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# Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (*Persea americana*) fruit

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**Abstract**

West Indian-type avocado (*Persea americana* Mill. cv. 'Simmonds') fruit were treated with two different concentrations (0.09 and 0.45 $\mu l\,l^{-1}$) of 1-methylcyclopropene (1-MCP) for three exposure times (6, 12, and 24 h) at 20 °C. The fruit were then stored at 20 °C in ethylene-free air for ripening assessment. Firmness, weight loss, respiration and $C_2H_4$ production, peel color, cell-wall enzymes (polygalacturonase (PG), pectinmethylesterase, α-,β-galactosidase, and $C_x$-cellulase) and cell-wall matrix polysaccharides (polyuronides and hemicellulose) were monitored during storage. 1-MCP treatment at 0.45 $\mu l\,l^{-1}$ for 24 h at 20 °C delayed the ripening of avocado fruit by 4 days at 20 °C. This delay was characterized by a significant reduction in the rate of fruit softening and in the timing and intensity of the ethylene and respiratory climacterics. Avocado treated with 1-MCP (0.45 $\mu l\,l^{-1}$) for 24 h at 20 °C also showed significantly less weight loss and retained more green color than control fruit at the full-ripe stage (10–20 N). The delay in avocado ripening was influenced by 1-MCP concentration, exposure duration, and exposure temperature. 1-MCP treatment affected the activity trends of all cell-wall enzymes measured and completely suppressed increases in PG activity for up to 12 days. Consistent with the activity trends of PG, polyuronides from 1-MCP treated fruit when fully ripe exhibited less extensive molecular mass downshifts compared with the control fruit. The data indicate that the primary phase of avocado fruit softening occurs in the absence of appreciable PG activity. 1-MCP treatment also delayed and slightly reduced the depolymerization of 4 M alkali-soluble hemicelluloses, including xyloglucan.


*Keywords:* 1-Methylcyclopropene; Avocado; Ripening; Softening; Polygalacturonase; $C_x$-cellulase; Pectinmethylesterase; α- and β-Galactosidase; Polyuronides; Hemicellulose

## 1. Introduction

The avocado (*Persea americana* Mill.) is a climacteric fruit that is characterized by a surge in ethylene production at the onset of ripening. This climacteric increase in ethylene production is asso-

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ciated with hastened ripening. Avocado is one of the most rapidly ripening fruits, often completing ripening within 5 to 7 days following harvest (Seymour and Tucker, 1993).

The importance of ethylene in regulating fruit ripening has been clearly demonstrated from analyses of fruits exhibiting suppressed ethylene biosynthesis or action. For example, melon fruit expressing a 1-aminocyclopropeneoxidase antisense construct retained only 0.5% of normal ethylene production (Ayub et al., 1996) and fruit softening was completely blocked (Guis et al., 1997). Similarly, tomato fruit expressing an antisense construct for aminocyclopropene synthase failed to ripen unless provided with exogenous ethylene (Oeller et al., 1991). The identification of the *Nr* tomato-ripening mutant as an ethylene receptor (Lanahan et al., 1994; Wilkinson et al., 1995) has provided evidence that ethylene receptors regulate a defined set of genes that are expressed during fruit ripening. *Nr* mutants are affected in ethylene perception (Payton et al., 1996) and exhibit greatly impaired ethylene response (Lanahan et al., 1994).

In addition to the use of fruit lines with suppressed ethylene synthesis or perception, the application of compounds that block ethylene action (Jiang and Jiarui, 2000; Sisler and Serek, 1997) has provided a facile approach for examining relationships between ethylene, fruit ripening, and senescence in a range of horticultural commodities. 2,5-Norbornadiene (NBD) was perhaps the first widely employed cyclic olefin utilized for its ability to compete with ethylene at the receptor level (Sisler et al., 1986); however, the effectiveness of NBD, a toxic and offensive-smelling compound (Sisler and Serek, 1999) required the continued presence of the gas. Sisler and Blankenship (1993) also reported the ethylene antagonism of diazocyclopentadiene (DACP), a photoaffinity label for the ethylene-binding site. DACP's effectiveness at inhibiting ethylene action is a result of a permanent attachment to the binding site (Serek et al., 1994). It is, however, explosive in high concentrations, which limits its commercial usefulness (Sisler and Serek, 1997).

Recently, 1-methylcyclopropene (1-MCP), a synthetic cyclopropene, has been shown to strongly block ethylene perception, preventing ethylene effects in plant tissues for extended periods (Sisler and Serek, 1997; Sisler et al., 1996a,b). This material is nontoxic, odorless, and effective when plants are treated at concentrations as low as 0.5 $nl\,l^{-1}$ (Sisler and Serek, 1997).

1-MCP has been shown to delay ripening and improve storage quality of climacteric fruits including pears (Lelievre et al., 1997b), bananas (Golding et al., 1998, 1999; Sisler and Serek, 1997), plums (Abdi et al., 1998), tomatoes (Nakatsuka et al., 1997; Sisler and Serek, 1997), apples (Fan and Mattheis, 1999; Watkins et al., 2000), and avocados (Feng et al., 2000). 1-MCP, therefore, has provided a valuable tool to investigate ethylene metabolism in ripening climacteric fruit and has the potential to extend the storage life of ethylene-responsive horticultural products.

The objectives of this study were to characterize the physiological and biochemical responses of avocado fruit to different concentrations and exposure periods of 1-MCP and to evaluate its ability as a postharvest tool for regulating the ripening of avocado fruit.

## 2. Materials and methods

### 2.1. Plant material

'Simmonds', an early season avocado (*P. americana* Mill.) variety, was selected for this experiment. It is a West Indian avocado type and is low-temperature sensitive (Crane et al., 1996; Hatton and Reeder, 1965). Recommended storage is 13 °C to avoid chilling injury (Seymour and Tucker, 1993). Mature avocado fruit were obtained from a commercial grower in Homestead, Florida, packed in fiberboard cartons, and transported to the Postharvest Horticulture Laboratory in Gainesville within 24 h of harvest. Fruit were selected for uniformity of weight ($757 \pm 31$ g) and shape (diameter at equatorial region, $10.5 \pm 0.7$ cm), and then were surface sterilized in a 90 mM NaOCl, rinsed, and dried.

### 2.2. 1-MCP treatment

Twelve fruit were placed in 18-l containers and exposed to 1-MCP by releasing the gas from a commercial powdered formulation (Ethyblock®, Floralife, Burr Ridge, IL). The concentrations selected, 0.09 and 0.45 $\mu l\, l^{-1}$, were achieved through addition of 1 or 5 mg of the powder, respectively, to 100 ml of Floralife buffer following manufacturer's instructions (Floralife product specification sheet). Following addition of the buffer to 1-MCP, the beakers were transferred to the 18-l containers, which were sealed immediately. 1-MCP treatment at each concentration was performed for three exposure periods (6, 12, and 24 h) at 20 °C and 85% relative humidity (RH). Immediately following treatment, the fruit were removed from the chambers and transferred to 20 °C storage facilities. Control fruit (not exposed to 1-MCP) were maintained under identical storage conditions. Samples of fruit from each treatment were evaluated for fruit quality on a daily basis until they reached the full-ripe stage (10–20 N). Fruit quality was assessed on the basis of fruit firmness, weight loss, $CO_2$ and $C_2H_4$ production, and peel color. Mesocarp tissue derived from the equatorial region of selected fruit was stored at −30 °C and used for the analysis of cell-wall enzymes and structural polysaccharides.

