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Effect of Methyl Jasmonate on Phenolic Compounds and Carotenoids of Romaine Lettuce (*Lactuca sativa* L.)

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The effect of exogenous methyl jasmonate (MeJA) on antioxidative compounds of romaine lettuce (*Lactuca sativa* L.) was investigated. Lettuces were treated with various MeJA solutions (0, 0.05, 0.1, 0.25, and 0.5 mM) before harvest. Total phenolic compounds content and antioxidant capacity of romaine lettuce significantly increased after MeJA treatments (0.1, 0.25, and 0.5 mM). The total content of phenolic compounds of the romaine lettuce treated with 0.5 mM MeJA (31.6 μg of gallic acid equivalents/mg of dry weight) was 35% higher than that of the control. The increase in phenolic compound content was attributed to a caffeic acid derivative and an unknown phenolic compound, which also contributed to increased antioxidant capacity. The induction of phenylalanine ammonia-lyase (PAL) activity by the MeJA treatment indicated that phenolic compounds were altered due to the activation of the phenylpropandoid pathway. Total content of carotenoids, including lutein and β-carotene, of the MeJA-treated lettuce did not change after 8 days of treatment, whereas the content of the control without MeJA decreased after 8 days. This research indicated that preharvest application of MeJA could increase the nutritional value of romaine lettuce under determined conditions discussed in this work.

KEYWORDS: Antioxidant; carotenoid; methyl jasmonate (MeJA); phenolic compound; phenylalanine ammonia-lyase (PAL); romaine lettuce; secondary metabolite

INTRODUCTION

The consumption of natural products with potential health benefits has been continuously growing at a rate of 5−10% per year (*1*), and more research has emphasized the discovery of novel bioactive compounds (*2*–*4*). Among various sources, plants are regarded as major sources of bioactive compounds with diverse biological activities such as anticancer, antioxidant, antimicrobial, and anti-inflammatory effects (*2*, *3*, *5*–*7*). Phytobioactive compounds commonly play important physiological roles in plants as secondary metabolites, which can change quantitatively and qualitatively as a result of environmental changes such as wounding and UV-light exposure (*8*–*13*). Under various stresses, levels of a number of secondary metabolites, including methyl jasmonate (MeJA) and jasmonate (JA), are altered through the octadecanoid pathway to enhance the defense system (*14*–*20*). Unlike JA, MeJA is released with various organic volatile compounds from stressed plants to the air to transfer a stress signal to healthy neighboring (receiver) plants, and the receiver plants may induce defense systems without being subjected to any stress factor (*21*). Due to this characteristic of MeJA, the application of exogenous MeJA treatment has been investigated to increase the contents of secondary metabolites in various plants such as sweet basil, *Nicotiana attenuate*, and raspberries (*17*, *19*, *20*).

Along with tomatoes, lettuce is the most important salad vegetable, known as a source of phytonutrients including vitamins, carotenoids, and antioxidants (*22*–*24*). It has been reported that the contents of these nutrients are altered by external factors that cause stress to the plant such as wounding, high atmospheric ozone levels, and drought (*24*–*26*). To our knowledge, however, the effect of preharvest application of exogenous MeJA treatment on bioactive compounds in romaine lettuce has not been examined. Therefore, in this study, the effect of various MeJA concentrations on phenolic compounds and antioxidant capacity of romaine lettuce was investigated. Further research was conducted to determine (a) antioxidant capacity and content of phenolics and carotenoids as affected by the interval between treatment and harvest and (b) any correlation between the response to MeJA and PAL activity.

MATERIALS AND METHODS

Chemicals. Methyl jasmonate (MeJA), *trans*-cinnamic acid, butylated hydroxytoluene (BHT), and 6-hydroxy-2,5,7,8-tetramethylchro-

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man-2-carboxylic acid (Trolox) were purchased from Aldrich Chemical Co. (Milwakee, WI). Bradford reagent, bovine serum albumin, β-mercaptoethanol, lutein, β-carotene, gallic acid, caffeic acid, chlorogenic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and Folin−Ciocalteu's reagent were purchased from Sigma Chemical Co. (St. Louis, MO). 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH•) was obtained from Alfa Aesar (Ward Hill, MA), and all HPLC analytical grade solvents were sourced from Fisher Scientific (Chino, CA).

