Effect of Selected Browning Inhibitors on Phenolic Metabolism in Stem Tissue of Harvested Lettuce

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Wound-induced changes in phenolic metabolism causes stem browning (butt discoloration) in harvested lettuce. Stem tissue near the harvesting cut exhibited increased phenylalanine ammonia-lyase (PAL) activity and accumulation of caffeic acid derivatives. These *o*-diphenols can be oxidized by the enzyme polyphenol oxidase (PPO) to produce brown pigments. This browning reaction can readily be followed by measuring *a** values. Browning was reduced by washing stem disks with solutions of 0.3 M calcium chloride, 1.0 mM 2,4-dichlorophenoxyacetic acid (2,4-D), or 0.5 M acetic acid. These browning inhibitors appear to act in different ways. Calcium chloride decreased PAL activity to 60% of the control, but did not substantially affect the accumulation of phenolic compounds. The mechanism of calcium action could be to decrease PPO activity or to preserve membrane structure. PAL activity was inhibited 60% by 2,4-D, and the biosynthesis of phenolic compounds was strongly inhibited but not suppressed. Acetic acid completely inhibited PAL activity and the production of wound-induced phenolics. PAL was irreversibly inhibited by acetic acid, and this may explain its role as a browning inhibitor.

Keywords: *Lettuce; Lactuca sativa; postharvest storage; butt discoloration; phenolic metabolism; PAL; browning inhibition*

INTRODUCTION

The shelf life of minimally processed fruits and vegetables is often limited by enzymatic browning (Vamos-Vigyazo, 1981). The organoleptic and biochemical characteristics of fruits and vegetables are also strongly modified by the appearance of brown pigments. Oxidative browning is mainly due to the enzyme polyphenol oxidase (EC 1.14.18.1) (PPO), a mixed function oxidase which catalyzes the hydroxylation of monophenols to *o*-diphenols and, in a second step, the oxidation of colorless *o*-diphenols to highly colored *o*-quinones (Sapers and Hicks, 1989; Janovitz-Klapp et al., 1990). The *o*-quinones condense spontaneously with other *o*-quinones and with many constituents of foods such as proteins, reducing sugars, etc. to form high molecular weight polymers which precipitate yielding the brown, red, or dark pigments characteristic of browned fruit and vegetable tissues (McEvily et al., 1992). PPO has a broad specificity toward different phenolic substrates, and the brown pigments resulting from these phenolics differ widely in color intensity (Nicolas et al., 1993).

Browning is one of the main causes of quality loss during the postharvest storage of head lettuce and minimally processed lettuce (Ilker et al., 1977; Mateos et al., 1993). Extensive investigations have been conducted to understand the biochemical basis of lettuce browning and to find physical or chemical treatments to prevent these discolorations (Hyodo et al., 1978; Ke and Saltveit, 1986; Siriphanich and Kader, 1985; Fujita et al., 1991; Heimdal et al., 1994; Chazarra et al., 1996). In a recent study we described the beneficial effect of washing the cut stem end (i.e., the butt) of lettuce heads with different organic acid solutions to prevent butt discoloration (Castañer et al., 1996). Prevention of ethylene-induced browning (russet spotting) by calcium and synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) has also been reported (Ke and Saltveit, 1986).

Wounding generally induces increased phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity and increased phenolic metabolism in many plant tissues (Dixon and Paiva, 1995). In addition, wounding induces cellular decompartmentalization which allows mixing of phenolic substrates and PPO, leading to the development of browning (Mayer, 1987). Browning can be delayed by storage at low temperatures, but it nevertheless occurs after a lag period.

Lettuce butt discoloration is a type of browning induced by wounding. Harvesting lettuce entails wounding the stem butt end, thus inducing all the above mentioned changes leading to the formation of brown pigments and butt discoloration. Similar changes also occur in iceberg lettuce midribs during the storage of minimally processed cut lettuce (Mateos et al., 1993). The objective of this work was to study changes in phenolic metabolism during the development of wound-induced lettuce stem browning and to understand how washing treatments with calcium chloride, 2,4-D, and acetic acid solutions can prevent browning through their effects on phenolic metabolism.

