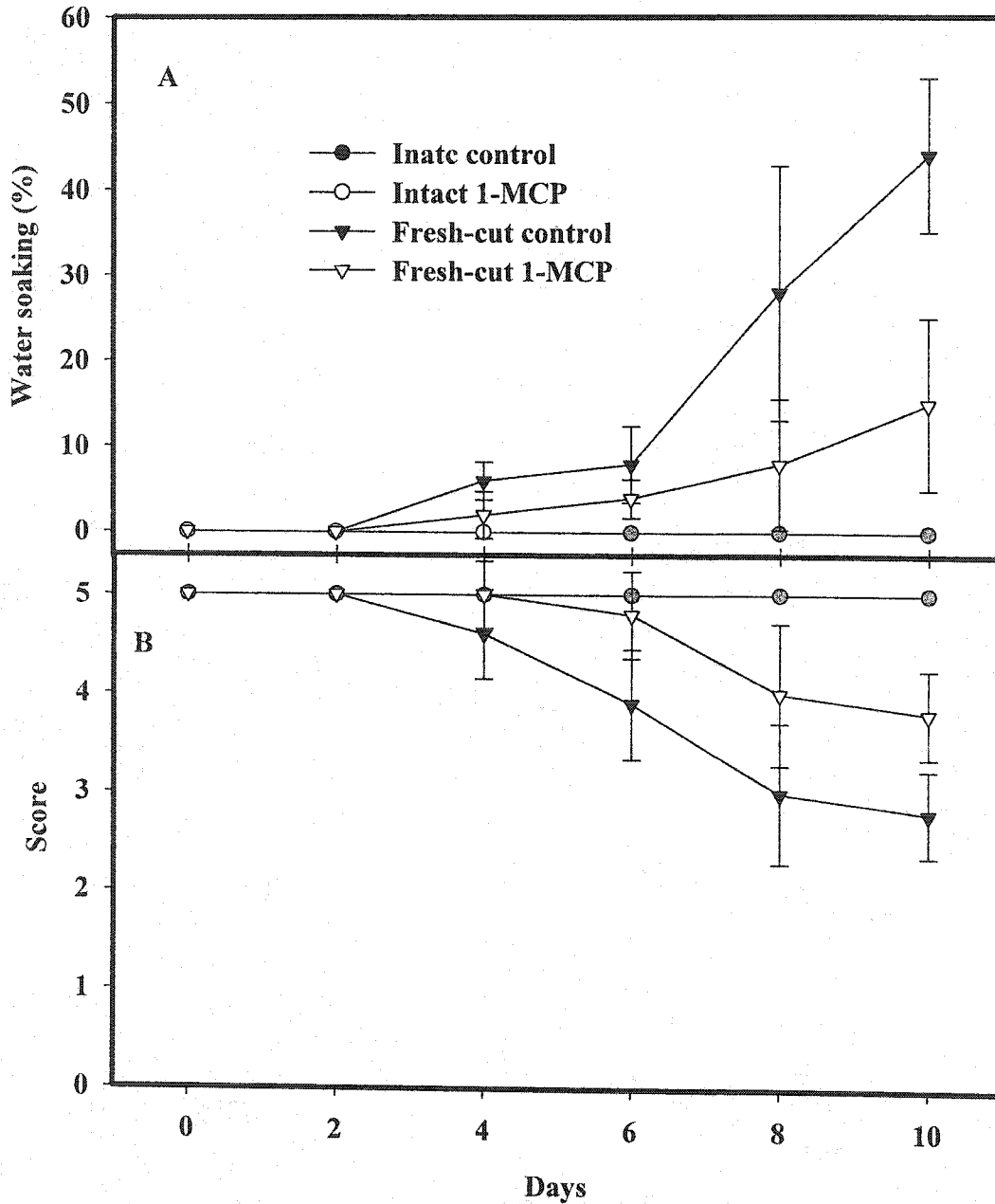


Figure 4-6. Mesocarp water soaking percentage (A) and sensory evaluation (B) for intact ripe 'Galia' fruit with and without 1-MCP and fresh-cut fruit derived from intact ripe fruit treated with and without 1-MCP during storage at 5 °C. Vertical bars represent standard deviation of the means (n = 5). When bars absent, the value for standard deviations was within the dimension of the symbol.

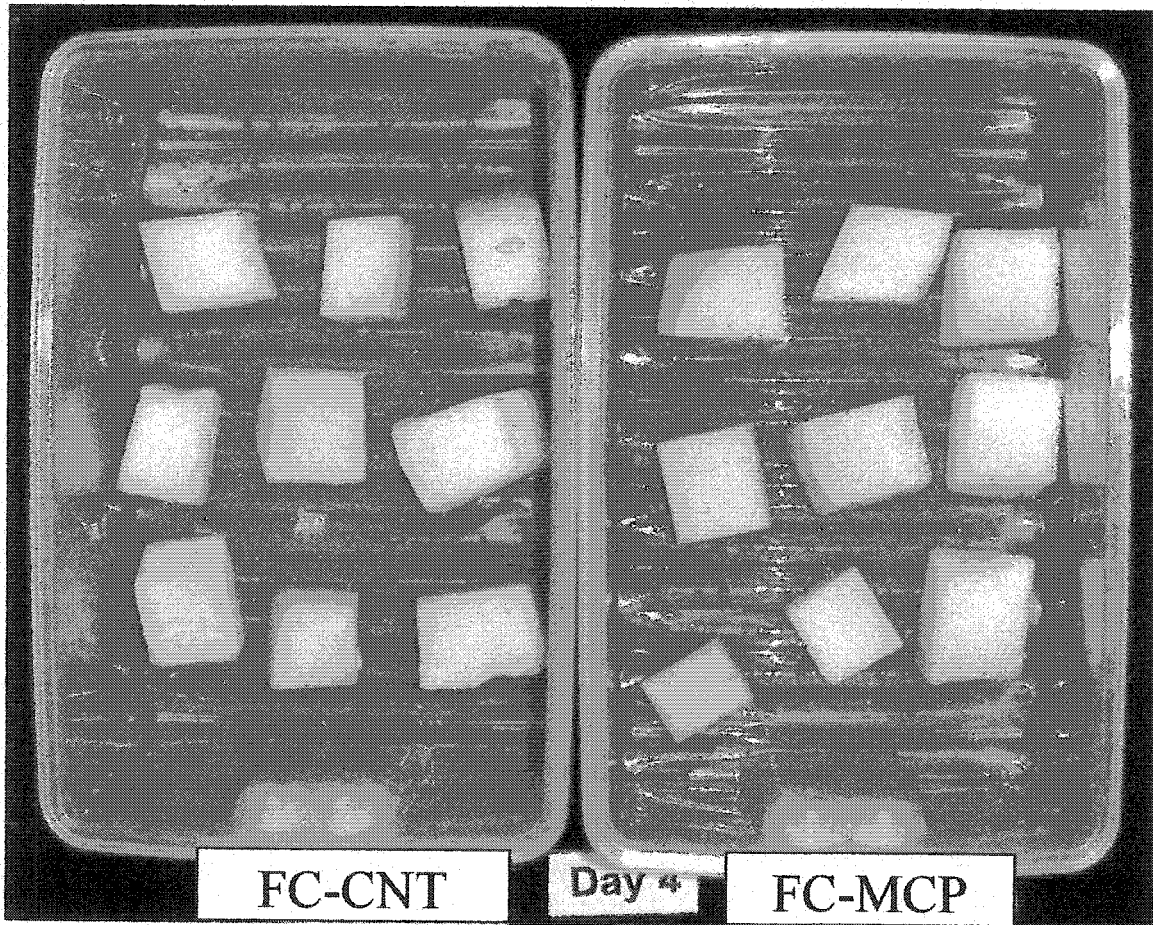


Figure 4-7. Ripe fresh-cut 'Galia' fruit derived from intact ripe fruit treated with (FC-1-MCP) and without 1-MCP (FC-CNT) and then stored for 4 days at 5 °C.



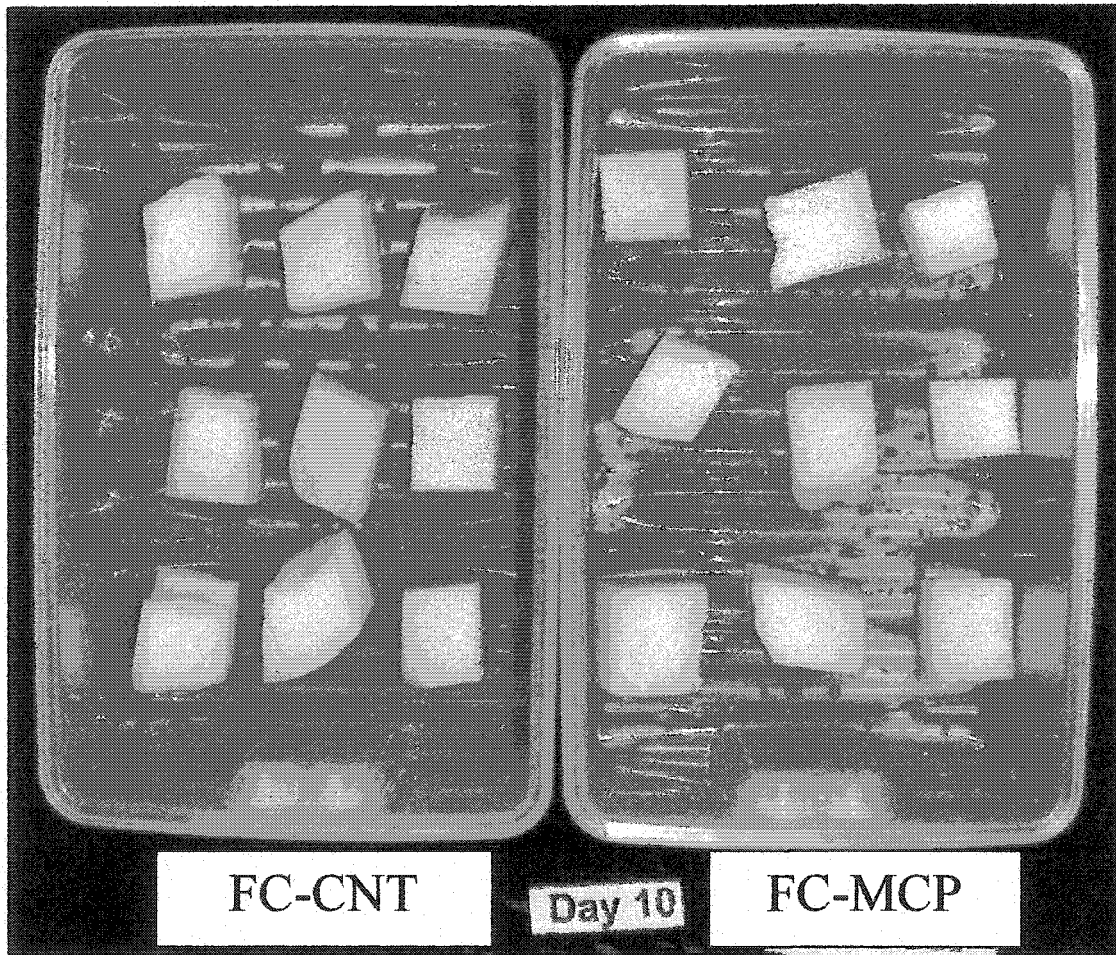


Figure 4-8. Ripe fresh-cut 'Galia' fruit derived from intact ripe fruit treated with (FC-MCP) and without 1-MCP (FC-CNT) and then stored for 10 days at 5 °C.

Table 4-1. Microbial counts (CFU g<sup>-1</sup> fresh weight) for intact ripe 'Galia' fruit with and without 1-MCP and fresh-cut fruit derived from intact ripe fruit treated with and without 1-MCP during storage at 5 °C. IF-CNT, intact control; IF-MCP, intact control with 1-MCP; FC-CNT, fresh-cut control fruit without 1-MCP; and FC-MCP, fresh-cut fruit derived from intact 1-MCP-treated fruit.

Treatment	Total aerobic count			Treatment	Total coliforms		
	Day				Day		
	0	5	10		0	5	10
IF-CNT	4.7 a	0.7 b	8.0 c	IF-CNT	0 a	0 a	3.3 a
IF-MCP	0 a	0 b	11.3 c	IF-MCP	0 a	0 a	3.3 a
FC-CNT	6.0 a	2.0 b	1.8 x 10 <sup>3</sup> a	FC-CNT	0 a	0 a	5.3 a
FC-MCP	6.0 a	15.3 a	1.5 x 10 <sup>3</sup> b	FC-MCP	0 a	0 a	4.0 a

Treatment	Enterobacteriaceae			Treatment	Lactic acid bacteria		
	Day				Day		
	0	5	10		0	5	10
IF-CNT	0 a	0 b	0.7 c	IF-CNT	0.7 a	0.7 a	6.7 b
IF-MCP	0 a	0 b	4.7 c	IF-MCP	0.7 a	0 a	22.7 a
FC-CNT	0 a	26.0 a	86.7 a	FC-CNT	0 a	0.7 a	6.7 b
FC-MCP	0 a	0 a	52.7 b	FC-MCP	4.7 a	2.0 a	22.0 a

Treatment	Yeasts			Treatment	Other fungi		
	Day				Day		
	0	5	10		0	5	10
IF-CNT	0 a	0 a	1.3 b	IF-CNT	0 a	0 a	2.7 a
IF-MCP	0 a	0 a	2.0 b	IF-MCP	0 a	0 a	1.3 a
FC-CNT	0 a	3.3 a	1.1 x 10 <sup>3</sup> a	FC-CNT	2.0 a	0 a	0 a
FC-MCP	0 a	0 a	1.1 x 10 <sup>3</sup> a	FC-MCP	0.7 a	0.7 a	4.0 a

Means (n = 3) followed by the same letter within a column are not significantly different,  $P \leq 0.05$ .

CHAPTER 5  
STORAGE LIFE EXTENTION OF PRE-RIPE AND RIPE 'SUNRISE SOLO'  
PAPAYA FRUIT BY 1-METHYLCYCLOPROPENE

**Introduction**

The storage life of papaya fruit under tropical conditions (30 °C) is limited due to their high respiration rate, delicate skin, and high water content (Sankat and Maharaj, 1997). Papaya fruit harvested at the color break stage can be kept for periods of up to 16 days at 10 to 16 °C (Sankat and Maharaj, 1997). Furthermore, papaya fruit can be harvested at the mature green stage; however, according to Hawaiian grade standards, the fruit must have at least 6% surface yellow coloration to meet the minimum grade requirement of 11.5% soluble solids (Akamine and Goo, 1971). For local markets, papaya fruit are harvested at the full-ripe stage (full yellowish-orange surface coloration; Morton, 1987) and stored at temperatures above 7 °C. Storage below 7 to 10 °C may cause low-temperature injuries depending on the variety and maturity stage (Paull and Chen, 1983; Sankat and Maharaj, 1997).

Approaches to extending the postharvest quality and duration of the chill-sensitive papaya have included the use of controlled-atmosphere storage, polymeric films and wax coating, and gamma irradiation (Sankat and Maharaj, 1997). For example, Maharaj and Sankat (1988) reported that papaya fruit harvested at the color break stage and stored under controlled atmosphere conditions of 1.5 to 2% O<sub>2</sub> and 5% CO<sub>2</sub> at 16 °C remained acceptable for 17 to 29 days. Another, more facile approach to extending the storage life and quality of harvested papaya fruit has been through the application of 1-

methylcyclopropene (1-MCP), a potent anti-ethylene compound (Sisler and Serek, 1997). In recent years some very effective agents for blocking ethylene action have been discovered by Sisler and coworkers, and four of them are extensively used in scientific investigation: 2,5-norbornadiene, *trans* cyclooctene, diazocyclopentadiene and 1-MCP (Sisler and Serek, 1999). Silver thiosulphate is used widely as commercial non-organic ethylene action inhibitor for cut flowers potted plants (Sisler and Serek, 1997). Since 1-MCP has no detectable odor, minimal phototoxic properties, and is stable and active at very low concentrations, it has been the favored compound among the inhibitors of ethylene responses (Sisler and Serek, 1997; Sisler and Serek, 1999). Recently, it is or has been reported that 1-MCP improved the storage life and quality of fruits treated prior to or during ripening including apple (Fan et al., 1999; Pre-Aymard et al., 2002) and tomato (Wills and Ku, 2002; Hoeberichts et al., 2002). 1-MCP (90 or 270 nL L<sup>-1</sup>) extended the storage life of 'Sunrise Solo' papaya fruit treated at an early stage of ripening from 4 to 6 days at 20 °C (Jacomino et al., 2002). Furthermore, 25 µL L<sup>-1</sup> 1-MCP treatment increased the number of days to ripening from approximately 5 to 20 days for 'Solo' fruit treated at commercial harvest maturity (Hofman et al., 2001).

The objective of this study was to examine the physiological responses and quality of papaya fruit treated with 1-MCP at the pre-ripe and full-ripe stages of maturation.

## Materials and Methods

### Plant Material

Papaya fruit (*Carica papaya*, L. var. 'Sunrise Solo') originating from Belize (no thermal or wax treatment) and were obtained from Brooks Tropicals Inc., Homestead, FL. 'Sunrise Solo' variety was chosen due to its year-round availability. After transfer to the postharvest facilities in Gainesville, fruit were selected on the basis of uniformity of

size, freedom from defects, and graded according to surface color. Fruit at the stage of pre-ripe (PRP; 10 to 20% surface yellow coloration) and ripe (RP; 70 to 80% surface yellow coloration) were employed in these studies. The fruit were gently brushed and washed with tap water, dipped in  $200 \mu\text{L L}^{-1}$  chlorinated water for 1 min, and then rinsed with tap water and dried.

### **1-MCP Preparation and Treatment**

Three g EthylBloc powder (Floralife Inc., Walterboro, SC) were dissolved in 50 mL deionized water in a 136-mL glass vial and sealed with a septum. The vial was placed on an oscillating shaker for 2 h. 1-MCP concentration in the vial headspace was measured using a gas chromatograph (Hewlett Packard-5890, Avondale, PA) equipped with an SP-1700 column (Supelco, Bellefonte, PA). Injector, oven, and detector (FID) were maintained at 150, 150 and 200 °C, respectively. Isobutylene gas, that has a FID detector response similar to that of 1-MCP (Jiang et al., 1999), was used as an external standard. Three g EthylBloc powder in 50 mL deionized water generated 1-MCP levels in the 137-ml vial of approximately  $27 \text{ mL L}^{-1}$ . Headspace samples (3.3 mL) of this gas were injected into sealed 18.9-L buckets having approximately 10-L free space, yielding a final 1-MCP concentration of  $9 \mu\text{L L}^{-1}$  and maintained for a total exposure period of 18 h at 20 °C (20 fruits per bucket). At 6-h intervals, the treatment containers were vented for 5 minutes, resealed, and injected with fresh 1-MCP. Air-treated fruit (1-MCP free, controls) were maintained under identical storage conditions.

1-MCP concentration and efficacy was investigated in a preliminary experiment in which fruit (20 to 30% skin yellowing) were treated with air (control), 0.9 and  $9 \mu\text{L L}^{-1}$  1-MCP for 24 h at 20 °C and stored at 15 °C.

### **Respiration and Ethylene Production**

Following the 1-MCP or air treatment, individual fruits (5 fruit/treatments) were sealed in 2-L airtight plastic containers for 1 h. CO<sub>2</sub> in 0.5 ml headspace samples was measured using a Gow-Mac GC (Bridge Water, NJ) equipped with a Porapak-Q column. Ethylene in 1 ml headspace samples was measured with a Hewlett Packard-5890 GC equipped with an activated alumina column.

### **Firmness Determination**

Firmness was measured at two equidistant points on the equatorial region of each fruit using an Instron Universal Testing Instrument (Model 4411-C8009, Canton, MA) fitted with a 5 kg load cell and an 8-mm convex probe. The probe was positioned at zero force contact with the pared fruit surface, and driven to a depth of 10 mm at a crosshead speed of 50 mm min<sup>-1</sup>. Firmness data are expressed as the maximum force (N) attained during penetration.

### **Electrolyte Efflux**

Five cylinders of mesocarp tissue were removed from the equatorial region of each fruit using an 8-mm diameter cork borer. From each cylinder, one disk (8 mm diameter and thickness) was excised from the centermost portion. Disks (5 per fruit) were rinsed briefly with deionized water to remove loosely adhering tissue and then blotted on moistened Whatman filter paper. The five disks were placed in 15 mL of 500 mM mannitol and the initial conductivity of the bathing solution was measured using an YSI-31A conductivity bridge equipped with a conductivity cell (Model 3403, Yellow Springs, OH). The disks and bathing solution were incubated on an oscillating shaker (1.4 cycles per second) at room temperature for 7 h and conductivity of the bathing solution was again measured. Total electrolyte content was determined after freezing (24 h at -20 °C),

thawing, and heating the disks and bathing solutions in a boiling water bath for 30 min.

Electrolyte efflux was expressed as a percentage of total tissue electrolytes.

#### **Soluble Solids Concentration, pH, and Titratable Acidity**

Frozen fruit samples (80 g) were ground using a mortar and pestle and centrifuged at 27,200 RFC for 10 min at 21 °C. Soluble solids concentration (SSC) in the supernatant was determined using a digital refractometer (Abbe Mark-10480, Buffalo, NY), and titratable acidity (TA) using a Fisher-395 dispenser and -380 electrometer (Pittsburgh, PA). Six g of juice were titrated with 0.1 N NaOH to an end point of pH 8.2. TA was calculated from the volume of mL NaOH added and expressed as % malic acid equivalents.

#### **Statistical and Informal Taste Analyses**

General linear model program of SAS (SAS institute, Carry, NC) and Duncan's multiple range tests were performed for Completely Randomized Designs. Informal taste analyses to determine the edible stage on fruit surface and flesh appearance, odor, flavor and texture quality were performed by untrained personnel of the postharvest research group of University of Florida.

### **Results**

#### **Effective 1-MCP Concentration**

Experiments employing different 1-MCP concentrations (0.9 to 9  $\mu\text{L L}^{-1}$ ) were conducted with fruit at 20 to 30% skin yellowing ripening stage to determine the effectiveness of 1-MCP at affecting the ripening metabolism of papaya, with emphasis on fruit firmness. Firmness retention of the papaya fruit treated with 1-MCP and stored at 15 °C was the highest in response to 9  $\mu\text{L L}^{-1}$  (5.2 N) followed by 0.9  $\mu\text{L L}^{-1}$  (5.1 N), and control (4.3 N) respectively, at the final day (day 19) of storage (Figure 5-1). Since

firmness retention was of particular interest, the present studies were performed using 1-MCP at  $9 \mu\text{L L}^{-1}$ .

### Respiration and Ethylene Production

Respiration showed an increase after day 5 for both treatments, reaching a maximum of  $23.8 \text{ mL kg}^{-1} \text{ h}^{-1}$  at day 6 for control and  $25.4 \text{ mL kg}^{-1} \text{ h}^{-1}$  at day 8 for 1-MCP-treated, representing a 2-day delay in the climacteric respiratory peak (Figure 5-2A). Ethylene production in PRP control fruit increased through day 5 and reached a peak of  $1.81 \mu\text{L kg}^{-1} \text{ h}^{-1}$  at day 5 while in 1-MCP-treated fruit remained unchanged during the first 4 days of storage, thereafter exhibiting a slow, continuous increase through day 10 (Figure 5-2A). Maximum ethylene production of PRP 1-MCP-treated fruit remained below  $1.5 \mu\text{L kg}^{-1} \text{ h}^{-1}$ ; however, as evident in Figure 5-2A, a clear ethylene climacteric peak was not observed. The onset of the ethylene rise and the maximum ethylene production for PRP fruit treated with 1-MCP was delayed 3 and 5 days, respectively, compared with the control.