### 2.3. Fruit firmness

Firmness was determined on whole, unpeeled fruit using an Instron Universal Testing Instrument (Model 4411, Canton, MA, USA) fitted with a flat-plate probe (5 cm diameter) and 50-kg load cell. After establishing zero force contact between the probe and the equatorial region of the fruit, the probe was driven with a crosshead speed of 10 $mm\, min^{-1}$. The force was recorded at 2.5 mm deformation and was determined at two equidistant points on the equatorial region of each fruit. The same four fruit of each treatment were measured repeatedly every other day until they reached the full-ripe stage.

### 2.4. Respiration and ethylene evolution

Respiration and ethylene production were measured every other day using the same four fruit of each treatment. Fruit were individually sealed for 30 min in 2-l plastic containers prior to sampling. A 0.5 ml gas sample was withdrawn by a syringe through a rubber septum, and carbon dioxide determined using a Gow–Mac gas chromatograph (Series 580, Bridge Water, NJ, USA) equipped with a thermal conductivity detector (TCD). Ethylene was measured by injecting a 1.0 ml gas sample into a HP 5890 gas chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with a flame ionization detector.

### 2.5. Peel color

Individual fruits were marked at the equatorial region (two regions per fruit), and color at the same location was recorded every other day as $L^*$, hue angle, and chroma value with a Minolta Chroma Meter CR-2000 (Minolta Camera Co Ltd, Japan). The chroma meter was calibrated with a white standard tile. The color was reported as hue angle (°), with a value of 90° representing a totally yellow color, and 180° a totally green color. The results are presented as lightness ($L^*$), chroma ($C^*$), and hue angle (°). The chroma and hue angle were calculated from the measured $a^*$ and $b^*$ values using the formulas $C^* = (a^{*2} + b^{*2})^{1/2}$ and $H^\circ = \text{arc tangent}(b^*/a^*)$ (McGuire, 1992).

### 2.6. Preparation of cell-free protein extract

Partially thawed mesocarp tissue (10 g) was homogenized with 40 ml of ice-cold 95% EtOH for 1 min in an Omnimixer (Model 17150, Newtown, CT, USA) and centrifuged at $7840 \times g$ for 10 min at 4 °C. The supernatant was discarded and the pellets were resuspended in 50 ml of ice-cold 80% EtOH for 1 min and again centrifuged at $7840 \times g$ for 10 min at 4 °C. The pellets were transferred to 50 ml of ice-cold acetone for 10 min followed by centrifugation ($7840 \times g$, 10 min, 4 °C). After two additional acetone washings, the pellets were suspended in 50

ml of ice-cold 80% EtOH, stirred with a spatula, kept for 10 min in an ice-cold water bath, and then centrifuged (7840 × *g*, 10 min, 4 °C). The pellets were transferred to 30 ml of 10 mM Na-acetate, pH 6.0, containing 1.8 M NaCl, for 30 min in ice-cold water bath and centrifuged. The supernatant was analyzed for enzyme activities as described below. Protein content was measured using the bicchinoninic method (Smith et al., 1985) with bovine serum albumin as a standard.

### 2.7. Enzyme assays

Polygalacturonase (PG, E.C. 3.2.1.15) activity was assayed reductometrically by incubating a 100 μl aliquot of the cell-free protein extract with 500 μl (2 mg) of polygalacturonic acid (from orange peel, Sigma Chemical Co., St. Louis, MO, USA) dissolved in 30 mM KOAc, pH 5.5, containing 100 mM KCl. After 30 min at 34 °C, uronic acid (UA) reducing groups were measured using the method of Milner and Avigad (1967). PG activity was expressed as molecular D-galacturonic acid equivalents produced per kilogram protein per minute. Pectinmethylesterase (PME, E.C. 3.1.1.11) was measured using modifications of the method of Hagerman and Austin (1986). A 0.5% (w/v) solution of citrus pectin (Sigma Chemical Co., St. Louis, MO, USA) was prepared in 0.1 M NaCl and adjusted to pH 7.5 with dilute NaOH. A 0.01% (w/v) solution of bromothymol blue was prepared in 0.003 M potassium phosphate, pH 7.5. In a cuvette, 2.0 ml of the 0.5% citrus pectin were mixed with 0.15 ml of bromothymol blue and 0.83 ml of water, pH 7.5. The reaction was initiated by adding 20 μl of the cell-free protein extract adjusted to pH 7.5 with dilute NaOH, and the decrease in $A_{620}$ was recorded. PME activity was expressed as $\Delta A_{620}$ per milligram protein per minute. $C_x$-cellulase (*endo*-1,4-β-glucanase; E.C. 3.2.1.4) activity was measured viscometrically. A 100 μl aliquot of the cell-free protein extract was added to 1.5 ml of a 2.5% solution of carboxymethylcellulose (CMC.7HSP, Fisher Scientific Co., Fair Lawn, NJ, USA) in 40 mM NaOAc, pH 5.0, with 0.02% $NaN_3$, and the mixture was incubated at room temperature for 30 min. The time required for the solution to pass through a calibrated portion of a 1-ml pipette was recorded. Activity was expressed as percentage change in viscosity per milligram protein per minute. α- and β-Galactosidase activities were measured using modifications of the method of Pharr et al. (1976). *p*-$NO_2$-phenyl α- and β-D-galactopyranosides (Sigma Chemical Co., St. Louis, MO, USA) were used as substrates. Substrates were prepared at 2 g $l^{-1}$ in 0.1 M NaOAc, pH 5.2. A 200 μl aliquot of the cell-free protein extract adjusted to pH 5.2 with dilute HCl was added to 200 μl of substrate, and the reaction mixture incubated at 37 °C for 15 min. The release of *p*-$NO_2$-phenol was measured spectrometrically at 400 nm. The activity was expressed as mole of $NO_2$-phenol equivalents released per kilogram protein per minute. $NO_2$-phenol concentration was determined using free $NO_2$-phenol (Fisher Scientific Co., Fair Lawn, NJ, USA).

### 2.8. Preparation of ethanol-insoluble solids

Approximately 20 g of partially thawed mesocarp were homogenized in 80 ml of cold 95% ethanol over ice for 3 min in a Polytron (Kinematica Gmbh Karens, Lunzern, Switzerland) at speed setting # 7. The homogenate was heated for 20 min in a boiling water bath. The EIS were filtered under vacuum through glass fiber filters (GF/C, Whatman) and washed with 100 ml of 95% ethanol. The EIS were transferred to 100 ml of chloroform–methanol (1:1, v/v) and stirred for 30 min at room temperature. The EIS were filtered under vacuum through glass fiber filters (GF/C, Whatman) and washed with 100 ml of acetone. EIS samples were dried in an oven at 40 °C for 5 h and stored in a desiccator at room temperature.