MATERIALS AND METHODS

Lettuce. Commercially grown and harvested crisphead (Iceberg) lettuce were obtained from a local wholesale market and transported to the laboratory where they were stored at 0 °C until used.

Preparation of Stem Disks. Stem cylinders were excised from lettuce heads with a 2.5 cm diameter stainless steel cork-borer, by pushing the cork-borer up the stem from the butt

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S0021-8561(96)00478-5 CCC: $14.00

end to the apex. The extracted cylinder was then cut with a stainless steel blade into disks of 0.5 cm height (the first disk, which was the closest to the butt end, was discarded). The disks were divided into four equal quarters, and each quarter was used for a different treatment. Eight to nine disks obtained from three lettuce heads were used for each replicate. At least three replicates were used for each experiment. The disk pieces were placed in 20 × 100 mm plastic Petri dishes (without lid) and held in 2-L glass jars flushed with humidified air (95% relative humidity) at a flow rate sufficient to prevent accumulation of CO_2 or ethylene. The jars were stored at 5 °C. Each experiment was repeated at least twice with similar results.

Browning Inhibitor Treatments. Aqueous solutions of 0.3 M $CaCl_2$ (Sigma), 1.0 mM 2,4-D (Sigma), and 0.5 M acetic acid (Merck) were prepared and kept at 5 °C. Immediately after excision, the disks were dipped in the solutions or water (control) for 5 min and then blotted with soft paper towels. Representative samples were stored at −80 °C until extracted.

Color Measurements. Lettuce stem browning was objectively measured with a Minolta Chromameter CR-300. This tristimulus color spectrophotometer was used to obtain the absorption spectra from which Hunter L^*, a^*, and b^* values were calculated using illuminant D65 and a 10° observer according to the CIELAB 71 convention. All measurements were done in triplicate (three different stem disks) just after cutting (initial time) and after different storage periods, and mean values are reported in the data. The color of the plant material remaining in the cheesecloth filter after phenolic extraction was also measured. The residue was collected and introduced into plastic wells of 1.8 cm diameter. The material was then pressed slightly with a glass rod, and the L^*, a^*, and b^* values measured with the Minolta CR-300 spectrophotometer through the bottom of the plastic well.

Extraction Procedure 1. The procedure of Ke and Saltveit (1989) was adapted to the extraction of lettuce stem disks. Disks (2−3 g) were homogenized with 5 mL of methanol (HPLC grade) in an Ultra-Turrax Tissue Homogenizer at 0 °C (on ice). The homogenates were filtered through four layers of cheesecloth. The residue was placed into 1.8 cm diameter plastic wells, and the color was measured as described above. The filtrate was centrifuged at 25000*g* for 20 min at 2 °C. The supernatant was then recovered and concentrated under reduced pressure (35 °C). The dried extracts were then redissolved in 0.5 mL of methanol−water (1:1, v:v) and filtered through 0.45 μm prior to HPLC analysis.

Extraction Procedure 2. The method used was modified from that described by Babic et al. (1993). Lettuce disks (5 g) were freeze-dried and homogenized in 20 mL of an ethanol−water mixture (4:1, v:v). The extract was filtered through four layers of cheesecloth, and the ethanol was removed by evaporation at 40 °C under vacuum. The aqueous extract was then taken to 20 mL with distilled water and extracted with petroleum ether (10 mL × 3) to remove pigments and lipids. Quinones and phenolic compounds were then extracted with ethyl acetate (10 mL × 3), the solvent was evaporated at 40 °C under vacuum, and the residue was redissolved in 1.5 mL of methanol for analysis. The extracts were filtered through 0.45 μm before spectrophotometric and HPLC analyses.

HPLC Analyses. Two HPLC systems were used. System 1 was used for phenolic compounds and quinones and used a Lichrochart RP-18, 12 × 0.4 cm, 5 μm particle size column, with a solvent flow rate of 1 mL min^{-1}. The mobile phases were water−formic acid (19:1, v:v) (A) and methanol (B). A linear gradient elution was used, starting with 10% B in A to reach 25% B at 25 min, 35% B at 30 min, and 80% B at 40 min.