Both control and 1-MCP-treated RP fruit exhibited nearly linear trends for respiration during storage, remaining under  $25 \text{ mL kg}^{-1} \text{ h}^{-1}$  (Figure 5-2B). The respiration of RP 1-MCP-treated fruit averaged about 20 to 25% lower than control fruit during the 6- to 8-day storage period. Ethylene production of fruit treated with 1-MCP at the RP stage was significantly reduced by 1-MCP through the initial 4 d of storage, thereafter attaining values comparable to the control (Figure 5-2B). In RP control fruit, ethylene production remained nearly constant during storage. Consistent with the fact that RP fruit were post-climacteric at the start of the experiment, ethylene production during storage did not exceed  $1 \mu\text{L kg}^{-1} \text{ h}^{-1}$ . In contrast, ethylene production in both control and fruit



treated with 1-MCP at the PRP stage reached maximum values of between 1.5 and 1.9  $\mu\text{L kg}^{-1} \text{h}^{-1}$ .

### **Mesocarp Firmness**

Mesocarp firmness of PRP control and 1-MCP-treated fruit declined through day 5 (Figure 5-3A). Thereafter, control fruit continued to soften while the firmness of the 1-MCP-treated fruit remained relatively constant through day 8. The firmness of PRP control fruit declined 52% (from 14.1 to 6.8 N) within 9 days of storage compared with 30% (from 15.1 to 10.5) for 1-MCP-treated fruit over the same time period. The firmness of PRP 1-MCP-treated fruit (8.8 N) at day 11 was comparable in magnitude to that of control fruit (8.1 N) at day 5. After 11 days, PRP 1-MCP-treated fruit retained about 58% of their original firmness. The consistent but insignificant differences in firmness at the first measurement (day 1) likely reflects a slight divergence in firmness values during the 24 h 1-MCP treatment, during which time fruit were held at 20 C.

Firmness of papaya fruit treated with and without 1-MCP at the RP stage gradually declined, but the decrease was significantly attenuated in the 1-MCP-treated fruit (Figure 5-3B). Within 2 days, the firmness of RP control fruit had declined about 22% (from 5.7 to 4.4 N); thereafter, the rate of softening declined through day 5 (4.09 N) with the rate again increasing through day 6 (2.91 N), at which time RP control fruit had lost approximately 50% of their initial firmness values. Firmness loss in 1-MCP-treated fruit was only about 15%. After 8 days, fruit treated with 1-MCP at the RP stage retained 84% of their initial firmness (5.76 N at day 1, 4.81 N at day 8).

### **Electrolyte Efflux**

Electrolyte efflux (% of total) of PRP control and 1-MCP-treated fruit gradually increased during storage (Figure 5-4A); however, in neither treatment were trends

indicative of significant leakage increases during ripening. Between PRP control and 1-MCP-treated fruit, total electrolyte leakage ranged from about 17 to 22%. Even so, PRP1-MCP-treated fruit displayed statistically lower leakage values from days 5 to 9.

Electrolyte leakage of RP fruit treated with or without 1-MCP showed no clear trend during storage and no differences between the treatments (Figure 5-4B).

### **Soluble Solids Concentrations, Titratable Acidity and pH**

Soluble solids concentration of PRP control and 1-MCP-treated fruit displayed similar trends during storage and no significant differences were noted between treatments (Figure 5-5A). The trend of SSC in RP fruit showed a similar pattern between control and 1-MCP-treated fruit (Figure 5-5B). TA of both PRP control and 1-MCP-treated fruit gradually increased until day 7, followed by a slight decline in control fruit and little further change in 1-MCP treated fruit (Figure 5-6A). At 5 days of storage and thereafter, the TA values were significantly higher in PRP 1-MCP treated fruit compared with control fruit. TA of RP control and 1-MCP-treated fruit also rose until day 4, and then decreased. The TA of RP control was significantly higher than that of RP 1-MCP treated fruit during most of storage (Figure 5-6B). PRP 1-MCP-treated fruit had significantly higher pH values relative to control fruit after day 1 of storage through day 9; however, the magnitude of the differences was unremarkable, with both treatments displaying pH values near 5 (Figure 5-7A). In RP fruit, pH gradually decreased though not significantly from values near 5.0 on day 1 to values from 4.77 to 4.87 on day 6 (Figure 5-7B).

### **Fruit Evaluation**

Based on informal quality analysis assessed from peel and pulp color, aroma, texture, and flavor (O'Connor-Shaw et al., 1994) by untrained laboratory personnel, the

period of table-ripe edibility (fruit suitable for consumption) persisted from days 4 through 7 for PR control fruit and on days 6 through 10 for 1-MCP-treated fruit, representing an average shelf-life extension of 25 %. RP control fruit were edible through 3 days of storage whereas fruit treated with 1-MCP at the RP stage were still edible on day 6, representing a doubling of useful shelf-life or table-ripe edibility. 1-MCP delayed the surface color change from green to yellow in both PRP and RP fruit treated with 1-MCP (Figure 5-8 and 5-9). No external decay was evident in either PRP control or 1-MCP-treated fruit until day 7; thereafter, some fruit displayed external decay. Five of 30 PRP control fruit (16.6%) and 3 of 30 PRP 1-MCP-treated fruit (10%) were removed from the experiment due to decay throughout storage. Decay was primarily evident as stem-end rot, which becomes more prominent in papaya as the fruit ripen (Alvarez and Nishijima, 1987). Decay incidence in RP fruit was negligible during storage.

### Discussion

The response of either PRP or RP 'Sunrise Solo' fruit to  $9 \mu\text{L L}^{-1}$  1-MCP was significant for most measured parameters. In a much lower concentration ( $90$  or  $270 \text{ nL L}^{-1}$  1-MCP, 12 h at  $20^\circ\text{C}$ ), 'Sunrise Solo' fruit at the breaker stage and pre-climacteric stage responded to 1-MCP by extending their storage life from 4 to 6 and from 2 to 4 days, respectively, at  $20^\circ\text{C}$  (Jacomino et al., 2002). Hofman et al. (2001) reported that 'Solo' fruit treated with  $25 \mu\text{L L}^{-1}$  1-MCP (14 h at  $20^\circ\text{C}$ ) at commercial harvest maturity increased the days to reach the edible soft stage from about 5 to 20 days at  $20^\circ\text{C}$ . Hofman et al. (2001) also speculated that effective 1-MCP concentrations might be lower than  $25 \mu\text{L L}^{-1}$ . In our findings, the storage life of PRP papaya treated with  $9 \mu\text{L L}^{-1}$  1-MCP was 11 days while the control 9 days. Thus, in terms of shelf-life extension, the response of papaya fruit to 1-MCP appears to be closely linked to concentration and appears to reflect

a high saturation level for maximum. Wills and Ku (2002) reported that higher concentration of 1-MCP (20 or 100  $\mu\text{L L}^{-1}$ ) achieved a greater increase in postharvest life of ripe tomato fruit at 20 °C relative lower 1-MCP concentrations (1, 5 or 10  $\mu\text{L L}^{-1}$ ). The maximum response of 1-MCP to papaya fruit in the upper concentration range (over 1  $\mu\text{L L}^{-1}$ ) might be related to features of the ethylene receptors: saturation of the ethylene receptors either requires higher concentrations of 1-MCP or exposures longer than 24 h.

PRP 'Sunrise Solo' fruit displayed a typical climacteric pattern, with a peak respiration rate of 23.8  $\text{mL kg}^{-1} \text{h}^{-1}$  and ethylene production rate of 1.81  $\mu\text{L kg}^{-1} \text{h}^{-1}$  at 20 °C. These values are in general agreement with those of Paull and Chen (1997), who reported similar respiration rates (approximately 20  $\text{mL kg}^{-1} \text{h}^{-1}$ ) and similar or slightly higher ethylene production (1 to 4  $\mu\text{L kg}^{-1} \text{h}^{-1}$ ) in 'Sunset' papaya at the color break stage at 22 °C. In the papaya cultivar 'Solo', the maximum respiration and ethylene production were documented at 71.5  $\text{mL kg}^{-1} \text{h}^{-1}$  and 5.5  $\mu\text{L kg}^{-1} \text{h}^{-1}$  at 20 °C (Wills and Widjanarko, 1995). Thus, the present data for respiration and ethylene production confirm previously published results for respiration and ethylene production of papaya fruit, and further report on the ethylene and respiration responses of 1-MCP-treated papaya fruit at the ripe stage

The onset of climacteric respiration and ethylene production in PRP fruit were significantly delayed by 1-MCP. Ethylene production of PRP papaya fruit treated with 1-MCP was delayed significantly, reaching production levels comparable to control fruit after about 10 days of storage. The recovery in ethylene production may be due to formation of new ethylene binding sites (Sisler and Serek, 1997) or release of 1-MCP from the receptors. Other explanations include the non-permanence dissociation of 1-

MCP from ethylene receptors, competition with ethylene, and potential binding of 1-MCP to receptors showing homology with the ethylene receptors (Able et al., 2002). Able et al. (2002) reported that multiple applications of 1-MCP had no further impact on storage life of broccoli florets. Delayed climacteric ethylene production and respiratory in PRP fruit in response to 1-MCP has been noted for various fruits treated before the onset of ripening including banana (Jiang et al., 1999;  $1 \mu\text{L L}^{-1}$ , 24 h at 24 °C) at 20 °C and avocado (Jeong et al., 2002;  $0.45 \mu\text{L L}^{-1}$ , 24 h at 20 °C) at 20 °C.

1-MCP suppressed both respiration and ethylene production in RP papaya fruit. Ethylene production of RP 1-MCP-treated papaya fruit recovered to control values after 5 days whereas respiration remained suppressed. Similarly, 'Golden Delicious' apple (Jiang and Joyce, 2002) and tomato (Wills and Ku, 2002) fruits treated with 1-MCP at a late stage of ripening displayed reduced ethylene production and respiration compared with non-1-MCP-treated fruit. The delayed respiratory climacteric peak in PRP and persistent respiration in RP papaya fruit treated with 1-MCP may imply that ethylene and respiration are not tightly linked during papaya fruit ripening. In detached tomato (Saltveit, 1993) and muskmelon (Bower et al., 2002) fruits, respiratory climacteric has been found to be not an essential part of ripening.

Treatment with 1-MCP delayed softening in both PRP and RP papaya, which indicates that softening in papaya fruit is dependent upon ethylene action throughout the ripening process. Lelievre et al., (1997) stated that softening of climacteric fruits is regulated by ethylene. Electrolyte efflux, an indication of membrane damage in senescing tissues (Marongoni et al., 1996), increased slightly in PRP papaya fruit but not in PR fruit during storage. Similar results regarding increased electrolyte efflux during papaya fruit

ripening was reported by Chan et al. (1985), who found that papaya fruit (harvested at the color break stage) initially stored at 10 °C followed by storage at 24 °C showed a significant increase in electrolyte leakage. 1-MCP significantly suppressed the increase in electrolyte leakage in PRP papaya fruit, which may serve as evidence that ethylene action is involved in mechanisms contributing to membrane catabolism (Kuo and Parkin, 1989; Faragher et al., 1986).

1-MCP had no influence on soluble solids concentration levels in either PRP or RP papaya fruit, indicating that ethylene responsiveness is not involved in soluble solids accumulation in ripening papaya fruit. Hofman et al. (2001) documented a small but significant increase in soluble solids of 'Solo' papaya treated with 1-MCP (control, 10.09%; 1-MCP, 11.47) at the edible soft stage. The control 'Solo' fruit reached the eating stage in 4 to 5 days, while 1-MCP-treated fruit required nearly in 21 days, which may explain the small but significance differences noted in their studies. Treatment of 'Delicious' and 'Fuji' apple with 1-MCP (0.8 to 1  $\mu\text{L L}^{-1}$  for 12 to 16 h at 20 to 24 °C) caused higher SSC compared to control at 0 °C after 6 to 7 months (Fan et al., 1999), treatment of 'Elberta' peach harvested pre-climacteric period with 1-MCP (0.5  $\text{mL L}^{-1}$  for 4h at 20 °C) resulted in higher SSC compared to control at 20 °C (Fan et al., 2002) as well. Ethylene balance could determine the way tissues respond to changes in soluble solid concentration, for example partitioning carbohydrate into metabolism or into storage. In muskmelon fruit a diminution of biosynthetic enzymatic activities sucrose synthesis and an increase invertase acid activity attributed to ethylene level (Hubbard et al., 1989), which further supports the idea of ethylene involvement in soluble solid accumulation.

The results indicate that 1-MCP suppressed the increase in TA in papaya fruit regardless of ripening stage. These findings are in contrast to reports where the TA of fruits including 'Gala' apple (Fan et al., 2001;  $0.5 \mu\text{L L}^{-1}$ , 23 h at  $20^\circ\text{C}$ ) at  $20^\circ\text{C}$  and 'Royal Zee' plum (Dong et al., 2002;  $1 \mu\text{L kg}^{-1} \text{h}^{-1}$ , 20 h at  $20^\circ\text{C}$ ) at  $0^\circ\text{C}$  was enhanced by 1-MCP when applied at an early stage of ripening, and where the decrease in TA of tomato (Wills and Ku, 2002) and 'Anna' apple (Pre-Aymard et al., 2002;  $0.1$  or  $1 \mu\text{L kg}^{-1} \text{h}^{-1}$  1-MCP, 24 h, room temperature) was either suppressed or unaffected by 1-MCP when applied at a late stage of ripening. Flores et al. (2001) reported that 1-aminocyclopropene-1-carboxylic acid oxidase antisense muskmelon displayed higher citric acid compared to wild type. The result from PRP and RP papaya fruit and others cited above indicate that ethylene may involve directly or indirectly organic acid metabolism.

The surface color change from green to yellow in both PRP and RP papaya fruit was delayed by 1-MCP. Similar results from fruits treated with 1-MCP at an early stage of ripening have been reported for banana (Golding et al., 1998;  $45 \mu\text{L L}^{-1}$  1-MCP, for 1 h at the room temperature) and avocado fruits (Jeong et al., 2002). Pre-Aymard et al. (2002) found that 1-MCP delayed the surface color change from green to yellow in 'Anna' apple treated with 1-MCP late in ripening. The delayed color change from green to yellow in papaya fruit treated with 1-MCP supports the conclusion of Lelievre et al. (1997) who documented that color production can be either ethylene-dependent or -independent according to the type pigments and the fruit species.

1-MCP significantly extended the acceptable edible period of papaya fruit irrespective of stage of maturity at the time of treatment. PRP control fruit reached the

acceptable edible stage at day 4 that persisted through day 7 while PRP control attained an edible condition at day 6, persisting through day 10, and a 25-percent increase in response to 1-MCP. The edible stage of RP fruit lasted 3 days while RP-1-MCP-treated fruit 6 days, indicating a 3-day extension in storage life. Jacomino et al. (2002) found papaya fruit treated with 1-MCP at the color break stage lasted longer compared with control fruit (control, 4 days; 1-MCP, 6 days). Additionally, Hofman et al. (2001) reported that 1-MCP treatment increased the number of days to the ripe stage, defined as the time at which fruit attained firmness readings of 5 to 7 N, of 'Solo' papaya-treated at commercial maturity (mature-green stage) by 325% (from 4.8 to 20.6 days).

1-MCP-treated PRP fruit showed less decay compared with non-1-MCP-treated PRP fruit. During storage 16.6% of PRP control fruit were eliminated from the experiment due to decay while only 10% of PRP 1-MCP. Stem-end rot, which can develop rapidly in ripe fruit, was the primary indication of decay (Alvarez and Nishijima, 1987). The reduced incidence of decay in fruit treated with 1-MCP at the PRP stage might be due to a consequence of the reduced rate of ripening. Decay incidence was unremarkably low in either RP control or RP 1-MCP-treated fruit, probably due to the fact that RP fruit had been selected based on external quality prior to the 1-MCP treatment. Hofman et al. (2002) noted that 'Solo' fruit treated with  $25 \mu\text{L L}^{-1}$  1-MCP at the commercial maturity stage showed higher disease symptoms (stem black rots, body black rots and anthracnose) after approximately 20 days of storage than non-1-MCP-treated fruit after 4 to 5 days of storage at 20 °C. The authors speculated that delaying ripening by 1-MCP may result in fruit close to full ripe stage having lower concentrations of endogenous antifungal compounds and higher incidence of decay.



1-MCP has been reported to reduce the rate of over-ripening by delaying firmness loss and color change in 'Anna' (Pre-Aymard et al., 2002) and 'Golden Delicious' (Jiang and Joyce, 2002) apple, and by extending postharvest storage life in the tomato fruit (Wills and Ku, 2002). Coriander leaf senescence, as assessed in terms of chlorophyll and protein loss, was significantly retarded by 1-MCP (Jiang et al., 2002). Furthermore, earlier work demonstrated that silver thiosulphate arrested tomato ripening (Tucker and Brady, 1987; Smith et al. 1989). 2,5-norbornadiene, a competitive ethylene action inhibitor, has been reported to interrupt petal senescence of carnation flower as well (Peiser, 1989; Wang and Woodson, 1989). The results from RP 'Sunrise Solo' fruit show quite conclusively that ethylene is required throughout the ripening process (Tucker and Brady, 1987; Smith et al., 1989; Hoerberichts et al., 2002).

In conclusion, our results indicate that inhibition of ethylene action in 'Sunrise Solo' papaya fruit by  $9 \mu\text{L L}^{-1}$  1-MCP is sufficient to extend the table-ripe edibility at either the pre-ripe or ripe stage by 25% and 100% at 20 °C, respectively. Use of 1-MCP in combination with low temperature and controlled atmosphere storage has potential to extend storage life of papaya fruit for a longer period. In consideration of the perishable nature and inherently short storage life of papaya and other tropical fruits, 1-MCP treatment should provide a number of alternative handling and shipping options. These data should be of considerable interest to the papaya and other tropical fruit industries, which is a tremendous development for tropical fruit industry.

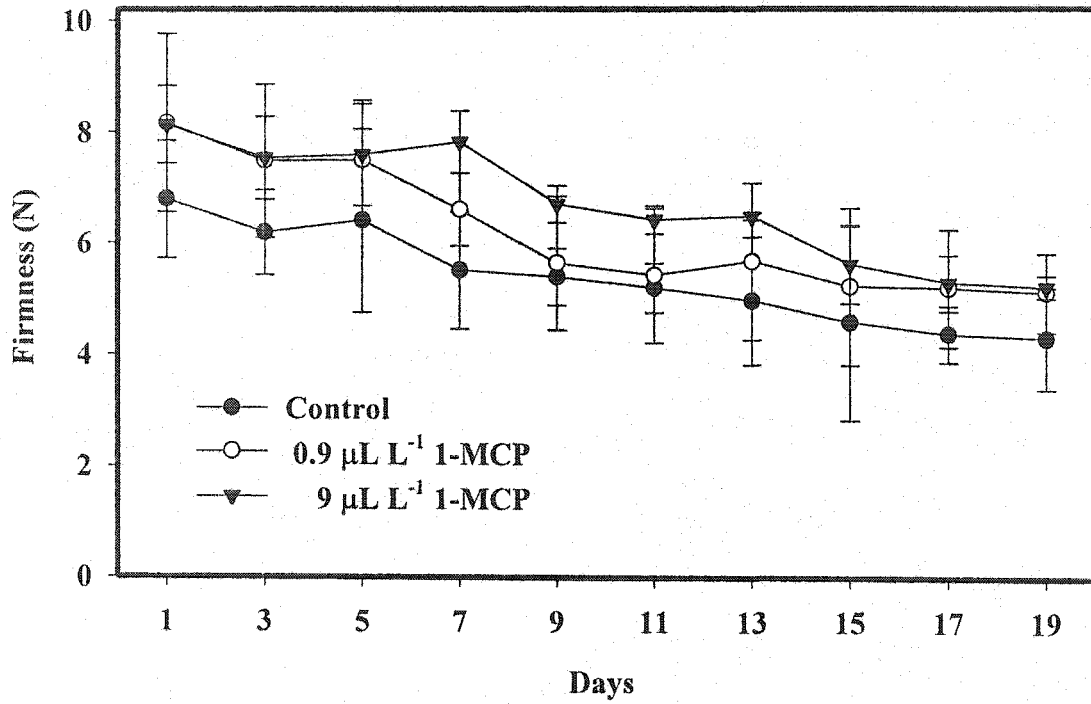


Figure 5-1. Mesocarp firmness for 'Sunrise Solo' fruit treated with air (control), 0.9 µL L<sup>-1</sup>, and 9 µL L<sup>-1</sup> 1-MCP at 20 to 30% skin yellowing ripening stage and subsequently stored for 19 days at 15 °C. Vertical bars represent standard deviations of the means (n = 5).

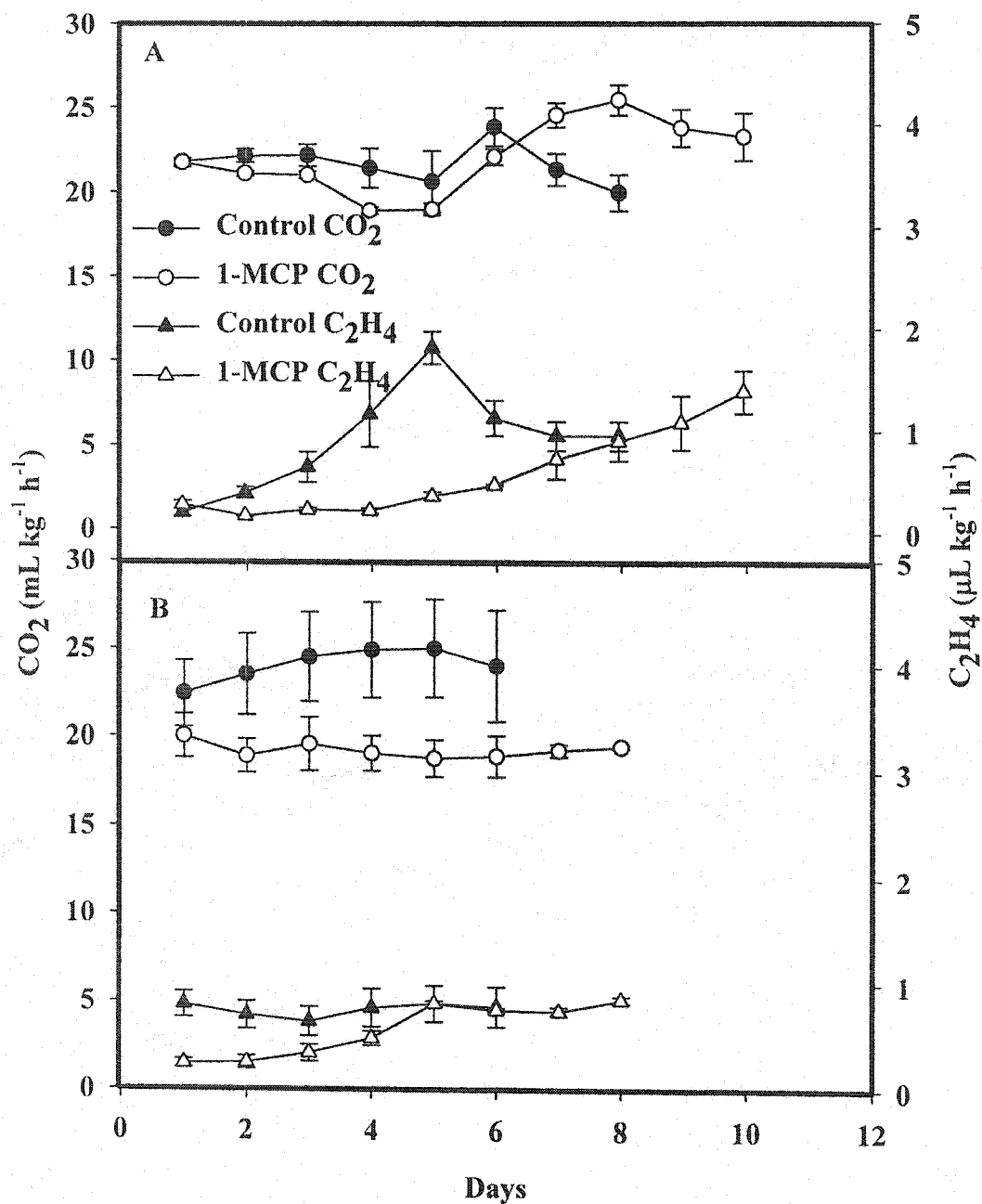


Figure 5-2. Respiration and ethylene production for 'Sunrise Solo' fruit treated with air (control) and 9  $\mu\text{L L}^{-1}$  1-MCP at the pre-ripe (A) and ripe (B) stage and subsequently stored at 20 °C. Vertical bars represent standard deviation of the means ( $n = 5$ ).