### 2.9. Pectin extraction and analysis

Water- and CDTA (1,2-cyclohexylenedinitrilotetraacetic acid)-soluble pectins were extracted from 30 mg of EIS incubated successively with 7 ml of distilled water and 7 ml 50 mM Na-acetate, pH 6.5, containing 50 mM CDTA. Extractions were carried out at 34 °C for 4 h and quantified in terms of extractable UA content.

UA was determined by the hydroxydiphenyl assay (Blumenkrantz and Asboe-Hansen, 1973) and expressed as microgram galacturonic acid equivalents per milligram EIS. Total UAs in the EIS preparations were determined using the method of Ahmed and Labavitch (1977).

### 2.10. Gel permeation chromatography of polyuronides

The water- and CDTA-soluble UAs were concentrated using rotary evaporation to a final UA concentration of approximately 500 mg $l^{-1}$. Two milliliters of aliquots (1 mg UA equivalents) were passed through a Sepharose CL-2B-300 (Sigma Chemical Co., St. Louis, MO, USA) column (1.5 cm wide and 30 cm high) operated with 200 mM ammonium acetate, pH 5.0 (Mort et al., 1991). Two milliliter fractions were collected at a flow rate of 40 ml $h^{-1}$, and aliquots (0.5 ml) were used for the determination of UA content. The column void ($V_o$) and total ($V_t$) volumes were identified by the elution positions of Blue Dextran (2000 kDa.) and glucose, respectively. The UA content in each column fraction was expressed as a percentage of the total UA recovered.

### 2.11. Hemicellulose extraction

Hemicelluloses were isolated using the method of Huber and Nevins (1981) as modified by De Vetten and Huber (1990). To remove the major portion of pectin prior to hemicellulose extraction, 200 mg of EIS were incubated in 100 ml of 40 mM Na-phosphate, pH 6.8, in a boiling water bath for 20 min. The suspension was filtered through Miracloth and washed with 1 l of distilled water. The residues were filtered under vacuum through glass fiber filters (GF/C, Whatman) and washed with 200 ml of 100% acetone. The phosphate-buffer-treated EIS were dried in an oven at 40 °C for 5 h. For hemicellulose extraction, 50 mg of EIS were incubated in 5 ml of 4 M KOH including 26 mM $NaBH_4$ for 12 h at room temperature. The suspension was filtered through glass fiber filters. The solution was neutralized over ice with concentrated acetic acid and then dialyzed (mwco 2000 Da.) overnight against running tap water followed by deionized water ($2 \times 4$ l, 12 h total). Total carbohydrates in the alkali-soluble extracts were determined by the phenol–sulfuric acid method (Dubois et al., 1956).

### 2.12. Gel permeation chromatography of hemicelluloses

Two milliliters of the hemicellulose extracts (approximately 1 g $l^{-1}$ glucose equivalents) were heated for 5 min in a boiling water bath (to disperse aggregates) before being applied to a column (2 cm wide, 30 cm high) of Sepharose CL-6B-100 (Sigma Chemical Co., St. Louis, MO, USA) operated with 200 mM ammonium acetate, pH 5.0, at room temperature. Fractions of 2 ml were collected and aliquots (0.5 ml) were assayed for total sugars (Dubois et al., 1956) and xyloglucan (XG) (Kooiman, 1960). The column $V_o$ and $V_t$ were identified by the elution positions of Blue Dextran 2000 and glucose, respectively. The column was calibrated with Dextran standards of 70, 40, and 10 kDa (Sigma, St. Louis, MO).

### 2.13. Statistical analysis

The experiments were conducted in a completely randomized design. Statistical procedures were performed using the PC-SAS software package (SAS-Insititute, 1985). Data were subjected to ANOVA using the General Linear Model (Minitab, State College, PA). Differences between means were determined using Duncan's multiple-range test.

## 3. Results

### 3.1. Avocado fruit firmness and weight loss

Changes in avocado fruit firmness following 1-MCP treatment are shown in Fig. 1A. Control fruit softened rapidly and completed ripening (softened to 10–20 N) within 8 days of storage. The firmness of the fruit treated with 1-MCP at 0.09 μl $l^{-1}$ was not significantly different from that of control fruit (data not shown). The firmness decrease of the fruit treated with the higher

concentration of 1-MCP (0.45 μl $l^{-1}$) was significantly delayed and varied with the duration of exposure to the gas. After 8 days of storage at 20 °C, fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 6, 12, or 24 h exhibited firmness values of 20.1, 28.5, and 41.6 N, respectively. Fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 6 and 12 h reached a full-ripe firmness (10–20 N) after about 10 days. Fruit treated with 0.45 μl $l^{-1}$ for 24 h required additional 2 days to reach the full-ripe stage.

Weight loss trends for avocado fruit during storage at 20 °C are shown in Fig. 1B. Control fruit and fruit treated with 1-MCP at 0.09 μl $l^{-1}$ showed no differences in the magnitude and rate of weight loss (data not shown). After 8 days, at which time fruit were fully ripe, the cumulative weight loss ranged between 6.5 and 7.0%. The rate of weight loss in fruit treated with 1-MCP at 0.45 μl $l^{-1}$ paralleled the effects of the gas on firmness. After 8 days of storage, fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 6, 12, or 24 h showed weight loss values of 5.0, 4.5, and 3.9%, respectively. Final weight loss values of fruit treated with the higher 1-MCP concentration (0.45 μl $l^{-1}$) were not significantly different from control fruit (Fig. 1B) and fruit treated with 1-MCP at 0.09 μl $l^{-1}$ (data not shown). Regardless of the treatment, fruit when ripe (10–20 N) showed overall weight loss values ranging from 6 to 7% (Fig. 1B).

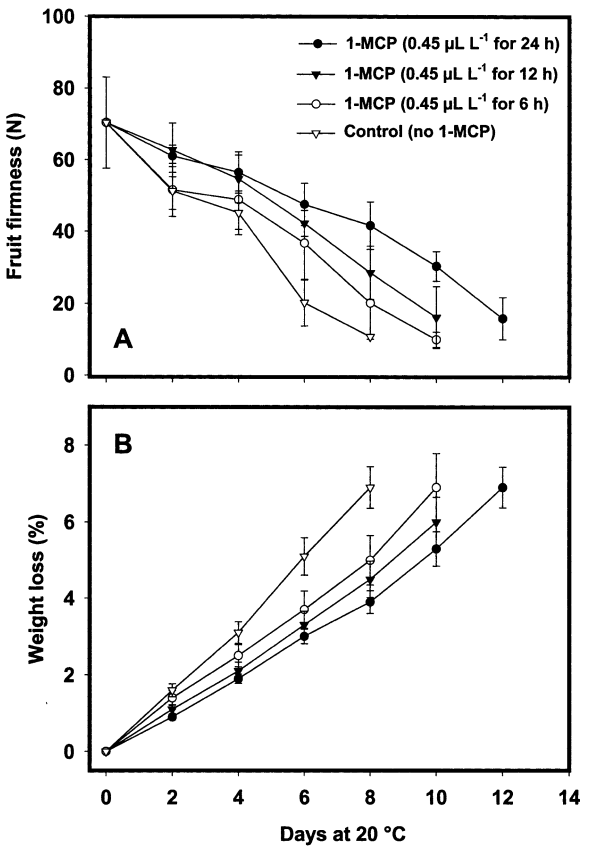


Fig. 1. Fruit firmness (N) and weight loss (%) of 'Simmonds' avocados stored at 20 °C following treatments with 1-MCP. Fruit were treated with 1-MCP concentration (0.45 μl $l^{-1}$) and three different exposure periods (6, 12, and 24 h). Vertical bars represent standard deviation of six independent samples.

