Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 1254–1262
Food Chemistry
www.elsevier.com/locate/foodchem

The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue

L. Fernando Reyes, J. Emilio Villarreal, Luis Cisneros-Zevallos *

Department of Horticultural Sciences, Texas A&M University, College Station, HFSB Bldg Rm 202, MS 2133, TX 77843-2133, United States

Received 24 January 2006; received in revised form 14 March 2006; accepted 16 March 2006

Abstract

Wounding of fresh produce may elicit an increase in antioxidant capacity associated with wound-induced phenolic compounds. However, there have been no reports on the wounding response of different types of fresh produce. Changes in antioxidant capacity, total soluble phenolics, ascorbic acid, total carotenoids and total anthocyanins were evaluated after wounding in zucchini, white and red cabbage, iceberg lettuce, celery, carrot, parsnips, red radish, sweetpotato and potatoes. Phenolic changes ranged from a 26% decrease to an increase up to 191%, while antioxidant capacity changes ranged from a 51% decrease to an increase up to 442%. Reduced ascorbic acid decreased up to 82%, whereas the changes in anthocyanins and carotenoids were less evident. In general, the wound response was dependent on the type of tissue and influenced by the initial levels of reduced ascorbic acid and phenolic compounds. Wounding may increase the antioxidant content towards the development of selected healthier fresh-cut produce.

Keywords: Wounding; Antioxidant capacity; Phenylalanine ammonia lyase activity; Phenolic compounds; Ascorbic acid; Reactive oxygen species

1. Introduction

There has been increasing interest in the characterization of antioxidant phytochemicals due to their distinct bioactive properties (Dillard & German, 2000; Shetty & McCue, 2003). Antioxidants scavenge reactive oxygen species (ROS) that can cause cell damage in plant tissues. ROS are generated in normal metabolic processes as by-products of cell metabolism and are also actively involved in the signalling and function of antioxidant systems within the cell to detoxify reactive products from oxidative stress (Mittler, 2002). Recent studies have suggested a more active involvement of ROS as signal mediators upon stresses (Desikan, Mackerness, Hancock, & Neill, 2001; Moon et al., 2003; Neill, Desikan, Clarke, Hurst, & Hancock, 2002; Saran & Bors, 1989). For example, ROS participate as signal messengers after wounding (Orozco-Cárdenas, Narváez-Vázquez, & Ryan, 2001). ROS also mediates the biosynthesis and polymerization of lignin (Razem & Bernards, 2002) and the cross-linking reactions of lignin in the formation of suberin (Ros, 1997), the main compound synthesized during wound healing. This novel role of ROS could further help understand the tissue responses upon abiotic stresses (Mittler, 2005).

Results from recent research have shown that the diverse phenolic compounds present in fruits and vegetables are responsible for the high antioxidant capacity shown by these produce (Proteggente et al., 2002). In view of this, an increase in the consumption of fruits and vegetables has been suggested so as to observe the beneficial effects of the antioxidant phytochemicals (Prior & Cao, 2000). Stresses such as light, temperature, water-stress and wounding, affect the physiology of fresh produce by triggering responses that could induce the accumulation of phenolic compounds or other secondary metabolites (Kays, 1997; Saltveit, 1997). For example, after wounding,

* Corresponding author. Tel.: +1 979 845 3244; fax: +1 979 845 0627.
E-mail address: lcisnero@taexgw.tamu.edu (L. Cisneros-Zevallos).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.03.032

the activation of phenylalanine ammonia lyase (PAL; EC 4.3.1.5) is followed by the synthesis of protective phenolic compounds to reduce water loss or pathogen attack (Rhodes & Wooltorton, 1978). Moreover, recent research has shown that wounding increases the antioxidant capacity of carrots (Heredia & Cisneros-Zevallos, 2002), lettuce (Kang & Saltveit, 2002) and purple-flesh potatoes (Reyes & Cisneros-Zevallos, 2003). This increase was found to correlate with the observed increase in total phenolic content after wounding.

Given that research has confirmed the increase in antioxidant content of produce after wounding, there is a need to better understand if these responses are tissue-dependent. The objective of this paper was to study the response of selected fruits and vegetables to wounding and its effects on different antioxidant phytochemicals. Changes in the content of phenolic compounds, carotenoids and ascorbic acid, were determined and the resulting changes in antioxidant capacity characterized. This paper will provide important information towards the selection and characterization of healthier fresh-cut produce.

2. Materials and methods

2.1. Plant material

Ten different produce were selected. Zucchini (*Cucurbita pepo* L.), white cabbage (*Brassica oleracea* L. convar. *capitata* var. *alba* DC.), red cabbage (*B. oleracea* L. convar. *capitata* var. *rubra* DC.), iceberg lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), carrot (*Daucus carota* L.), parsnips (*Pastinaca sativa* L.), red radish (*Raphanus sativus* L.), sweetpotato (*Ipomoea batatas* L.) and potatoes (*Solanum tuberosum* L. cv Russet Norkotah), were purchased from a local supermarket. Produce were washed with water to remove excess dirt.

2.2. Sampling procedure and experimental setup

The antioxidant changes in fresh produce were studied under intense wounding stress conditions (shredding) and storage at 15 °C to accelerate the tissue's response. Samples and utensils were disinfected in chlorinated water (250 ppm) before the experiment. External leaves from lettuce, white cabbage or red cabbage heads were removed and internal leaves used for sampling. Green tissue from lettuce leaves was removed. Whole leaves from white and red cabbage were used. The ends of celery stems were removed and the main stem section used (~15–20 cm). The stem end of zucchini, carrots, parsnips and radish were cut to remove the presence of stem or leaves. Potato tubers and sweetpotato roots were used as is. Samples were cut with a knife and shredded using a West Bend® High Performance Food Processor (The West Bend Co.; West Bend, WI). The number of shredded pieces obtained with the food processor was 72, 53, 23, 21, 13, 12, 10, 6, 5 and 5 pieces/g for white cabbage, red cabbage, lettuce, celery, parsnips, carrot, radish, zucchini, sweetpotato and potato, respectively.