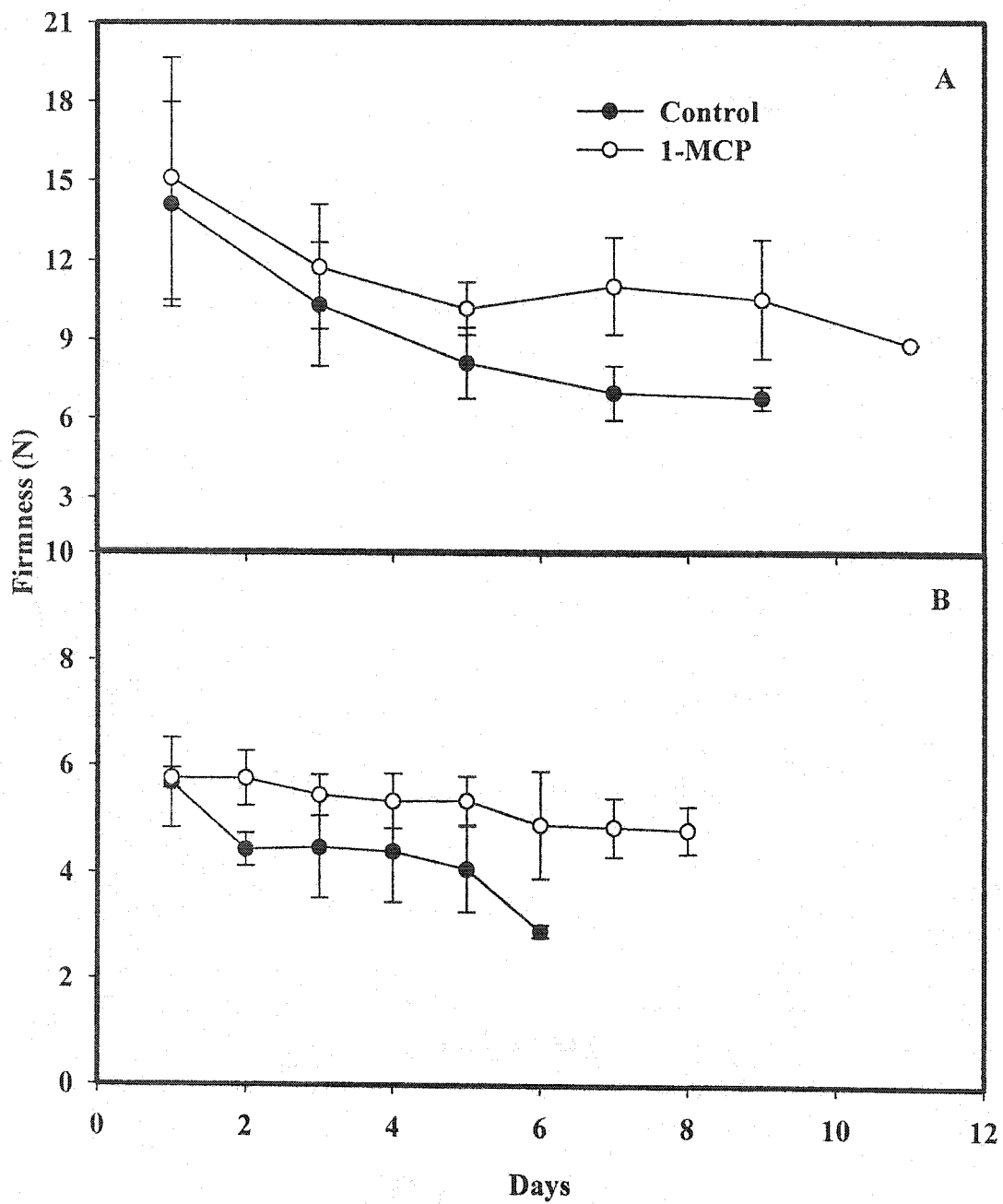


Figure 5-3. Mesocarp firmness for 'Sunrise Solo' fruit treated with air (control) and  $9 \mu\text{L L}^{-1}$  1-MCP at the pre-ripe (A) and ripe (B) stage and subsequently stored at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

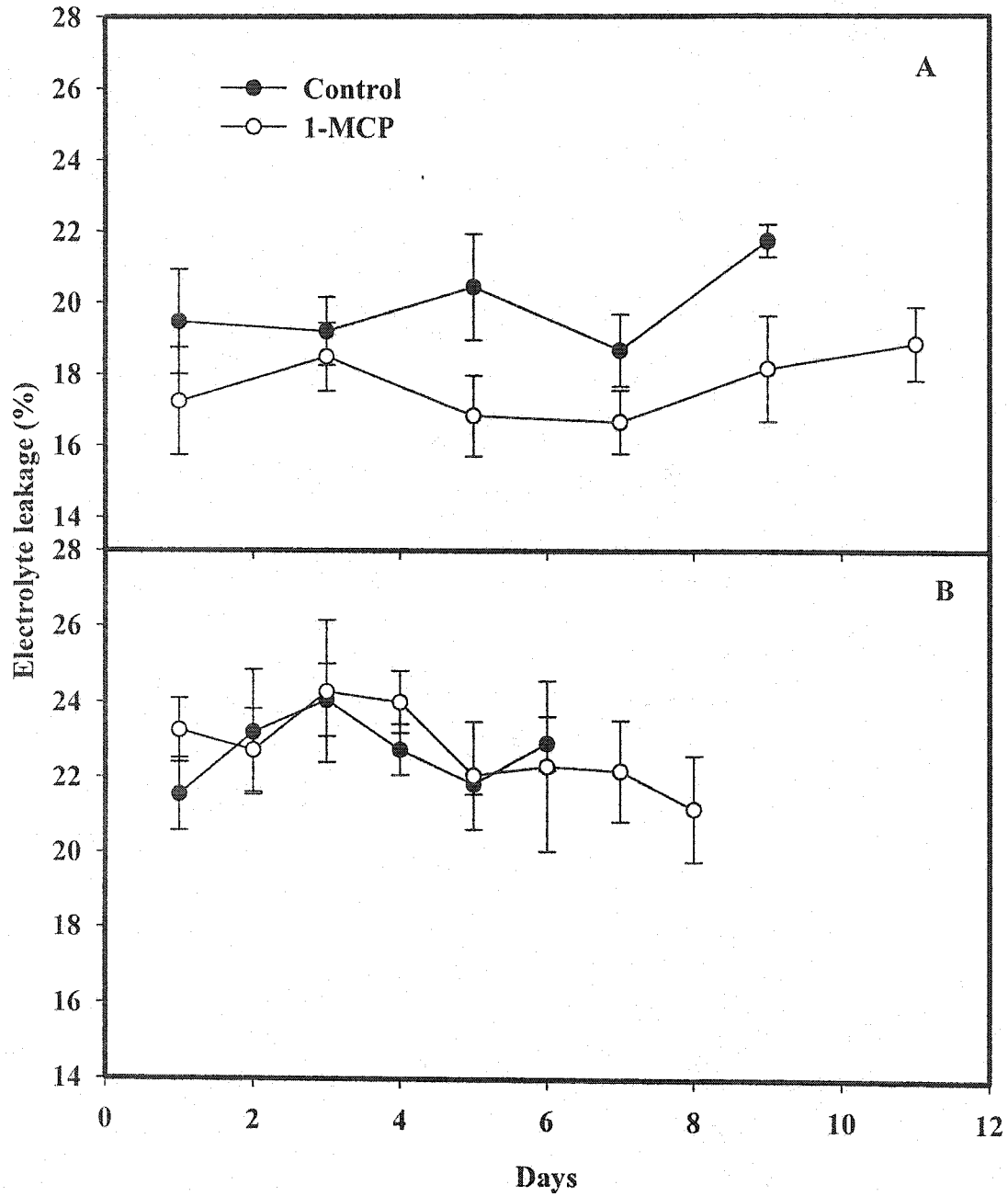


Figure 5-4. Electrolyte leakage for 'Sunrise Solo' fruit treated with air (control) and  $9 \mu\text{L L}^{-1}$  1-MCP at the pre-ripe (A) and ripe (B) stage and subsequently stored at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

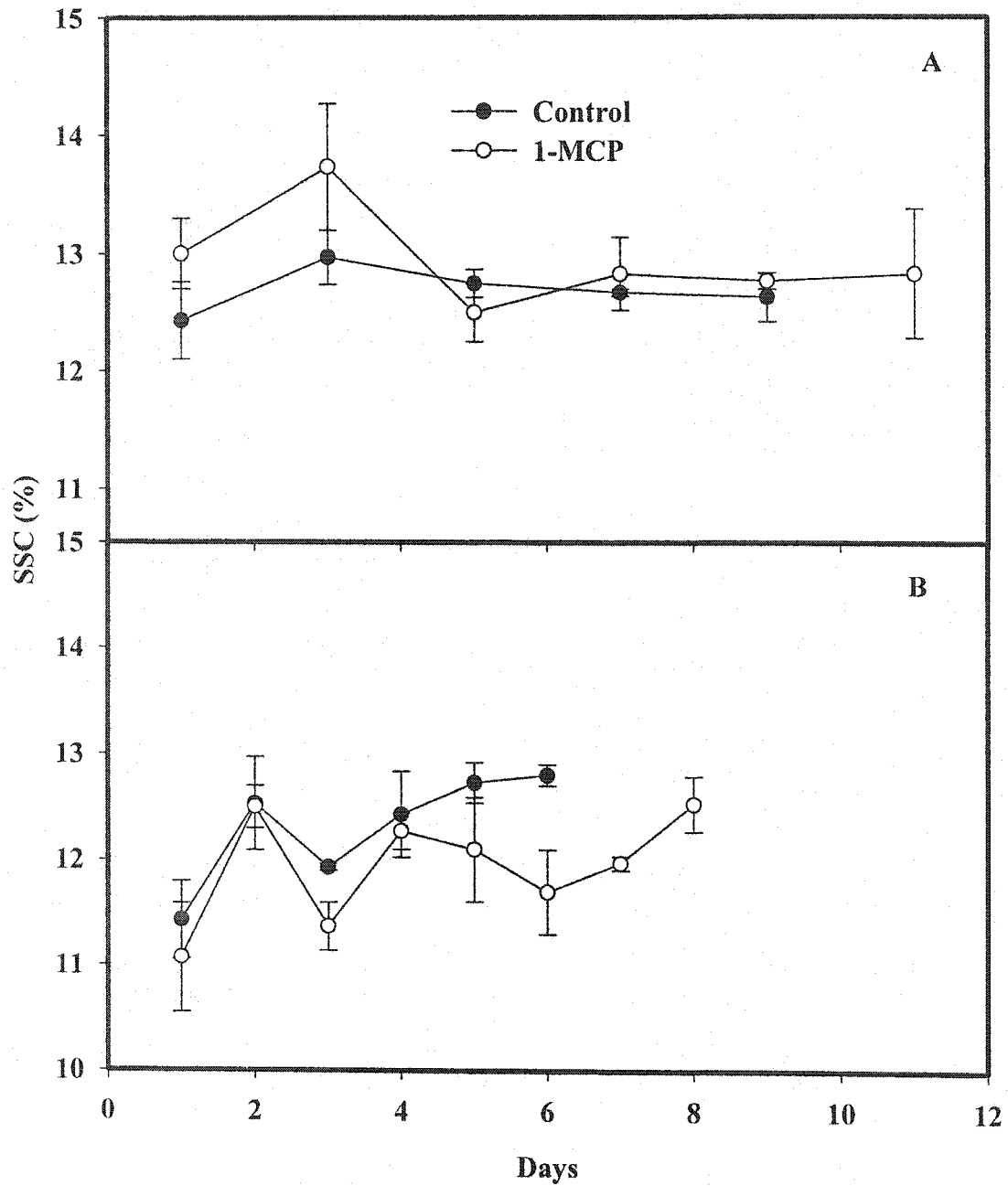


Figure 5-5. Soluble solids concentration (SSC) for 'Sunrise Solo' fruit treated with air (control) and  $9 \mu\text{L L}^{-1}$  1-MCP at the pre-ripe (A) and ripe (B) stage and subsequently stored at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

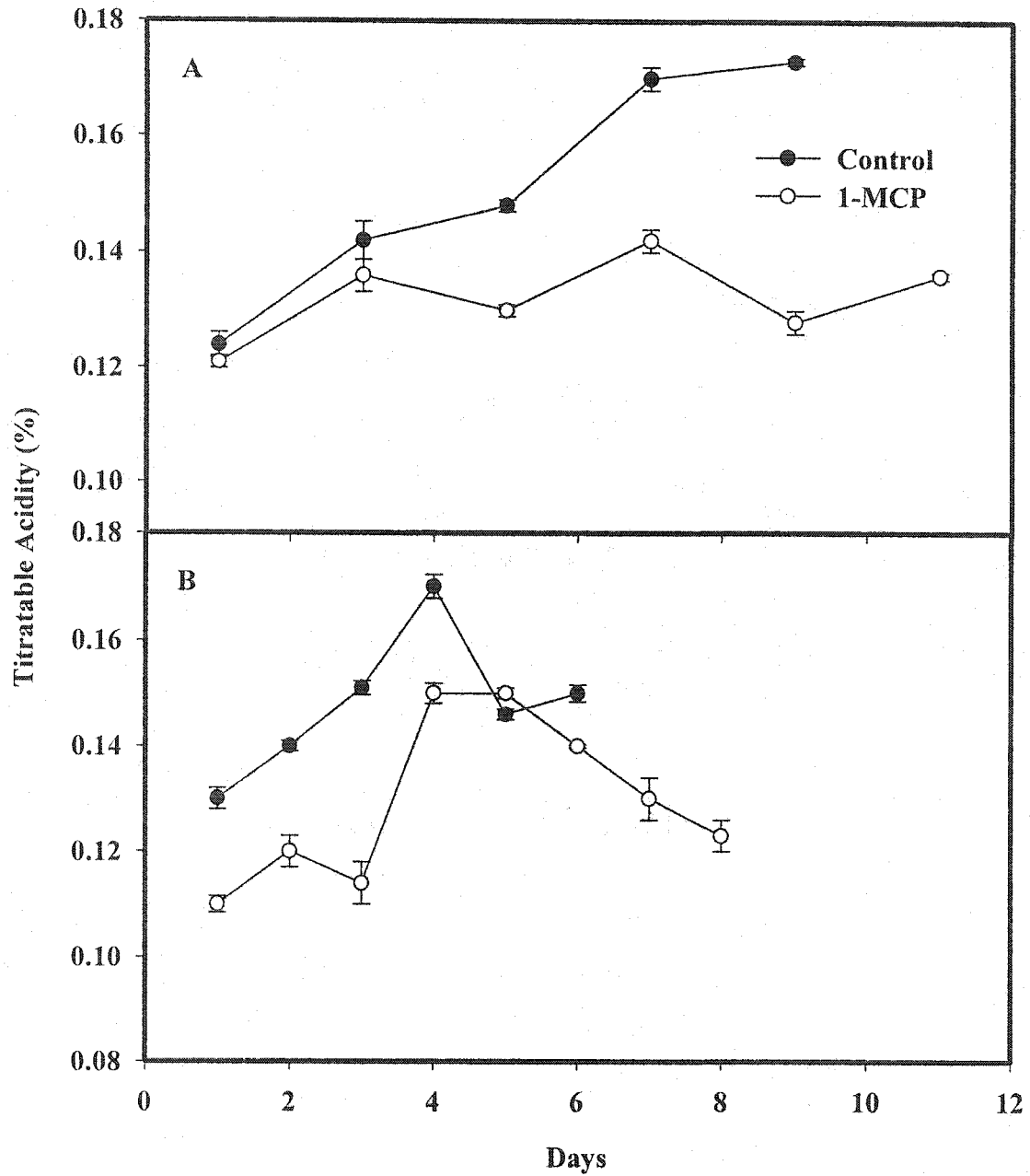


Figure 5-6. Titratable acidity for 'Sunrise Solo' fruit treated with air (control) and  $9 \mu\text{L L}^{-1}$  1-MCP at the pre-ripe (A) and ripe (B) stage and subsequently stored at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ).

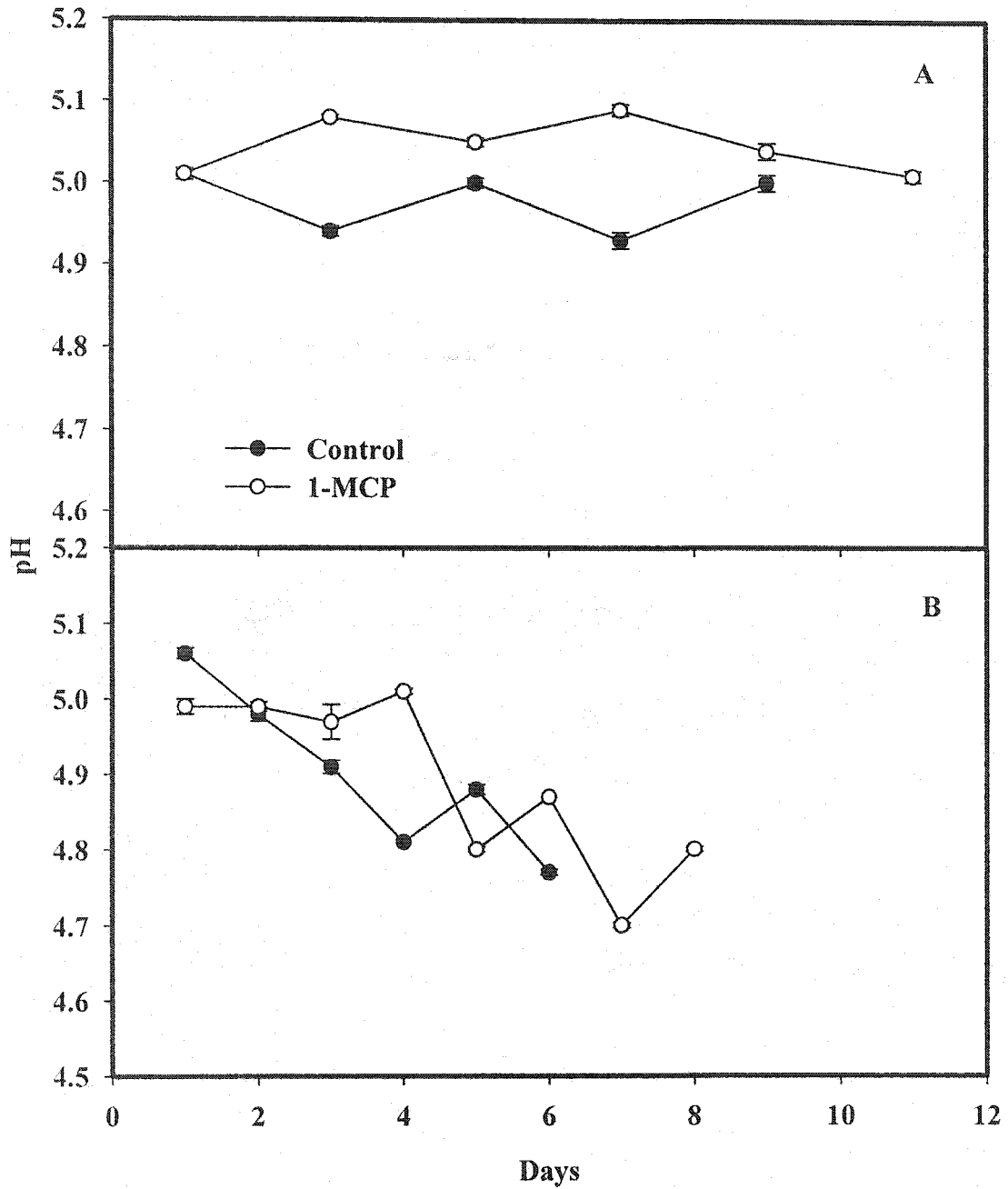


Figure 5-7. The pH for 'Sunrise Solo' fruit treated with air (control) and  $9 \mu\text{L L}^{-1}$  1-MCP at the pre-ripe (A) and ripe (B) stage and subsequently stored at  $20^\circ\text{C}$ . Vertical bars represent standard deviation of the means ( $n = 5$ ). When bars absent, the value for standard deviations was within the dimension of the symbol.



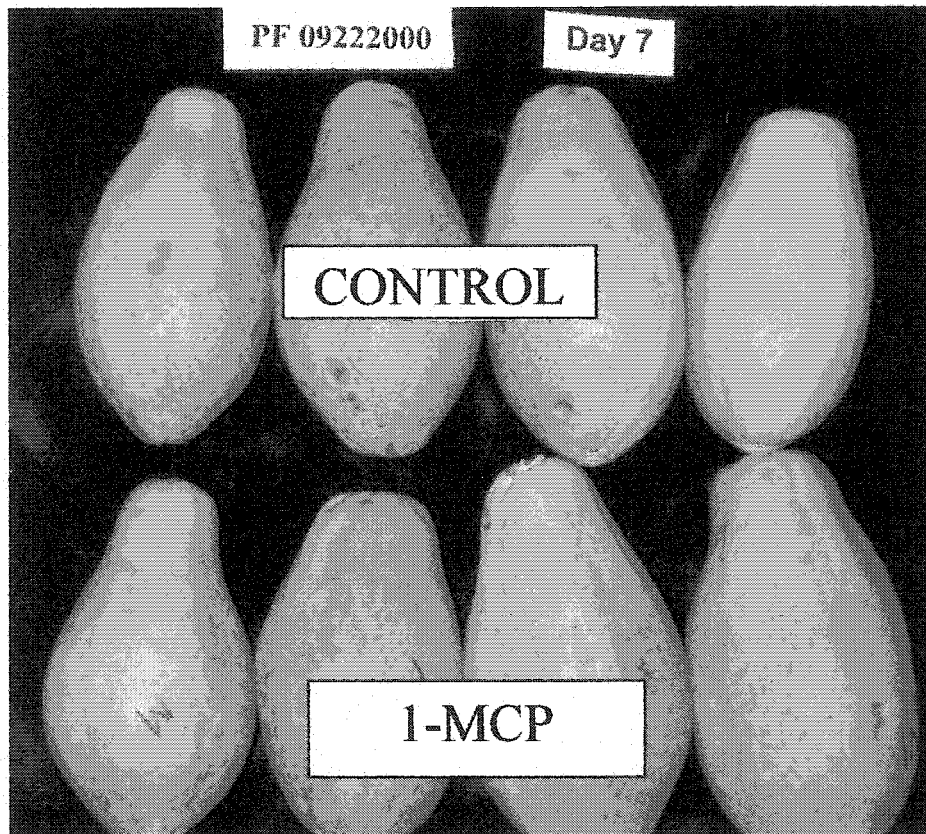


Figure 5-8. Pre-ripe 'Sunrise Solo' fruit treated with  $9 \mu\text{L L}^{-1}$  1-MCP or air (control) and then stored for 7 days at  $20^\circ\text{C}$ .