The second system (system 2) was used to increase the resolution of the different phenolic acid derivatives eluting in the first part of the chromatogram. A Bio-Sil ODS-5S (25 × 0.4 cm, 5 μm particle size) column was used. The gradient started with 3% B to reach 5% B at 10 min and 30% B at 30 min. The absorbance of the different compounds were recorded at 265, 280, 325, and 420 nm with a diode array detector. The different caffeic acid derivatives were quantified as chlorogenic acid (monocaffeoyl derivatives) from Sigma and as cynarin (1,3-dicaffeoylquinic) (dicaffeoyl derivatives) from artichoke kindly provided by Dr. Marie Jo Amiot, Avignon, France.

Table 1. Effect of Lettuce Stem Disk Position on the Concentration of Wound-Induced Phenolic Compounds[a]

disk (cm)	chlorogenic	chicoric	isochlorogenic	total
0.0−0.5	14.73	3.05	2.39	20.16a
0.5−1.0	2.13	0.10	1.67	3.90b
1.0−1.5	0.20	nd	0.10	0.30c
1.5−2.0	0.06	nd	nd	0.06d
2.0−2.5	0.05	nd	nd	0.05d
2.5−3.0	0.05	nd	nd	0.05d

[a] Whole heads of Iceberg lettuce were stored at 2.5 °C for a week in glass jars with a sufficient flow of humidified air to maintain the CO_2 levels below 0.05%. Extraction by procedure 1. [b] Values are μg/g fresh weight stem disk (mean values, $n = 3$), and values with different letters are signicantly different (Student's *t*-test, $P < 0.05$). nd, compound not detected.

PAL Activity. PAL was analyzed as previously reported (Ke and Saltveit, 1986). One unit of PAL activity equals the amount of PAL that produces 1 μmol of cinnamic acid in 1 h under the specific conditions and is expressed as μmol/g fresh weight per h.

Hydrolysis of Extracts. The methanol−water (1:1) phenolic extracts obtained from disks previously washed with 1 mL of 0.5 M acetic acid were hydrolyzed at 80 °C for 30 min after addition of 1 mL of 2N HCl. During hydrolysis all of the methanol was removed. The hydrolysates were then successively extracted first with ethyl acetate (2 mL) and then with *n*-butanol (2 mL). The extracts were concentrated under reduced pressure (30 °C ethyl acetate and 45 °C *n*-butanol), redissolved in methanol−water (1:1; v:v), filtered through 0.45 μm, and analyzed by HPLC.

RESULTS

Soluble Phenolic Metabolites in Brown Stems. The butt end of harvested lettuce first becomes yellow and then reddish-brown, finishing with an intense brown pigmentation. This browning is known as lettuce butt discoloration. These changes in color are accompanied by changes in the tissue's phenolic composition. Preliminary HPLC analyses of methanol extracts obtained from brown control iceberg lettuce stem disks (i.e., the last 0.5 cm of the stem) showed that they contained large amounts of phenolic compounds (i.e., caffeic acid derivatives, with a characteristic UV spectrum). These compounds were identified as 5-caffeoylquinic (chlorogenic acid), dicaffeoyltartaric (chicoric acid), and 3,5-dicaffeoylquinic acids (isochlorogenic acid) by chromatographic comparisons with authentic markers and green coffee beans (Clifford, 1986) and with chicory extracts (Winter and Herrmann, 1986). Their percentages of the absorbance at 320 nm in the HPLC chromatogram were 42%, 20%, and 38%, respectively.

Phenolic compounds were extracted (procedure 1) from stem disks taken from different positions of the stem cylinder from harvested lettuce stored for 1 week at 2.5 °C. Methanol soluble phenolic compounds were mainly located in the first disk. They were also present in much smaller concentrations in the second disk, and only in trace amounts in the rest of the disks. This spatial distribution suggests that these compounds had been induced by wounding (Table 1). In addition, some compounds absorbing in the visible region (420−440 nm) were also detected in the extracts of the first disk, although at very low concentrations.

