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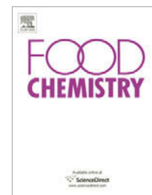

The effects of exogenous ethylene and methyl jasmonate on the accumulation of phenolic antioxidants in selected whole and wounded fresh produce

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ABSTRACT

Selected fruits and vegetables were exposed to wounding, methyl jasmonate (MJ) or ethylene (ET) stress and effects on the phenylpropanoid metabolism were determined. Lettuce, cilantro, cabbage, green beans, apples, plums, peaches, table grapes, strawberries, bell peppers, asparagus, celery, carrots, radishes, potatoes, and jicama were evaluated for phenolic content and antioxidant capacity (AOX). The phenolic synthesis response to the stresses was tissue-dependent, including decreases, increases or no effects. The use of phytohormones enhanced the wound response on some crops, confirmed by an increase in phenylalanine ammonia lyase (PAL) activity and HPLC phenolic profiles. Several reasons could explain the phenolic accumulation, including the plant genetic machinery, the presence of a common signalling response and differences in phenolic synthesis and degradation kinetics. The synthesized phenolics increased the overall AOX (μg trolox/g FW) of the tissue. Furthermore, the specific AOX (μg trolox/mg phenolics) of the synthesized phenolic compounds was influenced by type of tissue and phytohormone used.

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1. Introduction

Epidemiological and clinical investigations have revealed that diets rich in fruits and vegetables are associated with a reduced risk of cardiovascular and neurological diseases and various forms of cancer (Temple, 2000). Phytonutrients associated with these health benefits may include phenolic antioxidants (Prior & Cao, 2000; Wu et al., 2004) which can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids. Cao, Sofic, and Prior (1996) studied a broad range of vegetables, which had high levels of polyphenols and antioxidant capacity (AOX). Other studies have shown that fruits are also an excellent source of phenol antioxidants (Sun, Chu, Wu, & Liu, 2002). There is indeed an enormous diversity of phenolic antioxidants found in fruits and vegetables, and their presence and roles can be affected or modified by several pre- and postharvest cultural practices and/or food processing technologies (Goldman, Kader, & Heintz, 1999; Tudela, Cantos, Espin, Tomas-Barberean, & Gil, 2002). These phenolics are mainly synthesized in plants through the malonic and/or shikimic acid pathways, including a very important role of the key enzyme phenylalanine ammonia lyase (PAL) within the latter pathway (Dangl, Dietrich, & Thomas, 2000).

The fundamental principle underlying quality of wounded fruits and vegetables is that they are living tissues and, as a consequence, show physiological response to diverse processing and handling procedures, as well as to the package environment in which they are enclosed (Toivonen & DeEll, 2002). The effect of wounding on produce varies with the variety, temperature, water content and degree of maturity (Lamikanra, 2005). Wounding seems to induce the expression of genes encoding defence-related proteins involved in wound healing (Saltveit, 2000). Therefore, plants can react to diverse stresses, e.g. mechanical injury or exposure to plant hormones, such as ethylene (ET) and methyl jasmonate (MJ), by activating a set of responses that include transcriptional activation of wound responsive genes (Lamikanra, 2005).

Ethylene (ET) can influence a diverse array of plant growth and development processes, including germination, senescence, cell elongation, fruit ripening, and plant defence mechanisms (Kieber, 1997). Slight ET applications can increase the anthocyanin accumulation in several crops (Awad & de Jager, 2002). Other studies have shown a positive correlation between an increase in respiration rate and the phenolic content of plant tissues exposed to ET (Lafuente, Lopez-Galvez, Cantwell, & Yang, 1996; Yokotani et al., 2004).

Methyl jasmonate (MJ) can interact with other phytohormones in eliciting biological activity, and play a prominent role in signalling plant defences (Fan, Mattheis, & Fellman, 1998). Leja, Mareczek, Wojciechowska, and Rozek (1997) and O'Donnell et al. (1996) showed that MJ and ET influence each other's concentration in plant

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tissues and act together to regulate wound-induced gene expression. Other important effects of MJ applications are related to the promotion of colour in tomato fruits (Saniewski, Nowacki, & Czapski, 1987) and in tulip bulbs (Saniewski et al., 1998).

Since exogenous ET and MJ can elicit the synthesis of secondary metabolites, the ability of combinations of wounding and MJ and ET applications to enhance the synthesis of health-promoting bioactive phenolic compounds was investigated. In our approach, we used a selected group of fruits and vegetables to determine whether the phenylpropanoid metabolism was activated in different types of tissues when exposed to these stresses.

2. Materials and methods

2.1. Plant materials and reagents

Lettuce (*Lactuca sativa*), cilantro (*Coriander sativum*), cabbage (*Brassica oleracea*), green beans (*Phaseolus vulgaris*), apples (*Malus* x domestica), plums (*Prunus salicina*), peaches and nectarines (*Prunus persica*), pears (*Pyrus communis*), strawberries (*Fragaria* x ananassa), table grapes (*Vitis vinifera*), tomatoes (*Solanum lycopersicum*), bell peppers (*Capsicum annum*), asparagus (*Asparagus officinalis*), onions (*Allium cepa*), celery (*Apium graveolens*), carrots (*Daucus carota*), radishes (*Raphanus sativus*), potatoes (*Solanum tuberosum*), and jicama (*Pachyrrhizus erosus*) were obtained from a local supermarket. All produce was washed with chlorinated water (250 ppm). For the wounded studies, tissues were cut as described in Table 1. Whole and ~150 g wounded samples were each placed inside one gallon glass jars, where MJ or ET treatments were continuously applied during the 4 d of storage at 20 °C. While ET was directly injected into the jars to obtain 1000 ppm, MJ was applied by wetting a filter paper over a petri dish to obtain 250 ppm in a headspace-saturated atmosphere.

