

1-Methylcyclopropene Application and Modified Atmosphere Packaging Affect Ethylene Biosynthesis, Fruit Softening, and Quality of 'Tegan Blue' Japanese Plum During Cold Storage

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ABSTRACT. This research was carried out to extend the postharvest storage of Japanese plum (*Prunus salicina* Lindl. cv. Tegan Blue), which has a short shelf life limiting its export potential. The effects of 1.0 $\mu\text{L}\cdot\text{L}^{-1}$ 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP), alone or in combination, on quality of mature Japanese plum fruit during storage ($0 \pm 1^\circ\text{C}$ and $90\% \pm 5\%$ relative humidity) were investigated. The activities of enzymes of ethylene biosynthesis [1-aminocyclopropane-1-carboxylic acid synthase (ACS), 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), and 1-aminocyclopropane-1-carboxylic acid (ACC) content] and those of cell wall-associated enzymes [exo-polygalacturonase (exo-PG), endo-polygalacturonase (endo-PG), pectin esterase (PE), and endo-1,4- β -D-glucanase (EGase)] were also measured. 1-MCP-treated fruit stored in normal atmosphere or in MAP had lower ACC content and inhibited ethylene production with reduced ACS and ACO activities compared with fruit stored in MAP and in normal atmosphere. Similarly, 1-MCP-treated fruit, stored either in normal atmosphere or in MAP, were firmer with reduced exo-PG, endo-PG, PE, and EGase activities compared with fruit stored in MAP and in normal atmosphere. During storage as well as during ripening, fruit stored in MAP exhibited a higher rate of respiration compared with other treatments. MAP exacerbated the effect of 1-MCP in reduction of ethylene production and fruit softening. 1-MCP application in combination with MAP after 5 and 7 weeks of storage delayed the fruit ripening by 10 and 8 days in contrast with control fruit, respectively. During storage, and as well as in ripe fruit, weight loss was reduced in fruit stored in MAP either with or without 1-MCP application. Control fruit and 1-MCP-treated fruit, stored in a normal atmosphere or in MAP, had the same values for the following parameters: chromaticity value L^* , C^* , and hue angle, titratable acidity, and concentrations of soluble solids, ascorbic acid, and total antioxidants. In conclusion, 1-MCP application in combination with MAP can be used effectively to reduce the ethylene biosynthesis and fruit softening during cold storage and to extend the storage life up to 7 weeks followed by 8 d of ripening without any adverse effects on the quality of ripe fruit.

The short shelf life of Japanese plum (*Prunus salicina* Lindl.) and European plum (*Prunus domestica* L.) fruit limits its export through sea freight. At 1°C , Japanese plum can be stored for only 3 to 5 weeks (Navarro et al., 2005). Different techniques to extend the postharvest storage life of various Japanese plum and European plum cultivars that have been tested include preharvest calcium application (Plich et al.,

2002); postharvest heat treatment (Serrano et al., 2004); pre- or postharvest application of polyamines (Serrano et al., 2003), aminoethoxyvinylglycine (Jobling et al., 2003), and 1-methylcyclopropene (1-MCP) (Khan and Singh, 2007; Watkins, 2006); edible coating (Navarro et al., 2005); cold storage (Robertson et al., 1991); controlled atmosphere storage (Wang and Vestreheim, 2003); and modified atmosphere (MA) storage (Turk and Ozkurt, 1994). However, the available information is inconclusive and sporadic.

Fruit softening is an important attribute associated with quality of Japanese plum and European plum fruit. Fruit softening involves compositional and structural changes in cell wall carbohydrates as a result of activities of cell wall enzymes (Fischer and Bennett, 1991). Pectins, hemicelluloses, and celluloses undergo depolymerization and structural modifications during fruit softening (Chin et al., 1999). Enzymes that are associated with fruit softening include polygalacturonase (PG), pectin esterase (PE), cellulase, and β -galacturonase (Fischer and Bennett, 1991). In papaya (*Carica papaya* L.), it has been reported that combination of MA packaging (MAP) and low temperature retarded the fruit firmness with decreased and suppressed activities of PG, PE, and β -galacturonase enzymes (Lazan et al., 1993) as well as ethylene biosynthesis enzymes (Latifah et al., 1997).

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We were interested in the effects of MAP and application of 1-MCP on japanese plum fruit quality, including its effects on dietary antioxidants that may play a role in human health (Huang et al., 2005). MAP has been reported to extend storage life of various climacteric and nonclimacteric fruit such as sweet cherry (*Prunus avium* L.) (Petracek et al., 2002), peach [*Prunus persica* (L.) Bastch.] (Fernandez-Trujillo et al., 1998), and european plum (Turk and Ozkurt, 1994) with variable results on extending storage life and maintaining fruit quality. However, depending on storage conditions and type of polyethylene film used under MA storage, off-flavor and off-odors associated with anaerobic respiration may accumulate and reduce fruit quality (Petracek et al., 2002).

1-MCP is a gaseous compound that binds irreversibly to ethylene receptors and thereby prevents ethylene-dependent response (Blankenship and Dole, 2003). Extensive research has been conducted on the use of 1-MCP to reduce ethylene production, fruit softening, and to extend storage and shelf life of climacteric fruit, including both japanese plum and european plum (Abdi et al., 1998; Khan and Singh, 2004; Watkins, 2006). Application of 1-MCP in combination with MAP has been reported to delay fruit ripening and extend the postharvest life of banana (*Musa acuminata* Colla.) and mango (*Mangifera indica* L.) (Jiang and Joyce, 2000; Jiang et al., 1999). No research work has been reported on the effects of MAP alone and MAP in combination with 1-MCP in extending storage life, regulations of ethylene biosynthesis, fruit softening, and quality parameters such as levels of ascorbic acid and total antioxidants. We hypothesized that 1-MCP in combination with MAP might be more effective in reducing ethylene production, fruit softening, and maintaining fruit quality compared with 1-MCP and MAP alone. These observations prompted us to investigate the role of 1-MCP alone and in combination with MAP in the regulation of ethylene biosynthesis and fruit softening enzymes as well as fruit quality during low-temperature storage and in ripe 'Tegan Blue' japanese plum fruit after low-temperature storage.

Materials and Methods

Plant materials and fruit

Nineteen-year-old 'Tegan Blue' japanese plum trees grafted on myrobalan (*Prunus cerasifera* Ehrh.) rootstock at Casuarina Valley Orchard, Manjimup (lat. 34°15' S, long. 116°09' E) in the southwest region of Western Australia (WA) were selected for the experiments. Experimental trees planted in north-south row direction (4.25 m between rows and 2 m within rows) were trained as a palmette. Fruit of uniform size, free from visual symptoms of disease or blemishes, were harvested at commercial maturity [rate of C₂H₄ production = 0.003 ± 0.001 μmol·kg⁻¹·h⁻¹, rate of CO₂ production = 0.56 ± 0.02 mmol·kg⁻¹·h⁻¹, soluble solids concentrations (SSC) = 16.3% ± 0.9%, and firmness = 60.3 ± 2.6 N] on 8 Mar. 2006.