Plant Culture and MeJA Treatment. Seeds of romaine lettuce (*L. sativa* L. cv. Clemente) were sown into a 0.4 L plastic pot containing a commercial potting soil (Uni-Gro, Yuma, AZ). Plants were grown in a greenhouse (day/night temperature, 28/12 °C; sunlight, 290 langley; day length, 10 h; Arizona Meteorological Network) at The University of Arizona Yuma Agricultural Research Center. Plants were irrigated once every three days with the addition of fertilizer (Miracle-Gro, N:P:K = 24:8:16, Scotts Miracle-Gro Co., Marysville, OH) once a week. Before harvest, five plants (50 days old) were sprayed with one of five different MeJA solutions (0, 0.05, 0.1, 0.25, and 0.5 mM) that were dissolved in 0.25% ethanol, covered with a plastic dome, and separately placed for 1 h. After removal of the plastic cover, each MeJA-treated lettuce was kept separately for 4 h to completely remove MeJA.

Extraction of Phenolic Compounds from Romaine Lettuce. The harvested romaine lettuce was dried using a freeze-dryer, ground, and kept at −80 °C before extraction. For the extraction of phenolic compounds, a ground sample (0.1 g) was mixed with 5 mL of 80% methanol, and the mixture was shaken at room temperature for 12 h. After centrifugation at 2000*g* for 10 min, the supernatant was used for the determination of phenolic compounds.

Determination of Total Content of Phenolic Compounds. The total amount of phenolic compounds in romaine lettuce was determined using Folin−Ciocalteu's reagent according to the method of Singleton and Rossi (*27*). Fifty microliters of the methanolic extract was mixed with 450 μL of distilled water and 250 μL of 2 N Folin−Ciocalteu reagent. The mixture added to 1.25 mL of 20% Na_2CO_3 was incubated at 25 °C for 20 min and then centrifuged at 2000*g* for 10 min. The absorbance of the supernatant was measured at 735 nm, and the standard curve was prepared using gallic acid (GA). The absorbance was converted to phenolic content in terms of milligrams of GA equivalent (GAE) per gram of dry weight (DW) of sample.

Separation and Identification of Phenolic Compounds. Separation of phenolic compounds in the lettuce extract was performed with a reverse phase C_{18} high-performance liquid chromatograph (HPLC). An uUltra C_{18} column (150 × 4.6 mm, 5 μm; Restek, PA) connected to the LC-10AT HPLC system (Shimadzu, Kyoto, Japan) was equilibrated with 0.05% aqueous trifluoroacetic acid (TFA). Twenty microliters of the methanolic extract was injected and eluted with 0.05% aqueous TFA and acetonitrile at a flow rate of 1 mL/min. The absorbance of the eluant was simultaneously measured at 280 and 320 nm. To identify major phenolic compounds, the eluant of each phenolic compound peak was collected and the collected fractions were hydrolyzed by 2 N hydrogen chloride (HCl) at 90 °C for 20 min. After incubation, the hydrolyzed free phenolic compounds were extracted with ethyl acetate, and the supernatant was dried under nitrogen gas. The residue was recovered with methanol, and free phenolic compounds were separated by C_{18} HPLC following the same conditions as previously described. Standard chemicals were used to identify phenolic compounds.

To confirm HPLC analysis for identification of acid-hydrolyzed compounds, free phenolic compounds were derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in the capped vial at 70 °C for 30 min and the volatile TMS-phenolics were applied to GC-MS analysis. A HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA) was equipped in an Agilent 7890A GC system connected with a 5975C mass spectrometer (MS) detector (Agilent Technologies Inc., Wilmington, DE). The GC oven temperature was programmed from 60 to 280 °C at a rate of 10 °C/min and held at 60 and 280 °C for 5 and 15 min, respectively. The MS source and inert-F temperatures were 230 and 250 °C, respectively. The detector voltage was set at 70 eV, and the MS spectra were obtained in the mass range of *m*/*z* 43–450. Helium was used as the carrier gas at a flow rate of 1 mL/min. One microliter of each derivatized sample was injected into the column in a split mode at 20:1. Identification of compounds was based on comparison with mass spectra of the authentic standards and with the mass spectra of Wiley and NIST mass spectral database.

Determination of Antioxidant Activity. The antioxidant capacity of romaine lettuce was determined by two methods: the DPPH• free radical scavenging assay and the $ABTS^{\bullet+}$ assay.

DPPH• Free Radical Scavenging Activity. The DPPH• free radical scavenging activity was determined according to the method of Yamaguchi et al. (*28*) with slight modification. The reaction mixture containing 0.1 mL of sample, 0.4 mL of 0.1 M Tris-HCl (pH 7.4), and 0.5 mL of 0.3 mM DPPH• was shaken and incubated in the dark at room temperature for 20 min. After incubation, the absorbance of the reaction mixture was measured at 517 nm, and the scavenging activity of DPPH• free radical was calculated by using the following formula:

$$\text{scavenging activity (\%)} = \left(1 - \frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}}\right) \times 100 \quad (1)$$

A standard curve was prepared using Trolox, and the DPPH• free radical scavenging activity was calculated using the above equation and reported as micromoles of Trolox equivalents (TE) per gram of DW of sample.