Jars were kept in dark conditions with periodic ventilation every 12 h to avoid CO_2 accumulation of >0.5%. Air was used for control samples. Sampling and analysis were done on the initial and the final day of the experiments.

Methyl jasmonate (95%), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), chlorogenic acid (CHA), vanillic acid (VA), ferulic acid, trolox and 2,2-diphenyl-1-picrylhydrazyl (DPPH·), sodium phosphate, polyvinylpolypyrrolidone (PVPP), boric acid, sodium hydroxide (NaOH), 2-mercaptoethanol, L-phenylalanine and sulfuric acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). The 1-aminocyclopropene-1-carboxylic acid (1ACC) was purchased from MP Biomedicals (Aurora, OH, USA). Ethylene (CP grade, 99.5%) and sample preparation supplies were obtained from Fisher Scientific (Houston, TX, USA), including filters, membranes and syringes. The solvents, methanol, ethanol, hexane, acetonitrile and water, were of quality HPLC-grade.

Table 1
Description of fresh cuts for the wounding studies. All samples were manually processed using disinfected stainless steel knives and plastic cutting boards.

Produce	Type of fresh-cut
Green celery	Cut stick in half (long way), then made pieces 4–5 mm thick
White onions	Peel and cut bulb in slices 4–5 mm thick, then made pieces 5 × 10 mm
Iceberg lettuce	Cut leaves to make pieces 10 × 10 mm
Orange carrots	Cut roots to make slices 3–4 mm thick
Jicama	Peel and cut in slices 10–12 mm thick, then made 8 triangular pieces
Bell peppers	Cut slices-rings 4–5 mm thick, then made pieces 5 × 10 mm
Red onions	Peel and cut bulbs in slices 4–5 mm thick, then made pieces 5 × 10 mm
Asparagus	Cut in slices 4–5 mm thick
Green cabbage	Cut leaves to make small pieces 10 × 10 mm
Red apples	Cut in slices 10–12 mm thick, then made 8 triangular pieces
Tomatoes	Cut in slices 10–12 mm thick, then made 4 triangular pieces
Nectarines	Cut in slices 10–12 mm thick, then made 8 triangular pieces
Radish	Cut in slices 2–3 mm thick, then made 4 triangular pieces
Red cabbage	Cut leaves to make small pieces 10 × 10 mm
White potatoes	Cut in slices 10–12 mm thick, then made 8 triangular pieces
Green pears	Cut in slices 10–12 mm thick, then made 8 triangular pieces

2.2. Analysis of total soluble phenolics

Phenolic content was evaluated, following the procedure of Swain and Hillis (1959). Samples of 5 g were homogenized with 20 ml of methanol using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington, NC, USA), for uniform consistency, and then incubated overnight at 4 °C. Homogenates were centrifuged (rotor JA-17; centrifuge J2-21; Beckman Coulter, Inc., Fullerton, CA, USA) at 29,000 *g* for 15 min at 4 °C. Sample aliquots of 150 µl were taken from the clear supernatant and then diluted with 2400 µl of nanopure water, followed by 150 µl of 0.25 N Folin-Ciocalteu reagents and incubated for 3 min at room temperature. The reaction was stopped by adding 300 µl 1 N Na_2CO_3 and the mixture was incubated for 10 min. Samples were again centrifuged at 29,000 *g* for 15 min at 4 °C. Absorbance readings at 725 nm of clear supernatant samples were taken, using the spectrophotometer with photo diode array detector (model 8452A, Hewlett Packard Co, Waldbronn, Germany). A blank prepared with methanol was used as control. The level of total phenolics for each sample was determined by using a standard curve developed with CHA.

2.3. Analysis of antioxidant capacity

The antioxidant or antiradical activity of phenolic compounds was evaluated following the procedure of Brand-Williams, Cuvelier, and Berset (1995). Samples were homogenized with methanol, to uniform consistency, and then refrigerated overnight. Homogenates were centrifuged at 29,000 *g* for 15 min at 4 °C. Sample aliquots of 150 µl were taken from the clear supernatants and then diluted with a 2,850 µl DPPH solution, previously prepared with methanol until reaching 1.1 units of absorbance at 515 nm. A blank was prepared with methanol to be used as a control and also to zero the spectrophotometer for further readings of all samples at 515 nm. The mixture was allowed to react in a shaker until no significant decrease in absorbance was obtained compared to the methanol-based control for antioxidant activity. The decrease in absorbance was recorded and the antioxidant activity was calculated as µg trolox equivalents, using a standard curve. In addition, we determined the specific AOX, defined as the ratio between AOX and phenolic content (µg trolox/mg phenolics) which provides information of the effectiveness of the phenolic compounds to neutralize free radicals. A higher specific AOX means that the mixture of phenolic compounds present in a tissue sample has a higher capacity to stabilize free radicals (Reyes, Villarreal, & Cisneros-Zevallos, 2007).