For each crop, whole and wounded samples were placed separately in 4-L clear glass jars and stored for 2 days at 15 °C. Analyses were performed using nine replicates for each of the whole and wounded samples. Samples were stored in three different jars containing three replicates each. One replicate consisted of one fruit (zucchini), three leaves (from a head of lettuce, white cabbage or red cabbage), three stems (celery), one root (sweetpotato, carrot, parsnips or radish), or one tuber (potato). Jars were closed and vented every 8 h to avoid CO_2 accumulation $\geqslant 0.5\%$. After 2 days, experiment was stopped and samples taken for the required analyses.

2.3. Quantification of PAL-activity

The assay was adapted from Ke and Saltveit (Ke & Saltveit, 1986). A 1-g sample was added with 0.2 g polyvinylpolypyrrolidone (to remove phenolic and pigment interferences) and homogenized with 25 mL of borate buffer (pH 8.5) containing β-mercaptoethanol (400 μL/L) in an Ultra-Turrax T25 homogenizer (IKA Labortechnik) at low speed until uniform consistency. Samples were kept in ice throughout the assay. Extracts were passed through 4-layers of cheesecloth and centrifuged at 32,000*g* for 15 min at 2 °C. Two sets of tubes containing 5 mL of enzyme were prepared in clean 10-mL test tubes. One set of samples was warmed at 40 °C for 5 min and 0.55 mL of nanopure water added (control samples). Absorbance readings were taken at 290 nm using 1-cm quartz cuvettes in a spectrophotometer (Hewlett–Packard 8452A photodiode array) previously blanked with the borate buffer. The other set of samples were warmed up, 0.55 mL of 100 mM L-phenylalanine was added and absorbance readings taken in a similar way. Both set of samples were then incubated at 40 °C for 1 h and absorbance readings taken again. PAL-activity was calculated as μmol t-cinnamic acid formed per hour from a standard curve developed for this chemical (0–0.15 μmol/mL).

2.4. Quantification of total soluble phenolics and antioxidant capacity

Soluble phenolics were extracted by homogenizing 5-g samples with 25 mL of methanol until uniform consistency, covered and stored overnight at 2 °C. The methanolic extracts were then centrifuged as described above; the collected supernatant was used to quantify the total phenolic content and antioxidant capacity of the extracts. Total soluble phenolics were quantified according to Swain and Hillis (1959) as follows: a 150-μL aliquot of the clear methanolic extract was diluted with 2400-μL nanopure water. Simultaneously, a blank sample was prepared with pure methanol and treated in the same way as the samples. After adding 150 μL of 0.25 N Folin–Ciocalteu, the diluted extracts were vortexed and allowed to react for 3 min. At

3 min, 300 μL of 1 N sodium carbonate was added and allowed to react for ~2 h. The spectrophotometer was blanked with the prepared methanol blank, the samples placed in 1-cm quartz cuvettes and measurements taken at 725 nm. Soluble phenolics were quantified as mg chlorogenic acid from a standard curve developed for this standard (0–0.35 mg/mL). Antioxidant capacity was measured according to Brand-Williams, Cuvelier, and Berset (1995) as follows: a 150-μL aliquot of the clear methanolic extract obtained for the quantification of phenolics was added 2850 μL of a 2,2-diphenyl-1-picrylhydrazyl solution with an absorbance of ~1.1 at 515 nm. Simultaneously, 150 μL of pure methanol was treated in the same way as the samples. The reacting samples were allowed to react until steady state conditions were reached (~24 h) (Cevallos-Casals & Cisneros-Zevallos, 2003). The spectrophotometer was blanked with methanol, the solutions placed in 1-cm quartz cuvettes and the absorbance at 515 nm recorded. Antioxidant capacity was calculated by measuring the decrease in absorbance of the samples as compared to the methanol samples, and quantifying as μg Trolox equivalents from a standard curve developed for Trolox (0–800 μM). In addition, specific antioxidant capacity was defined in this study as the ratio of total antioxidant capacity/total soluble phenolics and expressed as μg Trolox equivalents/mg chlorogenic acid. The specific antioxidant capacity provides information of the effectiveness of phenolics to neutralize free radicals. A higher specific antioxidant capacity means phenolic compounds have a higher capacity to stabilize free radicals.

2.5. Quantification of ascorbic and dehydroascorbic acid

Five-gram samples were homogenized with 3% citric acid until uniform consistency. Samples were kept in ice and in the dark throughout the assay. Extracts were filtered through four layers of cheesecloth and centrifuged using the conditions described above. The supernatant was filtered through 0.22 μm syringe filters. A disposable Waters SepPak Plus Cartridge (Waters Corp., Milford, MA) was activated with 4 mL methanol followed by 10 mL nanopure water; 4-mL of the sample was passed through the cartridge to remove interfering compounds and the last 1-mL collected and used for HPLC analysis. An additional cartridge was used in this purification step for sweetpotato, red cabbage and radish, due to interfering phenolic compounds. Ascorbic acid was separated isocratically using a mobile phase of ACN:H_2O (70:30) containing 0.01 M $(NH_4)H_2PO_4$ (pH 4.3 adjusted with o-H_3PO_4), at a flow rate of 2.0 mL/min (Wimalasiri & Wills, 1983). Samples were injected into a 20 μL loop connected to a Waters μ-Bondapack/NH_2 (3.9 mm × 30 cm, 10 μm) column at room temperature fitted with a Waters μ-Bondapack/NH_2 (3.9 mm × 20 mm, 10 μm) guard column. The HPLC system consisted of Waters 6000 pump and a Spectra Physics Spectra 100 variable wavelength UV/Vis detector set at 254 nm. Data was collected with a Hewlett Packard 3394 Integrator. Ascorbic acid (AA) was quantified from a standard curve in 3% citric acid prepared for this compound (0–100 μg/mL).