Expt. 1: Effects of 1-methylcyclopropene or modified atmosphere packaging alone and in combination on ethylene biosynthesis, fruit softening, and fruit quality during low-temperature storage

Fruit were subjected to various treatments, including: 1) control, 2) 1.0 μL·L⁻¹ 1-MCP, 3) MAP only, and 4) 1.0 μL·L⁻¹ 1-MCP in combination with MAP. Fruit were kept in hermetically sealed plastic drums (68 L) and 1.0 μL·L⁻¹ 1-MCP was maintained by injecting the required amount through a rubber septum following the method of Lalel et al. (2003). 1-MCP was obtained from EthylBlock powder (0.43% a.i. 1-MCP; Bio-

Technologies for Horticulture, Waterboro, SC). Fruit were treated with 1-MCP for 24 h at 20 ± 1 °C. To apply MAP, fruit were packed in LifeSpan (AMCOR Packaging, Ptv. Ltd., Melbourne, Australia) polyethylene bags (710 cm long × 500 cm wide × 30 μm thick), commercially used for storage of japanese plums and european plums during low temperature in WA. Bags were sealed after 4 h storage at 0 ± 1 °C, when the temperature of fruit and the storage room had reached the equilibrium. After the previously described treatments, fruit were stored for 60 d at 0 ± 1 °C and 90% ± 5% relative humidity (RH). Fruit samples were taken on days 0, 15, 30, 45, and 60 of storage to determine ethylene production, respiration rate, fruit firmness, weight loss, SSC, titratable acidity (TA), SSC : TA ratio, levels of ascorbic acid and total antioxidants, activities of ethylene biosynthesis, and fruit softening enzymes. A two-factor (treatments and storage period) factorial design was used for the experiment. All treatments were replicated three times with six fruit as an experimental unit for each parameter studied.

Expt. 2: Effects of 1-methylcyclopropene or modified atmosphere packaging alone and in combination on quality of ripe fruit after 5 and 7 weeks of low-temperature storage

For the second experiment, the same four treatments were applied as for the first experiment and the fruit were stored at 0 ± 1 °C and 90% ± 5% RH. After 5 and 7 weeks of storage, ethylene production and respiration rate were recorded daily during the fruit ripening period at 20 ± 1 °C. However, fruit quality parameter such as weight loss, SSC, TA, SSC : TA ratio, fruit skin and pulp color, levels of ascorbic acid, and total antioxidants were determined at the fully ripe stage (easting soft).

RESPIRATION RATE. One fruit (in duplicate) per experimental unit was sealed in an airtight jar (1 L) fitted with a rubber septum for 1 h at room temperature (20 ± 1 °C). The respiration rate was measured as CO₂ production by injecting 2-mL gas samples from the headspace into an infrared gas analyser (Servomex, Gas Analyser, Analyser Series 1450; Servomex Ltd., East Sussex, UK).

ETHYLENE PRODUCTION. To determine the ethylene production, 1-mL gas samples were taken from the headspace of the same jar used for respiration rate and were injected into a gas chromatograph (6890 N Network GC system; Agilent Technologies, Palo Alto, CA) fitted with a 2-m-long stainless steel column (Porapak-Q, 3.175 mm, mesh size 80/100; Supelco, Bellefonte, PA) and a flame ionization detector. Ethylene production during cold storage and during japanese plum fruit ripening was determined as described by Khan and Singh (2007).

ACTIVITIES OF ETHYLENE BIOSYNTHESIS ENZYMES AND 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID CONTENT IN PULP TISSUES. Activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzymes as well as 1-aminocyclopropane-1-carboxylic acid (ACC) content from pulp tissue were determined as described by Khan and Singh (2007).

FRUIT FIRMNESS. An electronic pressure tester (model EPT-1 pressure tester; Lake City Technical Products, Kelowna, BC, Canada) fitted with an 8-mm spherical tip was used to determine fruit firmness. A small slice of fruit skin was removed and firmness was recorded from both sides of individual fruit. Means were expressed as Newtons.

ACTIVITIES OF FRUIT SOFTENING ENZYMES IN PULP TISSUES. Activities of exo-polygalacturonase (exo-PG), endo-polygalacturonase (endo-PG), pectin esterase (PE), and endo-1,4-β-D-glucanase

(EGase) were determined from fruit pulp tissues as described by Khan and Singh (2007).

PROTEIN DETERMINATION. Protein from fruit pulp tissue was determined using the method of Bradford (1976).

FRUIT COLOR. Hunterlab ColorFlex 45°/0° Spectrophotometer (HunterLab ColorFlex; Hunter Associates, Reston, VA) was used to measure the changes in fruit color parameters, including chromaticity values L^* , a^* , and b^* in skin and pulp of fruit after 5 and 7 weeks of storage and during fruit ripening. Measurements were taken at opposite sides of each fruit using the head of 15-mm diameter. For measuring changes in pulp color, a thin slice of skin (3 mm) was removed and color parameters were measured. The chroma value (C^*) and hue angle (h°) were calculated from chromaticity values a^* and b^* as reported earlier by McGuire (1992).

SOLUBLE SOLIDS CONCENTRATIONS, TITRATABLE ACIDITY, AND SOLUBLE SOLIDS CONCENTRATIONS:TITRATABLE ACIDITY RATIO. To determine the SSC of fruit juice, a digital refractometer (Atago-Palette PR 101; Atago Co., Tokyo) was used and SSC was expressed as percent soluble solid. To determine the TA, juice was titrated against 0.1 N NaOH using phenolphthalein as an indicator to pH 8.2 and was expressed as percent malic acid. SSC : TA ratio was calculated by dividing SSC with the corresponding TA value.

ASCORBIC ACID. Ascorbic acid concentration from fruit pulp was determined following the method of Malik and Singh (2005) with some modifications. Pulp samples (5 g) were homogenized in a glass pestle and mortar using 300 mg white quartz sand (−50 + 70 mesh; Sigma Aldrich, Sydney, Australia) with 20 mL (6%) metaphosphoric acid solution containing 0.18% disodium salt of ethylene diamine tetra-acetic acid. After homogenization, the content was centrifuged at 3186 g_n for 10 min, and 400 μ L supernatant was mixed with 200 μ L (3%) metaphosphoric acid, 1.4 mL distilled water, and 200 μ L diluted Folin's reagent (5 mL distilled water : 1 mL Folin's reagent, by volume). An ultraviolet/visible spectrophotometer (model 6405; Jenway Ltd., Felsted, UK) was used to measure the absorbance of mixed sample after 10 min at 760 nm using 2-mL disposable plastic cuvettes. Ascorbic acid concentration was calculated against 100% ascorbic acid standard curve.

WEIGHT LOSS. Weight loss percent was determined by following the formula: $(A - B)/A \times 100$, in which A is the fruit weight just before storage and B was the fruit weight after storage period.

TOTAL ANTIOXIDANTS. The level of total antioxidants in pulp tissue during fruit ripening was estimated by using the method of Brand-Williams et al. (1995).

STATISTICAL ANALYSIS. The data were subjected to analysis of variance (ANOVA) using Genstat (release 9.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK) by using two-way ANOVA including treatments and storage period. The effects of various treatments were assessed within ANOVA and Fisher's least significant differences were calculated following a significant ($P \leq 0.05$) F test. All the assumptions of analysis were checked to ensure validity of statistical analysis.