2.4. HPLC phenolics profile

2.4.1. Extraction and analysis

Fresh samples of 5 g were extracted with 20 ml of methanol, using the Ultra-Turrax homogenizer. Homogenates were centrifuged at 29,000 *g* for 15 min at 4 °C. Supernatant was passed through nylon membranes (0.2 µm), prior to injection into the chromatography system. Except for the SpectraPhysics SP8792 column heater (San Jose, CA, USA), all the HPLC system was composed

of Waters Co instruments (Milford, MA, USA). These are two 515 binary pumps, one 717-*plus* auto-sampler, and one 996 photodiode array detector. The column used to separate the phenolic compounds was a 4.6 × 150 mm, 5 μm, C-18 reverse-phase column (Waters Atlantis, Milford, MA, USA), which was maintained at 40 °C. The injection volume was 10 μl. Samples were analyzed under gradient conditions with two mobile phases, consisting of acidified water–HCl, pH 2.3 (solvent A) and acetonitrile (solvent B). The gradient system was 0/85, 5/85, 30/0, 35/0 (min/% solvent A). Data were processed by using Waters Millennium software v3.2. The levels of individual phenolics of carrots, including the most predominantly found chlorogenic (CHA), vanillic (VA) and ferulic acids (FA), were determined by using standard curves developed with high-purity HPLC-grade commercial phenolic compounds. In the case of isocoumarin (ISO) and dicaffeoylquinic acid (diCQA) compounds, due to unavailability of commercial standards, these were synthesized, HPLC-separated, isolated, collected and identified by mass spectrometry.

2.4.2. Synthesis, collection and confirmation test of diCQA and ISO

The synthesis, isolation, collection and confirmation tests of ISO and diCQA were done based on the methodology described by Heredia & Cisneros-Zevallos (2009). The confirmation tests for diCQA and ISO were done on a time of flight (TOF) mass spectrometer equipped with an electron spray ionization (ESI) in negative ion mode $(M–H)^-$ (PE Sciex API QStar Pulsar, Concord, Ontario, Canada). The TOF–MS procedure was done in collaboration with the Department of Chemistry at Texas A & M University (College Station, TX, USA). Levels of diCQA were expressed as CHA equivalents.

2.5. Phenylalanine ammonia lyase (PAL) enzyme activity

PAL was extracted from 1 g of fresh produce in 25 ml of borate buffer (pH 8.5) according to Ke and Saltveit (1986). Samples were placed inside ice and homogenized using an Ultra-Turrax homogenizer, under low-light conditions and at low speed to prevent protein denaturation. Homogenates were filtered through cheesecloth and then taken to centrifuge at 29,000 *g* for 15 min at 4 °C. The enzyme activity of PAL was assayed by following the accumulation of cinnamic acid at 290 nm, using 100 mM L-phenylalanine as PAL substrate and water for control samples. Units were reported as μmol of *t*-cinnamic acid/h g fresh weight.

2.6. Statistical analysis

The experiment followed a completely randomized design. Analyses were done using 9 replicates, unless otherwise indicated. Means, standard deviations, graphs and linear regressions were obtained using Microsoft Excel 2000. For means comparisons at the 5% significance level, ANOVA and LSD multiple-range tests were performed, using SAS (Raleigh, NC, USA).

3. Results and discussion

3.1. Effect of MJ and ET on the phenolic content of whole tissues

Results indicated that there were non significant differences in the total phenolic content between initial day controls vs. air control samples after 4 d of storage at 20 °C. The only exception was cilantro which showed a slight decrease in total phenolic content. The postharvest exposure of asparagus, potatoes, apples, peaches, strawberries and table grapes to MJ and ET plant hormones did not induce an increase in the phenolic content in each crop when compared to initial day evaluations or air control after 4 d (Table 2). Only, carrots with ET, plums with MJ and lettuce and green beans with MJ and ET showed significant increases in the phenolic content of ∼10–15%. Although there is limited comparative evidence of the role of both phytohormones as postharvest abiotic stresses, the concentration of phenolics found in these commodities coincides with those reported in the literature (Wu et al., 2004). Furthermore, the response of phenolic compounds in the ET-stressed whole carrots and lettuce studies, showed similarities to previous reports (Campos-Vargas & Saltveit, 2002; Lafuente et al., 1996).

3.2. Effect of MJ and ET on the AOX of whole tissues

The AOX showed no significant differences between initial day and air control evaluations, with the exception of slight decreases (∼15%) for cilantro and asparagus (Table 3). Whole lettuce and plums exposed to MJ, and strawberries exposed to ET showed increases of ∼50%, 25% and 20%, respectively, after 4 d of storage and compared to initial day or air control samples. The rest of the crops did not show an increase in AOX after exposure to MJ or ET. Lettuce and carrots showed positive correlations ($R^2 = 0.91$ and $R^2 = 0.90$, respectively) between the newly synthesized phenolics and the AOX, which coincides with previous reports (Kang & Saltveit, 2002; Heredia & Cisneros-Zevallos, 2002). In the case of the red-coloured tissues, e.g. plums and strawberries, previous reports also show high values of AOX, perhaps due to the high anthocyanin content (Cevallos, 2001; Wu et al., 2004).