$ABTS^{\bullet+}$ Assay. To generate the radical cation ($ABTS^{\bullet+}$) needed for the determination of antioxidant activity, 7 mM ABTS dissolved in distilled water was allowed to react with 2.45 mM potassium persulfate. The reaction solution was left in the dark at room temperature for 16 h, after which it was diluted with 5 mM sodium phosphate buffer (pH 7.4) to an absorbance of 1.00 ± 0.02 at 734 nm. Ten microliters of the methanol extract was added to 1 mL of the diluted free radical solution ($ABTS^{\bullet+}$), and the reaction mixture was incubated at 30 °C for 7 min. After incubation, the absorbance of the reaction mixture was spectrophotometrically measured at 734 nm (*29*). The standard curve was prepared using Trolox, and the free radical scavenging activity (percent) was reported as micromoles of Trolox equivalent antioxidant capacity (TEAC) per gram of DW of sample.

Determination of Phenylalanine Ammonia-lyase (PAL) Activity. To determine the activity of PAL, a key regulatory enzyme of secondary metabolites in plants, the crude enzyme of the lettuce was extracted with 0.1 N sodium borate buffer (pH 8.8) containing 15 mM β-mercaptoethanol. After centrifugation at 15000*g* for 10 min, the supernatant was used as source of the crude enzymes. Five hundred microliters of the crude enzymes was mixed with 1 mL of 10 mM L-phenylalanine dissolved in 0.1 N sodium borate buffer (pH 8.8). The reactant was incubated at 37 °C for 6 h, and cinnamic acid produced by the reaction was extracted using toluene. The amount of cinnamic acid recovered by toluene was measured at 290 nm with a spectrophotometer (*30*), and the PAL activity was expressed in picokatals per milligram of protein. One katal was defined as the enzyme activity producing 1 mol of cinnamic acid equivalents per second. The amount of protein in the crude enzyme was determined by using the Bradford assay (*31*) with bovine serum albumin as standard.

Extraction of Carotenoids from Romaine Lettuce. Carotenoids from romaine lettuce were extracted following the procedure of Moros et al. (*32*), with modifications. The dried sample powder (0.2 g) was mixed with 6 mL of ethanol containing 0.1% BHT, and the sealed mixture solution was preincubated at 85 °C for 5 min. For saponification, 120 μL of 80% KOH was added to the preheated mixture solution, and the reaction solution was incubated at the same temperature for 10 min. The reacted solution then was immediately placed in ice, and 3 mL of distilled water and 3 mL of hexane were added to the solution. After centrifugation, the hexane layer containing the crude carotenoids was collected. The separation step by hexane was repeated once. The hexane layers were combined and completely dried with nitrogen gas. The residue recovered with methanol/MTBE (1:1) was used as the crude carotenoids of the lettuce.

Determination of Total Carotenoids Content and Lutein Content by HPLC. HPLC separation of carotenoids was achieved following conditions used by Moros et al. (*32*). A YMC-Carotenoid S-5 column (250 × 4.6 mm, 5 μm; Waters, Milford, MA) was connected to the LC-10AT HPLC system and equilibrated with the mixed solvent of

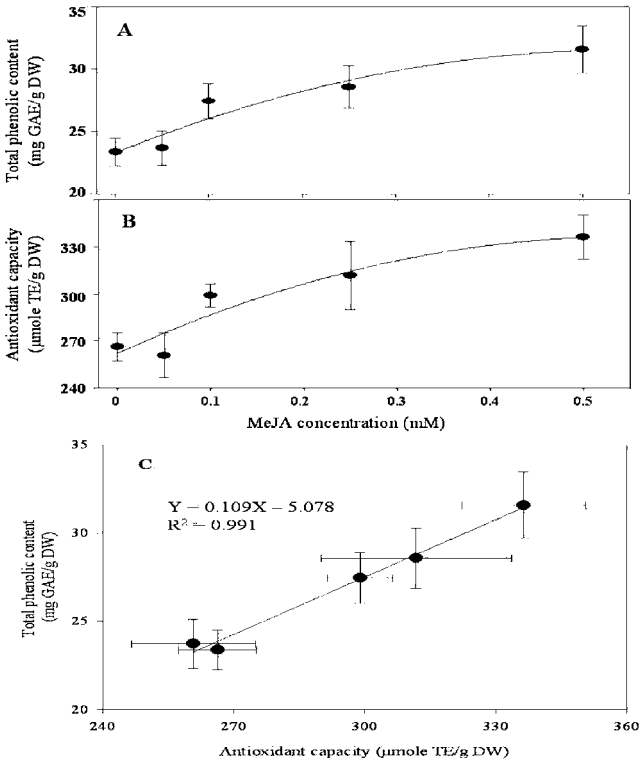

Figure 1. Total phenolic content (**A**) and antioxidant capacity (**B**) of the romaine lettuce treated with various MeJA concentrations and their correlation (**C**). The total amount of phenolic compounds was determined at 735 nm. Gallic acid was used as a standard compound. Antioxidant capacity was measured at 517 nm by DPPH$^{\bullet}$ free radical scavenging assay, and Trolox was used as a standard antioxidant. Error bars are standard deviations of triplicate experiments.

