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Methyl jasmonate treatments influence bioactive compounds and red peel color development of Braeburn apple

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Abstract: The present study was carried out to determine the effects of methyl jasmonate (MeJA) treatments on the fruit peel color parameters, anthocyanin content, ethylene biosynthesis, phenolic content, and antioxidant capacity of Braeburn apples. Experimental trees were treated with 1120, 2240, or 4480 mg L–1 MeJA at 1- and 2-week intervals between the 105th and 175th days of growth after full bloom. L* and hue decreased linearly with increasing MeJA concentrations in both experimental years. While MeJA treatments did not cause significant changes in chroma, except for in the final harvest in 2010, it decreased chroma values significantly on all sampling dates of 2011. Anthocyanin content, internal ethylene concentration, phenolic content, and antioxidant capacity increased linearly with increasing MeJA treatments with 1-week intervals were found to be as effective as those with 2-week intervals. Although MeJA treatments increased ethylene biosynthesis, they did not cause any softening; on the other hand, fruit firmness increased linearly with increasing MeJA concentrations. Preharvest MeJA treatments can be a good management strategy for red peel color development and fruit firmness preservation in Braeburn apples. Although both application regimes of MeJA significantly increased red color development, the treatments with 1-week intervals were found to be more effective than 2-week intervals with regard to phenolic content and antioxidant capacity.

Key words: Anthocyanin, antioxidant, firmness, ethylene, phenolics

1. Introduction

Red peel color is a significant quality parameter in apples and can provide significant contributions to the market value of the fruits. Anthocyanins are the main compounds responsible for red color formation in apples (Bae et al., 2006). Flavonols (quercetin 3-O-glycosides) and proanthocyanidins also have some influence on red peel color development (Lister et al., 1994), and phenolics are important compounds with antioxidant impacts. A diet that includes plants and fruits with high antioxidant capacity decreases the risk of cardiovascular and neurologic diseases and some cancers in humans (Karadeniz et al., 2005; Scalbert et al., 2005; Francini and Sebastiani, 2013). Biosynthesis of anthocyanin and red peel color development are greatly affected by environmental factors such as temperature, irradiance, light quality (Saure, 1990), and management practices like crop load (Cmelik et al., 2006), pruning (Saure, 1990), the use of a hail net and reflective foil (Blanke, 2008; Jakopic et al., 2010), wounding (Chalmers and Faragher, 1977), bagging (Liu et al., 2013), and cooling with microsprinklers (Iglesias

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et al., 2002). Many of these practices influence fruit light exposure and, ultimately, anthocyanin production. Light is an essential factor for anthocyanin biosynthesis. With light exposure, anthocyanin is accumulated in the epidermal and hypodermal cells of fruit peels during ripening (Kim, 1990).

Some nutrient or growth regulator treatments can affect red color development in apples. Two foliar applications of Phostrade Ca (containing high concentrations of phosphorus and minor amounts of calcium and nitrogen) late in the growing season was reported to be an effective way to improve the color of Braeburn apples (*Malus domestica* Borkh.) at commercial harvest (Bizjak et al., 2013). Fruit color and biosynthesis of anthocyanins can be regulated by ethylene, daminozide, and paclobutrazol treatments (Saure, 1990).

Jasmonates, including jasmonic acid and methyl jasmonate (MeJA), are a family of cyclopentanone compounds that modulate a wide range of plant responses (Sembdner and Parthier, 1993; Creelman and Mullet, 1997), including promotion of chlorophyll degradation (Ueda and Kato, 1980; Abeles et al., 1989; Hung and Kao, 1996). MeJA has been shown to enhance anthocyanin accumulation in soybean seedlings (Franceschi and Grimes, 1991), peach shoots (Saniewski et al., 1998), and apple fruits (Kondo et al., 2001). Kondo et al. (2001) showed that jasmonates effectively stimulated anthocyanin biosynthesis and that such effect was independent of ethylene. Similarly, MeJA was found to enhance rate of color change in Fuji apples independent of ethylene action, suggesting that the effect of MeJA on color development on apples may partly be independent of ethylene (Fan and Mattheis, 1999).

Braeburn is classified as a striped red-colored apple cultivar. Striped fruits have a lighter red color than blushed fruits (Telias et al., 2008). Poor and erratic red blush incidence over the fruit surface of Braeburn apples causes serious economic losses, especially in regions and years with poor coloring conditions. Therefore, in the present study, the effects of MeJA treatments at different intervals on the development of fruit peel color, anthocyanin biosynthesis, total phenolics, total antioxidant capacity, and ethylene biosynthesis of Braeburn apples were investigated.