To determine the AOX of the phenolic compounds present in each tissue, the term specific AOX was used and defined as the ratio between total-AOX and total phenolics (AOX on phenolic basis). When plotting the calculated specific AOX values, the results showed that these commodities have different specific antioxidant capacities due very likely to different phenolic profiles (e.g., fruits

Table 2
Total phenolic content of selected whole fresh produce. Samples were evaluated on the initial day before treatment (control) and after 4 d of storage at 20 °C (AC, 250 ppm MJ and 1000 ppm ET). Results show means of 9 replicates and their standard deviations. Letters in parentheses show mean comparisons among treatments of each produce (LSD $P > 0.05$).

Whole produce	Total phenolics [mg CHA/100 g FW]			
	Initial day control	Air control	Methyl jasmonate	Ethylene
Iceberg lettuce	18 ± 2(b)	19 ± 4(b)	26 ± 5(a)	23 ± 4(a)
Cilantro	392 ± 21(a)	346 ± 28(b)	355 ± 20(b)	392 ± 13(a)
Green beans	48 ± 10(b)	52 ± 6(b)	59 ± 11(a)	62 ± 10(a)
Asparagus	175 ± 12(a)	163 ± 19(a)	169 ± 23(a)	169 ± 20(a)
Orange carrots	69 ± 12(b)	72 ± 10(b)	74 ± 6(ab)	79 ± 11(a)
White potatoes	84 ± 6(a)	85 ± 6(a)	90 ± 17(a)	88 ± 9(a)
Red apples	358 ± 18(a)	348 ± 19(a)	365 ± 23(a)	364 ± 23(a)
Plums	248 ± 27(b)	250 ± 36(b)	284 ± 16(a)	256 ± 37(ab)
Peaches	86 ± 10(a)	90 ± 7(a)	86 ± 10(a)	84 ± 10(a)
Red table grapes	150 ± 11(ab)	146 ± 15(b)	146 ± 10(b)	157 ± 5(a)
Strawberries	383 ± 40(ab)	376 ± 31(b)	409 ± 42(ab)	416 ± 37(a)

Table 3
Antioxidant capacity of selected whole fresh produce. Samples were evaluated on the initial day before treatment (control) and after 4 d of storage at 20 °C (AC, 250 ppm MJ and 1000 ppm ET). Results show means of 9 replicates and their standard deviation. Letters in parentheses show mean comparisons among treatments of each produce (LSD $P > 0.05$).

Whole produce	Antioxidant capacity [μg trolox eq/g FW]			
	Initial day control	Air control	Methyl jasmonate	Ethylene
Iceberg lettuce	58 ± 16(c)	76 ± 18(bc)	101 ± 35(a)	77 ± 22(bc)
Cilantro	2865 ± 266(ab)	2417 ± 342(c)	2623 ± 240(bc)	3016 ± 461(a)
Green beans	228 ± 74(a)	211 ± 43(a)	205 ± 66(a)	216 ± 49(a)
Asparagus	1527 ± 185(a)	1319 ± 244(b)	1327 ± 205(ab)	1298 ± 227(b)
Orange carrots	436 ± 110(ab)	523 ± 157(ab)	406 ± 66(b)	534 ± 159(a)
White potatoes	434 ± 83(a)	439 ± 40(a)	452 ± 70(a)	428 ± 31(a)
Red apples	3319 ± 347(a)	3321 ± 338(a)	3223 ± 383(a)	3191 ± 192(a)
Plums	2139 ± 236(b)	2278 ± 371(b)	2597 ± 95(a)	2363 ± 374(ab)
Peaches	740 ± 119(a)	780 ± 98(a)	739 ± 106(a)	699 ± 127(a)
Red table grapes	1835 ± 458(a)	1710 ± 334(a)	1851 ± 257(a)	1991 ± 318(a)
Strawberries	3668 ± 617(b)	3453 ± 357(b)	3992 ± 719(ab)	4343 ± 566(a)

rich in red pigments, which showed the highest ratios) (Fig. 1). Whole produce can be arranged according to the specific AOX in the following decreasing order: red table grapes > strawberries ≈ apples > asparagus ≈ plums ≈ peaches > cilantro > carrots > potatoes ≈ green beans > lettuce. Based on this ranking, phenolics from table grapes have a higher radical-scavenging capacity than have phenolics from the other studied crops.

3.3. Effect of MJ and ET on the phenolic content of wounded tissues

Celery, white and red onions, lettuce, carrots and jicama were the only samples to show an increase in phenolic content due to wounding stress (∼15–80%) (Table 4). Other crops were not affected by wounding. Wounded celery, lettuce and carrots showed a larger increase in phenolic content with applications of MJ and ET (∼72–130%), when compared to wounded tissue alone. Wounded jicama, bell peppers and red onions only increased with ET (∼10–30%) but not with MJ.

Wounding is signalling the phenylpropanoid metabolism, as observed by the increase in phenolic content of some tissues. Furthermore, the increase in total phenolic content of wounded lettuce and carrots indicates that plant hormone stresses have a greater effect in the presence of wounding stress (Table 4) compared to whole tissues (Table 2). The reason for the observed synergistic effect between wounding and hormones in both crops is not clear. One possible explanation would be related to the fact that the two stresses may be sharing a common signalling molecule which, when added, may reach a certain threshold that amplifies the response. For example, signalling molecules generated, such as superoxide radicals, may trigger the production of further superoxide radicals by a feedback mechanism through an increase in activity of NADPH oxidase (Mittler, Vanderauwera, Gollery, & Van Breusegem, 2004). In addition, an increased respiration rate may produce more ROS through the electromagnetic process of moving electrons across the cell membrane (Murphy & DeCoursey, 2006; Rakwal & Agrawal, 2003).