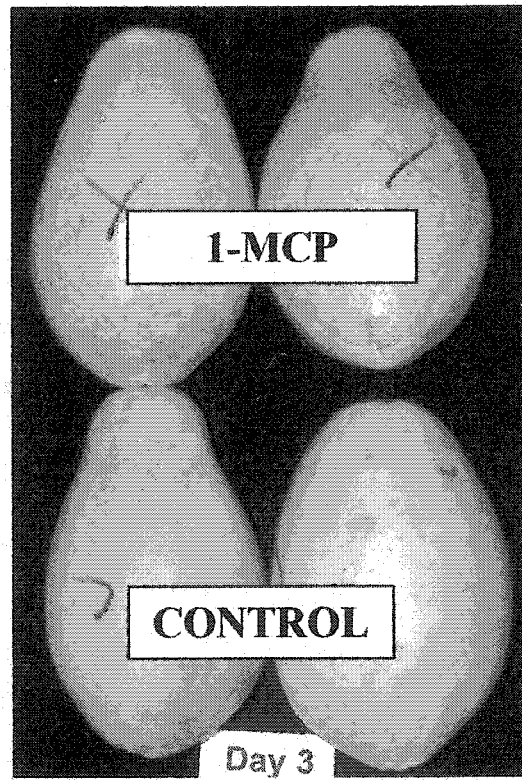


Figure 5-9. Ripe 'Sunrise Solo' fruit treated with  $9 \mu\text{L L}^{-1}$  1-MCP or air (control) and then stored for 3 days at  $20^\circ\text{C}$ .

CHAPTER 6  
QUALITY AND STORAGE LIFE OF INTACT AND FRESH-CUT PAPAYA FRUIT  
TREATED WITH 1-MCP AT THE POSTCLIMACTERIC STAGE OF  
DEVELOPMENT

**Introduction**

Fresh fruit processing, described as cutting, slicing, dicing, peeling, trimming of agricultural commodities in a fresh-like stage (O'Connor-Shaw et al, 1994) has increased in popularity over the past 9 years and continues to increase in popularity along with fresh produce in general (International Fresh-cut Produce Association, 2002). In the United States, the sales of fresh-cut fruits and vegetables have increased from \$3.3 billion in 1994 to \$11 billion in 2000, with sales projected to increase to \$15 billion in 2005 (International Fresh-cut Produce Association, 2002). Due to the extensive tissue damage involved in fresh-cut processing, fresh-cut produce can deteriorate rapidly, becoming unacceptable in a few days (Rolle and Chism, 1987; King and Bolin, 1989). This represents a dramatic loss in storage life compared with the intact commodity, and illustrates the need to explore alternative procedures for prolonging the storage life of fresh-cut fruits and vegetables. The inherent high perishability of certain commodities including ripe tropical fruits is particularly problematic and they are high-priority commodities to be explored as fresh-cut products. For example, papaya fruit are very susceptible to mechanical damage and diseases, and papaya fruit harvested at the mature green stage typically exhibit a storage life of less than one week under ambient tropical conditions (Sankat and Maharaj, 1987). For local markets, papaya fruit are harvested at the full-ripe stage (full yellowish-orange surface coloration; Morton, 1987) and stored at

temperatures above 7 °C. Storage below 7 to 10 °C may cause low-temperature injuries depending on the variety and maturity stage (Paull and Chen, 1983; Sankat and Maharaj, 1997). Since papaya fruit have a limited shelf life, their fresh-cut products also exhibit a short storage life depending on temperature and maturity stage, ranging from 2 to 7 days at 3, 4 or 6 °C (O'Connor-Shaw et al., 1994; Teixeira et al., 2001). Paull and Chen (1997) reported that 'Solo' papaya fruit at 55-80% skin yellowing was judged as the best stage for fresh-cut processing based on flesh firmness, edible flesh and seed separation from placenta.

Wounding due to fresh-cut processing can induce certain physiological and biochemical changes that result in a reduction in storage life of. For instance, ethylene production increases significantly in many fresh-cut fruits and vegetables (Rolle and Chism, 1987). Another consequence of wounding is increased susceptibility to pathogenic microorganisms (Varoquaux and Wiley, 1994), most likely caused by removal of protective outer tissues. The tissue response to microorganisms may also contribute to fermentative alcohol or lactic acid production (Varoquaux and Wiley, 1994), which may contribute to the reduction in storage life of fresh-cut produce.

The exact role or consequence of the increased ethylene production in fresh-cut commodities is not known, but in view of the requirement for continued ethylene responsiveness throughout ripening (Watada et al., 1990), it seems logical to assume that fresh-cut fruits should also respond to the gas. If ethylene does play a role, either adverse or beneficial, in the deterioration of fresh-cut produce, effective inhibitors of ethylene action, including 1-methylcyclopropene (Sisler and Serek, 1997), provide excellent tools for addressing these questions. 1-MCP is a volatile cyclic olefin, effective in the ppb to

ppm range and leaving undetectable residual levels in fumigated tissues (Sisler and Serek, 1997). 1-MCP has been reported to reduce ethylene responses and improve the storage life of fruits at advanced stages of ripening including apple (Mir et al., 2001; Pre-Aymard et al., 2002; Jiang and Joyce, 2002) and tomato (Wills and Ku, 2002; Hoeberichts et al., 2002). Since fresh-cut fruit will typically not continue to ripen post processing, in large part due to the low temperatures employed for storage, optimum quality can be achieved only with fruits that are nearly ripe at the time of processing.

The objectives of the current study were to determine the storage life of fresh-cut papaya tissue derived from fruit treated with 1-MCP at the full-ripe, postclimacteric stage of development.

## **Materials and Methods**

### **Plant Material**

Papaya (*Carica papaya*, L. var. 'Sunrise Solo') fruit originating from Brazil (no thermal and wax treatment) were obtained from C-Brand Tropicals Inc., Homestead, FL. Fruit were transferred to the postharvest storage facilities in Gainesville, FL on the day of receipt and stored at 20 °C for 1 day. The fruit were then selected on the basis of uniformity of size and freedom from defects and graded according to surface color as an estimate of ripeness. Afterward, the fruit were gently brushed, washed with tap water, immersed in 200  $\mu\text{L L}^{-1}$  chlorinated water for 1 min, dried, and transferred 20 °C. Fruit at the postclimacteric stage (based on data from chapter 5; approximately 70 to 80% surface yellow coloration) were employed in this study. The firmness, ethylene production, and respiration of the fruit measured at 20 °C immediately prior to 1-MCP treatment was 6.8 N, 0.8  $\mu\text{L kg}^{-1} \text{h}^{-1}$  and 22  $\text{mL kg}^{-1} \text{h}^{-1}$ , respectively.

### **1-MCP Treatment**

1-MCP as Agrofresh commercial powder (active ingredient, 0.14% 1-MCP) was acquired from the manufacturer (Agrofresh, Philadelphia, PA). Three g of Agrofresh powder were placed in a 136-mL glass vial along with 50 mL of deionized water. The vial was sealed with a septum and incubated on an oscillating shaker for 2 h. The concentration of 1-MCP in the vial headspace was measured using a gas chromatograph (Hewlett-Packard; Model 5890, Avondale, PA) equipped with a 1/8" 80-100 mesh Chromosorb PAW stainless steel column (1.8 m x 3.18 mm i.d.; Supelco, Bellefonte, PA). Injector, oven and detector (FID) were set to 150, 150 and 200 °C, respectively. Isobutylene gas, which has a FID response similar to that of 1-MCP (Jiang et al., 1999), was used as a standard. 1-MCP levels in the stock preparation were approximately 7,500  $\mu\text{L L}^{-1}$  in the headspace. Headspace gas sample (3.3 mL) were injected into a 18.9-L plastic, airtight bucket having 10-L void volume (20 fruit per bucket), yielding a 1-MCP concentration of 2.5  $\mu\text{L L}^{-1}$ . The final 1-MCP concentration of 2.5  $\mu\text{L L}^{-1}$  was maintained for a total exposure period of 24 h at 20 °C. The bucket was vented and reinjected with fresh 1-MCP gas at 6-h intervals. Control fruit were sealed in similar containers but without 1-MCP.

### **Fruit Preparation and Treatment Design**

Papaya fruit following treatment with 1-MCP or air (control) were transferred to a 5-°C room that had been sanitized (200  $\mu\text{L L}^{-1}$ -chlorinated water) prior to fresh-cut processing. After a 1 h period at 5 °C to allow temperature equilibration, both the blossom and pedicel ends of each fruit were removed, and the fruit longitudinally cut into 1.5 cm thick slices (from the pedicel end to the stem end). The two outermost slices were peeled, and cut into pieces approximating slices, with approximate dimensions of 1.5 x

3.5 x 4 cm and weighing 15 to 20 g using a plastic Bread Slicer (Coupe-Pain). Afterward, the slices were rinsed quickly with a sterile isotonic mannitol solution (500 mM) using a squeeze bottle, and then placed in 1.7-L vented plastic containers that had built-in grids on the bottom lifts (FridgeSmart, Tupperware Co., St. Paul, MN).

The treatments included fresh-cut tissue derived from intact fruit pre-treated with air (control, FCC), fresh-cut tissue derived from intact fruit treated with  $2.5 \mu\text{L L}^{-1}$  1-MCP (FCM), intact fruit pre-treated with air (IC), and intact fruit pre-treated with 1-MCP (FCM). At selected intervals during storage at 5 °C, ethylene production, mesocarp firmness, electrolyte leakage, color, sensory changes, and microbial growth were measured for both fresh-cut and intact fruit.

### **Ethylene Production**

Ethylene production was measured by placing individual fruit or fruit slices (4 slices per fruit) in 1.9-L and 950-mL plastic containers, respectively. The containers were sealed for 2 h at 5 °C, and  $\text{C}_2\text{H}_4$  in the containers was measured using a Hewlett Packard gas chromatograph (5890) equipped with an activated alumina SS column and flame ionization detector at room temperature. The carrier gas (Nitrogen) was  $30 \text{ mL min}^{-1}$ . Oven, injector and detector temperature was 70, 200 and 250 °C, respectively

### **Firmness**

Fruit firmness in mesocarp tissue of intact and fresh-cut papaya, kept in a cooler, was measured with an Instron Universal Testing Instrument (Model 4411, Canton, MA) fitted with an 8-mm convex probe and 5-kg load cell at 20 °C. Intact fruit prior to firmness measurements were sliced using the procedures described for obtaining fresh-cut slices from intact fruit. The probe was placed at zero force contact with the fruit surface, and penetrated to a depth of 10-mm at a crosshead speed of  $50 \text{ mm min}^{-1}$ . Data

are reported as the maximum force (Newton) generated during penetration of the tissue slices.

### **Electrolyte Leakage**

Five cylinders of mesocarp tissue were removed from the equatorial region of each fruit or from a fresh-cut slice using an 8-mm diameter cork borer. From each cylinder, one disk (8 mm diameter and 8 mm thickness) was excised from the centermost portion. Disks (5 slices per fruit) were rinsed with deionized water and blotted on a slightly moistened Whatman filter paper. The disks were then incubated in 15 mL of 500 mM mannitol on an oscillating shaker at room temperature for 1 h, followed by a conductivity measurement of the bathing solution. The aliquot removed from the bathing solution for the conductivity measurement was returned to the bathing solution. Conductivity was measured using a conductivity bridge (Y-31A, Yellow Springs, OH) equipped with a conductivity cell (model 3403, Yellow Springs, OH). Afterward, the bathing solution and disks were stored at -20 °C for at least 24 h, thawed and placed into a boiling water bath for 30 min, cooled to room temperature, and conductivity of the bathing solution was measured again. Electrolyte leakage was expressed as percentage of the total tissue electrolytes, estimated from the frozen/heated samples.

### **Color and Sensory Evaluation**

Fruit skin (equatorial region) and flesh (centermost mesocarp) color were assessed as lightness ( $L^*$ ; representing the lightness or grey scale), hue angle (the dimension of color that specifies a position in a color wheel of 360°, with 0°, 90°, 180° and 270° representing the hues red, yellow, green and blue, respectively) and chroma (distinguishing the difference from a pure hue to a grey shade) values using a chromameter (Minolta-CR-200, Japan). Informal descriptive analysis by untrained



personnel was used to profile the quality of fresh-cut and intact fruit, evaluating appearance, odor, texture, and flavor (O'Connor-Shaw et al., 1994) according to the following hedonic scale: 1, poor; 2, poor-good; 3, fair; 4, good-excellent; and 5, excellent. Intact fruit showing surface-pitting were graded from 0 to 20 by 5% increments, 0 for 0%, 1 for 5%, 2 for 10%, and so on. Water soaking on the flesh was expressed as the percentage of areas of a slice (upright surface) with a 5% interval (total 5 slices for each treatment). Intact fruit expressing water soaking were graded from 0 to 20 indicating a 5% increase, 0 for 0%, 1 for 5%, and so on. Five intact fruit or five slices of a container from each treatment was evaluated for mesocarp water soaking. Informal descriptive analysis was used to profile the quality of either fresh-cut cubes and intact fruit flesh by untrained personnel, evaluating appearance, odor, texture and flavor (O'Connor-Shaw et al., 1994) according to the following hedonic chart: 1, poor; 2, poor-good; 3, fair; 4, good-excellent; and 5, excellent. The informal descriptive analyses undertaken immediately after removing fresh-cut and intact fruit from the 5-°C cold room at days 0, 2, 4, 6, 8 and 10 at under white light and the room temperature, 23 °C, with testing at least 3 samples for each treatment.

### **Microbial Count**

Fruit tissue (5 g) was removed with a flame-sterilized cork borer (21.5 mm diameter) and knife from innermost part of a fruit or with a flame-sterilized knife from a fruit slice (approximately 1/3 to 1/4 of a cube) on sterilized aluminum foil in an air-circulated fume. The fruit tissue then was incubated in a 45-mL sterile phosphate buffered solution (PBS), pH 7. The PBS and fruit tissue were then vortexed at high speeds for 1 min using a vortex (Fisher-Genie 2, Scientific Industries Inc., Bohemia, NY), followed by subsequent 10-fold dilutions using sterile PBS as needed. Total

aerobic, Enterobacteriaceae, yeasts and other fungi, total coliforms, and lactic acid bacteria counts were made using 1 mL inoculum of the PBS. The plates and incubation conditions for each count were: total aerobic count, 3M Petrifilm aerobic count plate (3M Microbiology Products, St. Paul, MN), 3 days at 30 °C; Enterobacteriaceae, 3M Petrifilm Enterobacteriaceae count plate, 1 day at 30 °C; yeasts and other fungi, 3M Petrifilm yeast and other fungi count plate, 5 days at 25 °C; total coliforms, 3M Petrifilm coliform count plate, 1 day at 30 °C; and lactic acid bacteria, 3M Petrifilm aerobic count plate, anaerobic, incubation for 2 days at 30 °C in a 1.9-L airtight plastic container with a Gas Pak anaerobic system envelope (Becton Dickinson and Co., Cockeysville, MD). The plates were prepared in an air circulated hood after 0 (immediately after dicing), 5 and 10 days at room temperature, and microbial counts were reported as colony forming units per gram of tissue (CFU g<sup>-1</sup>).

### **Statistical Analysis**

General linear model program of SAS (SAS institute, Carey, NC) and Duncan's multiple range test were performed for randomized complete block design in which treatments (FCC, FCM, IC and IM) were blocks.

## **Results and Discussion**

### **Ethylene Production**

Differences in ethylene production rates (at 5 °C) were insignificant among treatments at day 1 as shown in Figure 6-1: IC, 38.4; IM, 24.7; FCC, 31.5; FCM, 31.4 nL kg<sup>-1</sup> h<sup>-1</sup>. The ethylene production rates increased during storage and reached values of 64.4 (IC), 47.8 (IM), 41.4 (FCC) and 34.7 nL kg<sup>-1</sup> h<sup>-1</sup> (FCM) at day 9. The increases in ethylene production rates from day 1 to 9 were 70% for IC, 94% for IM, 32% for FCC, and 11% for FCM. The ethylene production of both fresh-cut postclimacteric and intact

postclimacteric 'Sunrise Solo' fruit at 5 °C was lower than the values reported by Paull and Chen (1997) for preclimacteric and postclimacteric 'Sunset' papaya cultivar. These authors reported that ethylene production of halved, unpeeled and deseeded preclimacteric and postclimacteric papaya fruit was approximately  $1 \mu\text{L kg}^{-1} \text{h}^{-1}$  at 4 °C. Postclimacteric 'Sunrise Solo' fruit produce less than  $1 \mu\text{L kg}^{-1} \text{h}^{-1}$  ethylene at 20 °C (Chapter 5), which is consistent with the observation that 'Sunrise Solo' is a low-ethylene producer. Paull and Chen (1997) noted that ethylene production of the halved and deseeded preclimacteric papaya fruit was approximately 5 fold higher than that of intact preclimacteric fruit after 2-day of storage at 22 °C. The ethylene production of FCC and FCM did not differ significantly nor did that of IC and IM, however, FCC and FCM had slightly lower ethylene production compare to IC and IM collectively. Similarly, Artes et al. (1999) reported slightly higher ethylene production in fresh-cut preclimacteric tomato fruit relative to intact preclimacteric tomato fruit at 2 °C but the authors noted a 5-fold higher ethylene production than intact fruit at 10 °C (Artes et al., 1999).

The ethylene production of either fresh-cut or intact postclimacteric 'Sunrise Solo' fruit was not affected by 1-MCP at 5 °C. However, postclimacteric 'Golden Delicious' apple treated with 1-MCP ( $10 \mu\text{L L}^{-1}$ , for 6 h at 20 °C) before or after fresh-cut processing showed lower ethylene production, measured at 20 °C, compared with fresh-cut fruit treated with air at 4 °C (Jiang and Joyce, 2002). Jacomino et al. (2002) reported that intact preclimacteric 'Sunrise Solo' treated with 1-MCP (90 or  $270 \text{ nL L}^{-1}$ , for 12 h at 20 °C) also showed suppressed ethylene production compared to non-1-MCP-treated fruit at 20 °C. The very low ethylene production (below  $65 \mu\text{L kg}^{-1} \text{h}^{-1}$ ) of either fresh-cut or intact 'Sunrise Solo' fruit at 5 °C may explain the insignificant ethylene production rates

between 'Sunrise Solo' fruit or slices with 1-MCP and fruit or slices without 1-MCP. Another reason for the insignificant ethylene production of 'Sunrise Solo' fruit with 1-MCP and without 1-MCP might be release of 1-MCP from the receptor sites. Papaya fruit are chill-sensitive; and the storage temperatures used in this study are below the chill threshold for papaya (Chen and Paull, 1986) Storage of either fresh cut or intact papaya at sub-threshold temperatures might perturb the cell membrane system, resulting in conformational changes in the ethylene receptors that may influence the tenacity of 1-MCP-receptor interactions. Postclimacteric 'Sunrise Solo' fruit treated with  $9 \mu\text{L L}^{-1}$  1-MCP for 24 h recovered their suppressed ethylene production (measured at 20 °C) in 5 days at 20 °C (chapter 5), which may support the idea that 1-MCP binding is not irreversible and is possibly released with time.

#### **Firmness Assessment**

Mesocarp firmness values for all treatments (IC, IM, FCC and FCM) decreased during storage (Figure 6-2). The extent of softening was consistently and significantly higher in the fresh cut compared with the intact fruit. During the 10-day storage period, IC softened from 5.6 to 4.1 N (a 26% decline) and IM from 7.1 to 5.9 N (a 15% decline). Firmness of FCC decreased approximately 50%, from 4.7 to 2.4 N, during the first 2 days of storage. Afterward, the rate of softening of FCC declined, with firmness reaching values of 1.22 N after 10 days. Firmness of fresh cut tissue from 1-MCP-treated fruit (FCM) was significantly retained, declining only 19% (from 5.6 to 4.6 N) during the first 2 days, reaching a low of 2.6 N at day 10. FCC lost 74% of their original firmness during the 10-day storage while FCM declined only 53%. Initially, FCM had 27% higher firmness than FCC, and the percentage of firmness difference between IC and IM

increased 92% at day 2. At day 10, the firmness value of fruit slices with 1-MCP was more 2-fold higher than that of fresh-cut control fruit.

The augmented softening of fresh-cut postclimacteric 'Sunrise Solo' papaya fruit treated with or without 1-MCP might be due to over activation of cell-wall enzymes. For example, wounding stimulated polygalacturonase, and  $\alpha$ - and  $\beta$ -galactosidase activities in fresh-cut 'Sunrise Solo' fruit (at 60 to 70% yellow surface color) compared with intact fruit (Karakurt and Huber, 2002). These enzymes in addition to xylanase (Paull and Chen, 1987) might collectively contribute to softening of low-temperature stored fresh-cut papaya and of intact papaya fruit during normal ripening as well. 1-MCP delayed or reduced the rate of softening in both intact and fresh-cut postclimacteric 'Sunrise Solo' papaya, which is similar to the case for fresh-cut, postclimacteric 'Golden Apple' treated with  $10 \mu\text{L L}^{-1}$  1-MCP (6 h at 20 °C) before or after slicing and stored at 4 °C (Jiang and Joyce, 2002). The delayed softening in intact postclimacteric fruit treated with 1-MCP or fresh-cut fruit derived from 1-MCP-treated intact postclimacteric fruit supports the fact that ethylene is involved in the softening of climacteric fruit (Lelievre et al., 1997), even under stress (e.g., storage below the critical minimum temperature, wounding caused by processing). The deferred softening as a result of 1-MCP treatment has been also reported for intact postclimacteric 'Redchief Delicious' apple fruit subjected to either one or multiple applications of 1-MCP ( $0.7 \mu\text{L L}^{-1}$ , for 16 h at 0, 5, 10, 15 and 20 °C; Mir et al., 2001) and tomato fruit treated with 1-MCP at the orange red stage ( $50$  to  $150 \text{ nL L}^{-1}$ , for 24 h at 20 °C; Hoeberichts et al., 2002) at 20 °C.

### **Electrolyte Leakage**

A continuous increase of electrolyte efflux in FCC and FCM tissue was observed during 10 days of storage at 5 °C (Figure 6-3). The increase in electrolyte leakage of FCC

and FCM followed similar trends until day 8, after which time leakage of FCC tissue was significantly higher than that of FCM. Total electrolyte leakage of FCC increased from 15.3% to 38.8% during the 10-day storage period while FCM increased from 15.3% to 25.0%. In sharp contrast to the fresh-cut tissues, intact fruit (IC and IM), showed no changes in ion leakage during storage (Figure 6-3). Between IM and IC, electrolyte leakage ranged from lows of 14.0% (IM at day 0), comparable to day 0 fresh cut tissue, to a high of 17.8% (IM at day 10) over the 10-day period. Electrolyte leakage is considered to be an indirect measure of cell membrane damage (Marangoni et al., 1996), which may contribute to fruit softening through a loss in cell turgor. Karakurt and Huber (2002) reported that fresh-cut papaya fruit at slightly less ripe stage (60 to 70% yellow color) caused increased activities of lipoxygenase and phospholipase D, suggesting that these enzymes may assist in membrane breakdown and, consequently, the more rapid softening of fresh-cut fruit. Increased ion leakage is considered to be a common symptom of chilling injury (CI) in papaya fruit (Chen and Paull, 1986). As is true for papaya fruit, most chill-sensitive fruits show increased ion leakage in response to prolonged low-temperature storage (Saltveit, 2000). The effects of 1-MCP on leakage of postclimacteric 'Sunrise Solo' fruit were minimal and significant only for fresh-cut fruit derived from 1-MCP treated intact fruit. The suppressed electrolyte leakage of fresh-cut tissue derived from 1-MCP-treated fruit through day 10 might be due to the inhibitory effects of 1-MCP upon chilling injury. Preclimacteric Charentais melon fruit exposed to  $1 \mu\text{L L}^{-1}$  1-MCP (for 24 h at 22 °C) stored at 2 °C for 16 days followed by transfer to 22 °C for 5 days were insensitive to low-temperature damage (estimated by visually rating the extent of surface pitting and browning) compared to non-1-MCP-treated fruit (Ben-Amor et al.,

1999). 1-MCP, furthermore, suppressed chilling injury symptom (internal browning) in pineapple ( $1 \mu\text{L L}^{-1}$  18 h at  $20^\circ\text{C}$ ) relative to non-1-MCP-treated fruit stored at  $10^\circ\text{C}$  for 4 weeks (Selvarajah et al., 2001) and avocado fruit ( $100 \mu\text{L L}^{-1}$  for 24 or 48 h at  $20^\circ\text{C}$ ) (mesocarp discoloration) compared with non-MCP-treated fruit at  $5^\circ\text{C}$  after 4 weeks (Pesis et al., 2002).