Dehydroascorbic acid (DHAA) content was quantified by reduction to AA in presence of dithiotreitol (DTT). Two-millilitres of 4 mM DTT and 100–140 μL of 6 M NaOH (to adjust pH ~6) were added to a 2-mL sample of the centrifuged supernatant. Samples were allowed to react at room temperature in the dark for 45 min and 100–140 μL of 6 M HCl were added to the reacting solution to stop the reaction (Odum, 1993). Samples were then passed through a SepPak cartridge as described above and total ascorbic acid (TAA) was quantified. DHAA content was quantified by difference of TAA − AA. No differences were observed between the standard curve obtained for AA (direct quantification) and DHAA (after 45 min reaction time as described herein). The standard curve of DHAA was prepared in 3% citric acid with methanol (4:1) to assist in solubilization.

2.6. Quantification of total anthocyanins and total carotenoids

Anthocyanins (in red cabbage) and carotenoids (in carrots and sweetpotatoes) were quantified following modifications of the methodologies by Fuleki and Francis (1968) and Talcott and Howard (1999), respectively. Anthocyanins were extracted by homogenizing 1-g samples with 15 g of 95% ethanol containing 1.5 N HCl (85:15 v/v). Samples were covered, stored overnight at 2 °C and then centrifuged using the conditions mentioned above. The supernatant was collected, an aliquot of the extract diluted with the extraction solvent to obtain a 1% solution, the spectrophotometer blanked with the solvent, and absorbance measurements taken at 535 and 700 nm in a 1-cm quartz cuvette. Anthocyanins were quantified as mg cyanidin-3-glucoside using a molar extinction coefficient of 25965/(cm M) and a molecular weight of 494 (Abdel-Aal & Hucl, 1999). Carotenoids were extracted from 1-g samples by homogenizing with 25 mL of acetone:ethanol (1:1) containing 200 mg/L BHT. The homogenate was filtered through a Whatman #4 filter, washed with the solvent (~60 mL) and diluted to 100 mL using the extraction solvent. Extracts were transferred to a plastic container, added 50 mL hexane, shaken and allowed to stand for 15 min. At 15 min, 25 mL of nanopure water was added, samples vigorously shaken and allowed separation of phases to take place for 30 min. The spectrophotometer was blanked with hexane and absorbance of samples measured at 470 nm in 1-cm quartz cuvettes. Carotenoids were quantified as β-carotene using a standard curve for this compound (1–4 μg/mL).

2.7. Dry matter and water loss

Dry matter content was determined by drying triplicate samples in an Isotemp® vacuum oven (model 285A; Fisher

Scientific; Pittsburg, PA) set at 70 °C and 7.5 mm Hg vacuum for 24 h. The dry matter content was 21%, 19%, 17%, 11%, 9%, 9%, 6%, 5%, 4% and 4% in sweetpotato, potato, parsnips, carrot, white cabbage, red cabbage, zucchini, radish, lettuce, and celery, respectively. Sample water loss was determined by comparing the sample weights at the beginning and at the end of the experiment. All results in this study were expressed on a fresh weight basis (FW), since the water loss of the samples was <1%.

2.8. Statistical analysis

Summary statistics (mean and standard deviation), graphs and linear regressions were obtained using Microsoft® Excel 2000 (Microsoft Corp., 1999). Statistical analyses were performed with the GLM procedure from The SAS® System for Windows (version 8.1) (SAS Institute Inc., 1999). Statistical differences were determined using Dunnett's Multiple Comparison Test ($P = 0.05$).

3. Results and discussion

3.1. Effect of wounding on PAL-activity, total soluble phenolic content and antioxidant capacity of fresh produce

Results indicated there were 6.0–73.3-fold increases ($P < 0.05$) in PAL-activity after wounding of all tissues (Table 1). However, the changes in phenolic content were tissue-dependent. Lettuce, celery, carrot, parsnips and sweetpotato had an increase ($P < 0.05$) in phenolic content by 81%, 30%, 191%, 13% and 17%, respectively (Fig. 1A), while zucchini, radish, potato and red cabbage showed a decrease ($P < 0.05$) in phenolic content by 26%, 7%, 15% and 9%, respectively. Only white cabbage showed non-significant changes in phenolic content ($P > 0.05$) after 2 days storage at 15 °C (Fig. 1B).

Table 1
Changes in PAL-activity of different fresh produce after wounding and storage at 15 °C for 2 days[a]

	PAL-activity[b] (μmol/(h g))	
	Whole	Wounded
Potato	0.02 ± 0.01	0.65 ± 0.1
Sweetpotato	0.02 ± 0.01	0.12 ± 0.04
Celery	0.02 ± 0.01	0.17 ± 0.04
White cabbage	0.05 ± 0.01	2.81 ± 0.4
Parsnips	0.05 ± 0.02	1.53 ± 0.2
Zucchini	0.06 ± 0.02	0.56 ± 0.2
Carrot	0.06 ± 0.03	4.20 ± 0.2
Lettuce	0.07 ± 0.02	0.70 ± 0.1
Radish	0.07 ± 0.02	1.56 ± 0.1
Red cabbage	0.08 ± 0.02	3.05 ± 0.5

[a] Data represents the mean ± SD ($n = 9$).
[b] Data expressed as t-cinnamic acid formed per hour per gram of tissue.