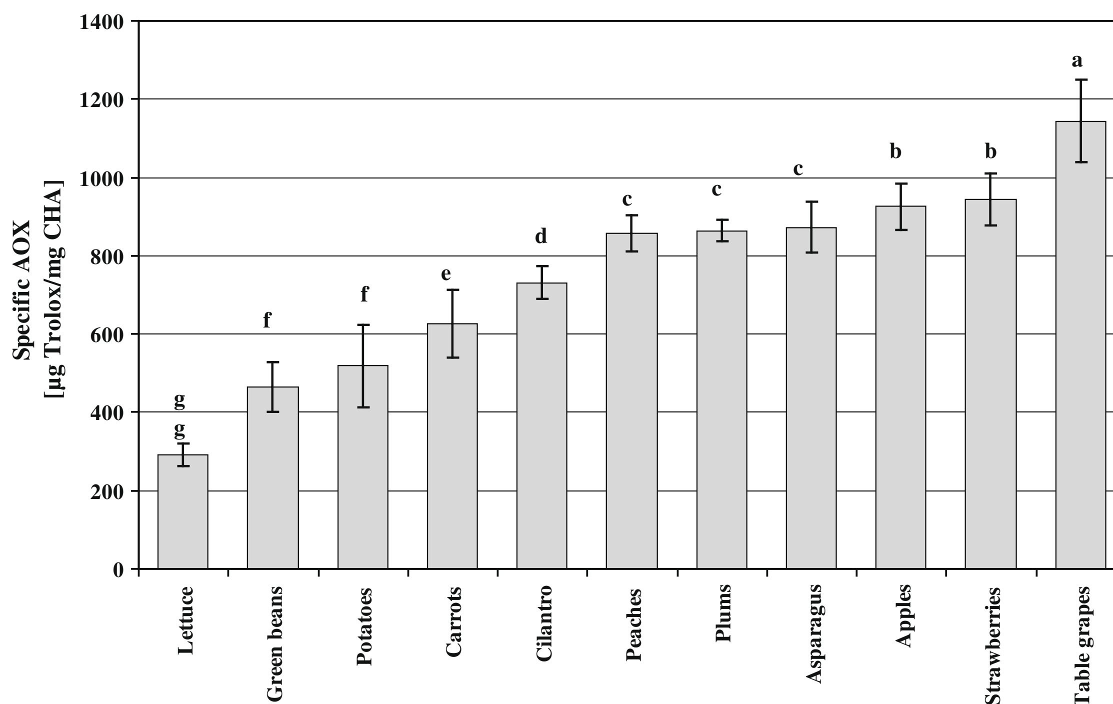

Fig. 1. Specific antioxidant capacity shown as the ratio between total-AOX and total phenolics present in each crop. Total phenolics and AOX analysis were done on the initial day. Results show means of 9 replicates, and bars show standard deviation. Letters over bars show mean comparison (LSD $P > 0.05$).

Table 4
Total phenolics content of selected wounded fresh produce. Samples were evaluated on the initial day before treatment (control) and after 4 d of storage at 20 °C (AC, 250 ppm MJ and 1000 ppm ET). Results show means of 9 replicates and their standard deviation. Letters in parentheses show mean comparisons among treatments of each produce (LSD $P > 0.05$).

Wounded produce	Total phenolics [mg CHA/100 g FW]			
	Initial day control	Air control	Methyl jasmonate	Ethylene
Celery	18 ± 2(c)	25 ± 3(b)	32 ± 2(a)	33 ± 4(a)
White onions	105 ± 5(c)	126 ± 8(ab)	120 ± 9(b)	128 ± 5(a)
Lettuce	22 ± 3(d)	34 ± 4(c)	38 ± 4(b)	48 ± 3(a)
Carrots	68 ± 9(d)	138 ± 7(c)	146 ± 7(b)	157 ± 4(a)
Jicama	60 ± 5(c)	71 ± 4(b)	69 ± 6(b)	80 ± 3(a)
Bell peppers	214 ± 17(b)	218 ± 23(b)	225 ± 16(ab)	237 ± 22(a)
Red onions	104 ± 9(c)	123 ± 5(b)	127 ± 6(ab)	132 ± 5(a)
Asparagus	170 ± 17(a)	162 ± 8(ab)	152 ± 9(b)	163 ± 16(ab)
Cabbage	101 ± 4(ab)	97 ± 3(bc)	94 ± 5(c)	104 ± 6(a)
Red apples	308 ± 26(a)	278 ± 23(b)	271 ± 9(b)	271 ± 22(b)
Tomatoes	94 ± 8(a)	95 ± 6(a)	96 ± 9(a)	91 ± 7(a)
Nectarines	85 ± 5(ab)	80 ± 5(b)	86 ± 7(a)	86 ± 4(a)
Radish	115 ± 9(a)	113 ± 5(a)	107 ± 5(b)	117 ± 5(a)
Red cabbage	341 ± 40(a)	341 ± 28(a)	347 ± 28(a)	336 ± 24(a)
White potatoes	115 ± 6(a)	116 ± 10(a)	116 ± 12(a)	116 ± 6(a)
Pears	88 ± 11(a)	76 ± 7(b)	75 ± 8(b)	75 ± 9(b)

Previous studies have shown that wounding elicits an increase in phenylalanine ammonia lyase activity (PAL), with a corresponding increase in phenolic content in tissues such as lettuce and carrots (Campos-Vargas & Saltveit, 2002; Christoffersen & Laties, 1982).