### 3.2. Respiration and ethylene evolution

Ethylene production in control fruit and fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 6 h showed characteristic climacteric patterns during storage at 20 °C. Ethylene production of control fruit began to increase after 2 days in storage (data not shown) and reached maximum values of 124.2 μl $kg^{-1}$ $h^{-1}$ after 6 days storage at 20 °C (Table 1). Ethylene production of fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 6 h began to rise after 4 days storage at 20 °C (data not shown), reaching a maximum of 117 μl $kg^{-1}$ $h^{-1}$ after 9 days. There were no significant differences in the maximum amount of ethylene production between control fruit and fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 6 h (Table 1).

Ethylene production trends in fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 12 or 24 h were atypical. In fruit treated with 1-MCP at 0.45 μl $l^{-1}$ for 12 or 24 h, ethylene production began to increase after 6 and 10 days of storage, respectively (data not shown). A distinct peak of ethylene production did not occur during the storage period, and maximum ethylene production rates were reduced over 50% compared with all other treatments (Table 1).

The time to attain the maximum respiratory rates closely paralleled that for ethylene production in all treatments (Table 1). Respiration in control fruit began to increase after 2 days storage at 20 °C (data not shown) and $CO_2$ production

Table 1
Days to peak and maximum $C_2H_4$ and $CO_2$ production for 'Simmonds' avocados stored at 20 °C following treatments with 1-MCP

| Treatments | $C_2H_4$ | | $CO_2$ | |
|---|---|---|---|---|
| | Days to peak | Maximum ($\mu l\,kg^{-1}\,h^{-1}$) | Days to peak | Maximum ($mg\,kg^{-1}\,h^{-1}$) |
| Control (no 1-MCP) | 6 | $124.2 \pm 39.0$ | 6 | $51.7 \pm 3.9$ |
| 1-MCP (0.45 $\mu l\,l^{-1}$ for 6 h) | 9 | $117.0 \pm 40.0$ | 9.3 | $43.6 \pm 8.3$ |
| 1-MCP (0.45 $\mu l\,l^{-1}$ for 12 h) | 10[a] | $45.9 \pm 64.6$[a] | 10[a] | $25.8 \pm 17.5$[a] |
| 1-MCP (0.45 $\mu l\,l^{-1}$ for 24 h) | 12[a] | $50.5 \pm 57.0$[a] | 12[a] | $30.3 \pm 13.5$[a] |

Fruit were treated with 1-MCP concentration (0.45 $\mu l\,l^{-1}$) for three different exposure periods (6, 12, and 24 h). Initial rates of $C_2H_4$ and $CO_2$ production were 0.5 $\mu l\,kg^{-1}\,h^{-1}$ and 61.3 $mg\,kg^{-1}\,h^{-1}$, respectively. Data are means ± standard deviation of four independent samples.

[a] Ethylene and respiratory climacteric peaks were not observed during storage at 20 °C, and data were measured when experiments were terminated.

reached maxima of 51.7 $mg\,kg^{-1}\,h^{-1}$ after 6 to 7 days storage at 20 °C (Table 1). $CO_2$ production of fruit treated with 1-MCP at 0.45 $\mu l\,l^{-1}$ for 6 h increased initially after 4 days storage at 20 °C (data not shown), reaching a maximum of 43.6 $mg\,kg^{-1}\,h^{-1}$ after 9.3 days storage at 20 °C (Table 1). The application of 1-MCP at 0.45 $\mu l\,l^{-1}$ for 6 h slightly suppressed the magnitude of the respiratory peak (Table 1); however, the differences in the maximum $CO_2$ production rate between control fruit and fruit treated with 1-MCP were not statistically significant.

The $CO_2$ production of fruit treated with 1-MCP at 0.45 $\mu l\,l^{-1}$ for 12 and 24 h showed a delayed and attenuated climacteric pattern. Increased respiration for fruit treated with 1-MCP at 0.45 $\mu l\,l^{-1}$ for 12 and 24 h was first evident at day 6 and day 10, respectively (data not shown). As with ethylene production, a respiratory peak was not observed during storage at 20 °C, and maximum $CO_2$ production rates were reduced nearly 40% compared with all other treatments when experiments were terminated (Table 1).

### 3.3. Peel color

The peel of avocado fruit prior to storage had a moderate green color (hue angle = 123.6°, where pure yellow = 90° and pure green = 180°). At the full-ripe stage, there were significant differences in the $L^*$ value, chroma value ($C$), and hue angle of the peel color among fruit from all treatments (Table 2). Changes in hue angle constituted the major alteration of color coordinates of fruit. The decline in hue angle represented the change from green to yellow, and the increase in chroma value reflected increasing intensity of yellow color.

At the full-ripe stage, fruit treated with 1-MCP at 0.45 $\mu l\,l^{-1}$ for 24 h had the lowest $L^*$ (44.1) and chroma values (26.0) and the highest hue angle (123.5) (Table 2). These data indicate that the peel of avocado fruit treated with 1-MCP at 0.45 $\mu l\,l^{-1}$ for 24 h retained moderate green color with low color intensity (light) during 12 days storage at 20 °C.

### 3.4. Enzyme activity

Based on the above studies employing a range of 1-MCP concentrations and treatment durations, experiments addressing the effects of the gas on selected cell-wall enzymes and polysaccharides were performed only with fruit exposed to the higher 1-MCP level (0.45 $\mu l\,l^{-1}$ for 24 h). The activities of cell-wall enzymes in cell-free protein extracts of avocado fruit treated with 1-MCP at 0.45 $\mu l\,l^{-1}$ for 24 h are shown in Figs. 2 and 3. The activity of PG was very low in freshly harvested, pre-climacteric fruit, increased during the climacteric period, and continued to increase during the postclimacteric phase (Fig. 2A). The pattern of PG accumulation in 'Simmonds' avocado fruit is consistent with the activity levels reported for 'Fuerte' avocado (Awad and Young, 1979).

PG activity in 'Simmonds' fruit treated with 0.45 $\mu l\,l^{-1}$ 1-MCP for 24 h remained at levels comparable to or slightly below those detected at harvest (Fig. 2A).

PME activity in control fruit declined from a maximum value at harvest to a minimum level at the full-ripe (day 8) stage (Fig. 2B). The trend for 1-MCP treated fruit paralleled that for control fruit, although the decline in activity was delayed. The levels of PME in 1-MCP treated fruit after 12 days were similar to those noted for control fruit at 8 days.