methanol/MTBE/water (81:15:4). Twenty microliters of the crude carotenoid extract was injected and eluted with methanol/MTBE/water (81:15:4) and methanol/MTBE (9:91) at a flow rate of 1 mL/min. The absorbance of the eluant was measured at 450 nm. Total content of carotenoids was calculated by total area of all peaks separated by HPLC, and the area was transformed to micrograms of β-carotene equivalents (CE) per gram of DW of sample. Authentic lutein and β-carotene were used to evaluate their quality and quantity in the romaine lettuce.

Experimental Design and Data Analysis. To investigate the effect of MeJA, romaine lettuce was treated with one of five different MeJA solutions (0, 0.05, 0.1, 0.25, and 0.5 mM) and harvested 1 day after treatment. On the basis of results from the first experiment, 0.5 mM MeJA, which showed the largest increase in total phenolic compounds, was selected for further experimentation to determine the extent of the effect of the preharvest MeJA treatment. The lettuces were harvested 0, 2, 4, 6, and 8 days after treatment with 0.5 mM MeJA. Five lettuces randomly selected were used in each treatment for each replicate. The experiments were conducted three times. The experimental data were subjected to analysis of variance (ANOVA) and analyzed (SAS 9.1, SAS Institute Inc., Cary, NC). Nonlinear regressions were obtained using Sigma plot with SPSS (2001; SPSS Inc., Chicago, IL). The least significant difference was used to find the differences among all sample means at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of MeJA on Total Phenolic Content and Antioxidant Capacity in Romaine Lettuce. The selection of an optimum concentration of MeJA that could induce phenolic compounds in romaine lettuce was achieved by treating plants with different MeJA concentrations 1 day before harvest. As shown in **Figure 1A**, total phenolic content significantly increased by MeJA treatments, whereas there was no significant

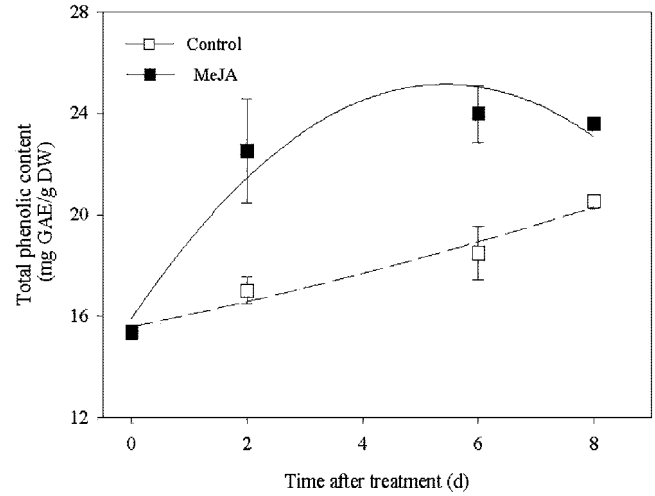

Figure 2. Time course of total phenolic content of the romaine lettuce treated with 0.5 mM MeJA. The total amount of phenolic compounds extracted with 80% methanol was spectrophotometerically determined at 735 nm. Gallic acid was used as a standard compound. Error bars are standard deviations of triplicate experiments.