Results

RESPIRATION RATE. Fruit stored in MAP exhibited a rapid increase in respiration rate during storage as well as during ripening of fruit that were stored for 5 and 7 weeks (Figs. 1A

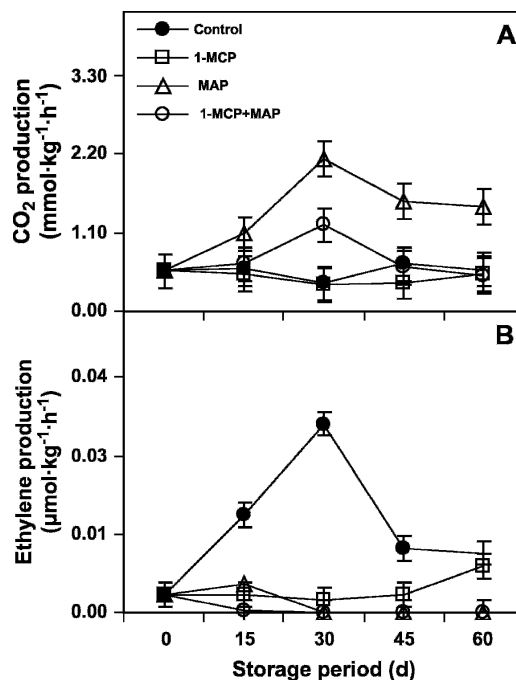


Fig. 1. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on (A) respiration rate and (B) ethylene production during low-temperature storage in 'Tegan Blue' Japanese plum ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for respiration rate: treatments (T) = 0.33, storage period (SP) = 0.37, $T \times SP = 0.73$. LSD ($P \leq 0.05$) for ethylene production: T = 0.003, SP = 0.002, $T \times SP = 0.006$.

and 2). The respiration rate of 1-MCP plus MAP-treated fruit increased after 15 d of storage and peaked on day 30 $\approx 43\%$ lower than fruit stored in MAP alone and control. Fruit treated with 1-MCP and stored in air did not show any rise in respiration throughout the storage period. After 5 weeks of storage, fruit stored in MAP exhibited 72% higher respiration rate than other treatments (Fig. 2A). Lowest respiration rate was recorded in 1-MCP-treated fruit compared with fruit stored in MAP, with or without 1-MCP treatment, and that of control fruit. On day 1 of fruit ripening, after 7 weeks of storage, 1-MCP-treated fruit showed 50% lower respiration rate than fruit stored in MAP either with or without 1-MCP treatment and control fruit (Fig. 2B). Fruit stored in MAP exhibited a fast increase in their respiration rate during fruit ripening and exhibited respiratory climacteric on day 10 of fruit ripening after 7 weeks of storage $\approx 28\%$, 38%, and 47% higher than the highest respiration rate of 1-MCP-treated fruit stored in MAP, control fruit, and 1-MCP-treated fruit, respectively.

ETHYLENE PRODUCTION AND ETHYLENE BIOSYNTHESIS ENZYMES. During low temperature storage, 1-MCP and MAP treatments significantly inhibited the ethylene production in 'Tegan Blue' Japanese plum (Fig. 1B). Control fruit exhibited an ethylene peak on day 30 of storage and later ethylene production decreased from day 45 to day 60. In the second experiment, fruit treated with 1-MCP and stored in MAP for 5 or 7 weeks exhibited reduced and delayed ethylene production during fruit ripening (Fig. 3). 1-MCP and MAP treatments significantly reduced the activities of ACS and ACO enzymes and as well as ACC content in pulp tissues during storage period. Reduction was more pronounced in 1-MCP-treated fruit

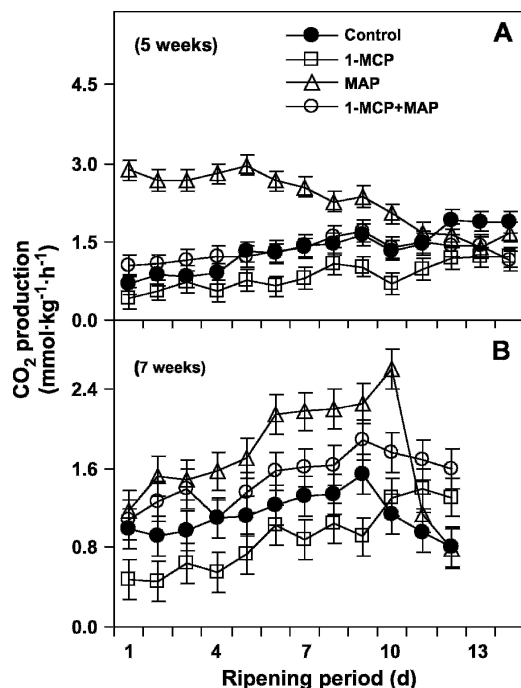


Fig. 2. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on respiration rate during fruit ripening in 'Tegan Blue' Japanese plum stored for 5 and 7 weeks ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for 5 weeks: treatments (T) = 0.14, ripening period (RP) = 0.27, $T \times RP = 0.54$. LSD ($P \leq 0.05$) for 7 weeks: T = 0.16, RP = 0.27, $T \times RP = 0.54$.

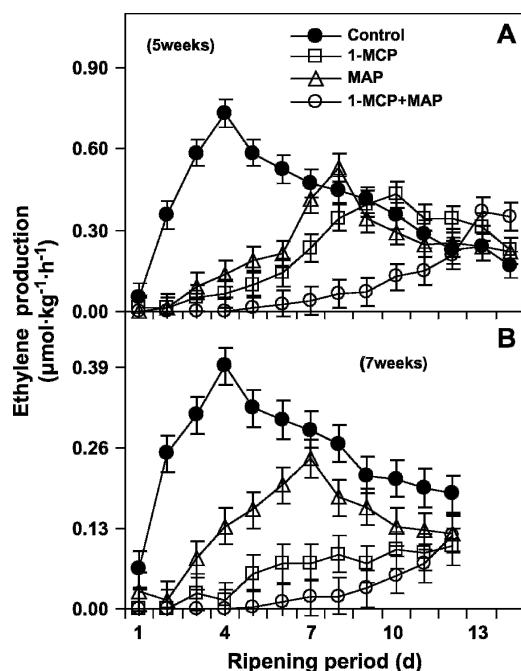


Fig. 3. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on ethylene production during fruit ripening in 'Tegan Blue' Japanese plum stored for 5 and 7 weeks ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for 5 weeks: treatments (T) = 0.03, ripening period (RP) = 0.06, $T \times RP = 0.11$. LSD ($P \leq 0.05$) for 7 weeks: T = 0.03, RP = 0.05, $T \times RP = 0.11$.

stored in MAP (Fig. 4), which shows that MAP exacerbates the effect of 1-MCP in the inhibition of ethylene production.