$C_x$-cellulase (*endo*-β-1,4-glucanase) activity was not detected in fruit measured within 24 h of harvest (Fig. 3A). In control fruit, $C_x$-cellulase levels increased significantly after day 4, reaching levels 13.4-fold higher than those noted at day 4 after 4 additional days of storage. $C_x$-cellulase in fruit treated with 0.45 $\mu l\,l^{-1}$ 1-MCP for 24 h was not detectable until day 4. $C_x$-cellulase levels increased slowly after day 4 and significantly after day 8, reaching levels 5.6-fold higher than levels at day 8 within 4 days.

Total α- and β-galactosidase activities are shown in Fig. 3B and C. α-Galactosidase activity of control fruit decreased significantly during storage and reached a minimum at day 8. The decline was less pronounced in fruit treated with 0.45 $\mu l\,l^{-1}$ 1-MCP for 24 h. α-Galactosidase in 1-MCP-treated fruit reached a minimum at day 8, thereafter remaining constant. β-Galactosidase activity of control fruit and fruit treated with 0.45 $\mu l\,l^{-1}$ 1-MCP decreased after harvest and reached a minimum at day 4 and day 8, respectively. β-Galactosidase activity in both control and 1-MCP treated fruit remained constant through the remaining storage period at 20 °C.

### 3.5. Solubility and molecular mass of avocado polyuronides

Polyuronide solubilility was markedly affected during ripening and in response to treatment with 1-MCP. Fig. 4 illustrates changes in water- and CDTA-soluble UA content, and total UA content for both the control fruit and the fruit treated with 0.45 $\mu l\,l^{-1}$ 1-MCP for 24 h. During the first 4 days of storage, total UA levels remained nearly constant in both the control and the 1-MCP-treated fruit. By the full-ripe stage in control fruit (8 days), total UA in EIS had declined by nearly 30% (Fig. 4A). In sharp contrast, total UA levels in 1-MCP-treated fruit remained constant throughout the entire storage period (12 days). Water-soluble UA in control fruit increased significantly after 4 days at 20 °C, and an additional 2.1-fold when fruit were fully ripe (Fig. 4B). The levels of CDTA-soluble UA in control fruit, though much lower than water-soluble levels, decreased significantly after 4 days at 20 °C (Fig. 4C). In 1-MCP treated fruit, water-soluble UA increased significantly after 8 days at 20 °C and reached levels 1.8-fold higher than those at day 0 (Fig. 4B). In contrast to control fruit, the levels of CDTA-soluble UA in 1-MCP-treated fruit remained constant through 12 days of storage at 20 °C (Fig. 4C).

Gel permeation profiles of water-soluble polyuronides from control and 1-MCP-treated avocado on days 0, 4, 8, and 12 are shown in Fig. 5. At the pre-ripe stage (day 0, Fig. 5A), water-soluble polyuronides, representing approximately 20%

Table 2
Peel color of 'Simmonds' avocados stored at 20 °C following treatments with 1-MCP

| Treatments | Time to full ripeness at 20 °C | $L^*$ | Chroma | Hue angle |
|---|---|---|---|---|
| Control (no 1-MCP) | 8 | $46.3 \pm 2.2$ | $33.2 \pm 3.6$ | $122.3 \pm 1.9$ |
| 1-MCP (0.45 $\mu l\,l^{-1}$ for 6 h) | 10 | $46.1 \pm 1.7$ | $32.4 \pm 2.9$ | $121.0 \pm 1.9$ |
| 1-MCP (0.45 $\mu l\,l^{-1}$ for 12 h) | 10 | $45.3 \pm 2.8$ | $31.6 \pm 4.2$ | $123.5 \pm 2.3$ |
| 1-MCP (0.45 $\mu l\,l^{-1}$ for 24 h) | 12 | $44.1 \pm 2.2$ | $26.0 \pm 3.4$ | $123.5 \pm 1.3$ |

Peel color was measured at the full-ripe stage. Fruit were treated with 1-MCP concentration (0.45 $\mu l\,l^{-1}$) for three different exposure periods (6, 12, and 24 h). Initial values for $L^*$, chroma, and hue angle were 46.5, 24.2, and 123.6, respectively. Data are means ± standard deviation of four independent samples.

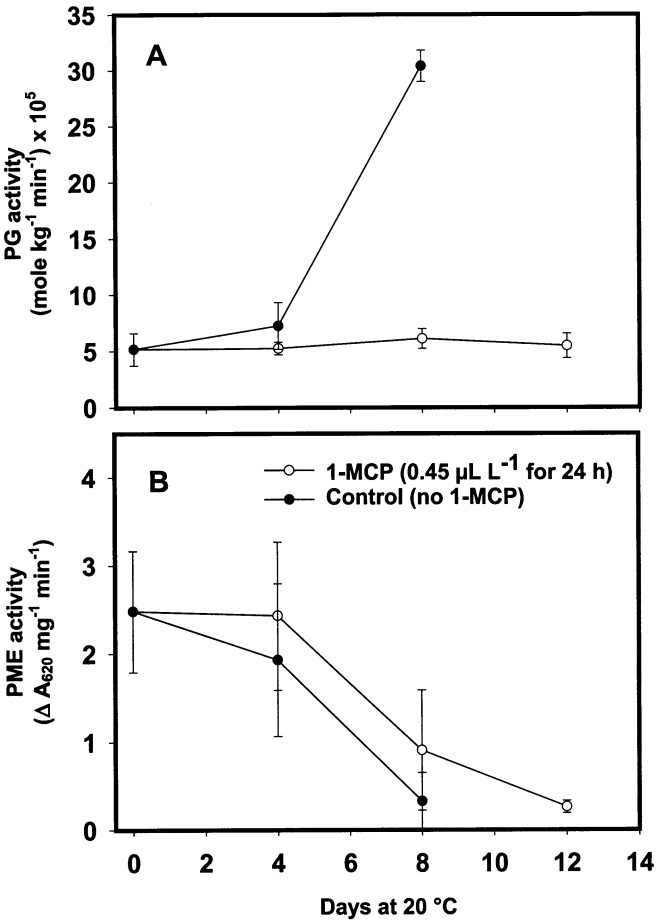


Fig. 2. Effect of 1-MCP on PG and PME activities of avocados stored at 20 °C. Fruit were treated with (○) or without (●) 1-MCP (0.45 µl $l^{-1}$ for 24 h). Vertical bars represent standard deviation of three independent samples.

of total EIS UA, eluted as a polydisperse population. As ripening proceeded (days 4 through 8; Fig. 5B and C), water-soluble polyuronides of control fruit exhibited molecular mass downshifts involving increases in the levels of intermediate and low molecular mass polymers, the latter eluting near the total column volume. Molecular mass downshifts of water-soluble polyuronides in 1-MCP-treated fruit were considerably more limited throughout 8 days of storage. At the full-ripe stage (day 12), the water-soluble polyuronides of 1-MCP-treated fruit showed evidence of further molecular mass downshifts (Fig. 5D); however, as evident from Fig. 5 A and B the levels of water-soluble polyuronides in 1-MCP-treated fruit represented a proportionally much lower percentage (40%) of total EIS UA compared with the 70% for control fruit.