The observed PAL-activity increase indicates that the biosynthetic pathway of phenolic compounds was activated after wounding in all tissues. This activation of the phenylpropanoid metabolism can be elicited by wounding through a signal of unknown nature (Saltveit, 1997) or through induced ROS (Orozco-Cárdenas et al., 2001). Since phenolics are synthesized after wounding in all tissues, the actual amounts measured result from a balance between the phenolic synthesis rate (K_s) and their decrease

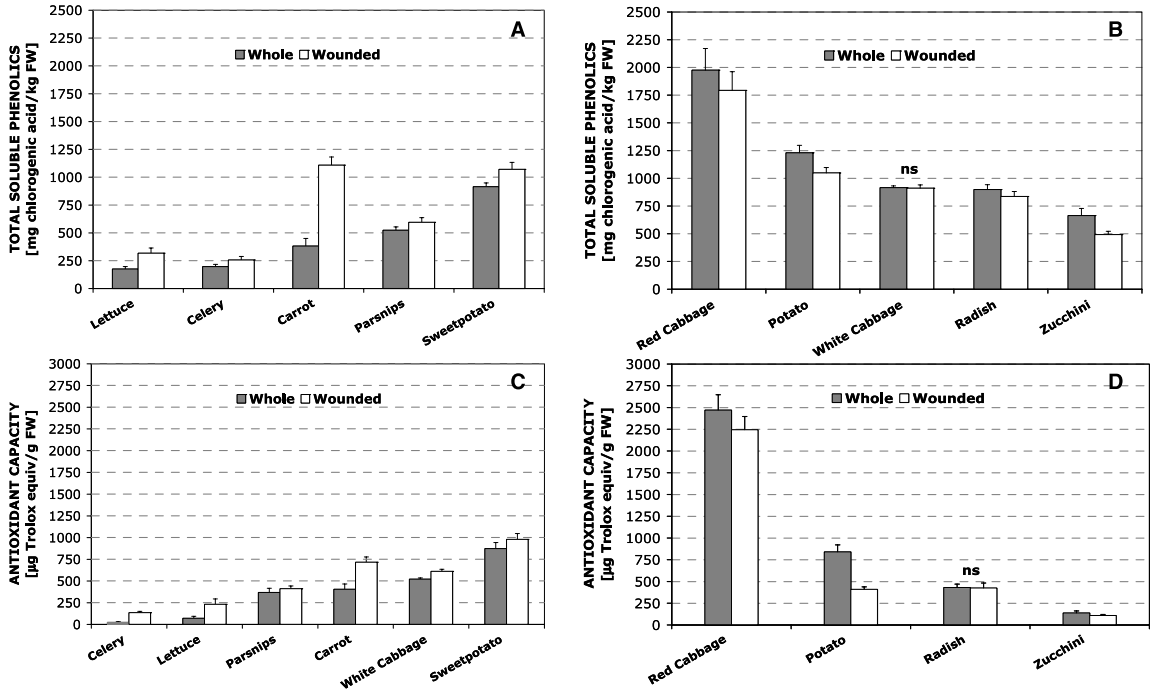

Fig. 1. Changes in total phenolic content (A and B) and antioxidant capacity (C and D) of different fresh produce after wounding and storage at 15 °C for 2 days. Vertical lines represent one-sided SD ($n = 9$). Non-significant differences in content after wounding are marked with "ns" ($P > 0.05$).

or utilization rate (K_d). Thus, the observed increase in soluble phenolic content would be associated to a $K_s > K_d$, with the resulting phenolics used possibly as defence mechanisms through the formation of phytoalexins or for the scavenging of ROS (Dixon & Paiva, 1995; Tamagnone et al., 1998). On the other hand, the observed decline in phenolic content would suggest that $K_s < K_d$, with the resulting phenolics being preferentially diverted towards the formation of insoluble phenolics, such as lignin and suberin (Amiot, Fleuriet, Cheynier, & Nicolas, 1997; Razem & Bernards, 2002), or to phenolic polymerization due to oxidation (Talcott & Howard, 1999). In the case of white cabbage samples with no apparent change in soluble phenolic content, the rate of phenolic synthesis and decrease would be similar ($K_s \approx K_d$).

After wounding, the antioxidant capacity of tissues showed similar trends to those observed with soluble phenolic content suggesting that phenolic compounds are likely the main contributors to the changes in antioxidant activity. There was a 442%, 233%, 12%, 77%, 17% and 12% increase ($P < 0.05$) in the antioxidant capacity of celery, lettuce, parsnips, carrot, white cabbage and sweetpotato, respectively (Fig. 1C), for wounded tissues; whereas zucchini, potato and red cabbage showed a decrease ($P < 0.05$) by 21%, 51% and 9%, respectively. On the other hand, wounded and whole radish showed similar antioxidant capacity ($P > 0.05$) (Fig. 1D). In addition to the tissue-dependent responses, the wounding response could also be cultivar-dependent. For example, the Russet potatoes used in the present study showed a decrease in both phenolic content and antioxidant capacity, whereas in a previous study, purple-fleshed All Blue potatoes showed a large increase in phenolic content and antioxidant capacity after slice wounding (Reyes & Cisneros-Zevallos, 2003).