In the present study, asparagus, cabbage, apples, tomatoes, nectarines, radishes, potatoes, and pears did not apparently respond to both stresses and some tissues even showed significant decreases in the phenolic content. In general, tissues that did not respond or showed a reduction in phenolic content show that the stresses do not affect these tissues at the gene level. However, there could be an alternative explanation. For the apparently non responsive produce, the lack of increase in phenolic content may be due to similar kinetics of phenolic synthesis and degradation, while for tissues with a reduction in phenolic content, this would be related to a greater phenolic degradation kinetic compared to phenolic synthesis. For example, the presence of reduction–oxidation reactions involving the polymerization and degradation of phenolic compounds (browning) through the activation of diverse antioxidant enzymes (i.e., peroxidases), may reduce the overall phenolic content of the tissue (Reyes et al., 2007).

3.4. Effects of MJ and ET on the AOX of wounded tissues

Similar trends to those of phenolics were obtained for the AOX of wounded produce, including celery, white and red onions, lettuce, carrots and jicama. When MJ and ET were applied to the above wounded tissues, a greater increase in AOX was observed with the exception of white onion and jicama (Table 5). Among the tissues, lettuce and carrots showed the largest increase in AOX with combinations of wounding and plant hormones (~300–400% over the control).

Among the commodities that responded to wounding or combination of wounding and hormones, wounded carrot and lettuce tissues showed marked increases in the specific AOX values (Fig. 2). For wounded lettuce tissues, applications of hormones further increased the specific AOX. However, for carrot tissues, hormones did not increase the specific AOX compared to wounding *per se*. This suggests that wounding stress or a combination of wounding and exogenous hormone applications may influence the phenolic profiles associated with the different specific AOX values of lettuce and carrot tissues. On the other hand, jicama did not show an increase in specific AOX after wounding or combination of wounding and hormones, indicating that the phenolic profiles were not

Table 5
Antioxidant capacity of selected wounded fresh produce. Samples were evaluated on the initial day before treatment (control) and after 4 d of storage at 20 °C (AC, 250 ppm MJ and 1000 ppm ET). Results show means of 9 replicates and their standard deviation. Letters in parentheses show mean comparisons among treatments of each produce (LSD $P > 0.05$).

Wounded produce	Antioxidant capacity [μg trolox Eq/g FW]			
	Initial day control	Air control	Methyl jasmonate	Ethylene
Celery	70 ± 12(c)	110 ± 12(b)	136 ± 9(a)	114 ± 28 (b)
White onions	291 ± 41(c)	423 ± 59(ab)	378 ± 52(b)	440 ± 42(a)
Lettuce	29 ± 10(d)	129 ± 28(c)	162 ± 28(b)	279 ± 16(a)
Carrots	459 ± 99(c)	2182 ± 230(b)	2320 ± 218(b)	2556 ± 266(a)
Jicama	182 ± 18(c)	216 ± 20(ab)	203 ± 28(bc)	227 ± 27(a)
Bell peppers	1479 ± 222(a)	1462 ± 161(a)	1513 ± 132(a)	1591 ± 170(a)
Red onions	349 ± 52(c)	390 ± 31(b)	397 ± 48(b)	450 ± 35(a)
Asparagus	1445 ± ± 148(ab)	1503±142(a)	1348 ± 107(b)	1555 ± 109(a)
Green cabbage	657 ± 30(a)	586 ± 31(b)	573 ± 67(b)	684 ± 48(a)
Red apples	2629 ± 207(a)	2523 ± 200(a)	2559 ± 87(a)	2589 ± 169(a)
Tomatoes	559 ± 63(b)	551 ± 69(b)	636 ± 79(a)	558 ± 59(b)
Nectarines	581 ± 89(a)	410 ± 48(b)	385 ± 36(b)	395 ± 48(b)
Radish	630 ± 68(a)	546 ± 45(b)	485 ± 32(c)	569 ± 15(b)
Red cabbage	2117 ± 380(a)	2036 ± 288(a)	2128 ± 285(a)	2113 ± 256(a)
White potatoes	609 ± 99(ab)	678 ± 85(a)	651 ± 78(a)	544 ± 29(b)
Pears	381 ± 68(ab)	371 ± 38(b)	397 ± 49(ab)	430 ± 51(a)