2. Materials and methods

2.1. Plant material

The study was carried out at the Horticultural Research Center of Gaziosmanpaşa University (40°20'02.19"N, 36°28'30.11"E; 623 m above sea level) in Tokat Province, in the middle Black Sea region of Turkey, during the years 2010 and 2011. A total of 42 5-year-old Braeburn apples grafted on M26 trees were selected and grouped into 3 blocks of 14 trees based on proximity in orchard and crop load.

The experiment was laid out in a randomized complete block design with 3 replicates and 2 trees per replication.

Experimental trees were spaced at 1.5×3.5 m and trained with the slender spindle system. Trees were manually thinned so as to have 1 fruit per cluster at the 42nd day after full bloom. Trees were treated with 1120, 2240, or 4480 mg L-1 MeJA (Sigma-Aldrich, Germany) at 1- and 2-week intervals between the 105th and 175th days after full bloom. MeJA solutions contained 0.077% (v v⁻¹) Triton X-100 (Sigma-Aldrich). Treatments were applied to 40 fruits on each tree by using hand-pump-actuated spray bottles. Each fruit was sprayed to drip point. For each treatment, one pair of trees in each block was used for treatments. Two trees in each block were sprayed only with 0.077% Triton X-100 and served as the control. Beginning at the 154th day after full bloom, 10 fruits were harvested randomly from each tree at 1-week intervals until the anticipated harvest time.

In each analysis period, color characteristics (L^* , chroma, and hue angle) were determined in 10 fruits

collected from each tree. A 5-fruit subsample was used for anthocyanin, phenolics, and antioxidant analysis for the year 2010. The other subsample of 5 fruits was used for fruit flesh firmness. To evaluate internal ethylene concentration, 10 fruits were randomly harvested from 2 trees in each block for each treatment of the year 2011.

2.2. Color characteristics

Peel color of the sun-exposed and shade-exposed sides of each fruit was analyzed by using a colorimeter (Minolta model CR-400, Japan) and expressed as an average. Measurements were obtained by using the CIE L* (light to dark) a* (green to red) b* (blue to yellow) color space, and then a* and b* values were converted into chroma and hue angle (McGuire, 1992).

2.3. Fruit firmness

Flesh firmness was determined on 3 sides of the equatorial line of each fruit by using a press-mounted Effegi penetrometer (FT 327; McCormick Fruit Tech, Italy) with an 11.1-mm tip and expressed in newtons.

2.4. Internal ethylene concentration

To measure internal ethylene concentrations, a 1-mL air sample from the core cavity of each fruit was injected into a gas chromatograph equipped with an active alumina column and flame ionization detector (PerkinElmer Clarus 500, USA) in accordance with the method specified by Bramlage et al. (1980). The resulting peaks were compared to that of 100 μ L L⁻¹ ethylene standard and the internal ethylene concentration was calculated.

2.5. Total phenolics, total antioxidant capacity, and total anthocyanin

Total phenolics were determined according to the procedure described by Singleton and Rossi (1965). Briefly, fruit slurries (only cortex) were extracted with the buffer containing acetone, water, and acetic acid (70:29.5:0.5, v v⁻¹) for 2 h in the dark. Samples were replicated 3 times. Extracts were combined with Folin–Ciocalteu phenol reagent and water and incubated for 8 min, followed by the addition of 7% sodium carbonate. After 2 h, the absorbance at 750 nm was measured in an automated UV-Vis spectrophotometer (Model T60U, PG Instruments, USA). Gallic acid was used as the standard. The results were expressed as μ g gallic acid equivalent g⁻¹ fresh weight (FW).

Total antioxidant activity was estimated using a standard procedure, the TEAC assay, as suggested by Ozgen et al. (2006). For the standard TEAC assay, 10 mmol L^{-1} 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was dissolved in an acetate buffer and prepared with potassium persulfate. The mixture was diluted using an acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability. For the spectrophotometric assay, 2.90 mL of the

ABTS⁺ solution and 100 μ L of fruit extract were mixed and incubated for 10 min. The absorbance at 734 nm was then determined. The results were expressed in μ mol Trolox equivalents (TE) g⁻¹ FW.

The total anthocyanins of fruit peel were estimated by a pH differential method (Giusti and Wrolstad, 2005) using a UV-Vis spectrophotometer (Model T60U, PG Instruments). Absorbance was measured at 533 and 700 nm in buffers with a pH of 1.0 and 4.5 with a molar extinction coefficient of 29.600. Results were expressed as μ g cyanidin-3-galactoside g⁻¹ peel weight equivalent.

2.6. Statistical analysis

All statistical analyses were performed with SAS 9.1 (SAS Institute Inc., USA). Analysis of variance was used in all experiments to determine significance of treatments. The means of treatments were separated by orthogonal polynomial comparison.