The molecular mass distributions and downshifts of CDTA-soluble polyuronides paralleled those evident for water-soluble polymers (Fig. 6). Molecular mass downshifts were extensive for ripe control fruit (day 8, Fig. 6C) and considerably less pronounced for ripe 1-MCP-treated fruit (12 days, Fig. 6D). CDTA-soluble polyuronides were a relatively minor component of avocado polyuronides, representing approximately 7% (control) or 9% (1-MCP treated) of total EIS UA levels.

### 3.6. Molecular mass of hemicelluloses and xyloglucan

Gel permeation profiles of 4 M alkali-soluble hemicelluloses and XG from control and 1-MCP-treated (0.45 μl $l^{-1}$ 1-MCP for 24 h) avocado fruit are shown in Fig. 7. In control fruit, hemicelluloses at each developmental stage were polydisperse and exhibited a gradual but limited molecular mass downshift during ripening. The change in polymer distribution was evident as a gradual peak sharpening, with peak elution volumes noted at 40, 42, and 44 ml for hemicelluloses from 0, 4, and 8-day (ripe) fruit, respectively. Molecular mass downshifts were delayed in 1-MCP treated fruit (polymer distribution peaks at

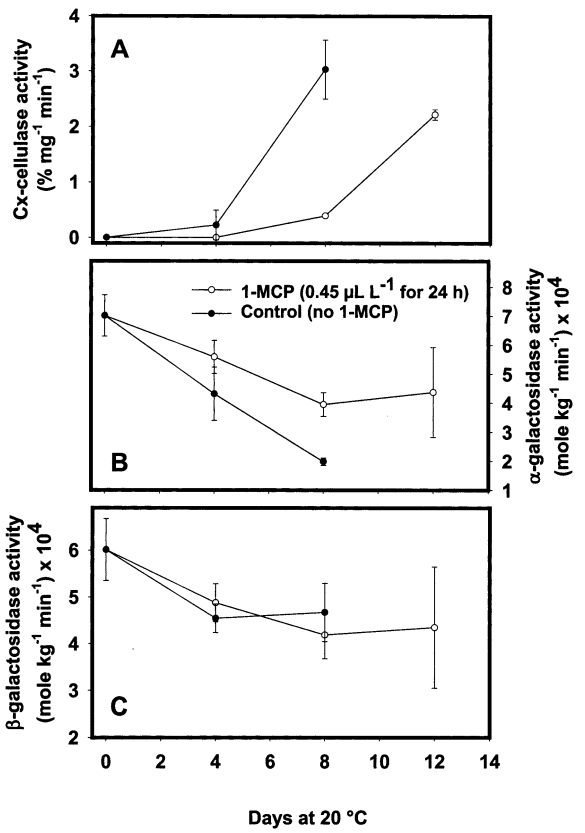


Fig. 3. Effect of 1-MCP on $C_x$-cellulase and α-,β-galactosidase activities of avocados stored at 20 °C. Fruit were treated with (○) or without (●) 1-MCP (0.45 μl $l^{-1}$ for 24 h). Vertical bars represent standard deviation of three independent samples.

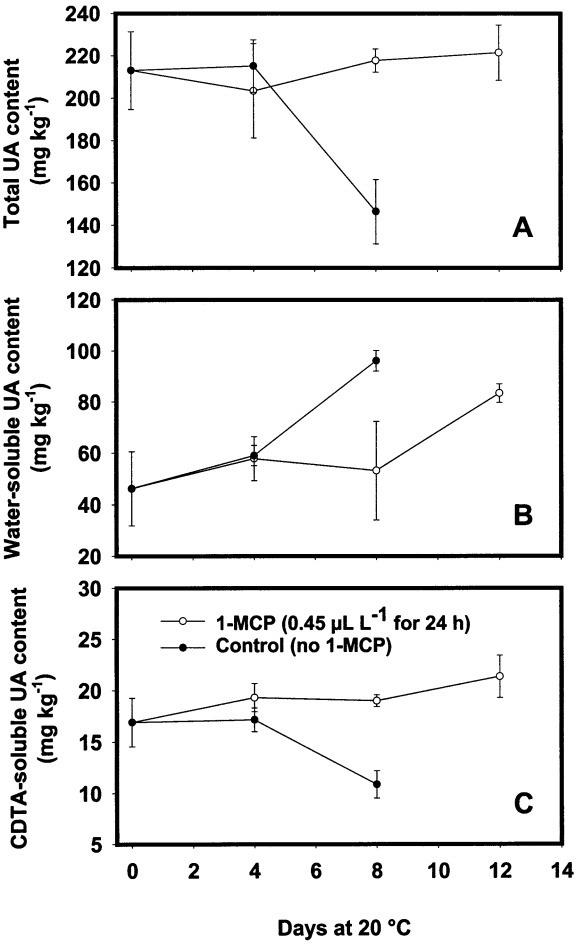


Fig. 4. Effect of 1-MCP on the levels of water-, CDTA-soluble, and total UA from avocados stored at 20 °C. EIS were incubated sequentially in distilled water and 50 mM CDTA in 50 mM Na-acetate, pH 6.5, each for 4 h at 34 °C. Suspensions were filtered and UA content determined. Fruit were treated with (○) or without (●) 1-MCP (0.45 μl $l^{-1}$ for 24 h). Vertical bars represent standard deviation of three independent samples.

day 0, 40 ml; day 4, 40 ml; day 8, 42 ml; day 10, 44 ml); however, profiles from full-ripe 1-MCP treated fruit (day 12, Fig. 7D) were nearly indistinguishable from those from full-ripe control fruit (day 8, Fig. 7C). Although hemicellulose molecular mass distributions in general were not markedly different between control and 1-MCP-treated fruit, the slight differences noted were similar for three independent replicates.

Changes in molecular mass of XG paralleled those observed for total hemicelluloses, although XG-enriched polymers eluted as larger molecular mass components of the total hemicelluloses (Fig. 7). As noted for total hemicelluloses, the change in XG distribution was evident as a gradual peak sharpening during ripening (Fig. 7). XG from 1-MCP-treated fruit at each developmental stage eluted as a polydisperse population with a slight decrease in average molecular mass. 1-MCP-treated fruit at the full-ripe stage (day 12, Fig. 7D) showed a slightly less pronounced downshift