A different extraction procedure with ethyl acetate (procedure 2) was used to record the UV−vis spectra of the soluble pigments generated during the development of browning. A characteristic chromatogram of these

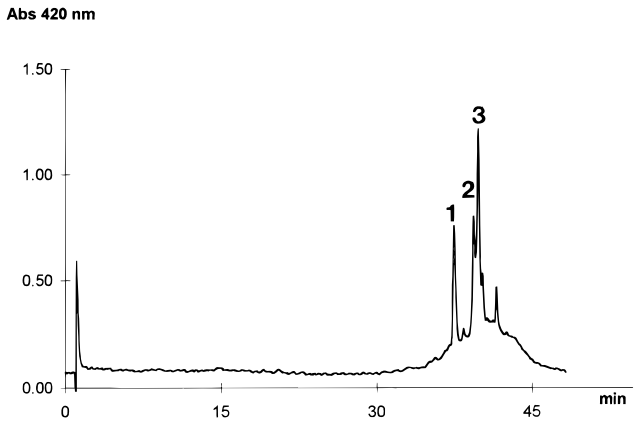

Figure 1. HPLC chromatograms of visible absorbing (420 nm) metabolites in iceberg lettuce stem disks after 7 days of storage at 5 °C. Extracts obtained by procedure 2 (Babic et al., 1993). HPLC conditions: System 1 in Materials and Methods.

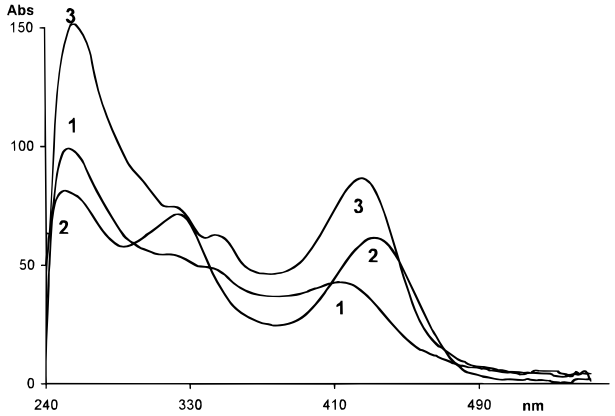

Figure 2. UV−vis spectra of visible absorbing metabolites in iceberg lettuce stem disks (after 7 days at 5 °C). (Main peaks in the chromatogram of Figure 1.)

pigments is shown in Figure 1. At least three different visible absorbing compounds were detected. All of the brown soluble compounds had similar spectral characteristics; a maximum between 420 and 440 nm and another maximum around 260 nm, with other shoulders between them (Figure 2). These spectra were similar to those previously reported for naturally occurring *o*-benzoquinones (Thomson, 1976). Since these visible absorbing compounds eluted in HPLC with 60−70% methanol, they are quite lipophilic in nature, especially when compared to the caffeic acid derivatives from which they were most likely produced by the action of PPO.

After extraction, however, the lettuce stem tissue residue was still brown. This indicates that some of the compounds responsible for the brown-reddish color are insoluble in methanol. These results agree with previous reports that these pigments are polymers produced by condensation of the *o*-quinones with themselves or with proteins or other plant constituents to give insoluble brown polymers (McEvily et al., 1992).

Wound-Induced Changes in Lettuce Stem Phenolic Metabolism. Preparation of lettuce stem disks induced PAL activity which started to increase after 12−24 h at 5 °C and reached maximal activity at 48 h (Figure 3). Wound-induced PAL activity led to an increase in soluble phenolic compounds. Therefore, the first 0.5 cm disk of the stem cylinder was discarded to avoid stem tissue in which the harvest wound had already induced changes in phenolic metabolism. The

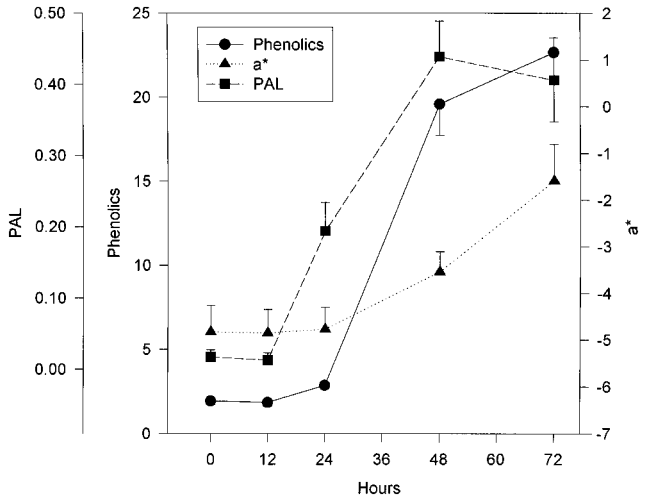

Figure 3. Wound-induced changes in phenolic composition (extracted by procedure 1), PAL activity, and *a** value of lettuce stem disks.