FRUIT SOFTENING AND FRUIT SOFTENING ENZYMES. 1-MCP-treated fruit stored in normal atmosphere or in MAP exhibited significantly higher fruit firmness during storage and in ripe fruit as compared with MAP and control fruit (Figs. 5 and 8A). After 45 d storage at $0 \pm 1^\circ\text{C}$, 1-MCP-treated fruit stored either in MAP or in normal atmosphere showed 42% and 51% reduction in fruit firmness compared with fruit stored in normal atmosphere and in MAP, respectively. 1-MCP-treated fruit stored for 5 weeks exhibited higher fruit firmness (23.1 N) followed by 1-MCP-treated fruit stored in MAP (22.3 N) as compared with all other treatments (Fig. 8A). Activities of exo-PG, endo-PG, PE, and EGase were inhibited during the storage period in fruit treated with 1-MCP and stored either in normal atmosphere or in MAP (Fig. 6). Lowest exo-PG activity during the storage period was determined in pulp tissue of fruit treated with 1-MCP and stored in MAP. On day 60 of storage, pulp tissue of fruit treated with 1-MCP and stored in MAP exhibited

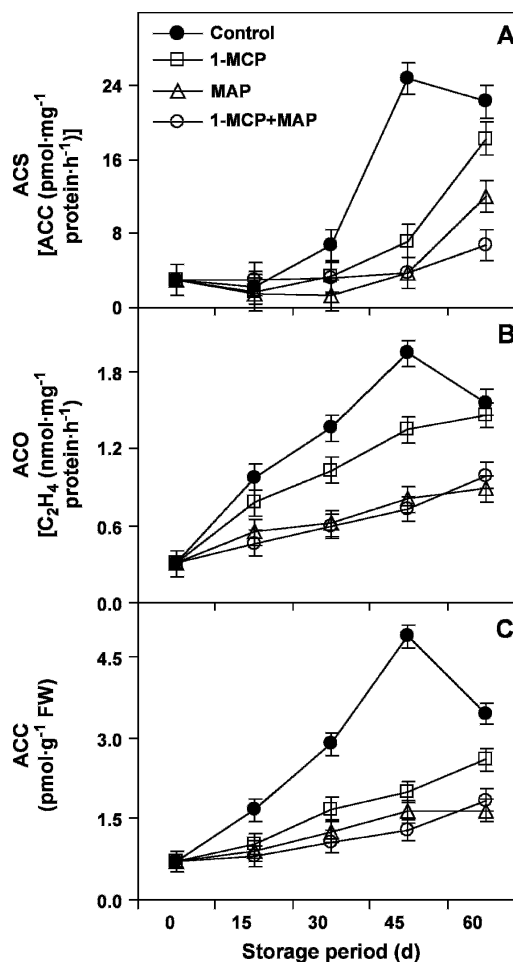


Fig. 4. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on activities of (A) 1-aminocyclopropane-1-carboxylic acid synthase (ACS), (B) 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzymes, and (C) 1-aminocyclopropane-1-carboxylic acid (ACC) content during low-temperature storage in pulp tissues of 'Tegan Blue' Japanese plum ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for ACS: treatments (T) = 2.22, storage period (SP) = 2.48, $T \times SP = 4.97$. LSD ($P \leq 0.05$) for ACO: T = 0.1, SP = 0.11, $T \times SP = 0.23$. LSD ($P \leq 0.05$) for ACC content: T = 0.24, SP = 0.26, $T \times SP = 0.53$.

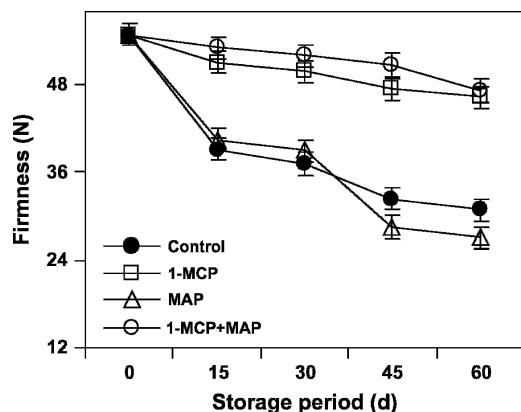


Fig. 5. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on fruit firmness during low-temperature storage in 'Tegan Blue' japanese plum ($n = 18$, three replications, six fruit per replication). Vertical bars represent SE. Least significant difference ($P \leq 0.05$) for fruit firmness: treatments (T) = 1.9, storage period (SP) = 2.12, $T \times SP = 4.24$.

57%, 51%, and 15% reduction in activity of exo-PG enzyme in contrast to MAP, control, and 1-MCP-treated fruit, respectively (Fig. 6A). Similarly, during storage, 1-MCP-treated fruit stored either in MAP or in normal atmosphere slowed the activity of endo-PG enzyme as compared with pulp tissues of fruit stored in normal atmosphere and in MAP (Fig. 6B). Activities of PE and EGase enzymes in the fruit stored in MAP and in normal atmosphere increased with increased storage period, whereas 1-MCP-treated fruit stored either with or without MAP did not show any significant increase in the activities of PE and EGase enzymes during the 60-d storage period (Fig. 6C–D).

WEIGHT LOSS AND DELAY IN FRUIT RIPENING. As expected, control and 1-MCP-treated fruit exhibited a continuous rise in

weight loss during the storage period as compared with fruit stored in MAP either with or without 1-MCP application. Highest weight loss was recorded in control fruit on day 60 $\approx 32\%$, 92% , and 96% higher as compared with 1-MCP-treated, MAP, and fruit treated with 1-MCP and stored in MAP, respectively (Fig. 7). In ripe fruit, weight loss was also reduced in MAP alone or in combination with 1-MCP application compared with other treatments. After 5 and 7 weeks of low-temperature storage, at the ripe stage, weight loss was highest in control fruit (6% and 7%) followed by 1-MCP-treated fruit (5% and 6.5%) as compared with other treatments (Fig. 8B). The effects of 1-MCP and MAP in delaying fruit ripening on 'Tegan Blue' japanese plum were synergistic. After 5 and 7 weeks of low-temperature storage, 1-MCP-treated fruit stored in MAP delayed the fruit ripening by 10 and 8 d ≈ 2 -fold more than their independent effects as compared with control fruit, respectively (Fig. 8C).

SOLUBLE SOLIDS CONCENTRATIONS, TITRATABLE ACIDITY, AND SOLUBLE SOLIDS CONCENTRATIONS : TITRATABLE ACIDITY RATIO.

During the first 15 d of storage, all treatments showed a reduction in SSC and later on 1-MCP-treated fruit stored either in normal atmosphere or in MAP and control fruit exhibited a rise in SSC (Fig. 9A). Fruit stored in MAP showed lowest SSC during storage, in contrast to other treatments, and on day 60, SSC of fruit stored in MAP was 16%, 17%, and 21% lower than 1-MCP-treated fruit stored either in MAP or in normal atmosphere and control fruit, respectively. TA in all treatments during the first 15 d of storage increased slightly and later decreased (Fig. 9B). Rate of decrease in TA was quick in fruit stored in MAP compared with other treatments. Fruit stored in MAP showed 55%, 64%, and 63% lower TA on day 60 in contrast to control and fruit treated with 1-MCP and stored either in MAP or in normal atmosphere, respectively. All treatments showed a slightly decreased SSC : TA ratio up to

15 d of storage and, later SSC : TA ratio increased with increased storage period (Fig. 9C). On day 60, fruit stored in MAP showed highest SSC : TA ratio (34) $\approx 43\%$, 57% , and 56% higher as compared with control and 1-MCP-treated fruit stored either in MAP or in normal atmosphere, respectively. Ripe fruit stored for 5 and 7 weeks in MAP exhibited significantly ($P \leq 0.05$) lower SSC (11% and 9.9%) as compared with all other treatments (Fig. 10A). Similarly, MAP fruit showed significantly ($P \leq 0.05$) lower TA (0.41% and 0.19%) in fully ripe fruit after 5 and 7 weeks of storage as compared with other treatments (Fig. 10B). 1-MCP-treated fruit maintained TA at a higher level in ripe fruit after 5 and 7 weeks of storage than control and fruit stored in MAP. SSC:TA ratio of fruit stored in MAP was higher after 5 and 7 weeks of low-temperature storage (Fig. 10C).