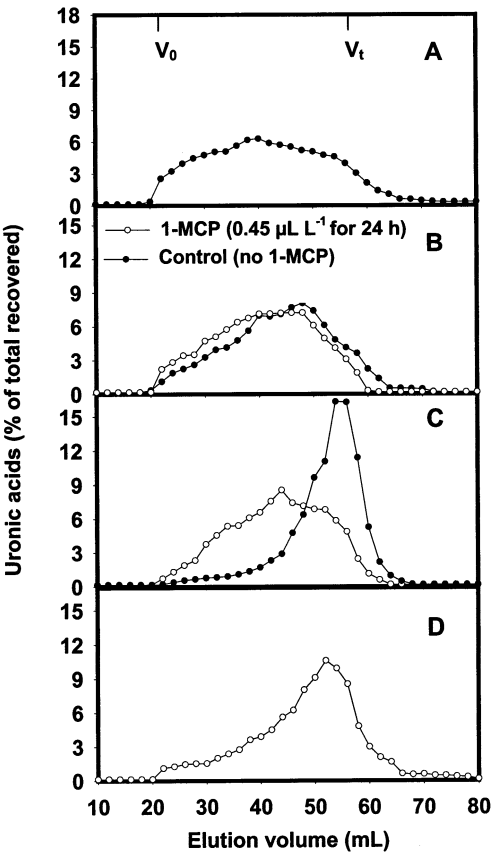


Fig. 5. Molecular mass distribution of water-soluble polyuronides from avocado treated with (○) and without (●) 1-MCP (0.45 $\mu l\,l^{-1}$ for 24 h). Polyuronides ($\approx 0.5$ mg galacturonic acid equivalents) were applied to a Sepharose CL-2B-300 column and individual fractions were measured for UA content. Data for each fraction expressed as a percentage of the total eluted UA. Day 0 (A); Day 4 (B); Day 8 (C); Day 12 (D). $V_o$, Void volume; $V_t$, total volume.

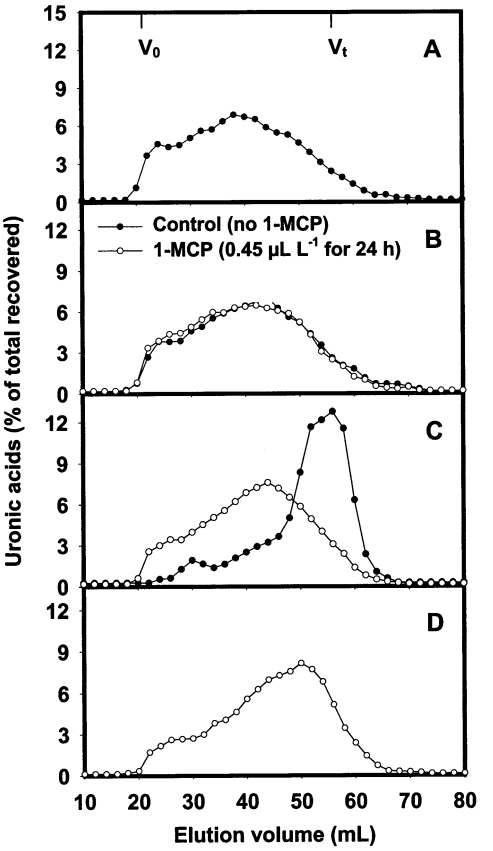


Fig. 6. Molecular mass distribution of CDTA-soluble polyuronides from avocado treated with (○) and without (●) 1-MCP (0.45 $\mu l\,l^{-1}$ for 24 h). Polyuronides ($\approx 0.5$ mg galacturonic acid equivalents) were applied to a Sepharose CL-2B-300 column and individual fractions were measured for UA content. Data for each fraction expressed as a percentage of the total eluted UA. Day 0 (A); Day 4 (B); Day 8 (C); Day 12 (D). $V_o$, Void volume; $V_t$, total volume.

in XG molecular mass (elution volume of XG peak = 36 ml) compared with control fruit (XG peak = 40 ml) (day 8, Fig. 7C).

## 4. Discussion

In the present study, several parameters (firmness, weight loss, respiration and $C_2H_4$ production, peel color, selected cell-wall enzymes activities and structural carbohydrates) were examined to determine the efficacy of 1-MCP in

delaying avocado fruit ripening. Avocado firmness was significantly retained in response to 1-MCP treatment, consistent with the fact that softening is one of the most sensitive ripening processes to ethylene (Lelievre et al., 1997a). Delayed softening by 1-MCP substantiates that ethylene is involved in augmenting the activity of softening-related metabolism. Similar effects of 1-MCP in attenuating fruit softening have been observed for 'Hass' avocado (Feng et al., 2000), apricot (Fan and Mattheis, 1999), and 'McIntosh' and 'Delicious' apple (Rupasinghe et al., 2000) fruits.

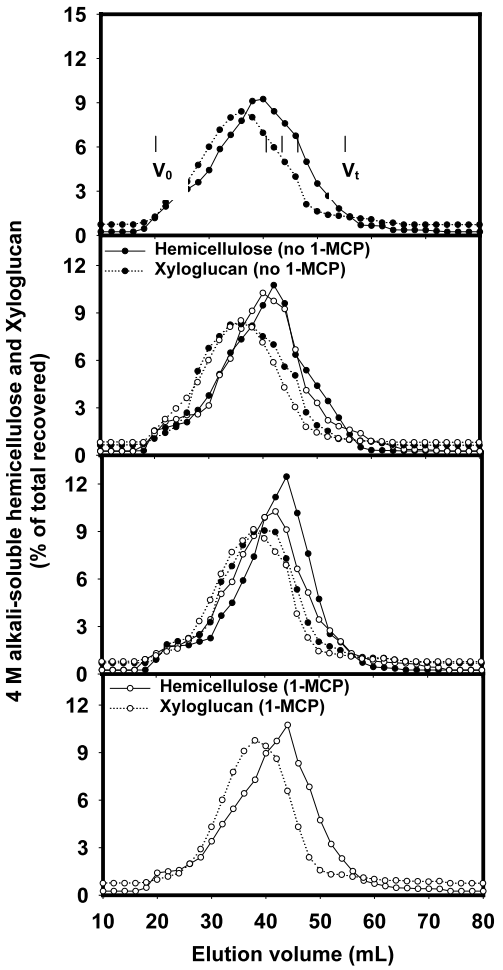


Fig. 7. Molecular mass distribution of 4 N alkali-soluble hemicelluloses and XG from avocado treated with (○) and without (●) 1-MCP. Two milliliters (containing 2 mg glucose equivalents) of hemicelluloses were applied to a Sepharose CL-6B-100 column and individual fractions were measured for total sugar and XG. Data for each fraction expressed as a percentage of the total eluted sugar. Day 0 (A); Day 4 (B); Day 8 (C); Day 12 (D). Tick marks at the top of the figure indicate, from left to right, the elution positions of Blue Dextran ($V_o$), Dextrans of 70, 40, 10 kDa, and glucose (right).

The concentration of and length of exposure to 1-MCP significantly influenced the effect of the gas in delaying avocado fruit ripening. Avocado fruit treated with 0.45 $\mu l\,l^{-1}$ 1-MCP for 24 h at 20 °C required 2 to 4 more days at 20 °C to reach the full-ripe stage compared with the fruit treated with 0.09 or 0.45 $\mu l\,l^{-1}$ 1-MCP for 6 or 12 h at 20 °C. These data are consistent with those of Feng et al. (2000), who reported that concentrations of 1-MCP to 30, 50, or 70 $nl\,l^{-1}$ caused a progressive delay in the softening of 'Hass' avocado.