### **Color and Sensory Evaluation**

$L^*$  and hue angle values for IC and IM did not change significantly during 10 days at  $5^\circ\text{C}$ , indicating no visible color changes over fruit surface (Figure 6-4A and 4B). Chroma, however, decreased slightly during 10 days storage for both IC and IM, suggesting that the epidermal color for both treatments became dull (Figure 6-4C). None of the skin color parameters ( $L^*$ , hue angle or chroma) in IC and IM papaya were significantly affected by 1-MCP.

No significant changes in mesocarp color, as evaluated by hue angle, were observed for any of the 4 treatments (IC, IM, FCC and FCM) during storage (Figure 6-4E).  $L^*$  and chroma of FCC and FCM, however, slightly decreased during the 10-day storage, implying that the color became duller during storage (Figure 6-4D and 4F). Through the last day of storage (day 10),  $L^*$  and chroma of FCC significantly decreased compared to FCM, meaning that the loss of color intensity or purity might have been delayed by 1-MCP (Figure 6-5). A beneficial effect of 1-MCP on the color of fresh-cut fruit was also reported for postclimacteric 'Golden Delicious' apple (Jiang and Joyce, 2002). These authors reported that the epidermal tissue of fresh-cut apple treated with 1-MCP ( $1$  or  $10 \mu\text{L L}^{-1}$  for 6 h at  $20^\circ\text{C}$ ) before or after cutting was greener after 10 days of storage at  $4^\circ\text{C}$  than fresh-cut fruit treated with air.

Pitting of the fruit surface, a chilling injury symptom in papaya (Chen and Paull, 1986), was observed in the present studies but was not influenced by 1-MCP (Figure 6-6). The overall average pitting percentage for IC and IM was 27% and 21.8%, respectively. Water soaked areas of mesocarp tissue of IC was 0% at day 0 and 6.5% at day 10, and those of IM 0% at day 0 to 2.5% at day 10 (Figure 6-7A). FCC and FCM mesocarp tissue displayed a 96% (from 0% to 96%) and 76% (from 0% to 76%) increase in water soaked areas, respectively, during the 10 day of storage (Figure 6-7A). The extent of water soaking of FCC mesocarp tissue was 54% at day 4 while the incidence in FCM was only 30%. FCC exhibited significantly higher water soaking incidence than FCM on days 8 through 10.

The flesh of IC and IM scored over 4 (good-excellent to excellent) for sensory evaluation during most of the storage period whereas FCC and FCM showed a dramatic decline to as low as 1.2 (FCC; poor to poor-good) at day 10 (Figure 6-7B). At day 2, sensory evaluation of FCM was 5 (excellent) whereas FCC was below 4 (fair to good-excellent). By day 6 of storage, the sensory scores for that of FCM was 3.2 (fair to good-excellent) compared with 2.2 (poor-good to fair) for FCC (Figure 6-8). Storage life FCC was limited to 2 days due to a dramatic decline in sensory evaluation while that of FCM limited 6 days for the same reason.

Advanced ripening of papaya is associated with development of yellowish-orange coloration of the surface (Akamine and Goo, 1971), involving primarily a loss of chlorophyll (Sanxter, et al., 1992). Sanxter et al. (1992) reported that mature green papaya skin had the highest chlorophyll and total carotenoids content, and chlorophylls in skin consistently decreased through the full ripe stage while total carotenoids showed a



minimal decrease. The initial skin color (estimated by hue angle) of intact, postclimacteric 'Sunrise Solo' fruit regardless of 1-MCP treatment remained unchanged during storage. Storage at 5 °C seems to be very effective at delaying color change irrespective of 1-MCP treatment by slowing down chlorophyll degradation. Pitting of the fruit surface was not extensive in either IC or IM postclimacteric 'Sunrise Solo' fruit, which may be due to immediate evaluation of fruit upon removal of the fruit from 5 °C since chilling injury symptoms are accelerated upon transfer of injured fruit to a higher temperature (Chan, 1998).

Fresh-cut 'Sunrise Solo' fruit regardless of 1-MCP treatment showed an increase in water soaking during storage. However, fresh-cut fruit derived from 1-MCP-treated fruit exhibited less increase in water soaking relative to fresh-cut control on days 8 through 10, which may support that ethylene contributes water soaking in papaya fruit. The ethylene-inducible water soaking was reported for watermelon fruit at 18 or 20 °C by Elkashif and Huber (1988), and Karakurt and Huber (2000). In contrast, Chatenet et al. (2000) reported water soaking in Charentais melon mesocarp during the late stage of ripening was not an ethylene-inducible event as evaluated due to no change in the expression of genes encoding ACS and ACO. Chatenet et al. (2000) also documented 1-MCP (1  $\mu\text{l L}^{-1}$  for 24 h) did not prevent water soaking in Charentais melon fruit, treated at the preclimacteric stage, after 35 days of storage at 14 °C. Water soaking increase in fresh-cut 'Sunrise Solo' fruit during storage at 5 °C may be caused by a depletion of cell wall calcium (Chatenet et al. 2000) and/or ethylene-inducible membrane permeability changes (Karakurt and Huber, 2000).

### Microbiological Counts

No changes were noted in intact fruit in terms of total aerobic count during storage, regardless of 1-MCP treatment (Table 6-1). However, population of aerobic organisms on fresh-cut fruit irrespective of 1-MCP were increased from  $1.73 \times 10^1$  (at day 0) to  $2.72 \times 10^3$  CFU g<sup>-1</sup> (at day 10) for FFC and from  $1.47 \times 10^1$  (at day 0) to  $3.04 \times 10^3$  (at day 10) for FCM (Table 6-1). The number of total coliforms, Enterobacteriaceae and other fungi were almost undetectable in all treatments during storage (Table 6-1). Both IC and FCC showed a slight but significant increase in lactic acid bacteria through day 5, then the two treatments displayed a decrease through day 10. However, lactic acid bacterium count of both IM and FCM initially decreased through 5 and then increased through day 10 (Table 6-1). Only FCC and FCM displayed significant yeast enumeration increase during storage, and the population of yeast were  $9.73 \times 10^1$  and  $9.13 \times 10^1$  CFU g<sup>-1</sup> for FCC and FCM, respectively, at day 10, with no significant differences between the two treatments (Table 6-1). Our microbial counts were low compared to findings of O'Connor-Shaw et al. (1994), who reported total aerobic counts of climacteric/postclimacteric papaya cubes (no 1-MCP and no chlorine treatment) varied from  $1.4 \times 10^4$  to  $1.7 \times 10^7$  CFU g<sup>-1</sup> after 4 days of storage at 4 °C. On the other hand, Teixeira et al. (2001) reported a total aerobic count of  $1 \times 10^3$  in fresh cut climacteric/postclimacteric papaya fruit (no 1-MCP; washed with 200- $\mu$ L L<sup>-1</sup> chlorinated water) after 7 days of storage at 9 °C. The lower microbial count in our experiment may be due to very strict sanitation practices during and after fresh-cut processing. Teixeira et al. (2001) also stated that hygienic care adopted during processing resulted in low microbial counts in fresh-cut papaya fruit. 1-MCP had no significant effect upon microbial growths except for lactic acid bacterium, the growth of which seemed to be

promoted by 1-MCP; however, the population of lactic acid bacteria was insignificantly low in all treatments, even at day 10. The 'Solo' variety treated with  $25 \mu\text{L L}^{-1}$  1-MCP  $20^\circ\text{C}$  at mature green stage and stored at  $20^\circ\text{C}$  for approximately for 20 days showed slightly higher symptoms of stem rots, body black rots, and anthracnose compared to untreated fruit lasted approximately 5 days (Hofman et al., 2001). Hofman et al. (2001) attributed the slight increase in the symptoms of the diseases in 1-MCP-treated fruit to a reduction in antifungal concentrations due to extended storage life. Jiang et al. (2001) reported that ripe 'Everest' strawberry fruit treated with 500 to  $1000 \text{ nL L}^{-1}$  1-MCP (24 h at  $20^\circ\text{C}$ ) displayed accelerated leak rot disease development at  $20^\circ\text{C}$  compared to untreated fruit; however, 1-MCP at 100 and  $250 \text{ nL L}^{-1}$  delayed the onset of the decay. Tomato plant lines expose to  $10 \text{ nL L}^{-1}$  1-MCP for 24 h at 18 to  $24^\circ\text{C}$  resulted in a significant increase in *Botrytis cinerea* frequency in the cultivars 'Moneymaker' and 'Castlemart' but no increase in 'Pearson' (Diaz et al., 2002). Hence, the effects of 1-MCP upon microbial growth are complex and depend on the type of microorganisms, 1-MCP concentration, cultivars, and tissue type and development.

To conclude, 1-MCP delayed quality loss of fresh-cut postclimacteric papaya fruit derived from intact postclimacteric fruit treated with 1-MCP, resulting in a 4-day extended shelf life. Thus, 1-MCP is a safe and inexpensive postharvest application for delaying quality loss and extending storage life of fresh-cut postclimacteric 'Sunrise Solo' fruit at  $4^\circ\text{C}$ . Microbial proliferation in fresh-cut postclimacteric papaya fruit would not be a problematic factor contributing either tissue softening or quality loss during the storage life if the necessary precautions are taken.

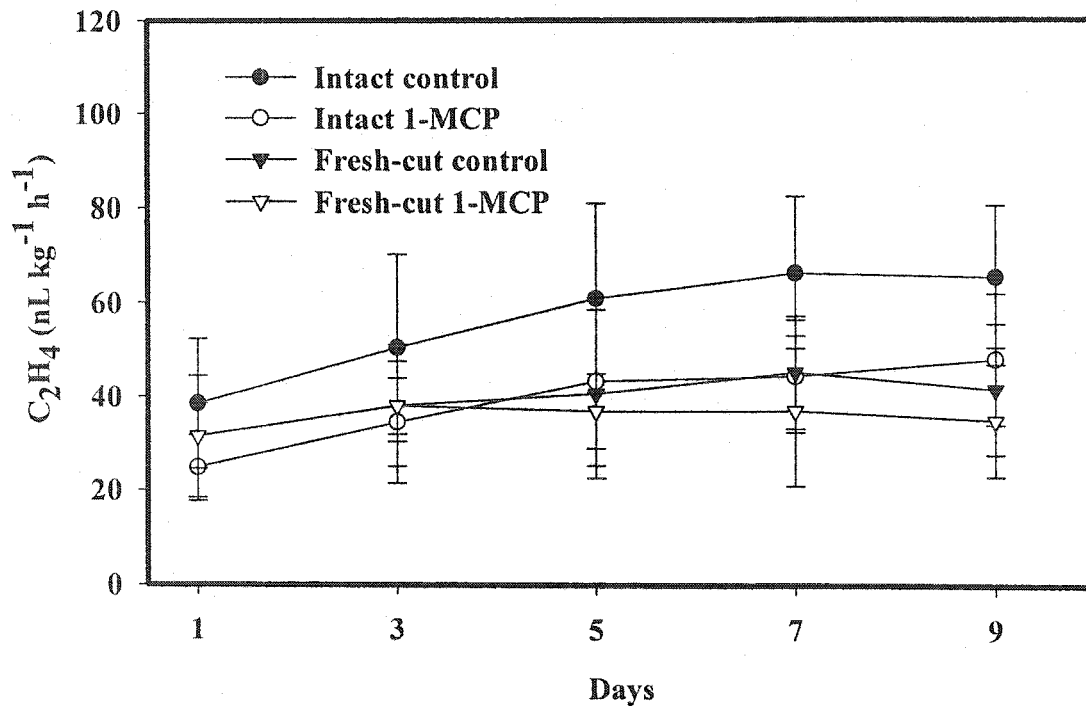


Figure 6-1. Ethylene production for intact postclimacteric 'Sunrise Solo' papaya fruit pretreated with  $2.5 \mu\text{L L}^{-1}$  1-MCP and air (control), and for fresh-cut postclimacteric fruit derived from the either intact air-treated or the intact 1-MCP-treated fruit during storage at  $5^\circ\text{C}$ . Vertical bars represents standard deviations of the means ( $n = 5$ ).

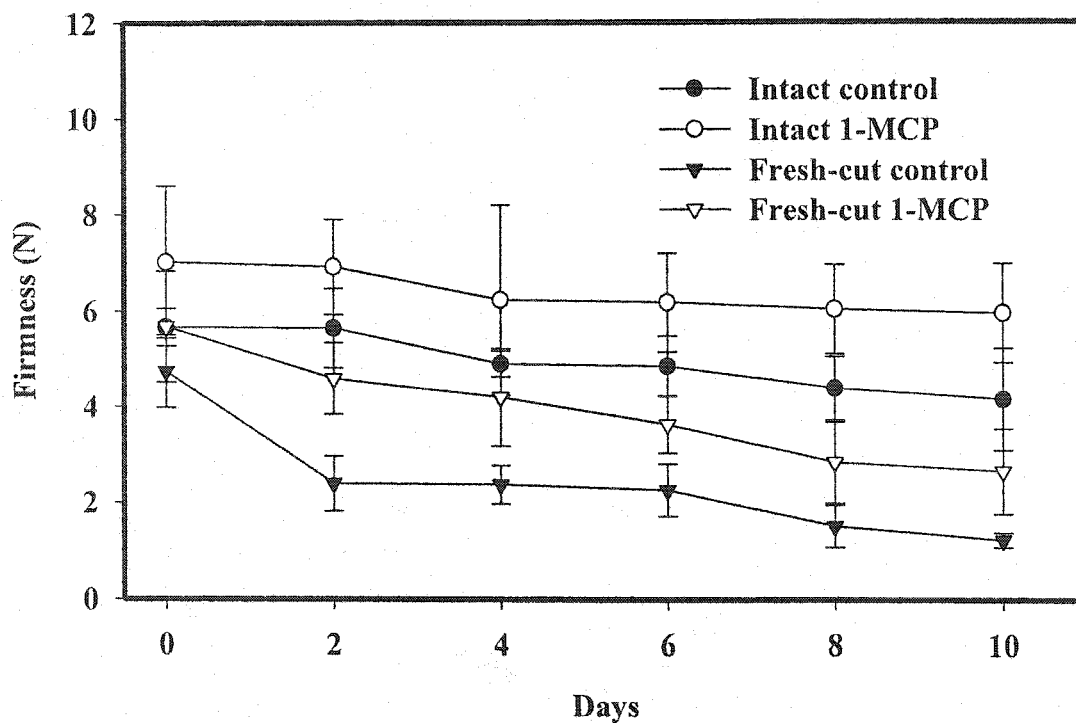


Figure 6-2. Mesocarp firmness for intact postclimacteric 'Sunrise Solo' papaya fruit pre-treated with  $2.5 \mu\text{L L}^{-1}$  1-MCP and air (control), and for fresh-cut postclimacteric fruit derived from either the intact air-treated or the intact 1-MCP-treated fruit during storage at  $5^\circ\text{C}$ . Vertical bars are standard deviations of means ( $n = 5$ ).

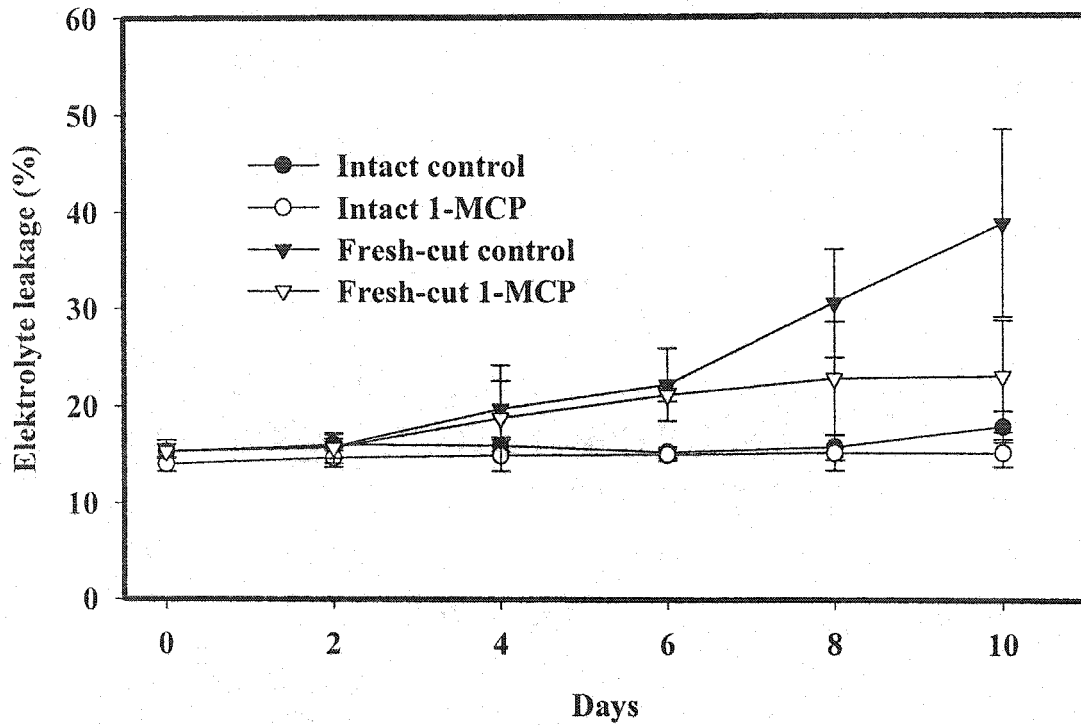


Figure 6-3. Electrolyte leakage (% of total) for intact postclimacteric 'Sunrise Solo' papaya fruit pre-treated with  $2.5 \mu\text{L L}^{-1}$  1-MCP and air (control), and for fresh-cut postclimacteric fruit derived from either the intact air-treated or the intact 1-MCP-treated fruit during storage at  $5^\circ\text{C}$ . Vertical bars are standard deviations of means ( $n = 5$ ).

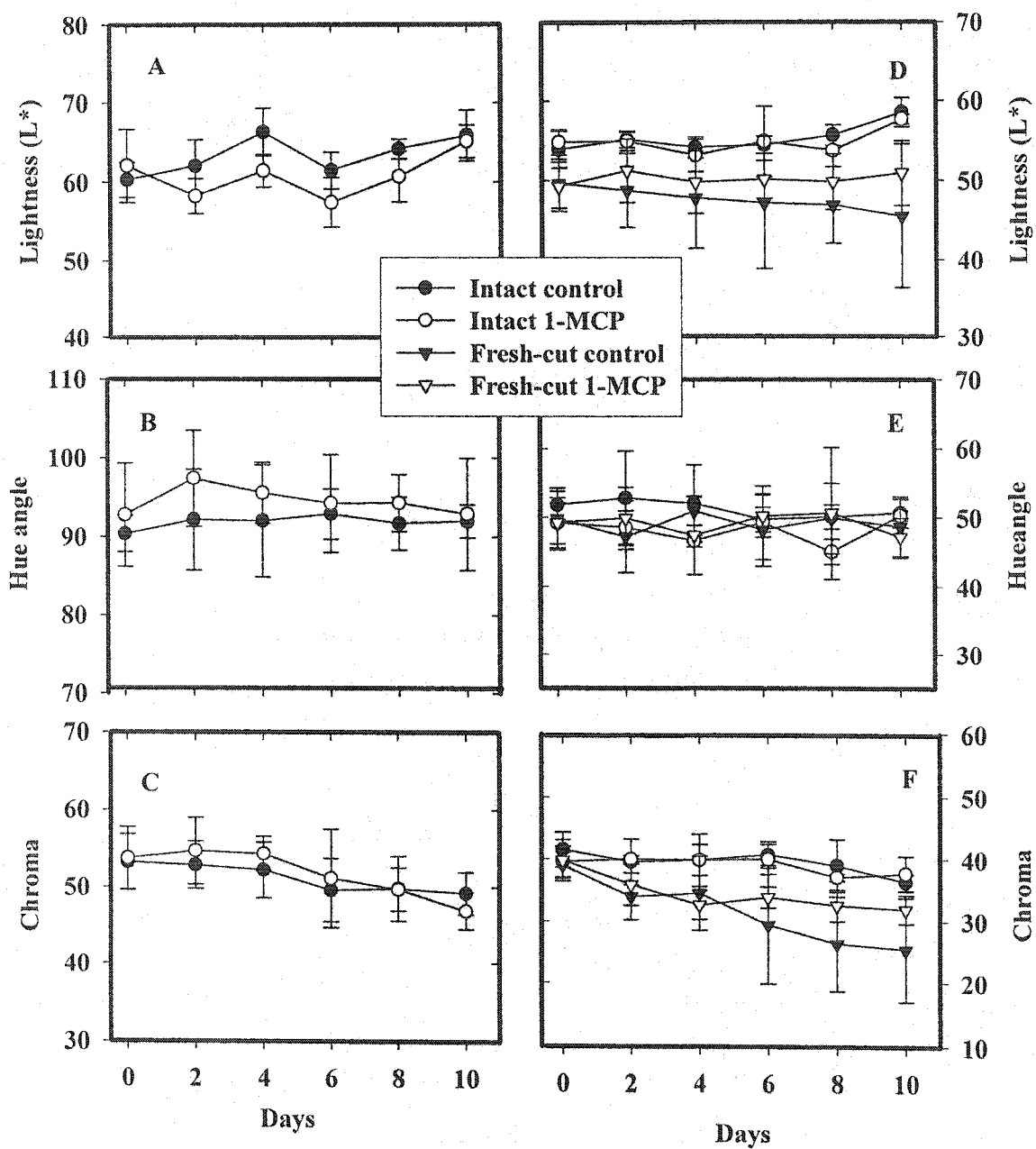


Figure 6-4. Color parameters for intact postclimacteric 'Sunrise Solo' papaya fruit skin pre-treated with  $2.5 \mu\text{L L}^{-1}$  1-MCP and air (control), and for the intact fruit flesh and fresh-cut fruit derived from either the intact air-treated or the intact 1-MCP-treated fruit during storage at  $5^\circ\text{C}$ . Vertical bars are standard deviations of means ( $n = 5$ ).