Phenolics encompass a wide range of compounds with distinct structural and functional properties (Shahidi & Naczk, 1995), such as antioxidant activity that depend on structural features like the number of available hydroxyl groups (Rice-Evans, Miller, & Paganga, 1996, 1997); therefore, the antioxidant capacity of a solution containing a mixture of phenolic compounds will depend on the specific phenolic profile which can be qualitative (type of phenolics present) or quantitative (the relative amounts or proportions of phenolics present). For example, plotting the antioxidant capacity versus phenolic content of a series of dilutions of quercetin (Q), chlorogenic acid (C), or Q/C mixtures of 75/25 and 25/75, will give linear relationships ($R^2 > 0.99$; Fig. 2) with slopes corresponding to the specific antioxidant activity (antioxidant capacity on phenolic basis) with values of 2638, 1470, 2172 and 1907 μg Trolox equivalents/mg chlorogenic acid, respectively. Similarly, since wounding could induce the synthesis or decrease of phenolic compounds in the tissues, we hypothesize that the resulting antioxidant capacity of wounded produce would depend on the specific phenolic profile present. A plot of antioxidant capacity versus total phenolic content shows that individual tissues have different linear trends. The slopes of these linear trends would be associated to different phenolic profiles and their specific antioxidant capacity (Fig. 3A). After wounding, the linear trend may remain the same for some tissues (Fig. 3A) indicating that the new phenolic profile of wounded samples have similar specific antioxidant capacity to those from whole samples or alternatively, the slope may shift for other tissues (Fig. 3B) indicating a change in the new phenolic profile of wounded samples and their respective specific antioxidant capacity. For whole celery, zucchini, lettuce, radish and white cabbage the specific antioxidant capacity were

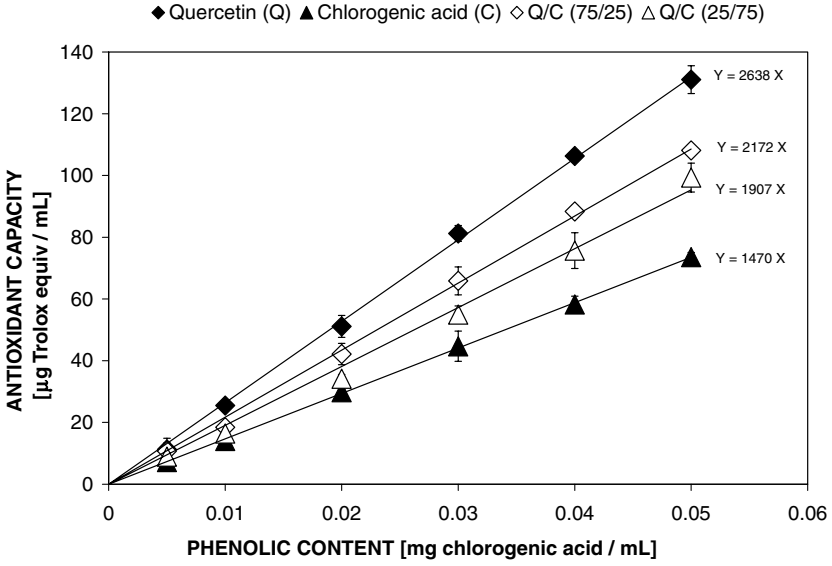

Fig. 2. Antioxidant capacity of solutions prepared with quercetin (Q), chlorogenic acid (C) and their mixtures (Q/C) in different proportions. Vertical lines represent SD ($n = 3$).

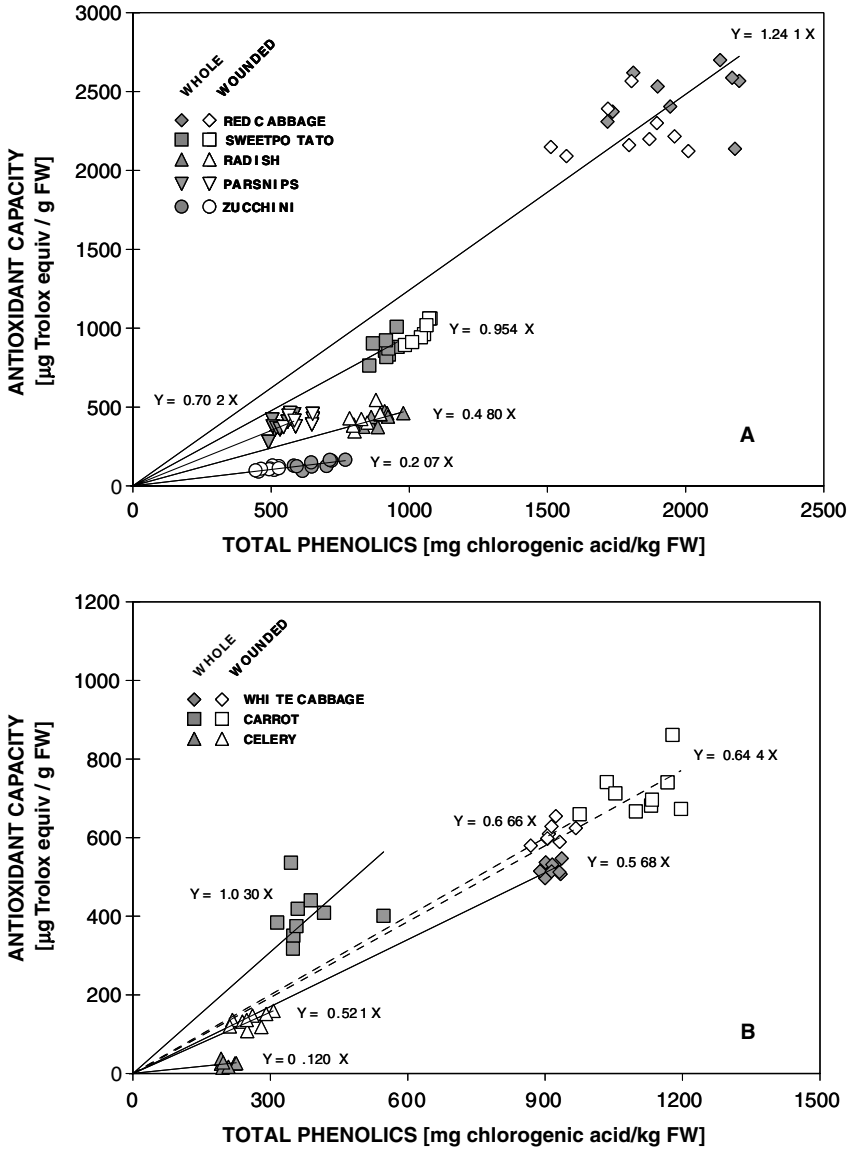

Fig. 3. Plot of antioxidant capacity versus total phenolic content of selected produce after storage at 15 °C for 2 days (A and B). Solid lines and markers represent whole samples while dashed lines and open markers represent wounded samples. In A, solid and dashed lines overlap.