1-MCP significantly delayed the onset of climacteric ethylene production and respiration in avocado fruit. The concentration and length of exposure to 1-MCP influenced the effect of 1-MCP at delaying ethylene production and respiration patterns of 'Simmonds' avocado fruit. Ethylene and $CO_2$ production did not fully recover in avocado fruit treated with 0.45 $\mu l\,l^{-1}$ 1-MCP for 12 or 24 h at 20 °C, with maximum production rates remaining 50 and 70% lower, respectively, than those for all other treatments. Delayed climacteric ethylene production and respiration has also been reported for apricot (Fan et al., 2000), apple (Fan et al., 2000), banana (Golding et al., 1998, 1999), and avocado (cv. Hass) (Feng et al., 2000) fruits.

Further increases in the concentration of 1-MCP (e.g. 4.5 $\mu l\,l^{-1}$ for 24 h) did not result in a further delay in ripening compared with treatment at 0.45 $\mu l\,l^{-1}$ 1-MCP for 24 h (data not shown). This observation indicates that treatment with 0.45 $\mu l\,l^{-1}$ 1-MCP for 24 h is sufficient to exert maximal delay of avocado ripening. This concentration is comparable to those found effective for delaying ripening of apricot (1 $\mu l\,l^{-1}$; Fan et al., 2000), and 'McIntosh' and 'Delicious' apples (1 $\mu l\,l^{-1}$; Rupasinghe et al., 2000). The gradual but incomplete recovery of $C_2H_4$ production in 0.45 $\mu l\,l^{-1}$ 1-MCP-treated avocados during storage at 20 °C suggests the synthesis of new receptor

proteins, metabolism of the 1-MCP receptor-protein complex, or dissociation of 1-MCP from the receptor sites (Sisler and Serek, 1999; Sisler et al., 1996a).

Consistent with the previous reports, the ripening of avocado fruit was accompanied by increases in $C_x$-cellulase and PG activities (Awad and Young, 1979; Christofferson et al., 1984; Pesis et al., 1978) and a decline in PME activity (Awad and Young, 1979, 1980). Of the cell-wall enzymes measured in 1-MCP-treated fruit, PG exhibited the strongest response, showing little or no recovery over the 12-day post-treatment storage period. Feng et al. (2000) found that 30, 50, or 70 nl $l^{-1}$ 1-MCP suppressed PG activity about 10 to 30% and delayed the increase in cellulase activity by 4 days in 'Hass' avocado.

Although PG activity did not recover in 1-MCP-treated avocado, the firmness ultimately reached values comparable to those of control fruit, indicating that PG is not required for the extensive softening of avocado fruit. This conclusion is consistent with the reports for tomato fruit, wherein antisense suppression of PG activity had minimal influence on fruit softening until the very late stages of ripening (Carrington et al., 1993; Kramer et al., 1992). Although not statistically significant, 0.45 μl $l^{-1}$ 1-MCP-treated avocados at the full-ripe stage remained slightly firmer than control fruit. This may indicate that PG in avocado fruit, as in tomato fruit, is more important in the late stages of ripening.

Consistent with the marked suppression of PG levels in 1-MCP-treated 'Simmonds' avocado fruit, the solubilization and degradation of polyuronides was significantly delayed and reduced in 1-MCP-treated fruit. At the pre-ripe stage (day 0), water- and CDTA-soluble UA constituted 22 and 7.9% of the total EIS UA content, respectively. At the full-ripe stage (10–20 N), water- and CDTA-soluble polyuronides of control fruit comprised approximately 65 and 7.5%, respectively, of the total UA content, whereas, those of 1-MCP treated fruit comprised approximately 38 and 9.7%, respectively, of the total UA content. The large difference in the levels of water-soluble polyuronides expressed as a percentage of total EIS UA was in large part due to the persistence of the total UA levels in EIS of 1-MCP-treated fruit and a 31% decrease in UA levels of control fruit. The 31% decrease in total EIS UA is consistent with the 28% decline in total UA reported for 'Lula' avocado fruit (Wakabayashi et al., 2000). The extensive depolymerization of polyuronides in ripening avocado (Huber and O'Donoghue, 1993; Sakurai and Nevins, 1997; Wakabayashi et al., 2000) possibly results in the production of pectic fragments that are soluble to various extents in 80% ethanol and, consequently, not recovered in EIS preparations. In contrast, the more limited molecular mass downshifts in the polyuronides of 1-MCP-treated fruit probably result in more quantitative recovery of these polymers in EIS preparations.

Both water- and CDTA-soluble polyuronides of control fruit exhibited the characteristic molecular mass downshifts reported in earlier studies, with polymers in ripe fruit eluting near the total column volume. Through 8 days of storage (Figs. 5C and 6C) 1-MCP treated fruit showed considerably less extensive breakdown of both water- and CDTA-soluble polyuronides. Further downshifts in polyuronide molecular mass were evident in 1-MCP-treated fruit after 12 days of storage. These data suggest that the low activity of PG in 1-MCP-treated fruit is sufficient to depolymerize avocado polyuronides; however, we note that the quantities of polyuronides extracted from 1-MCP treated fruit at 12 days are proportionally much lower than those from control fruit. Additionally, Wakabayashi et al. (2000) have shown that limited molecular mass downshifts in avocado polyuronides, as evident from gel filtration analyses, can be brought about by deesterification, independently of PG action. Consequently, the relatively normal levels of PME in these fruit might have influenced the gel filtration behavior of polyuronides from 1-MCP-treated fruit.

Total extractable α- and β-galactosidase activities decreased during avocado ripening; however, the use of total protein extracts in our assays would have masked differential responses of specific isozymes of these proteins. For example, Pressey (1983) and Carey et al. (1995) reported that total β-galactosidase activity in tomato remained relatively constant throughout ripening,

whereas the levels of one isozyme (β-Gal II) increased about fourfold. Smith and Gross (2000) found that transcript accumulation for tomato β-gal II, one of the seven β-gal transcripts detected, was significantly impaired in *rin*, *nor*, and *Nr* fruit relative to wild-type accumulation, indicating that β-gal II may be upregulated by ethylene.

The molecular mass distribution of hemicelluloses from 'Simmonds' avocado was similar to that of hemicelluloses from 'Lula' avocado fruit (O'Donoghue and Huber, 1992). During ripening of 'Simmonds' avocado at 20 °C, hemicelluloses exhibited a gradual but limited molecular mass downshift. 1-MCP treatment did not significantly affect the quantities of 4 M alkali-soluble hemicellulose during ripening (data not shown). 1-MCP treatment, however, significantly reduced molecular mass downshifts in 4 M alkali-soluble hemicelluloses and XG (Fig. 7).

## 5. Conclusions

Avocado fruit treated with 1-MCP at 0.45 $\mu l\, l^{-1}$ for 24 h at 20 °C exhibited delayed ripening, softening, and ethylene and respiratory climacterics. Fruit also showed significantly less weight loss and retained more green color than control fruit at the full-ripe stage. The differential effects of 1-MCP on specific cell-wall enzymes and polysaccharide metabolism indicate that the gas has the potential as a tool to elucidate the involvement of ethylene in the progression of cell-wall changes contributing to fruit softening. Further work on the effects of 1-MCP will allow its application, in conjunction with appropriate harvesting, handling, and environmental conditions, to extend the storage life of avocado fruit.

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