3. Results

To evaluate the effect of MeJA applications on red coloration during ripening, the changes in the colorimetric parameters (L*, chroma, and hue angle) were monitored. In 2010, MeJA treatments applied at 1-week intervals did not cause significant changes in the L* parameter on the first sampling date (19 September), whereas L* value decreased linearly with increasing MeJA concentrations on 26 September, 3 October, and 10 October. Similarly, while MeJA treatments applied at 2-week intervals did not cause significant changes on the first 2 sampling dates, they linearly reduced parameter L* on 3 and 10 October. The differences between treatments applied at 1- and 2-week intervals were insignificant for L* values on the first 2 sampling dates. On the other hand, the L* value was lower in MeJA treatments with 1-week intervals than in the treatments with 2-week intervals on the subsequent dates. In 2011, the L* value decreased linearly with increasing MeJA concentrations applied both in 1- and 2-week intervals on all sampling dates. In this year of the experiment, L* values were lower in MeJA treatments with 1-week intervals on all sampling dates than the values of the treatments with 2-week intervals (Table 1).

MeJA treatments did not cause significant changes in chroma on the first 3 sampling dates in the year 2010. On the last sampling date, chroma values linearly decreased with increasing MeJA concentrations, regardless of the application intervals. The differences between treatments made at 1- and 2-week intervals were insignificant for chroma values on all sampling dates. In 2011, the linear effect of increasing MeJA concentrations on chroma appeared on the first (1-week intervals) or second (2week intervals) sampling date and was maintained until the last sampling date. The differences between application regimes were insignificant on the first 2 sampling dates, but on the subsequent dates, chroma values of MeJA treatments applied at 1-week intervals were lower than those of MeJA treatments with 2-week intervals (Table 2).

As with L^* and chroma values, hue angle was also significantly influenced by MeJA treatments. Hue angle deceased linearly with increasing MeJA concentrations at almost all sampling dates in both experimental years regardless of the application regime. In the first year, there were insignificant differences between the 1- and 2-week intervals on the first 2 sampling dates (19 and 26 September). On the subsequent dates (3 and 10 October), hue angles of 1-week intervals were lower than those of 2-week intervals. In the second year, there were insignificant differences between 1- and 2-week intervals on all sampling dates except for the last sampling date (Table 3).

Fruit internal ethylene concentration was determined only in the second year (2011). Compared to the control treatment, all MeJA treatments significantly increased fruit internal ethylene concentration. There were positive linear relationships between MeJA treatment concentrations and internal ethylene concentrations of fruits on all sampling dates. On some sampling dates, the quadratic relationship between MeJA treatment concentrations and ethylene concentrations was also found to be significant. In terms of internal ethylene concentration, the differences between application regimes were significant on 29 September and 6 and 20 October, but not on 22 September or 13 October (Table 4).

Total anthocyanin, total phenolic content, and total antioxidant capacity of apples were measured only in the first year (2010). The results showed an obvious linear increase in anthocyanin content with increasing concentrations of MeJA on all sampling dates. Anthocyanin contents in 1-week intervals were higher than those in 2-week intervals. On the last sampling date (10 October), while total anthocyanin content was 239.0 μ g cyanidin 3-galactoside g⁻¹ peel weight for the control treatment, it was respectively observed as 857.5 and 534.3 μ g cyanidin 3-galactoside g⁻¹ peel weight for 4480 mg L⁻¹ MeJA applied at 1- and 2-week intervals (Table 5).

The increasing MeJA concentrations also caused linear increases in total phenolics content and total antioxidant capacity of Braeburn apples. The linear relationship between MeJA concentrations and total phenolics contents was found to be significant on all sampling dates regardless of application intervals. Although both MeJA application regimes increased total antioxidant capacity, treatments with 1-week intervals had higher total antioxidant capacity than treatments with 2-week intervals (Table 6).

As with phenolics and anthocyanin content, total antioxidant capacity also increased linearly on all sampling dates with increasing concentrations of MeJA