FRUIT COLOR. Fruit stored in MAP at the fully ripe stage after 5 and 7 weeks of storage exhibited

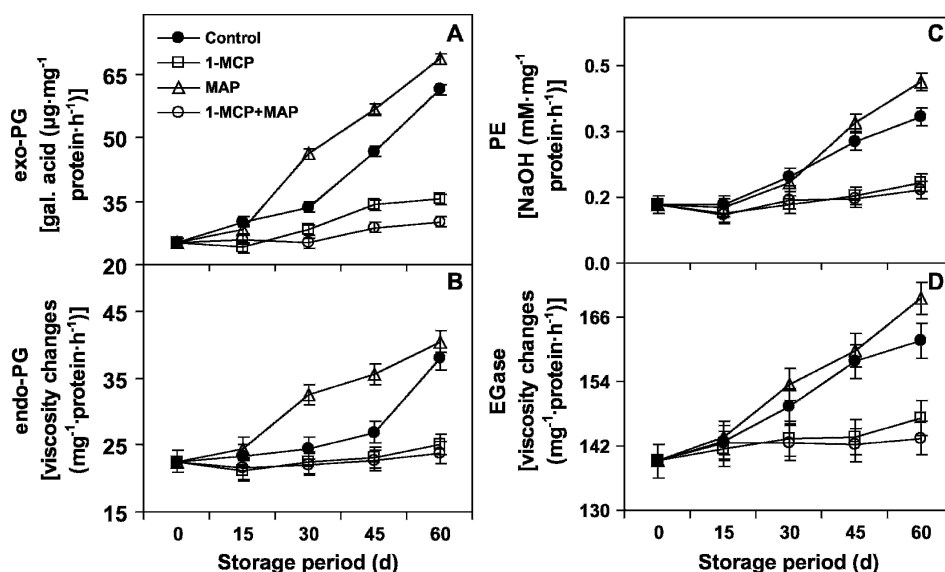


Fig. 6. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on (A) activities of exo-polygalacturonase (exo-PG), (B) endo-polygalacturonase (endo-PG), (C) pectin esterase (PE), and (D) endo-1,4- β -D-glucanase (EGase) enzymes during low-temperature storage in pulp tissues of 'Tegan Blue' japanese plum ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for exo-PG: treatments (T) = 1.39, storage period (SP) = 1.55, $T \times SP = 3.1$. LSD ($P \leq 0.05$) for endo-PG: $T = 2.1$, $SP = 2.32$, $T \times SP = 4.63$. LSD ($P \leq 0.05$) for PE: $T = 0.01$, $SP = 0.01$, $T \times SP = 0.03$. LSD ($P \leq 0.05$) for EGase: $T = 7.7$, $SP = 8.6$, $T \times SP = 17.4$.

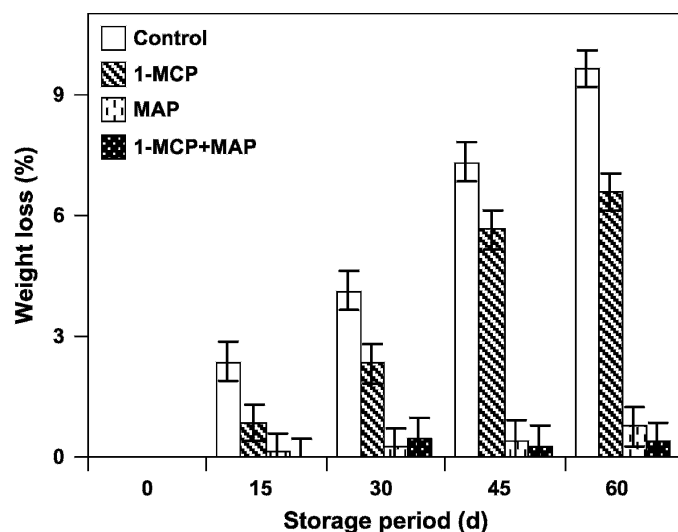


Fig. 7. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on weight loss during low temperature storage in 'Tegan Blue' japanese plum ($n = 3$, three replications). Vertical bars represent SE. Least significant difference ($P \leq 0.05$) for weight loss: treatments (T) = 0.61, storage period (SP) = 0.69, $T \times SP = 1.37$.

lowest chromaticity value L^* of fruit skin as compared with other treatments (Fig. 11A). A similar trend in chromaticity value L^* for fruit pulp was observed in fully ripe fruit after 5 and 7 weeks of storage, whereas MAP fruit showed lowest pulp L^* value as compared with other treatments (Fig. 11D). 1-MCP-treated ripe fruit after 5 and 7 weeks of storage exhibited higher chroma value of fruit skin compared with other treatments (Fig. 11B). Chroma value of pulp tissues was reduced in MAP fruit, whereas control and 1-MCP-treated fruit did not show any significant difference in pulp chroma value at the fully ripe stage after 5 and 7 weeks of storage (Fig. 11E). Fruit stored with MAP bags exhibited reduced hue angle at the fully ripe stage as compared with other treatments (Fig. 11C). Similarly, hue angle of pulp tissues was low in fruit that were stored in MAP than other treatments (Fig. 11F).

ASCORBIC ACID AND TOTAL ANTIOXIDANTS. During low-temperature storage, the level of ascorbic acid in all treatments decreased with increase in the storage period (Fig. 12A). Fruit stored in MAP exhibited a continuous decrease in level of total antioxidants, and on day 60 of storage, these fruit showed 24%, 27%, and 17% reduction in the level of total antioxidants as compared with control and 1-MCP-treated fruit stored either in MAP or in normal atmosphere, respectively (Fig. 12B). After 5 and 7 weeks of low-temperature storage and fruit ripening, ascorbic acid level of control and 1-MCP-treated fruit pulp tissues was significantly higher as compared with fruit stored in MAP (Fig. 13A). Pulp of 1-MCP-treated fruit exhibited 5%, 13%, and 26% higher level of total antioxidants in contrast to control and fruit stored in MAP either with or without 1-MCP treatment, respectively (Fig. 13B).

Discussion

During cold storage as well as during fruit ripening, fruit stored in MAP showed a rapid increase in respiration rate than other treatments. In the present study, the rate of respiration was determined as CO_2 production by the fruit itself, whereas in