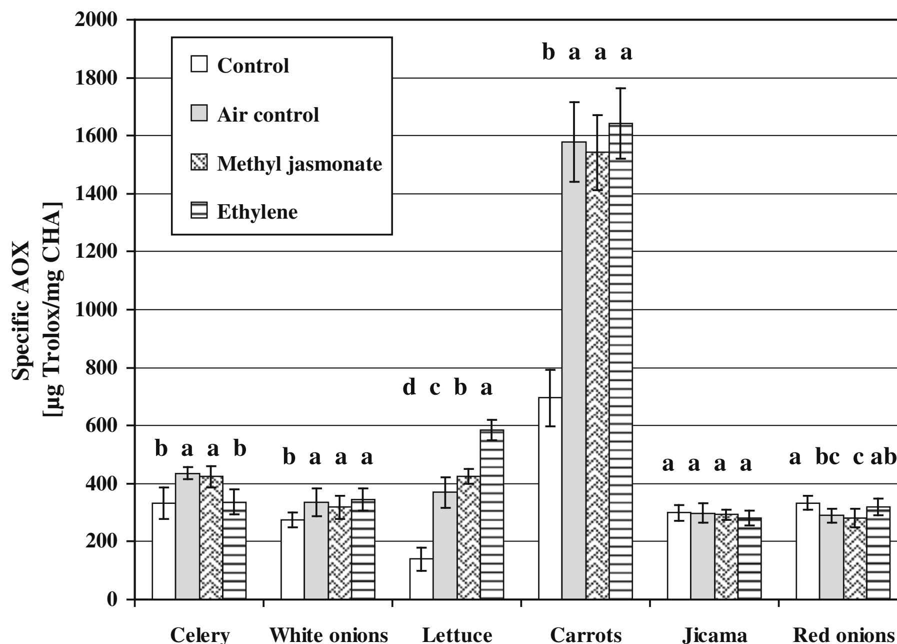

Fig. 2. Specific antioxidant capacity for wounded produce exposed to MJ (250 ppm) and ET (1000 ppm) for 4 d of storage at 20 °C. Results show means of 9 replicates, and bars show standard deviation. Letters show mean comparisons within each produce (LSD $P > 0.05$).

altered. If a combination of stresses (e.g., wounding and hormones) alters the phenolic profiles, this would imply that individual stresses are triggering the synthesis of specific enzymes of different branches in the phenylpropanoid metabolism.

3.5. Effects of MJ and ET on PAL activity of wounded tissues

The increase in total phenolic content of carrots, lettuce and jicama corresponds to an increase in PAL activity (Fig. 3). The wounding stress caused a synthesis of the main enzyme of the phenylpropanoid pathway and was further enhanced by both hormones in carrot and jicama but not in lettuce tissues. PAL activity has previously been shown to increase in lettuce after applying MJ (Campos-Vargas & Saltveit, 2002) and ET (Lopez-Galvez, Saltveit, & Cantwell, 1996). Lettuce response in the present study differs from previous reports and could be related to differences in lettuce cultivars and wounding intensities used, among other factors. In general, each tissue studied showed high linear correlations between total phenolics and PAL activity ($R^2 > 0.65$) (Fig. 4); however, each tissue had different slopes, indicating that a certain increase in the amount of PAL activity will produce different increases in the amounts of synthesized phenolics.

3.6. Effects of MJ and ET on the HPLC phenolic content of wounded carrot tissues

The HPLC provided chromatograms with the major peaks found for all treatments (Fig. 5). In order of retention time, the phenolic compounds and their derivatives were chlorogenic acid (CHA, 5.6 min), vanillic acid (VA, 9.8 min), ferulic acid (FA, 14.8 min), dic-

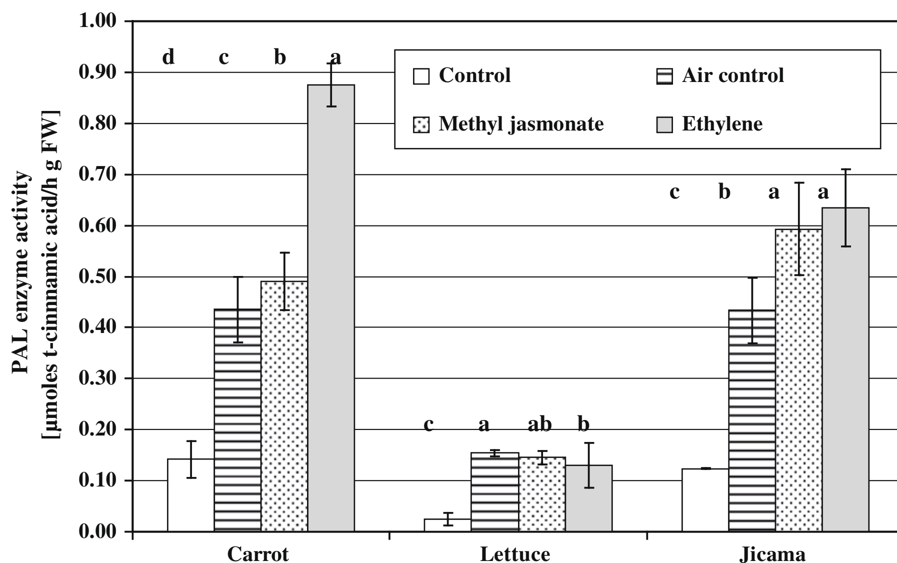

Fig. 3. PAL enzyme activity from selected wounded commodities. Samples were evaluated on the initial day before treatment (control) and after 4 d of storage at 20 °C (AC, 250 ppm MJ and 1000 ppm ET). Results show means of 9 replicates, and bars show standard deviation. Letters show mean comparisons within each produce (LSD $P > 0.05$).