| | | L* | L* | | | | | | |
|--|--------------------------|--------|------------|-------|----------|--------|--|--|--|
| intervals | Treatments $(mg L^{-1})$ | 2010 | | | | | | | |
| Treatments intervals (weeks) 1-week Significance 2-week 1-week Significance 2-week 2-week | (8 -) | 19 Sep | 26 Sep | 3 Oct | 10 Oct | 17 Oct | | | |
| | 0 | 61.55 | 59.72 | 58.10 | 57.15 | _X | | | |
| 1-week | 1120 | 60.95 | 58.96 | 56.55 | 51.04 | - | | | |
| | 2240 | 59.45 | 57.40 | 55.96 | 47.39 | - | | | |
| | 4480 | 59.27 | 56.95 | 51.78 | 44.49 | - | | | |
| Significance | MeJA concentration | NS | L^* | L** | L*** | - | | | |
| | 0 | 61.55 | 59.72 | 58.10 | 57.15 | - | | | |
| 2-week | 1120 | 62.37 | 59.58 | 57.29 | 53.82 | - | | | |
| | 2240 | 61.41 | 58.17 | 56.86 | 48.60 | - | | | |
| | 4480 | 60.51 | 57.24 | 55.12 | 46.61 | - | | | |
| Significance | MeJA concentration | NS | NS | * | L**** | - | | | |
| | 1-week vs. 2-week | NS | NS | * | * | - | | | |
| | | 2011 | | | | | | | |
| | | 22 Sep | 29 Sep | 6 Oct | 13 Oct | 20 Oct | | | |
| | 0 | 64.86 | 62.93 | 60.22 | 58.59 | 54.80 | | | |
| 1-week | 1120 | 62.34 | 59.78 | 56.70 | 52.48 | 51.13 | | | |
| | 2240 | 60.49 | 57.25 | 54.99 | 50.22 | 47.61 | | | |
| | 4480 | 58.79 | 55.29 | 50.48 | 48.27 | 45.04 | | | |
| Significance | MeJA concentration | L*** | L^{****} | L**** | L**** Q* | L*** | | | |
| | 0 | 64.86 | 62.93 | 60.22 | 58.59 | 54.80 | | | |
| 2-week | 1120 | 64.81 | 60.02 | 58.99 | 57.34 | 52.62 | | | |
| | 2240 | 62.42 | 57.61 | 56.01 | 53.14 | 48.72 | | | |
| | 4480 | 61.79 | 56.27 | 54.42 | 51.54 | 47.81 | | | |
| Significance | MeJA concentration | L^* | L^* | L** | L*** | L**** | | | |
| | 1-week vs 2-week | ** | * | * | **** | * | | | |

Table 1. Effects of application intervals of methyl jasmonate on the L* values of Braeburn apples.

NS, *, **, ***, and ****: nonsignificant or significant at P = 0.05, 0.01, 0.001, or 0.0001, respectively; L = linear and Q = quadratic response. X = no evaluated fruit parameters for this harvest date. n = 60 (10 fruits × 3 replicates × 2 measurements for each fruit) for L*.

| Tuestas ente | | Chroma | | | | | | |
|--------------|--------------------------|--------|--------|-------|--------|--------|--|--|
| intervals | Treatments $(mg L^{-1})$ | 2010 | | | | | | |
| (weeks) | × 0 , | 19 Sep | 26 Sep | 3 Oct | 10 Oct | 17 Oct | | |
| | 0 | 46.09 | 44.63 | 42.15 | 42.96 | _x | | |
| 1-week | 1120 | 45.41 | 43.03 | 41.28 | 40.64 | - | | |
| | 2240 | 44.56 | 42.89 | 40.95 | 39.42 | - | | |
| | 4480 | 44.13 | 42.20 | 39.56 | 38.53 | - | | |
| Significance | MeJA concentration | NS | NS | NS | L*** | - | | |
| | 0 | 46.09 | 44.63 | 42.15 | 42.96 | - | | |
| 2-week | 1120 | 45.97 | 43.98 | 42.28 | 41.71 | - | | |
| | 2240 | 45.37 | 43.32 | 41.73 | 40.14 | - | | |
| | 4480 | 44.73 | 43.07 | 40.19 | 39.74 | - | | |
| Significance | MeJA concentration | NS | NS | NS | L** | - | | |
| | 1-week vs. 2-week | NS | NS | NS | NS | - | | |
| | | 2011 | | | | | | |
| | | 22 Sep | 29 Sep | 6 Oct | 13 Oct | 20 Oct | | |
| | 0 | 45.07 | 44.54 | 43.66 | 42.81 | 41.75 | | |
| 1-week | 1120 | 44.40 | 43.50 | 41.14 | 40.70 | 39.63 | | |
| | 2240 | 43.36 | 40.82 | 40.50 | 39.39 | 38.66 | | |
| | 4480 | 42.98 | 40.23 | 39.66 | 38.85 | 38.56 | | |
| Significance | MeJA concentration | L^* | L** | L** | L** | L^* | | |
| | 0 | 45.07 | 44.54 | 43.66 | 42.81 | 41.75 | | |
| 2-week | 1120 | 45.11 | 43.76 | 43.13 | 42.58 | 41.16 | | |
| | 2240 | 44.50 | 42.16 | 41.62 | 40.86 | 40.22 | | |
| | 4480 | 44.30 | 41.84 | 41.28 | 40.37 | 39.27 | | |
| Significance | MeJA concentration | NS | L^* | L^* | L** | L^* | | |
| | 1-week vs. 2-week | NS | NS | * | * | * | | |

Table 2. Effects of application intervals of methyl jasmonate on the chroma values of Braeburn apples.