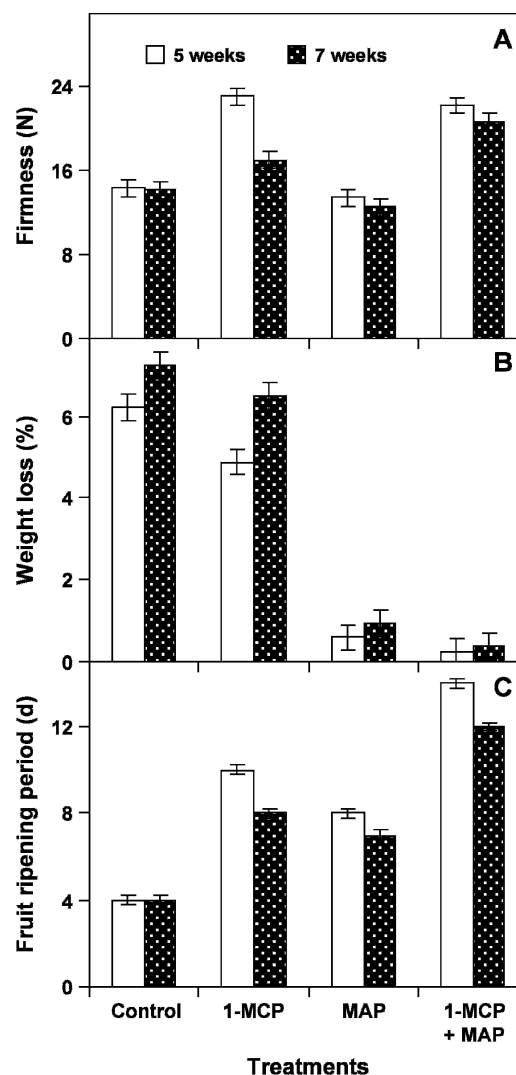


Fig. 8. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on (A) fruit firmness, (B) weight loss, and (C) fruit ripening period in 'Tegan Blue' japanese plum stored for 5 and 7 weeks ($n = 18$, three replications, six fruit per replication for fruit firmness; $n = 3$, three replications for fruit ripening period and weight loss). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for fruit firmness: treatments (T) = 1.89, storage period (SP) = 1.33, $T \times SP = 2.67$. LSD ($P \leq 0.05$) for weight loss: T = 0.67, SP = 0.48, $T \times SP = 0.95$. LSD ($P \leq 0.05$) for fruit ripening period: T = 0.52, SP = 0.51, $T \times SP = 0.62$.

other studies, the changes in the in-package gas concentrations have been monitored and higher CO_2 and lower O_2 levels have been found within MAP around the fruit commodities (Ding et al., 2002; Meir et al., 1997; Pesis et al., 2000). Higher respiration rate of fruit stored in MAP may be the result of lower O_2 levels inside the bags creating anoxic conditions that switched on the anaerobic respiration during storage and as well as during fruit ripening. Similarly, Jayas and Jeyamkondan, (2002) reported that reduced O_2 levels in the MA retard the overall metabolic activities and extend the storage life. Oxygen levels below the threshold limit have been reported to initiate anaerobic respiration with production of acetaldehyde, ethanol, lactates, and off-flavors in fruit with unacceptable eating quality (Pesis et al., 2002). In fermentative metabolism without uptake of O_2 , ethanol production involves the decarboxylation

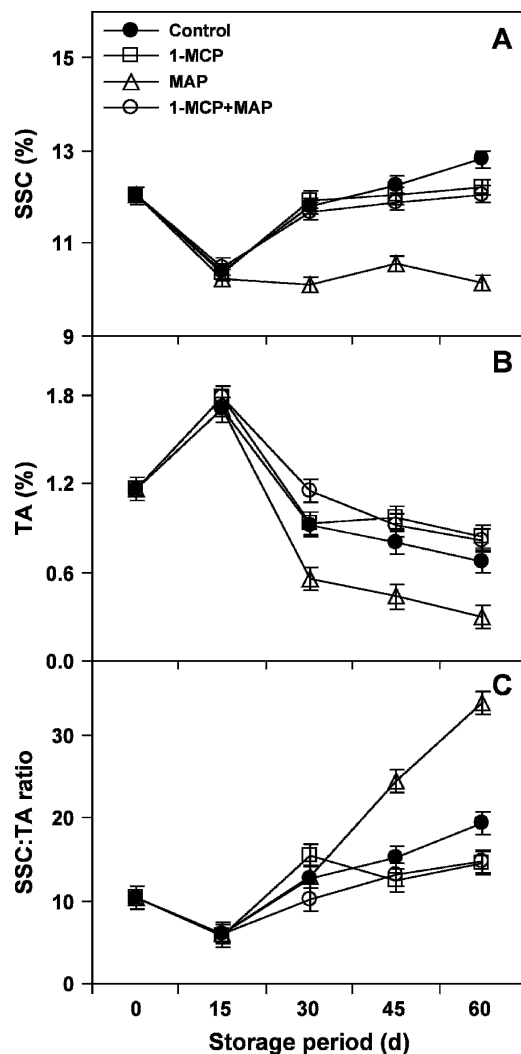


Fig. 9. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on (A) soluble solids concentration (SSC), (B) titratable acidity (TA), and (C) SSC : TA ratio during low-temperature storage in 'Tegan Blue' japanese plum ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for SSC: treatments (T) = 0.23, storage period (SP) = 0.26, $T \times SP = 0.52$. LSD ($P \leq 0.05$) for TA: T = 0.08, SP = 0.09, $T \times SP = 0.18$. LSD ($P \leq 0.05$) for SSC : TA ratio: T = 1.75, SP = 1.96, $T \times SP = 3.9$.

of pyruvate to CO_2 (Fonseca et al., 2002). Stimulated respiration rates have also been observed in lemon (*Citrus limon* Burm.), lettuce (*Lactuca sativa* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), and potato (*Solanum tuberosum* L.) exposed to CO_2 -enriched environments (Fonseca et al., 2002).

The reduction in ethylene production in 1-MCP-treated fruit is ascribed to irreversible blocking of the ethylene-binding site (Sisler and Serek, 1997) and reduction in the activities of ethylene biosynthesis enzymes (Khan and Singh, 2007). Similarly, during low-temperature storage, 1-MCP treated fruit resulted in reduced endogenous ethylene production in some japanese plum and european plum cultivars (Khan and Singh, 2004; Valero et al., 2005). A MA richer in CO_2 and poorer in O_2 can potentially reduce ethylene sensitivity and production in fruit and vegetable crops (Kader et al., 1989). Reduced ethylene production of fruit stored in MAP bags may be the result of a

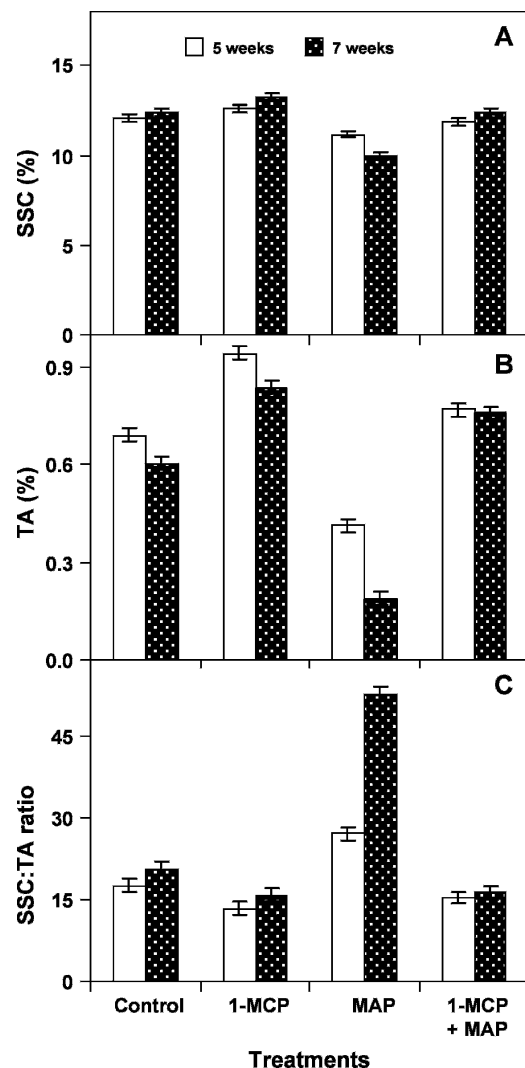


Fig. 10. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on (A) soluble solids concentration (SSC), (B) titratable acidity (TA), and (C) SSC : TA ratio in 'Tegan Blue' japanese plum stored for 5 and 7 weeks ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for SSC: treatments (T) = 0.13, storage period (SP) = nonsignificant, $T \times SP = 0.2$. LSD ($P \leq 0.05$) for TA: T = 0.04, SP = 0.03, $T \times SP = 0.06$. LSD ($P \leq 0.05$) for SSC:TA ratio: T = 2.5, SP = 1.76, $T \times SP = 3.52$.