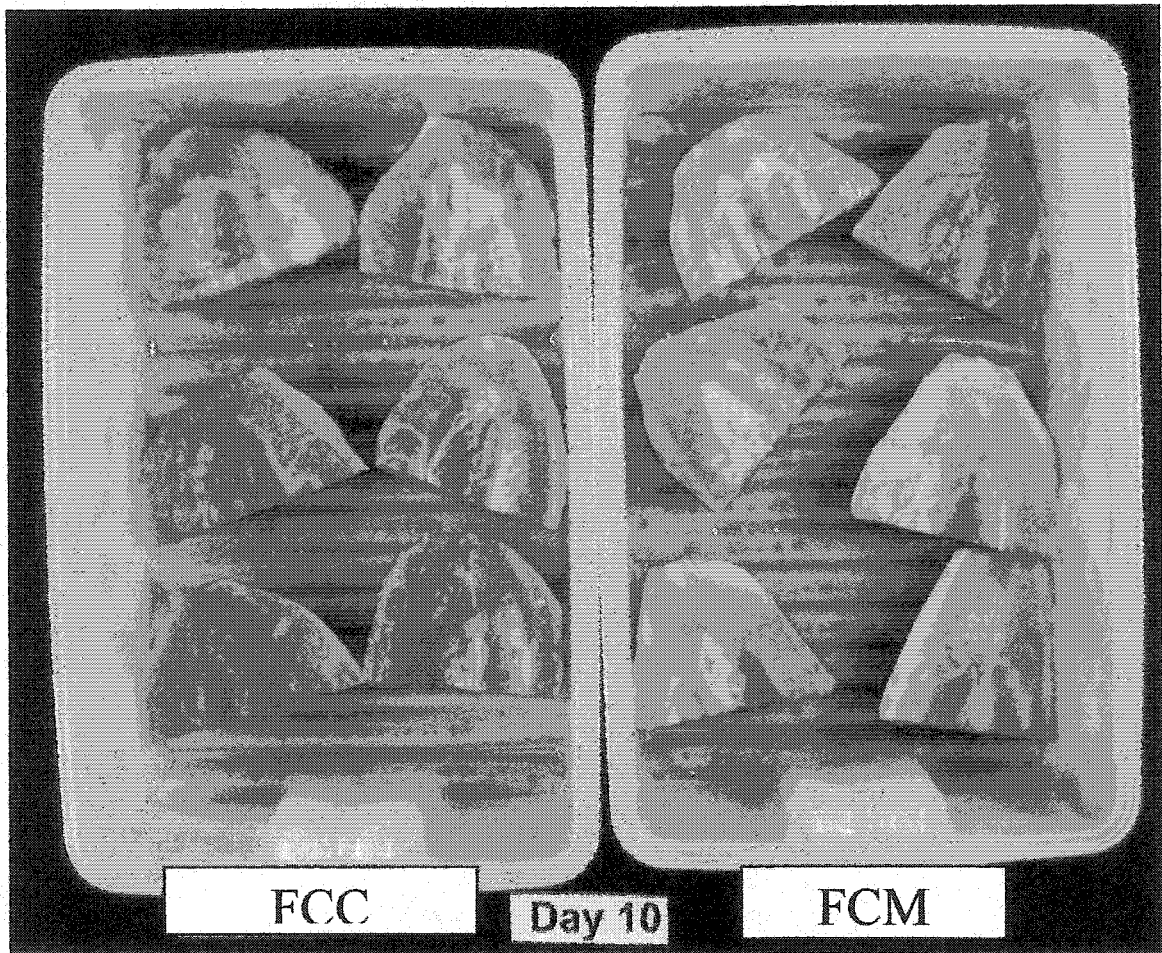


Figure 6-5. Fresh-cut postclimacteric 'Sunrise Solo' papaya fruit derived from either intact postclimacteric air-treated (FCC) or intact postclimacteric 1-MCP-treated fruit (FCM) and then stored for 10 days at 5 °C.



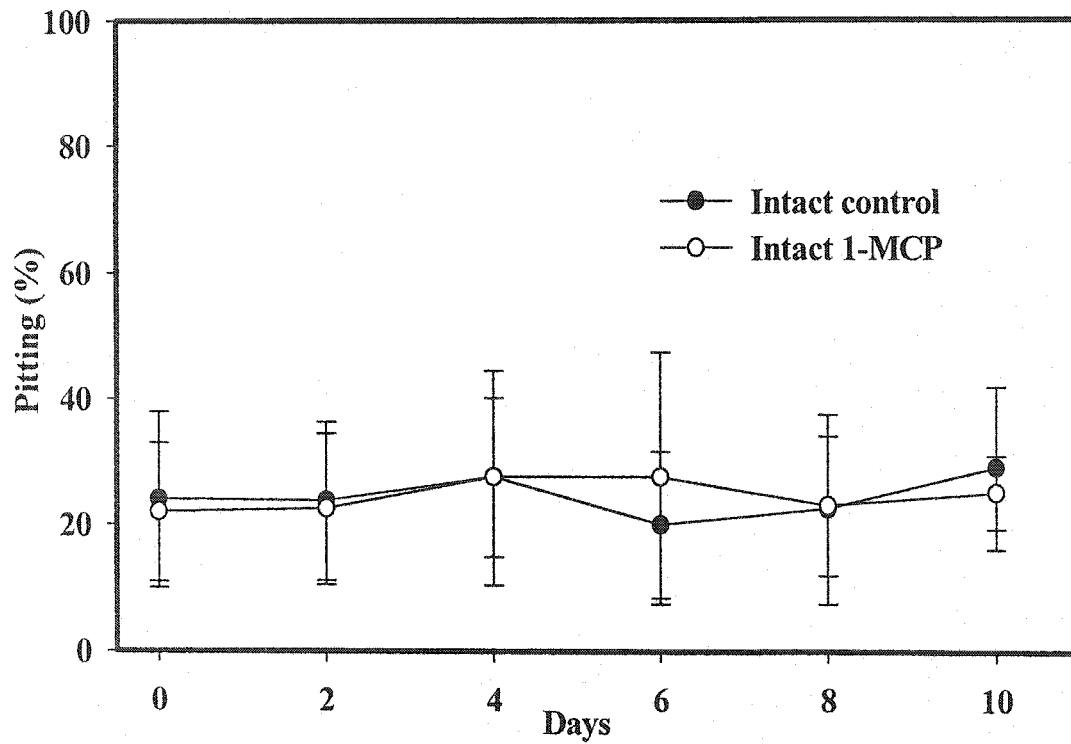


Figure 6-6. Pitting of postclimacteric 'Sunrise Solo' papaya fruit pre-treated with  $2.5 \mu\text{L L}^{-1}$  1-MCP and air (control) during storage at  $5^\circ\text{C}$ . Vertical bars are standard deviations of means ( $n = 5$ ).

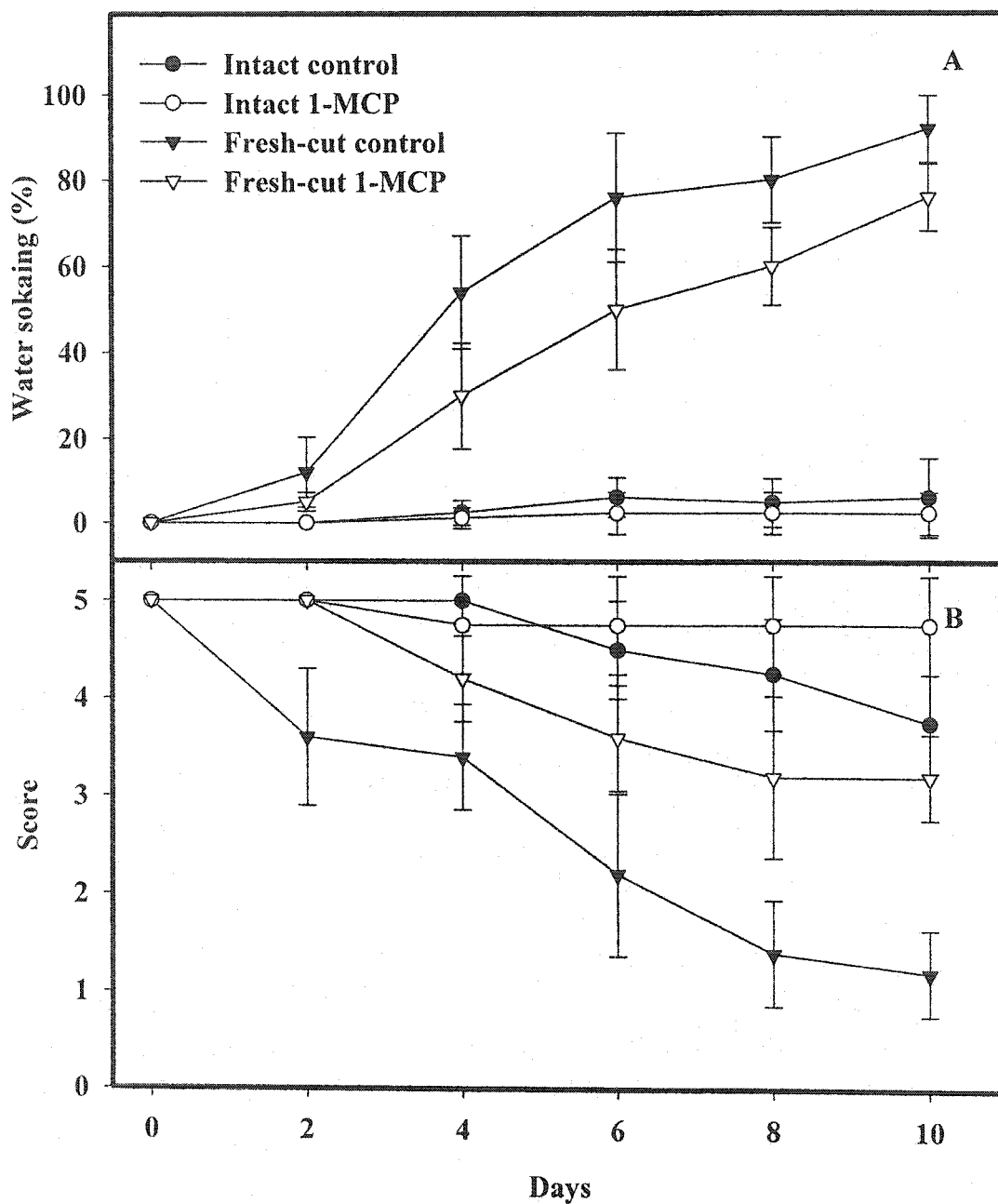


Figure 6-7. Water soaking (A) and sensory evaluation (B) for intact postclimacteric 'Sunrise Solo' papaya fruit pre-treated with  $2.5 \mu\text{L L}^{-1}$  1-MCP and air (control), and for fresh-cut postclimacteric fruit derived from either the intact air-treated or the intact 1-MCP-treated fruit during storage at  $5^\circ\text{C}$ . Vertical bars are standard deviations of means ( $n = 5$ ).

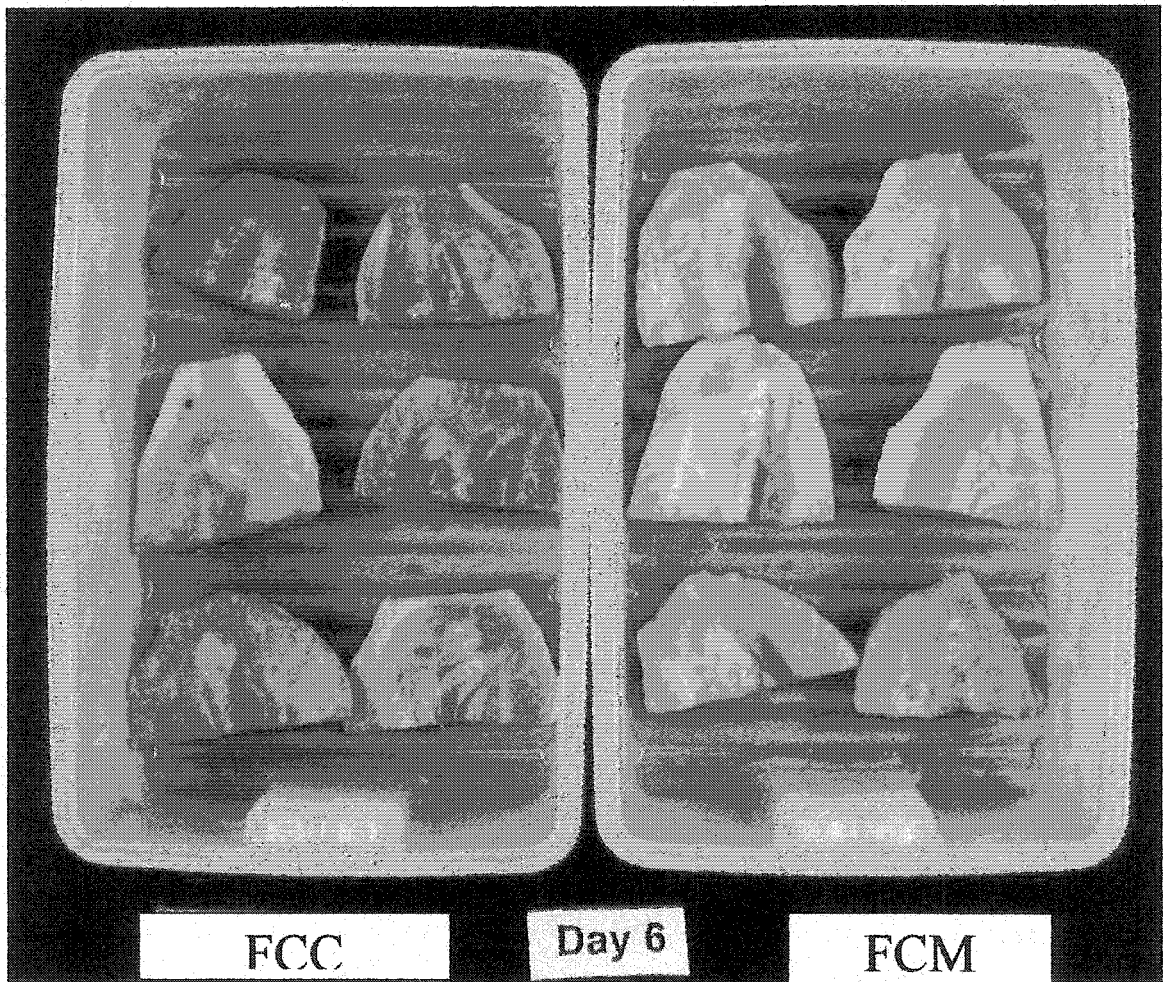


Figure 6-8. Fresh-cut postclimacteric 'Sunrise Solo' papaya fruit derived from either intact postclimacteric air-treated (FCC) or intact postclimacteric 1-MCP-treated fruit (FCM) and then stored for 6 days at 5 °C.

Table 6-1. Microbial counts (CFU g<sup>-1</sup> fresh weight) for intact postclimacteric 'Sunrise Solo' papaya fruit pre-treated with 2.5 µL L<sup>-1</sup> 1-MCP (IM) and air (control, IC), and for fresh-cut postclimacteric fruit derived from either the intact air-treated (FCC) or the intact 1-MCP-treated (FCM) fruit during storage at 5 °C

Total aerobic counts				Total coliforms			
Treatment	Day			Treatment	Day		
	0	5	10		0	5	10
IC	0 a	0 a	0.7 a	IC	0 a	0 a	0 a
IM	0 a	0 a	0.7 a	IM	0 a	0 a	0 a
FCC	17.3 b	42.0 b	2.7 x 10 <sup>3</sup> b	FCC	0 a	0 a	3.3 a
FCM	14.7 b	36.0 b	3.0 x 10 <sup>3</sup> b	FCM	0 a	0 a	0.7 a

Enterobacteriaceae				Lactic acid bacteria			
Treatment	Day			Treatment	Day		
	0	5	10		0	5	10
IC	0 a	0 a	0 a	IC	2.0 a	5.3 a	0.7 a
IM	0 a	0 a	0 a	IM	1.3 a	0.7 a	6.7 b
FCC	1.3 a	0 a	4.0 a	FCC	11.3 b	26.7 b	12.0 c
FCM	0.7 a	0 a	0 a	FCM	9.3 b	2.7 a	18.0 c

Yeasts				Other fungi			
Treatment	Day			Treatment	Day		
	0	5	10		0	5	10
IC	0 a	0 a	0 a	IC	0 a	0 a	0 a
IM	0 a	0 a	0 a	IM	0 a	0 a	0 a
FCC	0 a	0.7 a	97.3 b	FCC	0 a	0 a	0.7 a
FCM	0 a	0 a	91.3 b	FCM	0 a	0 a	0 a

Means (n = 3) followed by the same letter within a column are not significantly different,  $P \leq 0.05$ .

CHAPTER 7  
CELL WALL MODIFICATION IN POSTCLIMACTERIC FRESH-CUT AND  
INTACT PAPAYA FRUIT WITH AND WITHOUT 1-METHYLCYCLOPROPENE

**Introduction**

Fresh-cut produce are extremely fragile and perishable produce relative to their intact counterpart. Fresh-cut produce exhibit significant differences in terms of physiological behavior relative to their intact counterpart even though the quality and sensory of fresh-cut produce are somehow similar to intact produce. The behavior includes enhanced ethylene and respiration rates, wound-healing processes (synthesis of secondary compounds, suberization and lignification), biochemical changes (membrane changes, browning and degreening) and physical changes (softening and water loss; Rolle and Chism, 1987; Miller, 1992; Brecht, 1995). This behavior can greatly influence quality maintenance of fresh-cut fruits associated mostly rapid texture loss. The rapid texture loss in fresh-cut produce has not been clarified yet. However, a number studies have been shown that the rapid texture loss possibly results from cell wall and/or membrane damage. For example, the activities of some cell wall enzymes have been reported to increase in response to wounding (Dumville and Fry, 2000; Huber et al., 2001; Karakurt and Huber, 2003). The senescence-delaying influence of  $\text{Ca}^+$  on shredded carrots (Picchioni et al., 1996) and increased juice leakage in fresh-cut melon fruit (Cartaxo et al., 1997) supports a role of cell wall and membranes in the deterioration of fresh-cut produce.

Textural modifications during fruit ripening are related to changes in cell wall structure (Huber, 1983; Tucker and Grierson, 1987). The changes are mostly correlated with structure and composition of pectic components (Seymour et al., 1987). Solubilization and depolymerization of both pectins (Fischer and Bennett, 1991) and hemicelluloses (Lashbrook et al., 1997) during ripening are frequently associated with cell wall loosening and disintegration. Cell wall modifications have been extensively studied in tomato fruit, and early reports indicated that pectin degradation by polygalacturonase (PG) represented the model of fruit softening (Crookes and Grierson, 1983); however, analysis of PG-antisense tomato fruit (Smith et al., 1988; Giavannoni, 1990) revealed that this enzyme was not a significant contributor to tomato softening. Brummel and Harpester (2001) suggested that pectin metabolism might contribute to textural changes during tomato fruit ripening whereas modifications to the cellulose/matrix glycan network mainly to softening while each obviously affects each other. Xyloglucan, the primary hemicellulose in dicotyledonous plants, also undergoes depolymerization in most fruits (Sakurai and Nevins, 1993). In addition to the depolymerization of both pectin and hemicellulose, fruit softening is accompanied by a loss of neutral sugars, primarily galactose and arabinose, from pectic and hemicellulosic polysaccharides (Tucker, 1993).

Papaya fruit softening is accompanied by pectin hydrolysis and modification of hemicelluloses (Zhao et al., 1996; Paull et al., 1999; Karakurt and Huber, 2002) and increases in the activities of polygalacturonase (PG, EC 3.2.1.15; Paull and Chen, 1983; Karakurt and Huber, 2002), xylanase (EC 2.4.1.207; Paull and Chen, 1983), and  $\beta$ -galactosidase (EC 3.2.1.23; Ali et al., 1998). The levels of water-soluble (Zhao et al.,

1996; Paull et al., 1999), chelator-soluble (Lazan et al., 1995; Paull et al., 1999) and alkali-soluble polyuronides have been reported to increase during papaya ripening (Lazan et al., 1995; Zhao et al., 1996; Ali et al., 1998; Paull et al., 1999). A continuous or temporary increase in the activities of PG, pectin methylesterase (PME. EC 3.1.1.11), xylanase, and  $\alpha$ - (EC, 3.2.1.22) and  $\beta$ -galactosidases has been cited during papaya fruit ripening as well (Paull and Chen, 1983; Lazan et al., 1995; Ali et al., 1998).

1-methylcyclopropene (1-MCP), an ethylene action inhibitor (Sisler and Serek, 1997), has been shown to delay ripening of postclimacteric apple (Pre-Aymard et al., 2002; Jiang and Joyce, 2002), tomato (Wills and Ku, 2002; Hoeberichts et al., 2002), and apricot fruits (Botondi et al., 2003). 'Sunrise Solo' papaya fruit treated with 1-MCP at an early stage of ripening displayed delayed softening and color change (Jacaomino et al., 2002). Recently, 1-MCP has been shown to modify cell wall enzyme activities in a number of fruits including avocado, nectarine, tomato and apricots. Accumulation of both PG and cellulase (EC 3.2.1.5) activities were delayed by 1-MCP treatment in avocado fruit (Feng et al., 2000; Jeong et al., 2002). Less extensive molecular mass downshifts of polyuronides and alkali-soluble hemicelluloses including xyloglucan application were reported for 1-MCP-treated avocado (Jeong et al. 2002). In 'Flavortop' nectarine, 1-MCP treatment suppressed transcript abundance and activities of PG and PME during ripening whereas accumulation of endoglucanase activity and transcript abundance was enhanced by 1-MCP (1  $\mu$ L L<sup>-1</sup> 1-MCP for 20 h at 20 °C, Dong et al., 2001). 1-MCP treatment decreased mRNA abundance of exp 1 in mature green or ripe tomato fruit (Hoeberichts et al., 2002), and 1-MCP slightly reduced the activities of PME,  $\alpha$ -mannosidase (EC

3.2.1.113) and  $\beta$ -glucosidase (EC 3.2.1.21) in 'San Castrese' apricot stored harvested at an advanced ripening stage at 20 °C (Botondi et al., 2003).

In the present study we have examined the cell wall disintegration of fresh-cut and intact postclimacteric papaya fruit in which ethylene perception was suppressed by 1-MCP.

## Materials and Methods

### Plant Material and 1-MCP Treatment

Papaya fruit (*Carica papaya* L. 'Sunrise Solo'), originated from Brazil were purchased from C-Brand Tropicals Inc., Homestead, FL. The fruit were transferred the Postharvest Horticulture Laboratory at the University of Florida within 24 h of arrival at the packinghouse. The fruit were maintained at 20 °C until the majority of the fruit reached the desired ripeness stage (postclimacteric, 70% to 80% yellow surface color (Wills and Widjanarko, 1995). The average firmness, ethylene production, and respiration of the fruit was 6.8 N, 0.8  $\mu\text{L kg}^{-1} \text{h}^{-1}$  and 22 mL  $\text{CO}_2 \text{ kg}^{-1} \text{h}^{-1}$ , respectively, at 20 °C. The fruit were gently brushed, dipped in 200  $\mu\text{L L}^{-1}$ -chlorinated water for 1 min, air-dried, and placed in 174-L metal cambers for 1-MCP treatment. The fruit were treated four times at 6-h interval with 2.5  $\mu\text{L L}^{-1}$  of 1-MCP generated from Agrofresh powder (active ingredient 0.14%; Philadelphia, PA) for 24 h at 20 °C. 1-MCP was measured using a gas chromatograph (Hewlett Packard-5890 II; Avondale, PA) equipped with a 80-100 mesh Chromosorb PAW stainless steel column (1.8 m x 3.18 mm i.d.; Supelco, Bellefonte, PA) with injector, oven, and detector (FID) set at 150, 150 and 200 °C, respectively. Isobutylene gas, which has an FID response similar to that of 1-MCP (Jiang et al., 1999), was used as a standard. Control fruit were kept under identical conditions with the exception of 1-MCP gassing. The fruit were then transferred to facilities at 5 °C



for fresh-cut processing. After 1 h at 5 °C to allow temperature equilibration, the blossom and pedicel ends of each fruit were removed, and the fruit longitudinally cut using a Bread Slicer (Coupe-Pain, China) into 1.5-cm thick slices. The two outermost slices were peeled and cut into pieces (1.5 cm x 3.5 cm x 4 cm) weighing 15 to 20 g. Afterward, the slices were rinsed with sterile isotonic mannitol (500 mM) using a squeeze bottle, and then the slices were placed in 1.7 L vented plastic containers (FridgeSmart, Tupperware Co., St. Paul, MN). The treatments included: fresh-cut tissue derived from intact fruit pre-treated with 1-MCP (FCM), fresh-cut tissue derived from intact fruit pre-treated fruit with air as fresh-cut control (FCC), intact fruit pre-treated with 1-MCP (IC), and intact fruit pre-treated with air as intact control (IM). The fresh-cut and intact fruit were stored for 10 days at 5°C.