NS, *,**, and ***: nonsignificant or significant at P = 0.05, 0.01 or 0.001, respectively; $L = \text{linear.}^{X} = \text{no}$ evaluated fruit parameters for this harvest date. n = 60 (10 fruits × 3 replicates × 2 measurements for each fruit) for chroma.

| Tuestas ente | | Hue angle (h°) | | | | | | |
|--------------|--------------------------|----------------|--------|------------|-----------|---------|--|--|
| intervals | Treatments $(mg L^{-1})$ | 2010 | | | | | | |
| (weeks) | | 19 Sep | 26 Sep | 3 Oct | 10 Oct | 17 Oct | | |
| | 0 | 86.23 | 80.49 | 78.39 | 72.65 | _x | | |
| 1-week | 1120 | 84.27 | 78.02 | 66.11 | 60.29 | - | | |
| | 2240 | 83.61 | 76.39 | 63.74 | 55.47 | - | | |
| | 4480 | 80.86 | 74.26 | 60.09 | 56.66 | - | | |
| Significance | MeJA concentration | L* | L** | L**** | L**** | - | | |
| | 0 | 86.23 | 80.49 | 78.39 | 72.65 | - | | |
| 2-week | 1120 | 85.65 | 79.19 | 67.37 | 61.51 | - | | |
| | 2240 | 84.95 | 77.03 | 65.34 | 60.41 | - | | |
| | 4480 | 82.71 | 75.43 | 63.33 | 57.76 | - | | |
| Significance | MeJA concentration | NS | L^* | L****Q*** | L****Q** | - | | |
| | 1-week vs. 2-week | NS | NS | * | * | - | | |
| | | 2011 | | | | | | |
| | | 22 Sep | 29 Sep | 6 Oct | 13 Oct | 20 Oct | | |
| | 0 | 90.38 | 88.78 | 82.06 | 77.31 | 69.73 | | |
| 1-week | 1120 | 88.75 | 85.33 | 67.37 | 63.68 | 57.20 | | |
| | 2240 | 88.61 | 80.19 | 64.86 | 61.39 | 55.45 | | |
| | 4480 | 84.69 | 78.37 | 62.44 | 59.54 | 52.59 | | |
| Significance | MeJA concentration | NS | L**** | L****Q* | L***Q*** | L***Q** | | |
| | 0 | 90.38 | 88.78 | 82.06 | 77.31 | 69.73 | | |
| 2-week | 1120 | 90.64 | 85.97 | 65.95 | 64.15 | 61.40 | | |
| | 2240 | 90.39 | 83.78 | 63.56 | 63.06 | 59.17 | | |
| | 4480 | 88.79 | 81.05 | 62.89 | 61.12 | 57.37 | | |
| Significance | MeJA concentration | NS | L*** | L****Q**** | L****Q*** | L****Q* | | |
| | 1-week vs. 2-week | NS | NS | NS | NS | *** | | |

Table 3. Effects of application intervals of methyl jasmonate on the hue angle values of Braeburn apples.

NS, *, **, ***, and ****: nonsignificant or significant at P = 0.05, 0.01, 0.001, or 0.0001, respectively; L = linear and Q = quadratic response. x = no evaluated fruit parameters for this harvest date. n = 60 (10 fruits × 3 replicates × 2 measurements for each fruit) for hue angle.

| Treatments | | Internal ethylene concentration (mg L ⁻¹) | | | | | | |
|------------------------|--------------------------|---|------------|------------|--------|-----------------|--|--|
| intervals | Treatments $(mg L^{-1})$ | 2011 | | | | | | |
| (weeks) | (| 22 Sep | 29 Sep | 6 Oct | 13 Oct | 20 Oct | | |
| | 0 | 15.18 | 27.75 | 40.30 | 44.28 | 57.77 | | |
| 1-week | 1120 | 18.17 | 28.38 | 43.28 | 51.63 | 61.38 | | |
| 1-week Significance | 2240 | 22.57 | 42.20 | 46.61 | 47.04 | 83.52 | | |
| | 4480 | 21.25 | 42.62 | 43.16 | 54.43 | 84.59 | | |
| Significance | MeJA concentration | L** | L**** | L****Q**** | L** | $L^{****}Q^{*}$ | | |
| | 0 | 15.18 | 27.75 | 40.30 | 44.28 | 57.77 | | |
| 2-week | 1120 | 17.45 | 33.14 | 48.29 | 50.10 | 59.22 | | |
| | 2240 | 27.44 | 28.09 | 52.82 | 49.53 | 73.01 | | |
| | 4480 | 20.77 | 46.73 | 53.06 | 56.15 | 78.15 | | |
| Significance | MeJA concentration | L****Q**** | L****Q**** | L** | L**** | $L^{****}Q^{*}$ | | |
| | 1-week vs. 2-week | NS | * | *** | NS | *** | | |

Table 4. Effects of application intervals of methyl jasmonate on the internal ethylene concentration of Braeburn apples in 2011.