low (120, 207, 384, 480 and 568 µg Trolox equivalents/mg chlorogenic acid, respectively), while for whole potato, parsnips, sweetpotato, carrot and red cabbage, these values were higher (682, 702, 954, 1030 and 1241 µg Trolox equivalents/mg chlorogenic acid, respectively). After wounding the specific antioxidant capacity values increased for celery, lettuce and white cabbage (334%, 92% and 17%, respectively), decreased for potato and carrot (43% and 37%, respectively), and remained the same for red cabbage, parsnips, sweetpotato, radish and zucchini.

3.2. Changes in ascorbic acid, total anthocyanin and total carotenoid content after wounding

Results showed that reduced ascorbic acid content in carrot, parsnips, celery, potato, zucchini and white cabbage, decreased after wounding ($P < 0.05$). However, for sweetpotato, radish and red cabbage, there were non-significant changes in ascorbic acid content ($P > 0.05$) (Table 2). On the other hand, the ascorbic acid levels in lettuce tissue were not detectable. The changes in reduced ascorbic acid ranged from ~13 to 66 mg/kg tissue. In spite of the large decrease in ascorbic acid, the antioxidant capacity (Figs. 1C and D) was not affected by these changes. These observations are in agreement with Proteggente et al. (2002) who reported that vitamin C did not contribute to antioxidant capacity as much as phenolic compounds. The total anthocyanin content of red cabbage (599 mg cyanidin-3-glucoside/kg FW) showed a slight decrease ($P < 0.05$) by 14% after wounding, whereas the total carotenoid content of carrots and sweetpotato was 124 and 78 mg β-carotene/kg FW and showed a non-significant decrease ($P > 0.05$) by 6% and 8%, respectively, after wounding. The slight changes in anthocyanin and caroten-

Table 2
Changes in reduced ascorbic acid content of different fresh produce after wounding and storage at 15 °C for 2 days[a]

	Ascorbic acid[b] (mg/kg of tissue)		
	Whole	Wounded	Change[c] (%)
Lettuce	Not detected	Not detected	Not detected
Carrot	16.7 ± 1.5	3.0 ± 0.2	−82
Parsnips	29.5 ± 4.0	7.2 ± 3.1	−76
Celery	33.6 ± 4.3	15.6 ± 4.2	−53
Potato	50.0 ± 11.0	33.8 ± 5.6	−32
Zucchini	125 ± 20	59 ± 4.0	−53
Sweetpotato	162 ± 28	143 ± 21	−12*
Radish	225 ± 13	210 ± 14	−7*
White cabbage	350 ± 28	310 ± 23	−11
Red cabbage	599 ± 24	633 ± 23	+6*

[a] Data represents the mean ± SD ($n = 9$).
[b] Data expressed as mg ascorbic acid per kg of tissue.
[c] Data with "−" sign represents a decrease, while a "+" sign represents an increase in ascorbic acid content.
[*] Non-significant changes after wounding ($P > 0.05$).

oid content after wounding could be due to oxidation mechanisms caused by polyphenoloxidases or lipoxygenases (Adams, 1991).

Plant tissue cells enclose natural antioxidant systems to regulate the production of ROS resulting from the cascade of reactions during normal cellular metabolism or induced by stresses. The antioxidant systems are constituted by antioxidant enzymes (e.g., superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase) and low molecular weight antioxidants (e.g., ascorbic acid, glutathione) (Blokhina, Virolainen, & Fagerstedt, 2003). From the many antioxidants present in the cell, ascorbic acid readily reacts with radical species to protect cell integrity upon redox imbalances (Buettner, 1993; Mittler, 2002). In the present study, the large decrease in reduced ascorbic acid observed was predominantly due to the protection of cells from redox imbalances caused by wounding and in less proportion by conventional aerobic degradation of ascorbic acid observed in processed products (Lee & Howard, 1999). For example, the DHAA content of carrots increased by 7% whereas that in zucchini decreased by 38%.

In general, we observed that produce with low levels of reduced ascorbic acid (up to 34 mg/kg tissue; e.g. lettuce, carrot, parsnips and celery) had lower soluble phenolic content (175–524 mg/kg tissue) and were associated to increased phenolic content after wounding. On the other hand, produce with higher levels of reduced ascorbic acid (50–599 mg/kg tissue; e.g., potato, zucchini, sweetpotato, radish, white cabbage and red cabbage) had higher soluble phenolic content (664–1975 mg/kg tissue) and were linked to decreased phenolic content after wounding (except for sweetpotato). We hypothesize that tissues with high levels of reduced ascorbic acid are prepared to control ROS, thus the phenolics being synthesized are used for other purposes (e.g., lignin and suberin formation). Conversely, in tissues with low levels of reduced ascorbic acid, ascorbic acid is consumed readily and phenolics are possibly synthesized to partly control ROS. Although the synthesized phenolic compounds are strong antioxidants, their free radical scavenging properties would not be exerted as readily as that of the other antioxidants already present in the cell, since phenolic biosynthesis takes place after wounding. In the literature, there is scarce information of in situ antioxidant activity of phenolic compounds in the plant cell (Phillpott, Gould, Lim, & Ferguson, 2004). This role of phenolic compounds has not yet been proven.