At the indicated intervals, fresh-cut and intact fruit were removed from storage and stored at -30°C until analyzed. Prior to freezing, intact fruit were peeled and cut into slices as described above.

#### **Ethanol-insoluble Solids**

Approximately 80 g of partially thawed mesocarp tissue derived from fresh-cut and intact papaya fruit were combined with 420 mL of 95% ethanol, macerated with a Polytron homonogizer (Kinematica, Kriens-Luzen, Switzerland) for 2 min, refluxed in a boiling water bath for 20 min, and filtered through glass fiber filter paper (Whatman GF/C) in an aspiration flask and washed with 95% cold ethanol. The residue was transferred to 200 mL of chloroform/methanol (1:1 v/v) and incubated with stirring for 30 min. The suspensions were filtered (GF/C) and washed 300 mL of acetone. The ethanol-insoluble solids (EIS) were oven-dried at 43 °C for 5 h and stored in a desiccator at room temperature.

### **Total Soluble Sugars and Polyuronides**

Partially thawed mesocarp tissue (2 g) derived from fresh-cut or intact fruit in 20 mL of 95% ethanol was homogenized with the Polytron homonogizer for 30 sec. The homogenate was held at -20 °C for a minimum of 2 h and were then centrifuged at 3430 g for 5 min. Aliquots of the supernatant (0.5 mL) were used for measuring total soluble sugar (TSS) levels using the procedure, the phenol-sulfuric assay, described by Dubois et al. (1956). Total polyuronide content in the EIS samples (7 g) was determined using the hydroxydiphenol assay (Blumenkrantz and Asboe-Hansen, 1973).

### **Sequential Fractionation of Cell Wall Materials**

Water-, CDTA-(1,2 cyclohexylenedinitrilotetraacetic acid) and Na<sub>2</sub>CO<sub>3</sub>-soluble pectins were extracted by suspending 30 mg of EIS in 7 mL distilled water, 50 mM CDTA plus 50 mM Na-acetate, pH 6.5, and 50 mM Na<sub>2</sub>CO<sub>3</sub>, sequentially, at room temperature. Suspensions incubated on an oscillating shaker (1.4 cycle min<sup>-1</sup>) for 4 h were filtered through a Whatman GF/C filter paper in an aspiration flask. Polyuronides in aliquots were measured (Blumenkrantz and Asboe-Hansen, 1973). Pectins solubilized in water, CDTA and Na<sub>2</sub>CO<sub>3</sub> (0.5 mg galacturonic acid equivalent) were run on a Sepharose CL-4B column (1.5 cm x 28 cm; Sigma Chemical Co., St. Louis, MO) equilibrated with 200 mM ammonium acetate at pH 5.0. Fractions of 2 mL were collected at a flow rate of 40 mL h<sup>-1</sup> and 0.5 mL of each fraction was used for the determination polyuronide content. Dextran 2,000 and glucose (Sigma St. Louis, MO) were used to determine the void ( $V_0$ ) and total ( $V_t$ ) volumes of the column.

### **Hemicellulosic Polysaccharide Extraction**

For pectin removal, approximately 200 mg EIS in 500 mL Na-phosphate (40 mM, ph 6.8) were heated in a boiling water bath for 20 min, cooled, filtered through Miracloth,

and washed with 1 L distilled water, sequentially. Excessive water was removed from the residue, and the residue was transferred into 200 mL of 80% ethanol, filtered through Miracloth, transferred into 200 mL of 50% chloroform/50% methanol and filtered through Miracloth, sequentially. The residue was then transferred into 200 mL of acetone to remove chloroform/methanol and filtered through GF/C under aspiration using Buchler funnel system with additional acetone wash. The residue was oven-dried at 43 °C for 5 h.

For hemicellulose extraction, the dried residues (50 mg) were suspended in 5 mL 4% KOH/0.02% NaBH<sub>4</sub> overnight at room temperature. The suspension was centrifuged at 1,300 g at room temperature for 10 min, and the supernatant was removed from the pellet and saved at 5 °C. The pellet was resuspended in 1 mL 4% KOH/0.02% NaBH<sub>4</sub>, centrifuged as described above, and supernatant was removed. The supernatant was added into the previous supernatant. The remaining pellet was subject to the same procedure described above while using 24% KOH/0.02% NaBH<sub>4</sub> instead of 4% KOH/0.02% NaBH<sub>4</sub>. The combined supernatants were neutralized over ice with concentrated acetic acid. Hemicellulosic polysaccharides in the neutralized samples were determined by using the hydroxydiphenol and phenol-sulfuric assay.

#### **Compositional Analysis of Cell Wall Polymers**

EIS (2 mg) were used for glycosyl composition analysis using a GC (Hewlett-Packard 5490 II, Avondale, PA) on a 25-m cross-linked 5% phenylmethyl silicone capillary column (Hewlett Packard, 0.2 mm i.d., 0.33 μM film thickness). *Myo*-inositol was added as internal standard. The EIS samples were hydrolyzed in 2 N trifluoroacetic acid for 1 h at 120 °C. The cooled hydrolytes were then reduced and acetylated as described below (Blakaney et al., 1983). The resulting monosaccharides were reduced with 0.66 M sodium borohydride in 1 N ammonium hydroxide overnight at 25 °C. The

samples were acidified with Dowex 50W (Sigma, St. Louis, MO), and the resin was removed by filtration through a syringe fitted with GC/C filter paper. The samples were dried and subsequently washed three times with methanol and once with ethanol before derivatization. The sugars were converted into acetyl derivatives in the presence of 0.2 mL of acetic acid anhydride and 0.2 mL of pyridine for 1 h at 100 °C. After cooling to room temperature, the samples were dried under a gentle stream of air, washed with toluene three times, solubilized in methylene chloride, and injected into a gas chromatography (Hewlett Packard 5890 II, Avondale PA). The chromatograph was run at 210 °C for 5 min, increased to 230 °C within 10 min, held at 230 °C for 5 min.

## **Results**

### **Ethanol-insoluble Solids and Total Soluble Sugars**

The EIS yield remained unchanged in all treatments, having no variations among treatments during storage (Table 7-1). Assay of total soluble sugars (TSS) using the phenol sulfuric acid method was used primarily to quantify glucose, sucrose and xylose in the ethanol homogenate. TTS significantly decreased in all treatments over the 10-day period (Table 7-1). The decreases in TS were approximately 30%, 33%, 36% and 35% for IC, IM, FCC and FCM, respectively.

### **Polyuronides and their Sequential Fraction**

The water-soluble fraction constituted the majority of pectins followed by CDTA and  $\text{Na}_2\text{CO}_3$ , as illustrated in Table 7-2. The pectic polymers recovered in solutions of water, chelator, and dilute alkali compromised approximately more than 50% of total polymers recovered in EIS at day 0, 2, 6 or 10. Water-soluble pectins, consisting of more than 30% of the total polyuronide content, increased in all treatments throughout storage, and by day 10 the levels of water-soluble pectins had increased 17% for IC, 14% for IM,

13% for FCC, and 8% for FCM compared to the levels at day 0. The increase in water-soluble pectins was similar for both fresh-cut and intact fruit, regardless of 1-MCP. The CDTA-soluble polyuronides of intact fruit (IC and IM) did not change during storage while that of fresh-cut fruit (FCC and FCM) increased significantly (8% for FCC and 22% for FCM) from day 0 to 10. On days 2 through 10, fresh-cut fruit (FCC and FCM) yielded higher chelator-soluble pectins compared to intact fruit (IC and IM). The alkali-soluble pectins increased in both intact (IC, 30%; IM, 27%) and fresh-cut (FCC, 28%; FCM, 19%) fruit over the 10-day period. At day 2, IM and FCM showed higher alkali-soluble pectin content relative to IC and FCC, collectively. Total polyuronide levels consistently decreased for all treatments during storage (IC, 15%; IM, 15%; FCC, 19%, and FCM, 20%), with both FCC and FCM exhibiting slightly higher decline compared with both IC and IM.

Gel permeation chromatography of water-soluble pectins from all treatments is shown in Figure 7-1. Water-soluble polyuronides from IM and FCM showed negligible changes in mol mass during storage while IC and FCC demonstrated slightly higher changes, especially in the levels of intermediate mol mass polymers. The mol mass of chelator-soluble pectins also exhibited significant changes during storage (Figure 7-2). As was noted for the water-soluble pectins, the decrease in the levels of intermediate mol mass polymers was slightly higher in FCC and FCM compared with IC and IM. Alkali-soluble polyuronides exhibited mol mass downshifts involving a decrease in the levels of intermediate mass polymers in all treatments and a more extensive decline in the recovery of higher molecular mass polymers in FCC and FCM (Figure 7-3).

### **Hemicellulosic Polysaccharides**

The levels of neutral hemicelluloses and pectins extracted with 4% and 24% KOH from EIS are shown in Table 7-3. Weakly bound neutral hemicellulosic polysaccharides (extracted with 4% KOH) significantly declined (IC, 24%; IM, 16%; FCC, 27%; and FCM, 26%) in all treatments while no differences were noted among treatments during storage. Pectin content of the 4% KOH extracted polysaccharides also decreased (25% for IC, 35% IM, 28% FCC, and 28% FCM) during storage. Strongly bound hemicelluloses (extracted with 24% KOH) showed no variations among treatments and no changes during storage. Pectin levels in the 24% KOH extract, however, declined significantly in IC (22%) and IM (24%) during storage whereas the levels did not change in FCC and FCM. The decrease in the pectin content of intact fruit (IC and IM) resulted in a significant difference at day 10 between intact (IC and IM) and fresh-cut (FCC and FCM) fruit.

### **Compositional Analysis of Cell Wall Polymers**

Noncellulosic neutral sugar composition of EIS derived from the treatments is shown in Table 7-4. Analysis of the neutral sugars in EIS revealed that the predominant non-cellulosic neutral sugar in ripe papaya fruit is galactose, followed by glucose, xylose, rhamnose, mannose, and arabinose, respectively. Rhamnose showed no changes in any treatments during storage whereas the proportional quantity of arabinose increased (30% for IC, 21% for IM, 32 for FCC, and 26% for FCM). Xylose levels decreased by 30% (IC), 24% (IM), 27% (FCC), and 26% (FCM). IM yielded higher xylose compared with IC at days 2 and 6, and FCM had higher xylose levels in its EIS than FCC on days 2 through 10. No significant changes in mannose levels were observed for any of the treatments. All treatments showed a decline in galactose level during storage: IC, 26%;

IM, 20%; FCC, 30%; and FCM, 30%. Galactose levels in FCC and FCM were lower than those measured for IC and IM. Glucose declined significantly during storage, and by day 10, the decline in glucose quantity was 13%, 10%, 17%, and 17% for IC, IM, FCC and FCM, respectively, compared with the day 0 values.

### Discussion

The EIS recoveries exhibited no changes during 10 days of storage and they were not affected by either fresh-cut processing or 1-MCP. However, it has been reported that the yield of cell wall material using ethanol decreased significantly during ripening of papaya fruit at 25 °C (from color break to 100% yellow skin color; Paull et al., 1999). The insignificant change in EIS content in the present study was possibly due to storage temperature (5 °C) plus the fact that fruit were nearly ripe (70-80% yellow skin color) at the start of the experiment.

Total polyuronide levels in EIS decreased significantly during 10 days of storage in both intact and fresh-cut fruit, and were not significantly affected in response to 1-MCP treatment. The decrease in total pectin content was more prominent in fresh-cut fruit, confirming the findings of Karakurt and Huber (2003) who reported a significant reduction in total pectin content of fresh-cut papaya fruit (60 to 70% yellow surface color) during storage at 5 °C. The decrease in total polyuronides suggests that at least some component of these polysaccharides are depolymerized to monomer and other small oligomers which, due to their solubility in ethanol, are not recovered in EIS preparations. Water-soluble polyuronides constituted the majority of polyuronides in EIS followed by the CDTA- and Na<sub>2</sub>CO<sub>3</sub>-soluble fractions, and all showed an increase during storage except for the CDTA-soluble polyuronides of intact fruit (IC and IM). The changes in all three pectic fractions of fresh-cut fruit (FCC and FCM) suggests a possible

role of polyuronide metabolism in the rapid tissue softening reported for fresh-cut papaya fruit (Karakurt and Huber, 2003). In addition to the increase in polyuronide solubility in the intact and fresh-cut fruit, polyuronides exhibited mol mass downshifts during storage. The lower mol mass of polyuronides from fresh-cut compared with intact fruit could arise from depolymerization or largely from increased solubility of inherently smaller polymers. Most fleshy fruits show an increase in pectin solubility during ripening (Huber et al, 2001) attributed primarily to cell wall including PGs. Karakurt and Huber (2003) reported greater levels of PG activity in fresh-cut compared with intact fruit, supporting the involvement of PG in polyuronide solubility and depolymerization in papaya fruit. Paull et al. (1999) have attributed the mol mass downshifts in papaya polyuronides during ripening to increases in PG activity.

Pectin solubility and depolymerization in stored intact and fresh-cut papaya fruit was not significantly altered in response to 1-MCP treatment. This observation suggests that that polyuronide solubility and depolymerization in papaya fruit is possibly ethylene-independent at the final stages of ripening. Nevertheless, PG activity during avocado fruit ripening was lowered by 1-MCP at as low as 30 nL L<sup>-1</sup> (Feng et al., 2002; Jeong et al., 2002). Consistent with the decrease of PG activity in 1-MCP treated avocado fruit, pectin solubility and depolymerization during ripening was greatly suppressed by 1-MCP (Jeong et al., 2002). 1-MCP also affected  $\alpha$ -galactosidase activity by delaying decline in the activity during avocado ripening while it did not affect  $\beta$ -galactosidase level (Jeong et al., 2002). 1-MCP, moreover, reduced levels of  $\alpha$ - and  $\beta$ -galactosidase in pre-ripe 'Ceccona' apricot fruit whereas 1-MCP did not affect the activities of  $\alpha$ - and  $\beta$ -galactosidase in ripe 'San Castrese' apricot fruit (Botondi et al., 2003). The data for ripe 'San Castrese' imply



that polyuronide solubility and depolymerization during advanced ripening may be ethylene-independent or no longer respond to 1-MCP. The works presented by Jeong et al. (2002) and Botondi et al. (2003) showed that pectin methyl esterase activity seemed to be not significantly affected by 1-MCP during either avocado or apricot ripening. 1-MCP effect upon expansin was also recorded by Hoeberichts et al. (2002) who found that 1-MCP decreased the mRNA abundance of expansin 1 (*EXP*) in mature green, breaker, orange, and red ripe tomato fruit (Hoeberichts et al., 2002).

Significant changes were evident in neutral hemicellulosic polysaccharides derived from papaya fruit during storage. The strongly bound hemicelluloses extracted with 24% KOH contained high levels of polysaccharides relative to the weakly bound hemicelluloses extracted with 4% KOH. The change in hemicellulosic polysaccharides might be due to the increase in  $\alpha$  and  $\beta$ -galactosidase activities. Beta-galactosidases/-galactans have been linked with pectin and hemicellulose solubility and depolymerization in several fruit during ripening including papaya (Lazan et al., 1995; Rose et al., 1998; Karakurt and Huber 2003), avocado (De Veau, 1993) and melon fruit (Ranwala et al., 1992). Neutral hemicelluloses showed no changes in response to fresh-cut processing or 1-MCP treatment. The pectin composition extracted with 4% KOH from EIS were higher than the pectin composition extracted with 24% KOH, and weakly pectins in both fresh-cut and intact fruit and strongly bound pectins in only intact fruit declined during storage. The decrease in pectin content is possibly due to PG activity which has been reported to increase during ripening of papaya fruit (Paull and Chen 1983; Lazan et al., 1995; Karakurt and Huber, 2003).

Consistent with the involvement of galactosidases/-galactanases in depolymerization and solubility of hemicelluloses, galactose, glucose and xylose, the predominant neutral sugars in EIS derived papaya fruit, decreased significantly during storage. Significant changes in neutral sugars in EIS composition were minimal in response to 1-MCP treatment. 1-MCP treatment enhanced the loss of xylose, but did not significantly affect the levels of glucose, galactose, mannose and arabinose. Since a marked decrease in galactose content was noted for fresh-cut fruit, it is possible that the enzymes contributing to degalactosidation of polyuronides and hemicelluloses may be up-regulated by wounding (Karakurt and Huber, 2003). Pectin solubilization may result from the loss of galactosyl residues in the form of the galactose-rich side chains of rhamnogalacturonans (Seymour et al., 1990, Redgewell et al., 1992). Loss of galactans has been demonstrated to accompany increased solubilization of polyuronides (Gross and Wallner, 1979, Kim et al., 1991).

It is concluded that modifications of cell wall polyuronides and hemicelluloses in fresh-cut ripe papaya fruit showed similar patterns to those in intact ripe papaya fruit; although, few minor differences were observed between fresh-cut and intact fruit. The effect of 1-MCP is minimal in inhibiting the solubility and depolymerization of polyuronides and hemicelluloses of both fresh-cut and intact ripe papaya fruit at 5 °C.

Table 7-1. Ethanol insoluble solids (EIS) and total soluble sugars (TSS) of intact three-quarter ripe papaya fruit treated with and without 1-MCP and fresh-cut fruit derived from intact three-quarter ripe fruit treated with and without 1-MCP papaya during storage. IC, intact control fruit; IM, Intact 1-MCP-treated fruit, FCC, fresh-cut control fruit; and FCM, fresh-cut fruit with 1-MCP.

Ethanol insoluble solids (EIS) (mg g <sup>-1</sup> f.w.)				
Day	IC	IM	FCC	FCM
0	28.03 a	26.43 a	27.44 a	27.12 a
2	25.94 a	25.69 a	25.54 a	26.36 a
6	25.24 a	25.43 a	25.76 a	26.18 a
10	26.34 a	25.53 a	25.65 a	25.65 a
Total soluble sugar (TSS) (mg g <sup>-1</sup> f.w.)				
0	147.31 a	144.45 a	140.58 a	142.12 a
2	106.27 a	104.92 a	105.86 a	112.50 a
6	98.81 a	96.19 a	99.86 a	116.98 a
10	102.58 a	98.03 a	93.12 ab	94.76 a

a: means (n = 3) in the same row with same letters were not significantly different at  $P \leq 0.05$ .

TSS were measured in glucose equivalents.

Table 7-2. Polyuronide composition of intact three-quarter ripe papaya fruit treated with and without 1-MCP and fresh-cut fruit derived from intact three-quarter ripe fruit treated with and without 1-MCP papaya during storage. IC, intact control fruit; IM, Intact 1-MCP-treated fruit, FCC, fresh-cut control fruit; and FCM, fresh-cut fruit with 1-MCP.

Water-soluble polyuronides ( $\mu\text{g mg}^{-1}$ EIS)				
Day	IC	IM	FCC	FCM
0	127.78 a	128.24 a	132.25 a	131.63 a
2	137.16 a	130.05 a	137.21 a	139.84 a
6	135.33 a	136.54 a	138.66 a	137.87 a
10	149.29 a	146.34 a	149.10 a	142.76 a
CDTA-soluble polyuronides ( $\mu\text{g mg}^{-1}$ EIS)				
0	54.65 a	53.70 a	56.75 a	53.01 a
2	51.14 b	49.55 b	63.41 a	63.57 a
6	54.33 b	49.70 b	60.47 a	61.43 a
10	53.53 b	52.21 b	61.14 a	64.65 a
$\text{Na}_2\text{CO}_3$ -soluble polyuronides ( $\mu\text{g mg}^{-1}$ EIS)				
0	24.70 a	25.88 a	23.10 a	26.74 a
2	23.73 b	32.30 a	25.80 b	30.29 a
6	30.00 a	33.17 a	28.10 ab	30.77 a
10	32.04 a	32.80 a	29.58 a	31.72 a

a: means ( $n = 3$ ) in the same row with same letters were not significantly different at  $P \leq 0.05$ .

Table 7-2. Continued

Day	Total polyuronides ( $\mu\text{g mg}^{-1}$ EIS)			
	IC	IM	FCC	FCM
0	432.67 a	428.65 a	425.25 a	422.23 a
2	381.35 a	387.35 a	368.15 a	362.50 ab
6	363.94 a	373.06 a	357.69 ab	356.92 ab
10	363.31 a	365.33 a	345.35 ab	339.67 b

a: means ( $n = 3$ ) in the same row with same letters were not significantly different at  $P \leq 0.05$ .

Table 7-3. Neutral hemicellulose and pectin residue composition of intact three-quarter ripe papaya fruit treated with and without 1-MCP and fresh-cut fruit derived from intact three quarter ripe fruit treated with and without 1-MCP papaya during storage. IC, intact control fruit; IM, Intact 1-MCP-treated fruit, FCC, fresh-cut control fruit; and FCM, fresh-cut fruit with 1-MCP.

Neutral hemicelluloses ( $\mu\text{g mg}^{-1}$ EIS)					
Fraction	Day	IC	IM	FCC	FCM
4% KOH	0	10.49 a	10.01 a	11.11 a	11.03 a
	2	10.30 a	10.89 a	10.01 a	10.05 a
	6	9.89 a	10.50 a	9.09 a	9.73 a
	10	7.99 a	8.40 a	8.16 a	8.19 a
Pectins ( $\mu\text{g mg}^{-1}$ EIS)					
	0	5.13 a	5.20 a	4.95 a	5.01 a
	2	5.21 a	4.17 b	4.80 a	4.32 a
	6	4.54 a	4.07 a	4.36 ab	4.06 a
	10	3.86 a	3.35 a	3.57 a	3.59 a
24% KOH Neutral hemicelluloses ( $\mu\text{g mg}^{-1}$ EIS)					
	0	24.90 a	23.12 a	22.34 a	22.00 a
	2	22.76 a	22.95 a	24.88 a	24.38 a
	6	22.24 a	22.24 a	22.73 a	23.00 a
	10	21.48 a	22.32 a	22.78 a	21.18 a

a: means (n = 3) in the same row with same letters were not significantly different at  $P \leq 0.05$ .

Table 7-3. Continued

Fraction	Days	Pectins ( $\mu\text{g mg}^{-1}$ EIS)			
		IC	IM	FCC	FCM
24% KOH	0	2.85 a	2.95 a	3.12 a	3.04 a
	2	2.26 a	2.41 a	2.80 a	2.71 a
	6	2.30 a	2.35 a	2.75 a	2.71 a
	10	2.21 b	2.26 b	2.68 a	2.65 a

a: means ( $n = 3$ ) in the same row with same letters were not significantly different at  $P \leq 0.05$ .

Table 7-4. Neutral sugar composition of intact three-quarter ripe papaya fruit treated with and without 1-MCP and fresh-cut fruit derived from intact three-quarter ripe fruit treated with and without 1-MCP papaya during storage. IC, intact control fruit; IM, Intact 1-MCP-treated fruit, FCC, fresh-cut control fruit; and FCM, fresh-cut fruit with 1-MCP.