NS, *, **, ***, and ****: nonsignificant or significant at P = 0.05, 0.01, 0.001, or 0.0001, respectively; L = linear and Q = quadratic response. n = 30 (10 fruits × 3 replicates) for internal ethylene concentration.

Table 5. Effects of application intervals of methyl jasmonate on the total anthocyanin content of Braeburn apples in2010.

| Treatments intervals | | Total anthocyanin (μg cyanidin 3-galactoside g ⁻¹ peel weight) | | | | | | |
|----------------------|-------------------------------------|---|------------|-----------|------------|--------|--|--|
| | Treatments (mg I ⁻¹) | 2011 | 2011 | | | | | |
| (weeks) | (ing E) | 19 Sep | 26 Sep | 3 Oct | 10 Oct | 17 Oct | | |
| | 0 | 142.7 | 157.6 | 182.7 | 239.0 | _x | | |
| 1-week | 1120 | 256.6 | 331.0 | 389.7 | 506.7 | - | | |
| | 2240 | 346.3 | 387.6 | 459.5 | 737.7 | - | | |
| | 4480 | 443.5 | 658.9 | 709.0 | 857.5 | - | | |
| Significance | MeJA concentration | L****Q* | L****Q**** | L****Q*** | L****Q**** | - | | |
| | 0 | 142.7 | 157.6 | 182.7 | 239.0 | - | | |
| 2-week | 1120 | 161.7 | 184.8 | 213.9 | 333.3 | - | | |
| | 2240 | 209.0 | 262.7 | 317.0 | 448.7 | - | | |
| | 4480 | 267.9 | 289.9 | 363.0 | 534.3 | - | | |
| Significance | MeJA concentration | L****Q*** | L**** | L****Q* | L^{****} | - | | |
| | 1-week vs. 2-week | **** | **** | *** | **** | - | | |

*, ***, and ****: significant at P = 0.05, 0.001, or 0.0001, respectively; L = linear and Q = quadratic response. X = N = 0 evaluated fruit parameters for this harvest date. n = 9 (3 replicates × 3 measurements for each application) for total anthocyanin.

| Treatments | | Total phenolics (µg GAE g ⁻¹ FW) | | | | | | | |
|--------------|----------------------------------|---|-----------|---------|-------------|----|--|--|--|
| intervals | Treatments (mg I ⁻¹) | 2011 | 2011 | | | | | | |
| (weeks) | (ing L) | Total phenolics (μ g GAE g ⁻¹ FW)Total phenolics (μ g GAE g ⁻¹ FW)2011201119 Sep26 Sep3 Oct246.6227.2198.020262.1242.6205.740266.1247.8227.030285.0252.0231.3EVA concentrationL L Q 20250.5225.3200.940252.5235.3202.530256.1242.1221.0EVA concentrationL L L EVA concentrationL ^{L^{EVA concentrationL}} | 3 Oct | 10 Oct | 17 Oct | | | | |
| | 0 | 246.6 | 227.2 | 198.0 | 140.7 | _X | | | |
| 1-week | 1120 | 262.1 | 242.6 | 205.7 | 152.4 | - | | | |
| | 2240 | 266.1 | 247.8 | 227.0 | 171.8 | - | | | |
| | 4480 | 285.0 | 252.0 | 231.3 | 193.9 | - | | | |
| Significance | MeJA concentration | L**** | L**** Q** | L*** | L*** Q** | - | | | |
| | 0 | 246.6 | 227.2 | 198.0 | 140.7 | - | | | |
| 2-week | 1120 | 250.5 | 225.3 | 200.9 | 147.9 | - | | | |
| | 2240 | 252.5 | 235.3 | 202.5 | 156.9 | - | | | |
| | 4480 | 256.1 | 242.1 | 221.0 | 164.7 | - | | | |
| Significance | MeJA concentration | L** | L*** | L*** Q* | L^{*****} | - | | | |
| | 1-week vs. 2-week | **** | **** | ** | **** | - | | | |

Table 6. Effects of application intervals of methyl jasmonate on the total phenolics of Braeburn apples in 2010.

*, **, ***, and ****: significant at P = 0.05, 0.01, 0.001, or 0.0001, respectively; L = linear and Q = quadratic response. x = no evaluated fruit parameters for this harvest date. n = 9 (3 replicates × 3 measurements for each application) for total phenolics.

applied either at 1- or 2-week intervals. The quadratic as well as linear effects of increasing MeJA concentration on antioxidant capacity of apples were found to be significant at all sampling dates. Treatments with 1-week intervals were considered more effective than treatments with 2-week intervals (Table 7).