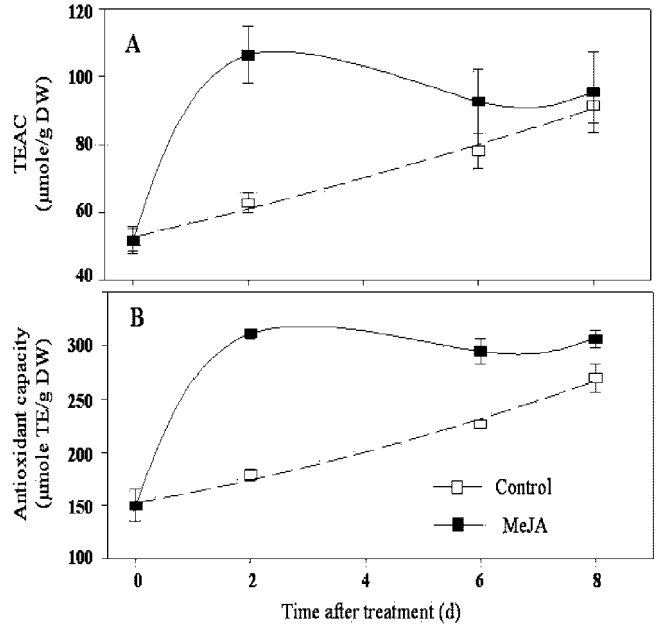

Figure 3. Time course of antioxidant capacity of the romaine lettuce treated with 0.5 mM MeJA. Two antioxidant assays were used to evaluate the antioxidant capacity of the romaine lettuce methanolic extract. Trolox equivalent antioxidant capacity (TEAC) was measured at 734 nm by ABTS$^{\bullet+}$ assay (**A**), and DPPH$^{\bullet}$ free radical scavenging capacity (**B**) was determined at 517 nm. Error bars are standard deviations of triplicate experiments.

difference in total phenolic content between the control (0.25% ethanol) and the 0.05 mM MeJA treatment. Although the difference between the 0.25 and 0.5 mM treatments was not significant, the total phenolic content of the romaine lettuce treated with 0.5 mM MeJA reached maximal amounts of 32.6 mg of GAE/g of DW, which was 37% higher than that of the control (23.4 mg of GAE/g of DW).

The antioxidant capacity of the romaine lettuce, determined by DPPH$^{\bullet}$ free radical scavenging assay, significantly increased with higher MeJA concentration, which coincided with the phenolic results. Lettuce treated with 0.5 mM MeJA had 31% higher antioxidant capacity than that of the control (**Figure 1B**).