Table 2. Wound-Induced Phenolic Compounds in Lettuce Stem Disks Stored at 5 °C[a]

time (h)	chlorogenic	chicoric	isochlorogenic	total
0	1.96 ± 0.21	nd	nd	1.96 ± 0.21
12	1.85 ± 0.18	nd	nd	1.85 ± 0.18
24	2.85 ± 0.12	nd	nd	2.85 ± 0.12
48	17.56 ± 2.02	0.97 ± 0.41	1.07 ± 0.58	19.60 ± 1.87
72	17.89 ± 3.94	2.12 ± 0.51	2.66 ± 0.99	22.67 ± 4.13

[a] Values are μg/g fresh weight stem disk (means of $n = 3$, ± standard deviations). nd, not detected. Extraction by procedure 1.

compounds detected by HPLC were similar to those found in lettuce butt end tissue. The biosynthesis of these compounds started after a lag of 24 h, rapidly increasing between 24 and 48 h, and then increasing slower between 48 and 72 h (Figure 3). The first phenolic which showed a sizable increase was chlorogenic acid, which accounted for the main changes observed between 24 and 48 h (Table 2). In contrast, the changes in dicaffeoyl derivatives concentration were the predominant ones between 48 and 72 h, and these compounds accumulated to much higher levels with longer storage periods. All of these *o*-diphenols compounds are susceptible to oxidation by PPO into *o*-quinones and then into brown polymers leading to the development of browning.

The development of disk browning can be objectively followed by measuring the changes in the *a** value (Castañer et al., 1996). Unlike changes in phenolic biosynthesis, however, the main changes in *a** occurred between 24 and 72 h (Figure 3). The progressive increase, first in PAL, then in phenolics and finally in *a**, show a sequence which clearly indicates the temporal relationships among these events in the wound-induced development of browning in harvested lettuce stems. It appears that wounding first induces an increase in PAL activity which leads to an increase in caffeic acid derivatives, which are rapidly transformed into brown compounds. A spatial distribution and compartmentalization of the browning reactants is also suggested by the observation that the rate of browning keeps increasing even when the rate of phenolic biosynthesis is decreasing (Figure 3).

If the changes in absorbance at 437 nm (maximum for soluble *o*-quinones) are followed in the methanol extracts during these 72 h, however, only very slight changes are observed. In fact, the absorbance of the

Table 3. Effect of Calcium, 2,4-D, and Acetic Acid on Wound-Induced Phenolics and PAL Activity of Stem Disks[a]

	phenolics				absorbance/g			
treatment	chlorogen	chicoric	ischlorogen	total	267 nm	320 nm	437 nm	PAL
control	12.7 ± 1.6	0.75 ± 0.36	2.93 ± 0.98	16.39 ± 2.08	0.65 ± 0.09	0.51 ± 0.10	0.023 ± 0.008	0.33 ± 0.01
calcium (0.3 M)	11.0 ± 2.4	0.72 ± 0.51	1.11 ± 0.93	12.79 ± 3.79	0.51 ± 0.04	0.30 ± 0.06	0.008 ± 0.001	0.19 ± 0.01
2,4-D (1 mM)	0.97 ± 0.05	0.09 ± 0.08	0.14 ± 0.06	1.20 ± 0.29	0.64 ± 0.07	0.09 ± 0.02	0.012 ± 0.004	0.18 ± 0.01
HOAc (0.5 M)	0.09 ± 0.08	nd	nd	0.09 ± 0.08	0.67 ± 0.08	0.06 ± 0.005	0.008 ± 0.006	0.016 ± 0.002

[a] Values of phenolics are μg/g fresh weight. nd, not detected. Values of absorbance of the extracts at the different wavelengths are expressed as absorbance/gram fresh weight. PAL activity is μmol of cinnamic acid/gram fresh weight per hour. In all cases, means (n = 3) ± standard deviations. Phenolics extracted by procedure 1.