higher concentration of CO_2 inside the bag because CO_2 has been reported to have an antagonistic effect on ethylene biosynthesis (Jayas and Jeyamkondan, 2002). During MA storage, similar reduction in ethylene production has been reported in avocado (*Persea americana* Mill), banana (*Musa* sp. group AAA subgroup Cavendish cv. Williams), and european plum (Jiang et al., 1999; Ke et al., 1991; Meir et al., 1997). However, during fruit ripening after 7 weeks of storage, an increased rate of ethylene production in fruit stored in MAP as compared with 1-MCP-treated fruit stored either with or without MAP could also be the result of accumulation of ethylene precursor in fruit stored in polyethylene bags during MA storage, and therefore when products are transferred to normal air, ethylene is produced rapidly (Wang, 1990). 1-MCP and MAP application to japanese plum fruit significantly ($P \leq 0.05$) reduced the activities of ACS and ACO enzymes and as

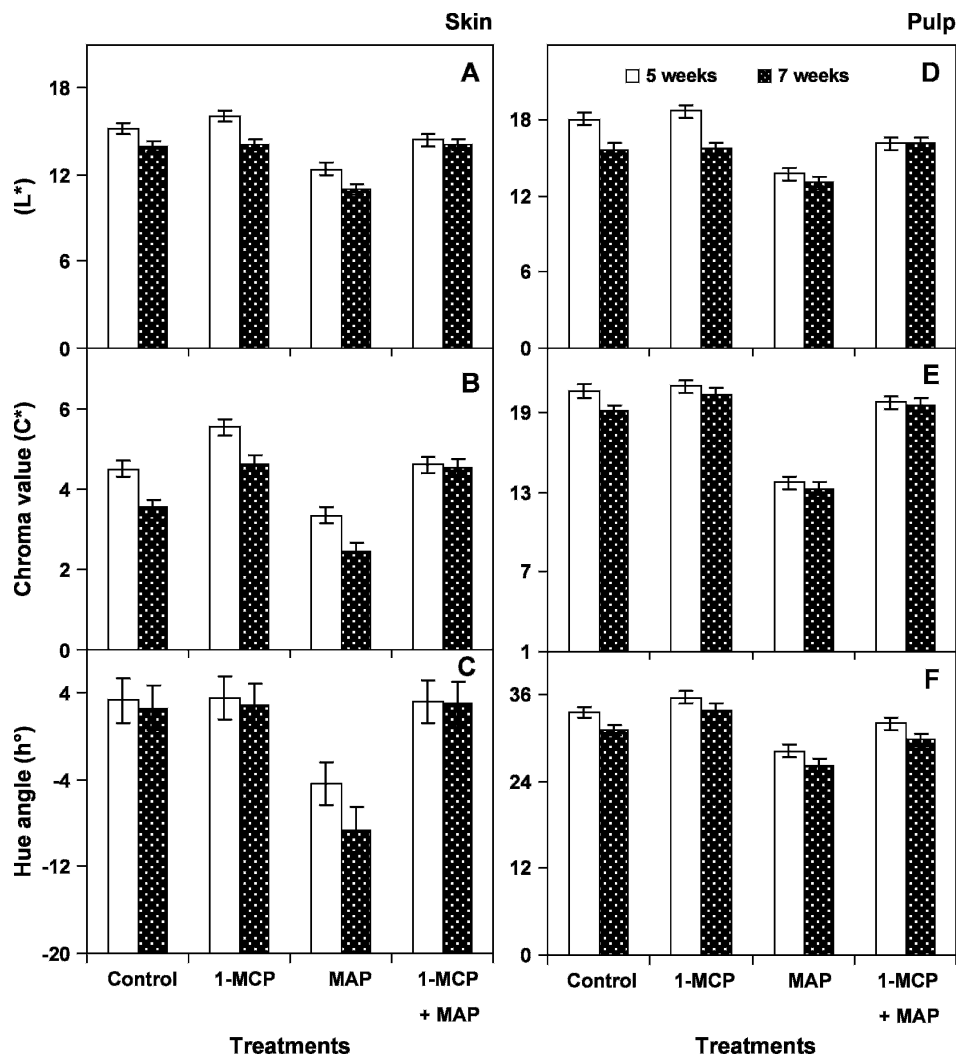


Fig. 11. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on chromaticity values L*, chroma value, and hue angle of fruit skin (A, B, and C), and pulp (D, E, and F) in 'Tegan Blue' japanese plum stored for 5 and 7 weeks (n = 18, three replications, six fruit per replication). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for skin L*: treatments (T) = 0.87, storage period (SP) = 0.62, $T \times SP = 1.24$. LSD ($P \leq 0.05$) for skin chroma value: T = 0.62, SP = 0.44, $T \times SP = 0.88$. LSD ($P \leq 0.05$) for skin hue angle: T = 5.57, SP = 3.93, $T \times SP = 7.88$. LSD ($P \leq 0.05$) for Pulp L*: T = 1.04, SP = 0.74, $T \times SP = 1.47$. LSD ($P \leq 0.05$) for pulp chroma value: T = 1.12, SP = nonsignificant, $T \times SP = 1.59$. LSD ($P \leq 0.05$) for pulp hue angle: T = 1.8, SP = 1.28, $T \times SP = 2.56$.

well as ACC content during storage and during fruit ripening. Reduced activities of ACS and ACO enzymes have been reported in 1-MCP-treated japanese plum fruit (Khan and Singh, 2007). A significant reduction in ethylene biosynthesis and activities of ACS, ACO enzymes, and their respective transcripts has also been reported in 1-MCP-treated apple (*Malus × domestica* Borkh.) fruit (Dal Cin et al., 2006). The reduction in the ACC content in pulp tissues may be ascribed to the reduction in the activities of ACS enzymes during storage or conversion of ACC to malonyl or glutamylamino derivatives instead of ethylene production (Lelievre et al., 1997). The mechanism through which MAP reduces the activities of ACS and ACO enzymes and ACC content in japanese plum and european plum is yet not well understood and warrants further investigations.

The reduction in japanese plum fruit softening with 1-MCP treatment may be attributed to reduction in ethylene production

with application of 1-MCP (Figs. 1A and 3) and consequent reduction in the fruit softening (Khan and Singh, 2007). Similarly, reduction in fruit softening with 1-MCP application has also been reported in some japanese plum and european plum cultivars (Khan and Singh, 2004; Valero et al., 2005). Fruit stored in MAP without 1-MCP application softened similar to those without treatment. Reduction in the activities of these enzymes with 1-MCP application may be the result of reduction in the endogenous ethylene production with 1-MCP treatment because ethylene has been reported to regulate the transcription of several ripening-related genes, including those related to fruit softening (Alexander and Grierson, 2002). Similarly, 1-MCP application has been reported to reduce the activities of fruit softening enzymes such as exo-PG, endo-PG, PE, and EGase in japanese plum (Khan and Singh, 2007). Fruit stored in MAP alone also showed reduction in the activities of exo-PG, endo-PG, PE, and EGase enzymes as compared with control fruit. However, reduction in these enzymes in a MAP bag was not as pronounced as observed in 1-MCP-treated fruit stored in MAP (Fig. 6). In papaya, MA storage retarded the decrease in the fruit firmness and reduced the activities of PG, PE, and cellulase enzymes (Lazan et al., 1993).