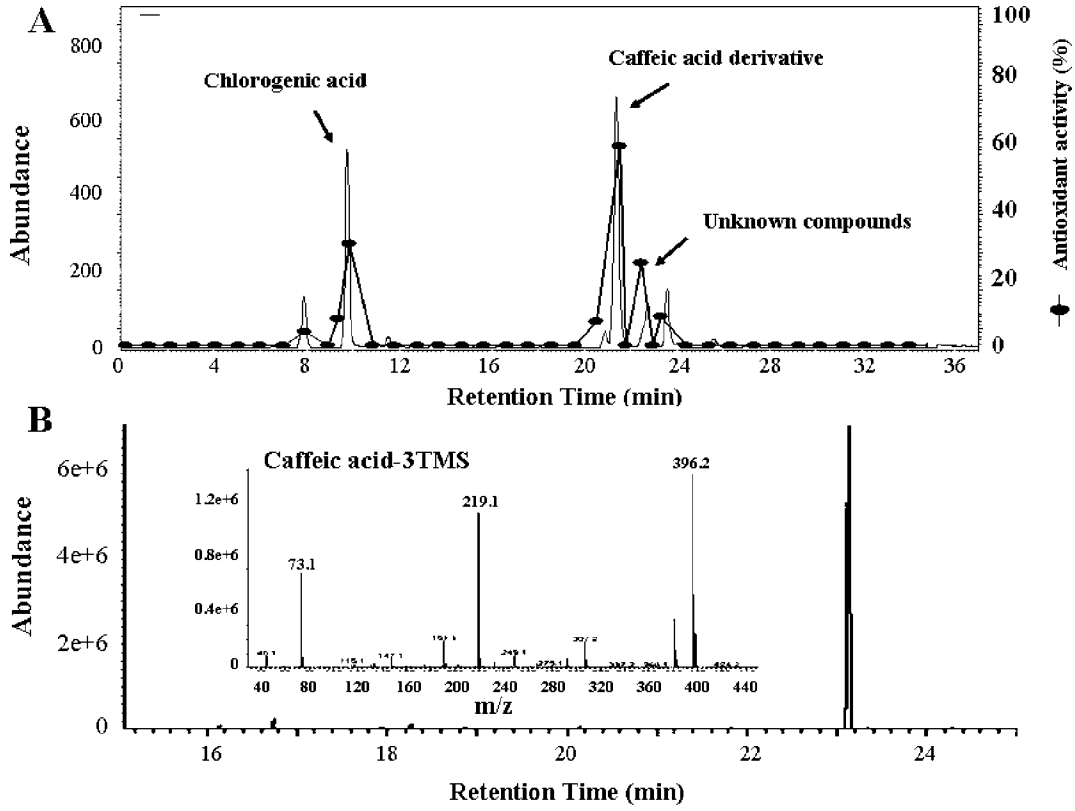

Figure 4. HPLC profile of phenolic compounds of the romaine lettuce and their antioxidant activity. The Ultra C_{18} column (150 × 4.6 mm, 5 μm) connected to the LC-10AT HPLC system was used to separate phenolic compounds in the romaine lettuce. Twenty microliters of the methanolic extract was injected and eluted with 0.05% aqueous TFA and acetonitrile at a flow rate of 1 mL/min. The absorbance of the eluant was measured at 280 and 320 nm. The hydrolyzed free phenolic compound was collected, and each phenolic compound peak was identified by the spike test with authentic standard compounds. Antioxidant activities of the collected fractions were measured by the DPPH• free radical scavenging assay. Free phenolic compounds were derivatized with BSTFA, and the volatile TMS-phenolics were applied to GC-MS with a HP-5MS capillary column The GC oven temperature was programmed from 60 to 280 °C at a rate of 10 °C/min and held at 60 and 280 °C for 5 and 15 min, respectively. The detector voltage was set at 70 eV, and the MS spectra were obtained in the mass range of *m*/*z* 43–450. Identification of compounds was based on comparison with mass spectra of the authentic standards and with the mass spectra of Wiley and NIST mass spectral database.

Table 1. Time Course of the Methyl Jasmonate (MeJA) Effect on Phenolic Compounds and Phenylalanine Ammonia Lyase (PAL) Activity in the Romaine Lettuce[a]

	phenolic compound							
	chlorogenic acid (mg of CA/g of DW)		caffeic acid derivative (mg of CAE[b]/g of DW)		unknown compound (mg of CAE[b]/g of DW)		PAL[c] (pkat/mg of protein)	
day	control	MeJA	control	MeJA	control	MeJA	control	MeJA
0	2.5 a	2.5 a	8.1 a	8.1 a	3.9 a	3.9 a	155.6 a	155.6 a
2	3.0 b	2.7 ab	8.3 a	25.9 b	4.2 ab	6.7 b	173.9 a	269.0 b
6	3.9 b	2.2 a	11.2 b	22.7 b	4.9 bc	7.5 b	165.1 a	260.7 b
8	5.5 c	3.4 b	12.9 b	22.8 b	5.4 c	5.9 b	163.8 a	160.5 a

[a] The contents of phenolic compounds were determined by HPLC chromatogram areas and the amount of cinnamic acid produced by the reaction of PAL was measured at 290 nm. Different letters in the same column indicate significant differences at $p \leq 0.05$. [b] Caffeic acid equivalent (CAE) was used to express the contents of a caffeic acid derivative and unknown compound. [c] PAL activity was expressed in picokatals per milligram of protein; 1 kat was defined as the enzyme activity producing 1 mol of cinnamic acid equivalents per second.

There are various compounds with significant antioxidant activity in plants including phenolic compounds, terpenoids, and carotenoids, but their antioxidant capacities differ depending on their structure and quantity in the plant (*33*). Among the potent antioxidants, phenolic compounds appear to be major antioxidants in the methanolic extract of romaine lettuce. To confirm this, the correlation between the total content of phenolic compounds and the DPPH• free radical scavenging capacity of the romaine lettuce methanol extract was examined (**Figure 1C**). The high correlation ($R^2 = 0.989$) indicated that the increase of the antioxidant capacity as a result of the MeJA treatment was due to the increase of phenolic compounds content. These results were similar to our previous research that showed a significant positive effect of MeJA on antioxidant phenolic compounds of sweet basil (*17*).

The 0.5 mM MeJA treatment, which produced the largest increment of phenolic content and antioxidant capacity, was also utilized to investigate the stability of induced metabolites. Most plants respond rapidly to stress by altering defense mechanisms, including secondary metabolites, but the induced levels are commonly not stable for a long time (*17*). Therefore, it was important to investigate the response of the plant throughout certain periods of time (0, 2, 4, 6, and 8 days after treatment).

Lettuce harvested 4 days after treatment was later excluded from this study due to contamination of the sample during processing.

The total phenolic content of romaine lettuce treated with 0.5 mM MeJA was significantly higher than that of the control at all harvest stages (**Figure 2**). Total phenolic content reached maximal values of 23.6 mg of GAE/g of DW 2 days after the MeJA treatment, which was 32% higher than that of the control (17.0 mg of GAE/g of DW) harvested the same day. No further increase in total amount of phenolic compounds was observed 6 and 8 days after MeJA treatment, because the plant possibly used phenolic compounds induced with MeJA treatment as sources of lignification (*34*, *35*). The total phenolic content of the control also increased with time. This was likely due to environmental changes; particularly the increase of temperature in the plastic dome may have induced accumulation of phenolics, as was observed in sugar cane sprouts exposed to heat-stress condition (*36*).