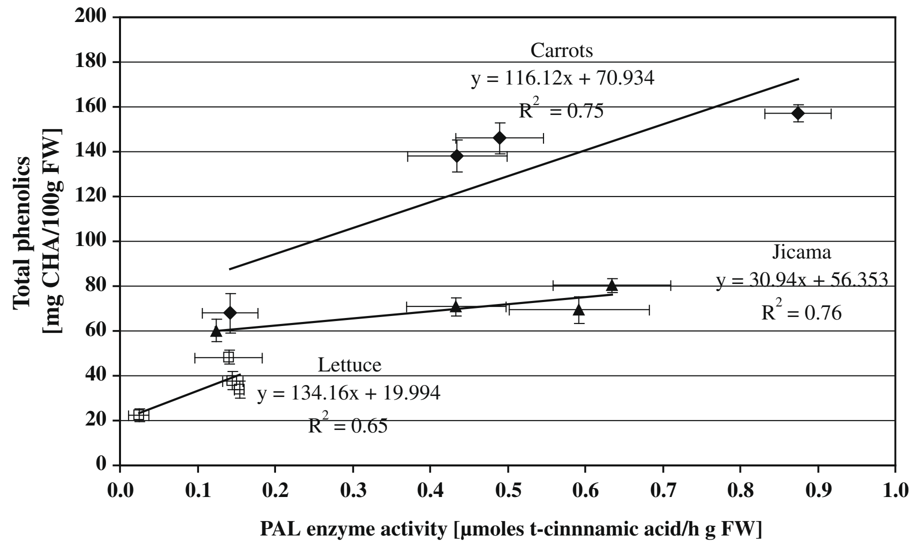

Fig. 4. Correlation between total phenolics and PAL enzyme activity from selected wounded commodities. Samples were evaluated on the initial day before treatment (control) and after 4 d of storage at 20 °C (AC, 250 ppm MJ and 1000 ppm ET). Each data point was obtained from 9 replicates, and bars show standard deviation. R^2 values refer to the coefficient of determination of the fit.

Fig. 5. Typical HPLC phenolic profile (shown at 320 nm) from methanolic extracts of hormone stressed wounded carrots. Control represents analysis at initial day, while AC, MJ (250 ppm) and ET (1000 ppm) were done after 4 d of storage at 20 °C. The identified peaks are chlorogenic acid (CHA, 5.6 min), vanillic acid (VA, 9.8 min), ferulic acid (FA, 14.8 min), dicaffeoylquinic acid (diCQA, 17.4 min) and isocoumarins (ISO, 23.9 min).

affeoylquinic acid (diCQA, 17.4 min) and ISO (23.9 min). The phenolic profile differed for each stressed carrot compared to the control on the initial day (Table 6). Results indicated that wounded stressed carrot samples accumulated mainly CHA (~80% increase)

Table 6
Main phenolic acids from methanolic extracts of wounded and wounded + hormone stressed carrots. Phenolics were obtained by reading abs at 320 nm, while isocoumarins were read at abs 267 nm. Samples were evaluated on the initial day (control) and after 4 d of storage at 20 °C (AC, 250 ppm MJ and 1000 ppm ET). Results show means of 9 replicates and their standard deviation. Letters in parentheses in each row show mean comparisons among treatments (LSD $P > 0.05$).

Phenolic acids	[mg phenolics/100 g FW]			
	Initial day control	Air control	Methyl jasmonate	Ethylene
Chlorogenic acid	14.5 ± 2.2(d)	51.7 ± 3.4(c)	47.3 ± 3(b)	69.5 ± 4.2 (a)
Vanillic acid	0.45 ± 0.13(c)	0.49 ± 0.20(b)	0.58 ± 0.35(c)	0.86 ± 0.43(a)
Ferulic acid	0.07 ± 0.01(c)	0.28 ± 0.06(b)	0.27 ± 0.05(b)	0.37 ± 0.08(a)
Dicaffeoyl-quinic acid	1.4 ± 0.43(c)	10.9 ± 2.8(a)	9.8 ± 1.1(a)	5.2 ± 2.3(b)
Isocoumarins	0.25 ± 0.02(d)	0.35 ± 0.06(c)	0.55 ± 0.17(b)	5.76 ± 1.68(a)

and its derivative diCQA (∼17% increase). These values were similar in MJ treated samples while, in ET-treated samples, there was a larger increase in CHA (∼85%) and a smaller increase in diCQA (∼6%). ET-treated samples showed an additional increase in the synthesis of the bitter compound ISO (∼450%) compared to the control. On the other hand, the use of MJ did not significantly increase the levels of ISOs, suggesting that MJ and ET trigger specific enzymes in the phenylpropanoid pathway other than PAL.

4. Conclusions

The selected whole produce studied did not show an increase in total phenolic content or in AOX after 4 d of storage at 20 °C. However, the use of MJ slightly enhanced the phenolic content of lettuce, green beans and plums, while ET caused an increase in lettuce, green beans and carrots. Whole cilantro, asparagus, potatoes, apples, peaches and table grapes were not affected by exogenous hormones.

Wounding stress enhanced the TP of carrots, lettuce, jicama, red onions, white onions and celery, with a corresponding increase in AOX, with the exception of wounded bell peppers. Other tissues, including asparagus, cabbage, apples, tomatoes, nectarines, radishes, potatoes and pears, did not show an increase in TP due to wounding stress.

The effects of ET and MJ on wounded lettuce, celery, red onions, carrots and jicama tissues enhanced the wounding stress response. The reason for the enhancement effect is not clear. However, we propose that both stresses may be sharing in part a common signalling molecule which, when added, may reach a certain threshold that amplifies the response. This possible synergistic effect may be influenced by hormone dose and wounding intensity. This observation requires further investigation.

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