MeJA treatments also significantly affected fruit firmness of Braeburn apples. In both years, the firmness increased linearly with increasing MeJA concentrations on all sampling dates. While the fruit firmness of the control treatment at the final harvest of the first year was 77.88 N, the value was measured as 91.32 N in the 4480 mg L^{-1} MeJA treatment with 1-week intervals. While there were no significant differences in fruit firmness between application regimes, except for the first sampling date of 2010, fruit firmness of MeJA treatments with 1-week intervals was higher than that of MeJA treatments with 2-week intervals in 2011 (Table 8).

4. Discussion

 L^* is the luminance or lightness component and ranges from 0 (dark) to 100 (light). Bizjak et al. (2013) reported that L^* values decreased during advanced maturity in Braeburn apples, indicating darker fruit peels. Decreasing L^* values were also reported with postharvest MeJA

treatments in Golden Delicious and Fuji apples (Fan et al., 1998; Fan and Mattheis, 1999) and with preharvest treatments in Cripps Pink apples (Shafiq et al., 2013). Similarly in the present study, parameter L* decreased linearly with MeJA treatments regardless of the application regime. Such a decrease was more prominent in fruits treated at 1-week intervals. By definition, chroma indicates the degree of departure from gray towards a pure chromatic color (McGuire, 1992). Contrary to the observations of Fan and Mattheis (1999) on Fuji and Shafiq et al. (2013) on Cripps Pink, in the current study, MeJA decreased the chroma value, as it was more pronounced in 2011. Such differences were mainly due to the differences in species and treatment concentrations. Hue angle is the best indicator of color changes during apple fruit development (Greer, 2005). Bizjak et al. (2013) reported that hue angle decreased during the advanced ripening in Braeburn apples, indicating a greater intensity of red color. In the present study, MeJA treatments increased (lower h°) red peel color development compared to the control treatment. Similar results were also reported by Rudell et al. (2005) and Rudell and Mattheis (2008) for Fuji apples.

Anthocyanins are the plant pigments responsible for the various shades of red color in fruits (Awad et al., 2000). As indicated in previous studies on different apple cultivars

| Treatments | | Total antioxidant capacity (μ mol TE g ⁻¹ FW) | | | | | | | |
|--------------|--------------------------------|---|------------|------------|------------|--------|--|--|--|
| intervals | Treatments $(m \sigma L^{-1})$ | 2011 | 2011 | | | | | | |
| (weeks) | (ing L) | 19 Sep | 26 Sep | 3 Oct | 10 Oct | 17 Oct | | | |
| | 0 | 5.54 | 4.53 | 3.63 | 3.19 | _X | | | |
| 1-week | 1120 | 6.38 | 5.68 | 5.22 | 4.16 | - | | | |
| | 2240 | 7.72 | 6.07 | 5.67 | 5.16 | - | | | |
| | 4480 | 8.11 | 6.24 | 5.95 | 5.42 | - | | | |
| Significance | MeJA concentration | L****Q**** | L****Q**** | L****Q**** | L****Q**** | - | | | |
| | 0 | 5.54 | 4.53 | 3.63 | 3.19 | - | | | |
| 2-week | 1120 | 5.65 | 5.19 | 5.02 | 3.71 | - | | | |
| | 2240 | 6.21 | 5.71 | 5.21 | 4.87 | - | | | |
| | 4480 | 6.71 | 5.85 | 5.41 | 5.17 | - | | | |
| Significance | MeJA concentration | L****Q*** | L****Q**** | L****Q**** | L****Q** | - | | | |
| | 1-week vs. 2-week | **** | **** | **** | **** | - | | | |

Table 7. Effects of application intervals of methyl jasmonate on the total antioxidant capacity of Braeburn apples in 2010.

, *, and ****: significant at P = 0.01, 0.001, or 0.0001, respectively; L = linear and Q = quadratic response. x = no evaluated fruit parameters for this harvest date. n = 9 (3 replicates × 3 measurements for each application) for total antioxidant capacity.

(Saniewski et al., 1987; Kondo et al., 2001; Rudell et al., 2005), ethylene and anthocyanin synthesis in Braeburn apples were clearly stimulated by MeJA treatments. It may be suggested that MeJA promoted anthocyanin production, and thus red skin development, by enhancing ethylene biosynthesis. Involvement of ethylene alone promotes anthocyanin production in apples (Awad and Jager, 2002; Li et al., 2002). However, in some other studies on anthocyanin accumulation and peel color development in apple fruits, it was documented that ethylene alone had little effect on anthocyanin production (Kondo et al., 2001; Rudell and Mattheis, 2008). Similarly, Fan et al. (1998) reported that MeJA promoted changes in L*, chroma, and hue angle, even in fruit without detectable ethylene production, indicating that some degree of MeJA response occurs independently of ethylene.