The antioxidant capacity of the romaine lettuce harvested at different days after the treatment of 0.5 mM MeJA is shown in **Figure 3**. The antioxidant capacities determined by $ABTS^{\bullet+}$ assay and $DPPH^{\bullet}$ assay increased by the application of 0.5 mM MeJA, and the capacities reached maximum values of 106 μmol of TEAC/g of DW in the $ABTS^{\bullet+}$ assay and 311 μmol of TE/g of DW in the $DPPH^{\bullet}$ assay 2 days after the treatment. These values were 69 and 74% higher than that of the control, respectively. However, no further significant change was observed 6 and 8 days after the application of MeJA, whereas the antioxidant capacity of the control continued increasing linearly until day 8, reaching values of 92 μmol of TEAC/g of DW with the $ABTS^{\bullet+}$ assay and 269 μmol of TE/g of DW with the $DPPH^{\bullet}$ assay.

Effect of MeJA on Individual Phenolic Compounds in Romaine Lettuce. To investigate the effect of MeJA on specific phenolic compounds, the methanolic extracts were analyzed with C_{18}-HPLC techniques, and all peaks were individually collected to determine their antioxidant activities (**Figure 4A**). Among the various peaks in the chromatogram, it was found that two large peaks and one small peak were altered with the MeJA application, all three containing significant antioxidant activity. The phenolic compounds in the two main peaks were identified as chlorogenic acid and a caffeic acid derivative (**Figure 4B**). Unfortunately, the compound(s) in the small peak could not be identified in this study.

In lettuce harvested 2 days after the MeJA treatment, the contents of caffeic acid and unknown compounds(s) were 26 and 7 mg of CAE/g of DW, respectively, which were 3.3- and 2-fold higher than that of the control. No further increase of caffeic acid derivative content was observed during the following 6 days, and the content of the unknown compound(s) was not significantly different from that of the control (**Table 1**) at 8 days after treatment. Contrary to the MeJA effect on total phenolic content, 8 days after the treatment the content of chlorogenic acid (3.4 mg/g of DW) was 59% lower than that of the control (5.5 mg/g of DW). Similarly, a previous study showed that the metabolic phenylpropanoid pathway in potatoes was induced by β-1,3-gluco-oligosaccharide, an elicitor. However, chlorogenic acid content declined, which was attributed to the use of chlorogenic acid as an intermediate for the formation of other induced compounds (*37*).

Despite the benefit associated with the antioxidant capacity of chlorogenic acid, this compound was regarded as a major trigger of browning tissue in lettuce (*24*). Moreover, MeJA has been indicated as an efficient treatment to extend the shelf life of produce (*38*). Our results suggest that the positive effect of MeJA on postharvest quality of lettuce (e.g., reducing browning of lettuce) may be due to the decline of chlorogenic acid; however, explanation of how the increase of other compounds positively affects the shelf life of lettuce awaits elucidation.

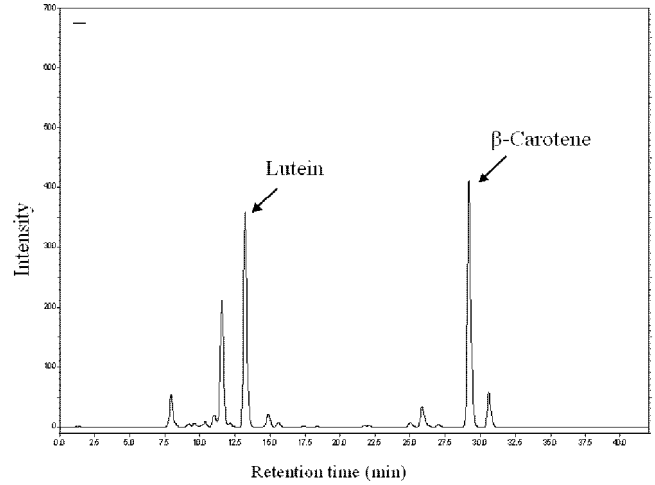

Figure 5. HPLC profile of the carotenoids of romaine lettuce. The carotenoids in romaine lettuce were extracted by hexane after saponification with 80% KOH and ethanol containing 0.1% BHT at 85 °C. The hexane extract was completely dried by nitrogen gas, and the residue recovered by methanol/MTBE (1:1) was used as the crude carotenoids of the romaine lettuce. A YMC-Carotenoid S-5 column (250 × 4.6 mm, 5 μm) was used to separate carotenoids of the romaine lettuce. Methanol/MTBE/water (81:15:4) and methanol/MTBE (9:91) were used as mobile phase. The flow rate of the eluant was 1 mL/min. The absorbance of the eluant was measured at 450 nm.