Reduction in weight loss is one of the major advantages of MAP resulting from water vapor accumulation within the bags and increase in the RH. A sevenfold reduction in weight loss has been reported in japanese plum stored in polyethylene packaging as compared with unwrapped fruit (Kluge et al., 1999). Reduction in weight loss has also been observed in loquat (*Eriobotrya japonica* Lindl.) and peach packed in MAP compared with control fruit (Ding et al., 2002; Fernandez-Trujillo et al., 1998). Application of 1-MCP in combination with MAP delayed the fruit ripening up to 10 d than other treatments (Fig. 8C). In banana and mango, application of 1-MCP in combination with the use of polyethylene bags has been reported to extend postharvest life (Jiang and Joyce, 2000; Jiang et al., 1999). Use of MAP has also been reported to extend the storage period in peach and european plum (Fernandez-Trujillo et al., 1998; Turk and Ozkurt, 1994).

During cold storage (Fig. 9) and after storage in ripe fruit (Fig. 10), lower SSC and TA were observed in fruit stored in MAP as compared with 1-MCP-treated and control fruit, which suggests that during storage and ripening, organic acids and sugar became the substrates for a higher rate of anaerobic

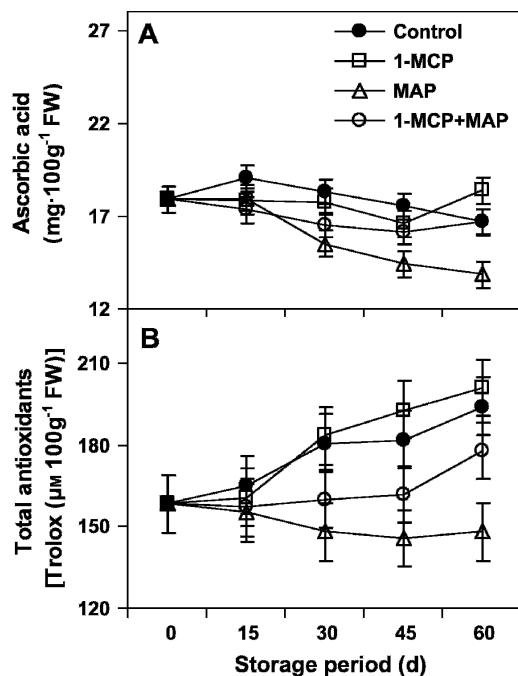


Fig. 12. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on (A) ascorbic acid and (B) total antioxidants during low-temperature storage in pulp tissues of 'Tegan Blue' japanese plum ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for ascorbic acid: treatments (T) = 0.76, storage period (SP) = 0.85, T \times SP = 1.71. LSD ($P \leq 0.05$) for total antioxidants: T = 13.5, SP = 15.1, T \times SP = 30.2.

respiration (Lurie, 1992) observed during low-temperature storage and during fruit ripening after storage (Figs. 1A and 2). Under elevated CO_2 concentrations in the MAP, the reduction in malic acid, the main acid in japanese plum, may be associated with inhibition of succinic acid dehydrogenase in Krebs cycle (Wankier et al., 1970). Higher SSC : TA ratio in fruit stored in MAP may be result of very low TA.

No significant differences were observed between ripe control fruit and fruit treated with 1-MCP and stored either in a normal atmosphere or in MAP for chromaticity value L^* , chroma value, and hue angle of fruit skin and pulp tissues (Fig. 11), which suggests that with these treatments, japanese plum storage and shelf life can be extended without any adverse effects on fruit skin and pulp color. Lower value of color parameters of ripe fruit stored in MAP bags revealed that after storage, these fruit exhibited fast ripening in contrast to 1-MCP-treated fruit. Similarly, storage atmosphere with higher CO_2 and lower O_2 reduced the L^* , chroma value, and hue angle in sweet cherry fruit as compared with fruit stored in air (Remon et al., 2004).

The reduction in the level of ascorbic acid may be the result of delayed biosynthesis or fast degradation of ascorbic acid in MAP fruit. An earlier reduced level of ascorbic acid has been reported in polypropylene and Pebax-C (Atofina, Tokyo)-packed papaya fruit as compared with low-density polyethylene-packed or unpacked fruit (Singh and Rao, 2005). There is very little information available regarding the effects of MAP on total antioxidant levels in general and particularly in japanese plum and european plum. The reduction in the level

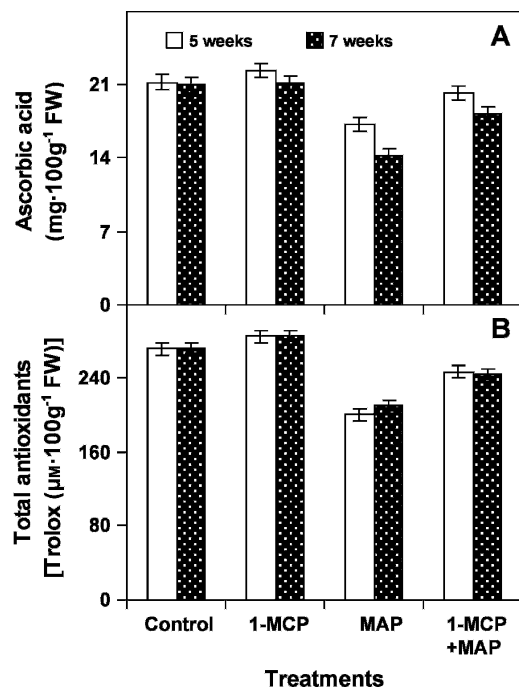


Fig. 13. Effects of postharvest application of 1-methylcyclopropene (1-MCP) and modified atmosphere packaging (MAP) alone or in combination (+) on (A) ascorbic acid and (B) total antioxidants in 'Tegan Blue' japanese plum stored for 5 and 7 weeks ($n = 3$, three replications). Vertical bars represent SE. Least significant difference (LSD) ($P \leq 0.05$) for ascorbic acid: treatments (T) = 1.47, storage period (SP) = 1.04, T \times SP = 2.09. LSD ($P \leq 0.05$) for total antioxidants: T = 13.5, SP = nonsignificant (NS), T \times SP = NS.

of total antioxidants in fruit stored in MAP may be the result of increased activities of cytochrome oxidase, ascorbic acid oxidase, and peroxidase enzymes (Rocha et al., 1995). No significant changes in level of total antioxidants were reported in pomegranate stored in MAP (Lopez-Rubira et al., 2005). The changes in level of total antioxidants in fruit stored in MAP warrant further investigations.

In conclusion, treatment of fruit with 1-MCP ($1.0 \mu\text{L}\cdot\text{L}^{-1}$) and storage in MAP inhibited the ethylene biosynthesis and fruit softening with substantial reduction in weight loss during low-temperature storage. This treatment can be used effectively to extend the storage life of japanese plum up to 7 weeks at $0 \pm 1^\circ\text{C}$ and $90\% \pm 5\%$ RH followed by 8-d ripening at $20 \pm 1^\circ\text{C}$ without any adverse effects on quality of ripe fruit.

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