Rhamnose ( $\mu\text{g mg}^{-1}$ EIS)				
Day	IC	IM	FCC	FCM
0	35.89 a	38.25 a	36.65 a	36.46 a
2	35.74 a	36.79 a	35.60 a	36.84 a
6	38.31 a	37.50 a	34.80 a	38.42 a
10	38.62 a	41.24 a	37.71 a	39.24 a
Arabinose ( $\mu\text{g mg}^{-1}$ EIS)				
0	18.65 a	21.37 a	19.19 a	17.48 a
2	17.45 a	20.34 a	20.46 a	17.66 a
6	22.60 a	21.68 a	24.47 a	18.27 a
10	24.08 a	25.78 a	25.26 a	22.06 a
Xylose ( $\mu\text{g mg}^{-1}$ EIS)				
0	88.46 a	85.23 a	90.56 a	85.27 a
2	73.40 a	61.23 b	69.00 a	55.19 b
6	70.46 a	64.32 b	68.26 a	54.95 c
10	62.04 a	65.33 a	66.33 a	49.08 b

a: means ( $n = 3$ ) in the same row with same letters were not significantly different at  $P \leq 0.05$ .



Table 7-4. Continued.

Mannose ( $\mu\text{g mg}^{-1}$ EIS)				
Day	IC	IM	FCC	FCM
0	24.52 a	25.89 a	23.97 a	24.49 a
2	22.71 a	23.92 a	24.76 a	22.81 a
6	22.06 a	24.94 a	24.63 a	25.58 a
10	25.12 a	27.72 a	26.32 a	25.42 a
Galactose ( $\mu\text{g mg}^{-1}$ EIS)				
0	114.21 a	110.42 a	108.08 a	107.16 a
2	85.04 a	88.33 a	75.48 c	75.38 c
6	87.74 a	89.54 a	80.79 b	80.48 b
10	88.76 a	86.96 a	83.36 b	81.45 b
Glucose ( $\mu\text{g mg}^{-1}$ EIS)				
0	102.27 a	104.04 a	106.18 a	107.00 a
2	96.80 a	99.32 a	95.35 a	97.24 a
6	95.74 a	98.54 a	90.79 a	88.48 a
10	89.25 a	94.07 a	88.84 a	89.72 a

a: means ( $n = 3$ ) in the same row with same letters were not significantly different at  $P \leq 0.05$ .

Figure 7-1. Molecular mass distribution of water-soluble polyuronides of intact three-quarter ripe papaya fruit treated with and without 1-MCP and fresh-cut fruit derived from intact three quarter-ripe fruit treated with and without 1-MCP at day 0 (o), 6 (●), and 10 (▼). Polyuronides (0.5 mg galacturonic acid equivalents) were applied to CL-4B-200 (1.5 x 28 cm) column operated with a mobile phase of 200 mM ammonia acetate, pH 5.0. Individual fractions were analyzed for polyuronides. Data for each fraction were expressed as a percentage of the total polyuronides.  $V_o$ , void volume;  $V_t$ , total volume.

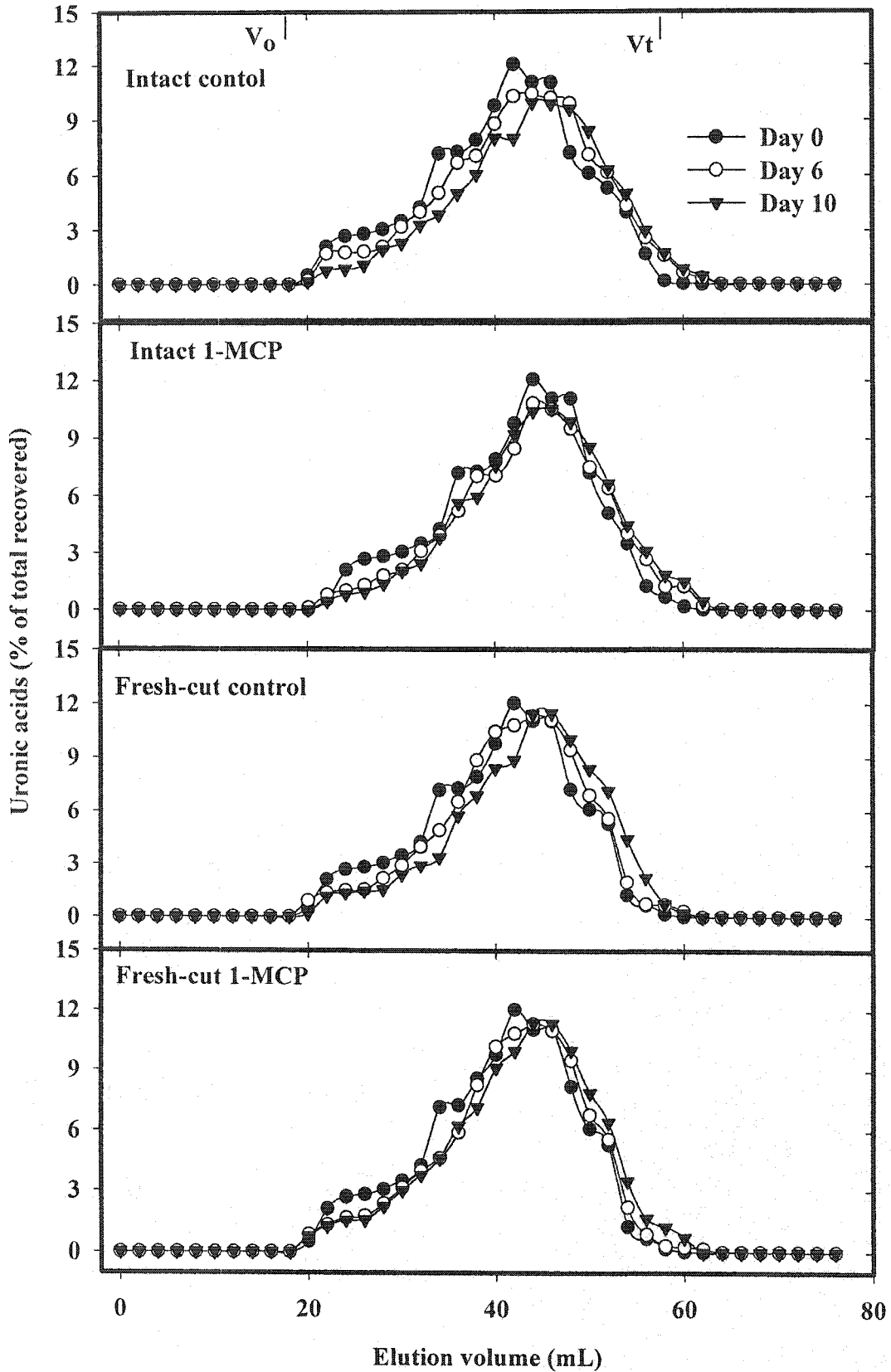


Figure 7-1. Molecular mass distribution of  $\text{Na}_2\text{CO}_3$ -soluble polyuronides of intact three-quarter ripe papaya fruit treated with and without 1-MCP and fresh-cut fruit derived from intact three quarter-ripe fruit treated with and without 1-MCP at day 0 (o), 6 (●), and 10 (▼). Polyuronides (0.5 mg galacturonic acid equivalents) were applied to CL-4B-200 (1.5 x 28 cm) column operated with a mobile phase of 200 mM ammonia acetate, pH 5.0. Individual fractions were analyzed for polyuronides. Data for each fraction were expressed as a percentage of the total polyuronides.  $V_o$ , void volume;  $V_t$ , total volume.

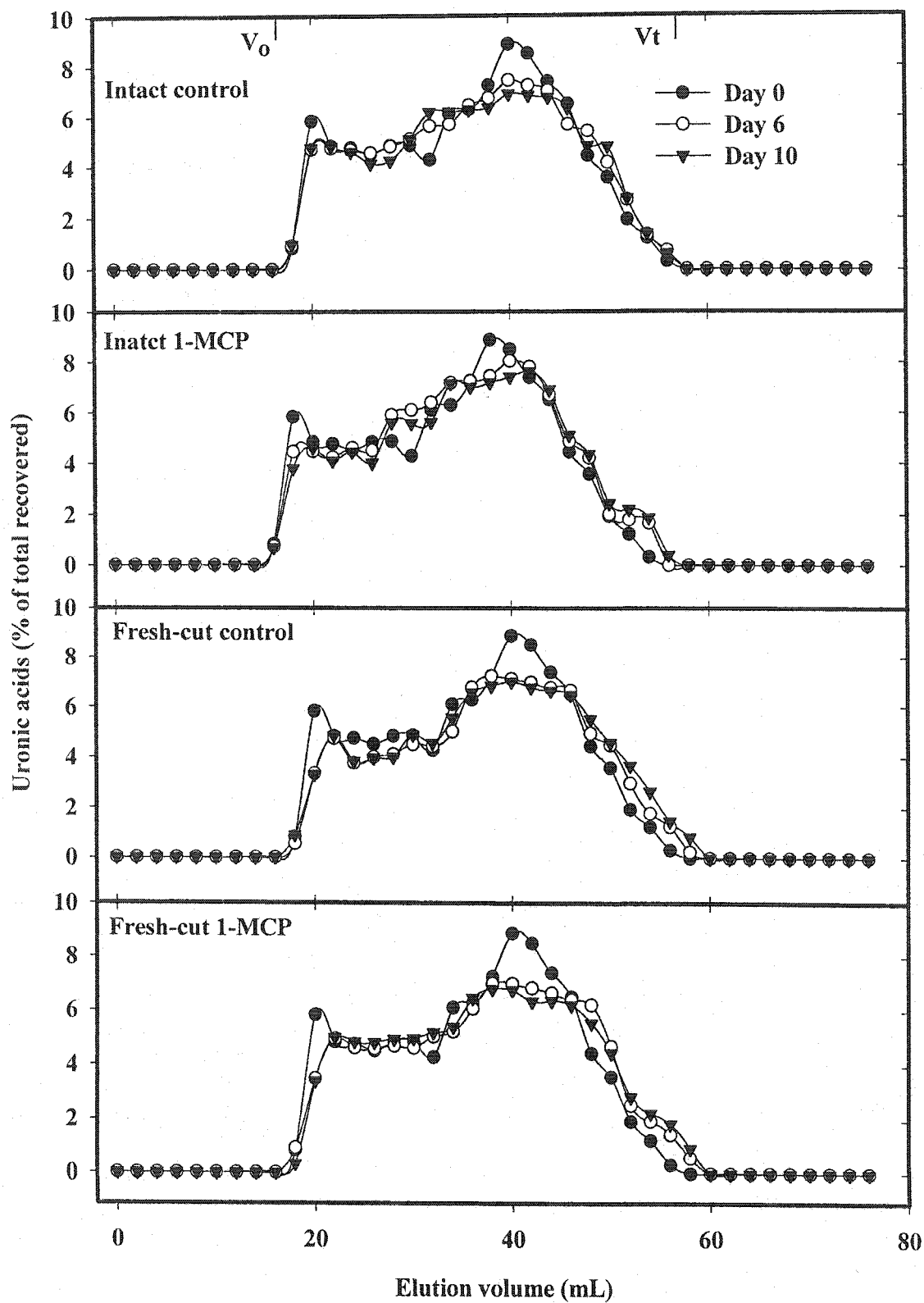
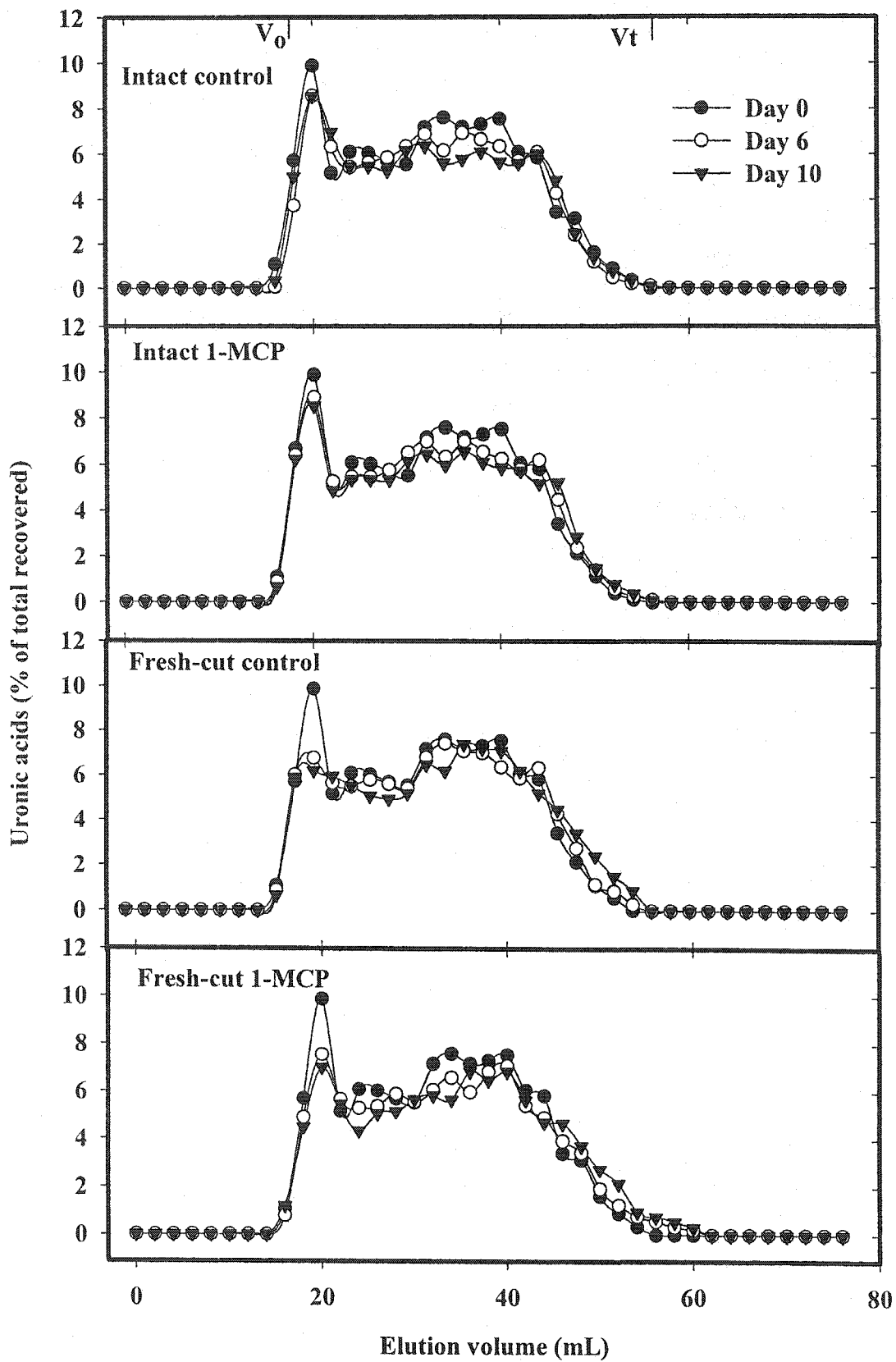


Figure 7-1. Molecular mass distribution of  $\text{Na}_2\text{CO}_3$ -soluble polyuronides of intact three-quarter ripe papaya fruit treated with and without 1-MCP and fresh-cut fruit derived from intact three quarter-ripe fruit treated with and without 1-MCP at day 0 (o), 6 (●), and 10 (▼). Polyuronides (0.5 mg galacturonic acid equivalents) were applied to CL-4B-200 (1.5 x 28 cm) column operated with a mobile phase of 200 mM ammonia acetate, pH 5.0. Individual fractions were analyzed for polyuronides. Data for each fraction were expressed as a percentage of the total polyuronides.  $V_0$ , void volume;  $V_t$ , total volume.



## CHAPTER 8 SUMMARY AND CONCLUSION

The objectives of the research reported in this study were to characterize physiological responses of both 'Galia' melon and 'Sunrise Solo' papaya fruits treated with the ethylene action antagonist 1-methylcyclopropene (1-MCP). The results indicated that inhibition of ethylene action in these fruits by 1-MCP delayed the rate of ripening of both pre-ripe fruit and fruit in which ripening had been initiated. In all cases, the use of 1-MCP significantly extended storage and shelf life. The study has also shown that most many of the physiological changes associated with 'Galia' and 'Sunrise Solo' fruit ripening, including softening, pigment changes, ethylene production and respiration, require functional ethylene responsiveness.

### **Influence of Ethylene-action Inhibition on Ripening of 'Galia' Melon Fruit**

'Galia' fruit is climacteric, with the ethylene climacteric peak occurring prior to the respiratory climacteric. 1-MCP delayed the onset and peak activity of both respiration and ethylene production and suppressed both respiration and ethylene production during ripening at 20 °C. Respiration and ethylene production of ripe fruit decreased during over-ripening at 20 °C. The decrease in ethylene production of ripe fruit was inhibited by 1-MCP while the decrease in respiration was not. Fresh-cut processing (wounding; in ripe fruit) initially promoted ethylene production at 5 °C that was not significantly affected by prior treatment with 1-MCP. Softening during ripening or over-ripening at 20 °C was significantly delayed by 1-MCP. 1-MCP also delayed softening in both fresh-cut and intact fruit stored at 5 °C. Membrane deterioration, measured by electrolyte efflux, was



evident during ripening and over-ripening and was partially inhibited by 1-MCP irrespective of ripeness stage. Soluble solids accumulation was not affected by 1-MCP during ripening; pH and titratable acidity were slightly or not affected by 1-MCP at 20 °C. The change in skin surface color of 'Galia' melon from green to yellow during ripening at 20 °C was delayed by 1-MCP, however, the color development at 5 °C was not influenced by 1-MCP. The effect of 1-MCP upon microbial growth was minimal, complex and dependent on ripening stage and type of microorganism. 1-MCP affected most of the physiological parameters measured in 'Galia' fruit, indicating that 1-MCP efficiently binds ethylene receptors, thereby restraining the positive feedback regulation of ethylene regardless of ripening stage. Therefore, 1-MCP significantly extended storage and storage life of both intact pre-ripe and ripe 'Galia' fruit and storage life of fresh-cut ripe 'Galia' fruit.

#### **Influence of Ethylene-action Inhibition on Ripening of 'Sunrise Solo' Papaya Fruit**

'Sunrise Solo' papaya fruit exhibited a typical climacteric pattern, with peak ethylene production occurring prior to the respiratory climacteric. 1-MCP delayed the initiation of the respiratory and ethylene climacteric, and suppressed ethylene production during ripening at 20 °C. 1-MCP had no influence, however, on ethylene production of either intact or fresh-cut fruit held at 5 °C. Wounding resulting from fresh-cut processing also did not affect ethylene production of fresh-cut fruit at 5 °C. 1-MCP deferred firmness loss in intact pre-ripe, and intact ripe and fresh-cut ripe fruit (at 5 °C). 1-MCP treatment delayed membrane damage during ripening but not during over-ripening. 1-MCP slightly suppressed membrane deterioration of wounded tissue through the end of storage at 5 °C as well. Titratable acidity of 1-MCP-treated fruit was significantly lower during ripening/over-ripening compared with non-1-MCP-treated fruit whereas soluble solids

levels were not influenced by 1-MCP. 1-MCP caused a slight increase in pH during ripening at 20 °C though the magnitude was minimal. The color change from green to yellow of the fruit surface was delayed by 1-MCP during ripening and over-ripening at 20 °C. The effects of 1-MCP upon flesh color were minimal at 5 °C and only significant at day 10 when 1-MCP-treated fresh-cut fruit displayed higher lightness and chroma values (more intense and brighter color) compared with control (no 1-MCP) fruit. 1-MCP did not have a significant effect upon microbial growth at 5 °C with the exception of lactic acid bacteria, whose growth appeared to be promoted by 1-MCP. The magnitude of this promotion, however, was so small as to not be a major concern in fresh-cut fruit maintained under proper temperature management. 1-MCP arrested ripening and over-ripening by restricting several physiological characteristics mentioned above, proving conclusively that ethylene responsiveness is required throughout ripening. Thus, inhibition of ethylene action by 1-MCP was sufficient to extend the postharvest life and windows of edibility for 'Sunrise Solo' regardless of ripening stage.

#### **Cell Wall Modification of 'Sunrise Solo' Papaya Fruit in Response to Fresh-cut Processing and 1-MCP**

The recoveries of ethanol insoluble solids did not change during 10 days of storage at 5 °C and was not influenced by either fresh-cut processing or 1-MCP treatment. On the other hand, total uronic acids decreased significantly during the 10 days of storage and were not affected by processing or 1-MCP treatment. Water-soluble polyuronides represented the primary pectic fraction followed by CDTA and Na<sub>2</sub>CO<sub>3</sub>, respectively. Water- and alkali-soluble polyuronides increased in both intact and fresh-cut fruit irrespective of 1-MCP during storage. Intact fruit did not exhibit an increase in CDTA-soluble polyuronides while fresh-cut showed an increase. Despite the increase in the

solubility of all pectic fractions, the mol mass of polyuronides changed only slightly during storage, indicating that inhibition of ethylene action (1-MCP) resulted in only minor effects on the depolymerization of water, chelator and alkali-soluble polyuronides. Significant changes were observed in hemicellulosic polysaccharides during storage. The 4% KOH fraction contained more than 30% uronic acids (UA); however, in the 24% KOH fraction UA content was minimal. The greatest proportion of total sugars was extracted by 24% KOH. However, consistent with the decrease in total polyuronides, UA content of the 24% KOH fraction decreased significantly with or without 1-MCP or processing. Consistent with the involvement of galactosidases/galactans in depolymerization and solubility of hemicelluloses, galactose, glucose and xylose, the predominant non-cellulosic neutral sugars in papaya, decreased significantly during storage. Significant changes in neutral sugars were also evident in response to 1-MCP treatment. 1-MCP treatment enhanced the loss of xylose, but did not significantly affect the change in glucose, galactose, mannose and arabinose. It is concluded that cell wall polyuronides and hemicelluloses show significant solubility and depolymerization in response to wounding, and the influence of ethylene action inhibition (1-MCP) is minimal at affecting the solubility and depolymerization of polyuronides and hemicelluloses.

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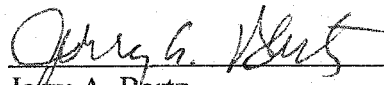
## BIOGRAPHICAL SKETCH

Muharrem Ergun was born on March 20 1971, in Konya, Turkey. He received his primary and secondary education in Konya, graduating from Gazi High School in 1988. He attended Ege University, Izmir, Turkey, and was awarded a BS degree in horticulture in 1992. In 1997, he entered a graduate program (in the Horticultural Sciences Department) at the University of Nebraska, Lincoln, where he was awarded an MS degree in 1998. Upon completing his PhD degree, he will be employed as an association professor at a university in Turkey.


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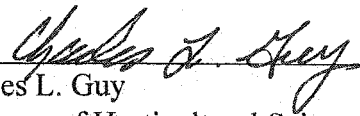
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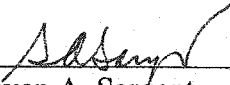
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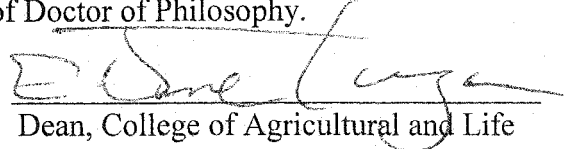
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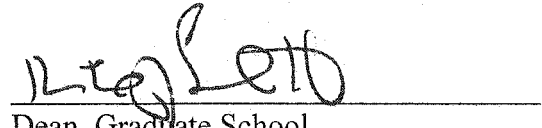
  
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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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