Determination of PAL Activity. The activity of PAL, a key regulatory enzyme of the phenylpropanoid pathway (*11*), was examined with the aim of better understanding the process involved in the plant's response to MeJA treatments (**Table 1**). The activity significantly increased in the MeJA-treated lettuce, whereas no significant difference in the PAL activity of the control was observed. Two and 6 days after the MeJA treatment, the PAL activities were 269 and 261 pkat/mg of protein, respectively, and these values were 55 and 58% higher than that of the control. However, 8 days after the treatment there was no significant difference between the activities of the MeJA-treated lettuce and the control lettuce. Similar to previous work which showed that phenylpropanoid metabolism in harvested lettuce was stimulated by physical injuries (*39*), our result revealed that exogenous MeJA treatments induce accumulation of phenolic compounds, such as the caffeic acid derivative, in romaine lettuce likely through the phenylpropanoid pathway.

Effect of MeJA on Carotenoid Content of Romaine Lettuce. Several carotenoids of romaine lettuce were separated using a HPLC-carotenoid S-5 column. Among carotenoids, lutein, regarded as an anti-colon cancer agent, antioxidant, and eye health promoter (*40*, *41*), and β-carotene, an important antioxidant (*42*), were identified as major carotenoids in romaine lettuce (**Figure 5**). Although it has been reported that the total content of carotenoids in barley leaf decreased 60 and 65% after 1 and 3 days of the MeJA treatment (*43*), the total amount of carotenoids in MeJA-treated lettuce did not change. However, the total carotenoid content of the control (6.0 mg of CE/g of DW) harvested 8 days after the treatment was lower than that of the MeJA treatment (7.8 mg of CE/g of DW) (**Figure 6A**). Similarly, the contents of lutein and β-carotene in the romaine lettuce were not significantly affected by the MeJA treatment (**Figure 6B**,**C**). Interestingly, lutein and β-carotene contents in the control declined

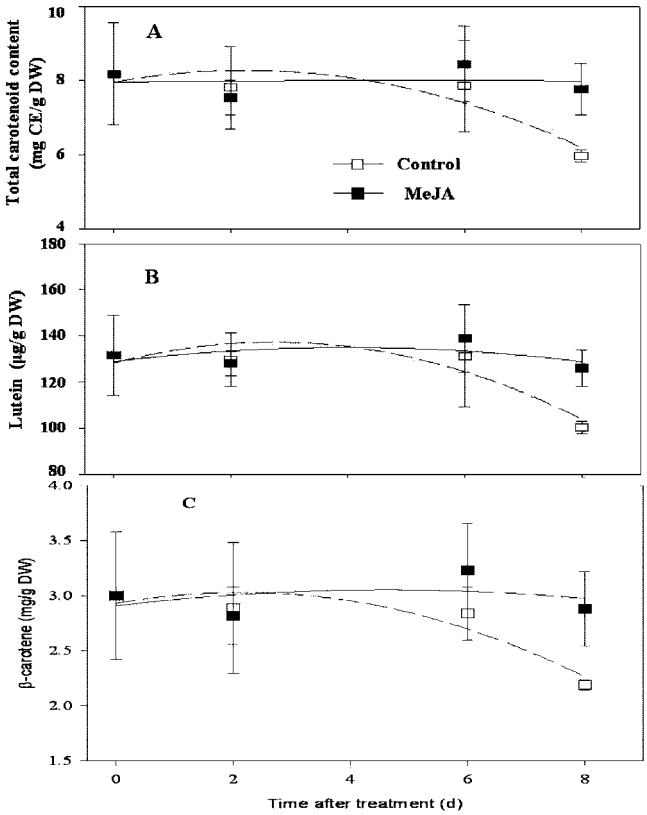

Figure 6. Time course of the MeJA effect on carotenoids in romaine lettuce. Total content of carotenoids was calculated by total area of all peaks separated by HPLC and expressed as micrograms of β-carotene equivalent (CE) per gram of DW of sample (**A**). Lutein (**B**) and β-carotene (**C**) were identified as major carotenoids in romaine lettuce. Error bars are standard deviations of triplicate experiments.

after 6 days, resulting in levels that were 26% (100.2 μg of lutein/g of DW) and 31% (2.2 mg of β-carotene/g of DW) lower than those of the MeJA treatment (126.0 μg of lutein/g of DW and 2.9 mg of β-carotene/g of DW).

Conclusions. In this study, the effect of exogenous MeJA treatment on phenolics and carotenoids of romaine lettuce was quantitatively and qualitatively evaluated. We found that MeJA can increase the amount of antioxidant phenolics in romaine lettuce. The increased content of phenolics, elicited by MeJA treatment, resulted in increased antioxidant capacities. The content of carotenoids, including lutein and β-carotene, in MeJA-treated lettuce changed until 8 days after treatments, whereas that of the control decreased during the same period. Despite the fact that more research is needed to elucidate any trade-off as a result of MeJA applications and the lack of information regarding degradation of the induced compounds, our study revealed that foliar applications of MeJA can improve some antioxidants in romaine lettuce.

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