It has been claimed that jasmonates, jasmonic acid, and MeJA are able to activate the enzymes responsible for biosynthesis of polyphenols such as the PAL enzyme. MeJA-induced PAL activation and the subsequent increase in total phenolics were reported in several fruits such as apples, plums, table grapes, and strawberries (Heredia and Cisneros-Zevallos, 2009). Similarly, in the present study, total phenolic content and antioxidant capacity were significantly affected by MeJA treatments and increased linearly with increasing MeJA concentrations. The increase in phenolic compounds and antioxidant capacity as a result of exogenous MeJA treatment was also reported for other apple cultivars (Rudell et al., 2002; Shafiq et al., 2011).

Although MeJA increased ethylene biosynthesis, it also increased fruit firmness compared to control treatment, indicating that MeJA differentially affected some other maturation-related processes. Such findings are consistent with the results of Rudell et al. (2005), where MeJA treatment enhanced both the ethylene production and fruit firmness of Fuji apples. On the other hand, Shafiq et al. (2013) reported that preharvest spray applications of MeJA did not significantly affect the fruit firmness in Cripps Pink apples.

In conclusion, current results suggest that preharvest application of MeJA can be a good management strategy for red peel color development over the surface of Braeburn apples and for the preservation of fruit firmness. Although both application regimes of MeJA significantly increased red color development, phenolic content, and antioxidant capacity, the treatments with 1-week intervals were observed as more effective than the treatments with 2-week intervals. Efficiency of MeJA linearly increased and such results may indicate better efficiencies at higher concentrations.

| Traatmonts | | Firmness (N) | | | | | |
|--------------|--------------------------|--|--------|--------|------------|--------|--|
| intervals | Treatments $(mg L^{-1})$ | 2010 | | | | | |
| (weeks) | (| Firmness (N) 2010 19 Sep 26 Sep 3 Oct 10 Oct 91.17 88.94 87.07 77.88 96.84 93.60 89.79 82.71 102.4 96.14 94.25 87.48 104.4 102.9 97.39 91.32 104.4 102.9 97.39 91.32 104.4 102.9 97.39 91.32 104.4 102.9 97.39 91.32 104.4 102.9 97.39 91.32 104.4 102.9 97.39 91.32 101 L" L" L" 93.74 91.30 89.47 82.08 98.27 95.17 92.20 88.04 104.0 101.2 94.80 90.23 104.0 101.2 94.80 82.07 vs. 2-week " NS NS 102.3 97.45 92.04 82.47 107.6 104.1 98.11 | 10 Oct | 17 Oct | | | |
| | 0 | 91.17 | 88.94 | 87.07 | 77.88 | _x | |
| 1-week | 1120 | 96.84 | 93.60 | 89.79 | 82.71 | - | |
| | 2240 | 102.4 | 96.14 | 94.25 | 87.48 | - | |
| | 4480 | 104.4 | 102.9 | 97.39 | 91.32 | - | |
| Significance | MeJA concentration | L** | L** | L*** | L**** | - | |
| | 0 | 91.17 | 88.94 | 87.07 | 77.88 | - | |
| 2-week | 1120 | 93.74 | 91.30 | 89.47 | 82.08 | - | |
| | 2240 | 98.27 | 95.17 | 92.20 | 88.04 | - | |
| | 4480 | 104.0 | 101.2 | 94.80 | 90.23 | - | |
| Significance | MeJA concentration | L** | L** | L**** | L**** | - | |
| | 1-week vs. 2-week | ** | NS | NS | NS | - | |
| | | 2011 | | | | | |
| | | 22 Sep | 29 Sep | 6 Oct | 13 Oct | 20 Oct | |
| | 0 | 102.3 | 97.45 | 92.04 | 82.47 | 79.11 | |
| 1-week | 1120 | 107.6 | 104.1 | 98.11 | 94.79 | 83.22 | |
| | 2240 | 113.5 | 107.2 | 101.9 | 96.27 | 86.87 | |
| | 4480 | 115.5 | 109.9 | 104.9 | 98.39 | 94.14 | |
| Significance | MeJA concentration | L*** | L**** | L**** | L****Q**** | L*** | |
| | 0 | 102.3 | 97.45 | 92.04 | 82.47 | 79.11 | |
| 2-week | 1120 | 104.5 | 99.26 | 94.73 | 88.09 | 80.69 | |
| | 2240 | 107.2 | 100.9 | 96.43 | 91.79 | 82.60 | |
| | 4480 | 113.4 | 103.2 | 97.59 | 93.63 | 85.56 | |
| Significance | MeJA concentration | L**** | L*** | L*** | L**** | L*** | |
| | 1-week vs. 2-week | * | **** | *** | **** | *** | |

Table 8. Effects of application intervals of methyl jasmonate on the firmness of Braeburn apples.

NS, *, **, ***, and ****: nonsignificant or significant at P = 0.05, 0.01, 0.001, or 0.0001, respectively; L = linear and Q = quadratic response. X = no evaluated fruit parameters for this harvest date. n = 45 (5 fruits × 3 replicates × 3 measurements for each fruit) for firmness.

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