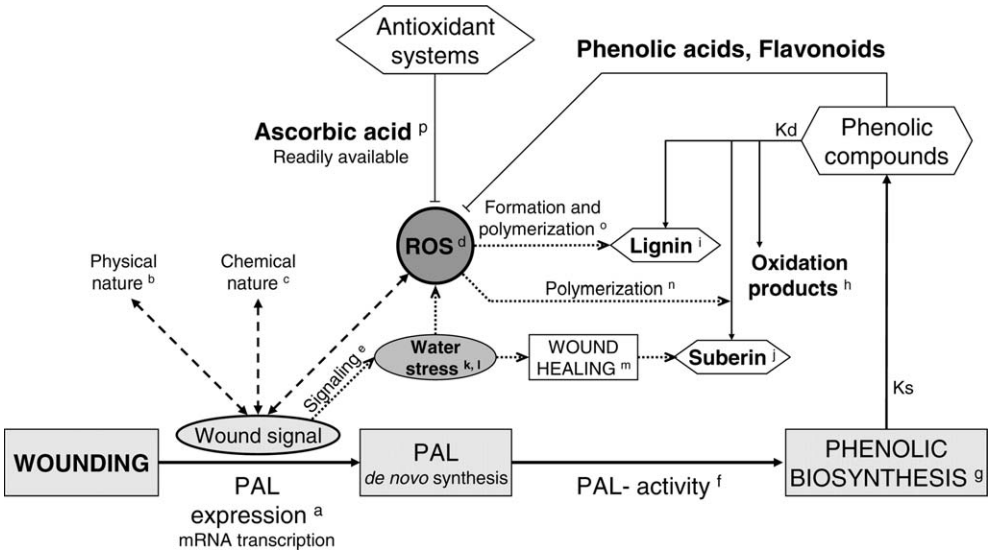

Fig. 4. Proposed mechanism of action of wounding stress and roles of reactive oxygen species (ROS) and antioxidants within the plant cell. K_s and K_d refer to phenolic synthesis and decrease rate, respectively (refer to Section 3.1). Superscripts a–p refer to literature references: (a) Kang and Saltveit (2003); (b) Malone and Alarcón (1995), Saltveit (1997); (c) Saltveit (1997), Campos-Vargas and Saltveit (2002); (d) Orozco-Cárdenas et al. (2001); (e) Reymond et al. (2000); (f) and (g) Dixon and Paiva (1995); (h) Talcott and Howard (1999); (i) Tamagnone et al. (1998); (j) and (m) Bernards et al. (1995); (k) Smirnoff (1993); (l) Jiang and Zhang (2002); (n) Razem and Bernards (2002); (o) Ros (1997); (p) Buettner (1993).

Based on literature information and results from the present study, we present in Fig. 4 a diagram proposing a hypothesis on the mechanism of action of wounding stress and ROS signalling by triggering the phenylpropanoid metabolism through induction of PAL activity, the modulating action of ROS by antioxidant systems present in the cell, the different roles and fates of newly synthesized phenolic compounds, the content of soluble phenolics as a result of a balance between the kinetics of phenolic synthesis and decrease, and the yet to prove role of phenolics as modulating agents of ROS. Further studies are needed to verify the role of newly synthesized phenolic compounds as possible ROS scavengers in the plant cell. The proposed diagram can be considered a starting point.

4. Conclusions

The present study indicates that health-promoting antioxidant phenolic compounds may be induced by wounding in selected fruits and vegetables. The amount and profile of wound-induced soluble phenolics is dependent on the type of tissue, initial levels of reduced ascorbic acid and soluble phenolic compounds. Reduced ascorbic acid is greatly affected by wounding. Further studies are needed to fully understand the effect of cultivar-dependent responses, time and temperature effects, wounding intensity (amount of area created), specific changes in phenolic profiles and bioactive properties after wounding. This information could be used by the fresh produce industry towards the promotion of healthier fruits and vegetables if detrimental quality changes may be overcome or by the nutraceutical industry for increasing yield and extraction of bioactive compounds.

Acknowledgement

The present work was funded through the Texas Agricultural Experiment Station and the Vegetable & Fruit Improvement Center with the Grant "Designing Foods for Health, USDA-CSREES 2001-34402-13647".

References

Abdel-Aal, E. S. M., & Hucl, P. (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chemistry, 76*, 350–354.

Adams, J. B. (1991). Significance of enzymes in individual vegetables. In P. F. Fox (Ed.). *Food enzymology* (Vol. 1, pp. 499–543). New York, NY: Elsevier Science Publishing Company Inc..

Amiot, M. J., Fleuriet, A., Cheynier, V., & Nicolas, J. (1997). Phenolic compounds and oxidative mechanisms in fruit and vegetables. In F. A. Tomás-Barberán & R. J. Robins (Eds.), *Phytochemistry of fruit and vegetables. Proceedings of the phytochemical society of Europe* (Vol. 41, pp. 51–85). New York, NY: Oxford University Press.

Bernards, M. A., López, M. L., Zajicek, J., & Lewis, N. G. (1995). Hydroxycinnamic acid-derived polymers constitute the polyaromatic domain of suberin. *The Journal of Biological Chemistry, 270*(13), 7382–7386.

Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany, 91*, 179–194.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie, 28*, 25–30.

Buettner, G. R. (1993). The pecking order of free radicals: lipid peroxidation, α-tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics, 300*, 535–543.

Campos-Vargas, R., & Saltveit, M. E. (2002). Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiologia Plantarum, 114*, 73–84.

Cevallos-Casals, B. A., & Cisneros-Zevallos, L. (2003). Stoichiometric and kinetic studies of phenolic antioxidants from Andean purple corn and red-fleshed sweetpotato. *Journal of Agricultural and Food Chemistry, 51*(11), 3313–3319.

Desikan, R., Mackerness, S. A. H., Hancock, J. T., & Neill, S. J. (2001). Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiology, 127*, 159–172.

Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture, 80*, 1744–1756.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell, 7*, 1085–1097.

Fuleki, T., & Francis, F. J. (1968). Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science, 33*, 72–77.

Heredia, J. B. & Cisneros-Zevallos, L. (2002). Wounding stress on carrots increases the antioxidant capacity and the phenolics content [abstract]. In *IFT annual meeting book of abstracts* (p. 180, Abstract 76C-14). Chicago, IL: Institute of Food Technologists.

Jiang, M., & Zhang, J. (2002). Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany, 53*(379), 2401–2410.

Kang, H., & Saltveit, M. E. (2002). Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agricultural and Food Chemistry, 50*, 7536–7541.

Kang, H., & Saltveit, M. E. (2003). Wound-induced increases in phenolic content of fresh-cut lettuce is reduced by a short immersion in aqueous hypertonic solutions. *Postharvest Biology and Technology, 29*, 271–277.

Kays, S. J. (1997). Stress in harvested products. In S. J. Kays (Ed.), *Postharvest physiology of perishable plant products* (pp. 335–407). Athens, GA: Exon Press.

Ke, D., & Saltveit, M. E. J. (1986). Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in Iceberg lettuce. *HortScience, 21*, 1169–1171.

Lee, Y., & Howard, L. (1999). Firmness and phytochemical losses in pasteurized yellow banana peppers (*Capsicum annum*) as affected by calcium chloride and storage. *Journal of Agricultural and Food Chemistry, 47*, 700–703.

Malone, M., & Alarcón, J. J. (1995). Only xylem-borne factors can account for systemic wound signaling in the tomato plant. *Planta, 196*, 740–746.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science, 7*, 405–410.

Mittler, R. (2005). Oxidative stress. Available from http://www.ag.unr.edu/mittler/oxistress.pdf (accessed September 2005).

Moon, H., Lee, B., Choi, G., Shin, D., Prasad, D. T., Lee, O., et al. (2003). NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. *Proceedings of the National Academy of Sciences USA, 100*, 358–363.

Neill, S. J., Desikan, R., Clarke, A., Hurst, R. D., & Hancock, J. T. (2002). Hydrogen peroxide and nitric oxide as signaling molecules in plants. *Journal of Experimental Botany, 53*, 1237–1247.

Odum, L. (1993). pH optimum of the reduction of dehydroascorbic acid by dithioerytritol. *Scandinavian Journal of Clinical Laboratory Investigation, 53*, 367–371.

Orozco-Cárdenas, M. L., Narváez-Vázquez, J., & Ryan, C. A. (2001). Hydrogen peroxide acts as a second messenger for the induction of

defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell, 13*, 179–191.

Phillpott, M., Gould, K. S., Lim, C., & Ferguson, L. R. (2004). In situ and in vitro antioxidant activity of sweetpotato anthocyanins. *Journal of Agricultural and Food Chemistry, 52*, 1511–1513.

Prior, R. L., & Cao, G. (2000). Analysis of botanicals and dietary supplements for antioxidant capacity: a review. *Journal of the Association of Official Analytical Chemists International, 83*, 950–956.

Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., et al. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research, 36*, 217–233.

Razem, F. A., & Bernards, M. A. (2002). Hydrogen peroxide is required for poly(phenolic) domain formation during wound-induced suberization. *Journal of Agricultural and Food Chemistry, 50*, 1009–1015.

Reyes, L. F., & Cisneros-Zevallos, L. (2003). Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes. *Journal of Agricultural and Food Chemistry, 51*, 5296–5300.

Reymond, P., Weber, H., Damond, M., & Farmer, E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. *The Plant Cell, 12*, 707–720.

Rhodes, J. M., & Wooltorton, L. S. C. (1978). The biosynthesis of phenolic compounds in wounded plant storage tissues. In G. Kahl (Ed.), *Biochemistry of wounded plant tissues* (pp. 243–286). Berlin, Germany: Walter de Gruyter & Co.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*, 933–956.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Ros, B. A. (1997). Lignification in plant cell walls. *International Review in Cytology, 176*, 87–132.

Saltveit, M. E. (1997). Physical and physiological changes in minimally processed fruits and vegetables. In F. A. Tomás-Barberán & R. J. Robins (Eds.), *Phytochemistry of fruit and vegetables. Proceedings of the phytochemical society of Europe* (Vol. 41, pp. 205–220). New York, NY: Oxford University.

Saran, M., & Bors, W. (1989). Oxygen radical acting as chemical messengers: a hypothesis. *Free Radical Research Communications, 7*, 312–320.

Shahidi, F., & Naczk, M. (1995). Food phenolics: An overview. In F. Shahidi & M. Naczk (Eds.), *Food phenolics: Sources, chemistry, effects and applications* (pp. 1–5). Lancaster, PA: Technomic Publishing Company Inc.

Shetty, K., & McCue, P. (2003). Phenolic antioxidant biosynthesis in plants for functional food application: integration of systems biology and biotechnological approaches. *Food Biotechnology, 17*, 67–97.

Smirnoff, N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist, 12*(1), 27–58.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 10*, 63–68.

Talcott, S. T., & Howard, L. R. (1999). Phenolic autoxidation is responsible for color degradation in processed carrot puree. *Journal of Agricultural and Food Chemistry, 47*, 2109–2115.

Tamagnone, L., Merida, A., Stacey, N., Plaskitt, K., Parr, A., Chang, C. F., et al. (1998). Inhibition of phenolic acid metabolism results in precocious cell death and altered cell morphology in leaves of transgenic tobacco plants. *Plant Cell, 10*, 1801–1816.

Wimalasiri, P., & Wills, R. B. H. (1983). Simultaneous analysis of ascorbic acid and dehydroascorbic acid in fruit and vegetables by high-performance liquid chromatography. *Journal of Chromatography, 256*, 368–371.