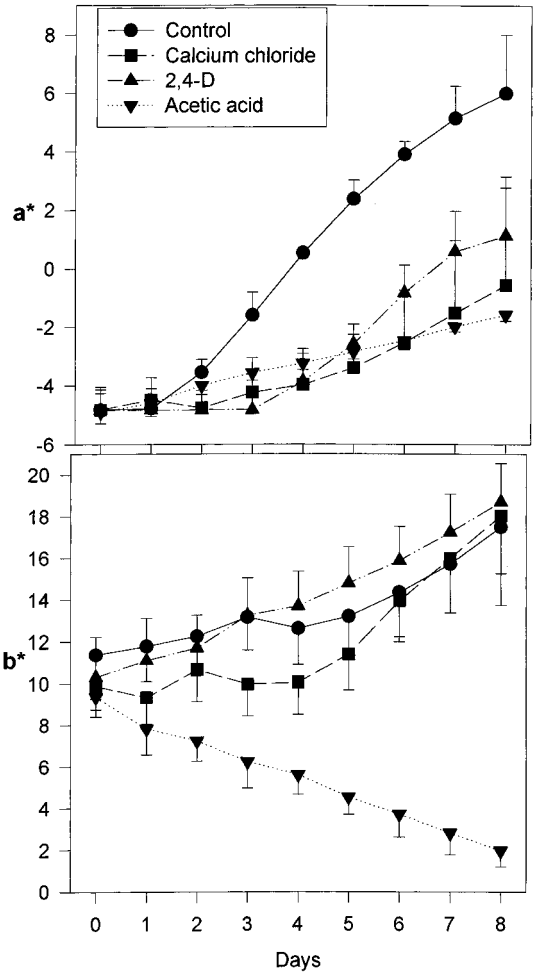

Figure 4. Effect of calcium chloride, acetic acid, and 2,4-D treatments on browning of lettuce stem disks (a* and b* values).

methanol extract is just 0.02 after 72 h, but increases to 0.2−0.3 after 7 days of storage. After extraction of the phenolics with methanol, the remaining plant material was still brown. The retained color indicates that the amount of soluble brown (yellow) compounds was only part of the brown polymers in the plant tissues.

Effect of Selected Browning Inhibitors on Stem Wound-Induced Phenolic Metabolism. Significant differences in browning between the control disks and those treated with the three inhibitors could be observed after 3 days of storage at 5 °C (Figure 4). After 7 days, the control had a* values close to 6, while acetic acid treated disks had values around −2. Calcium and 2,4-D treated disks kept their a* values below 0 and 1, respectively. Some differences were observed in the visual appearance of the disks after 7 days of storage. Acetic acid treated disks had a white appearance, while 2,4-D and $CaCl_2$ treated disks developed a yellowish tint. This difference was also reflected in the b* values which objectively evaluates yellow intensity (Figure 4). Acetic acid treated disks had b* values around 3, while the other two treatments and the control had b* values higher than 15 after 7 days of storage. The b* values observed in the acetic acid treated disks were even lower than those found in the initial disks (i.e., 12) indicating that some bleaching of the pigments had occurred.

Studies of the three browning inhibitors focused on changes occuring during the first 3 days since that is when PAL activity is induced and phenolic compounds start to accumulate (Figure 3). The UV−vis spectra of tissue extracts showed very significant differences among the treatments. Control tissue had the highest absorbance at 437 nm (i.e., o-quinones), an important absorption band in the region of caffeic acid derivatives (320 nm), and an additional band of absorption at 267 nm (Table 3). Similar spectra were observed in the $CaCl_2$ treated disks, indicating that $CaCl_2$ does not prevent browning by preventing the biosynthesis of the substrates susceptible of oxidation by PPO. On the other hand, 2,4-D and acetic acid, prevented the biosynthesis of caffeoyl derivatives, as shows the lack of an absorption band at 320 nm. In these cases, only a maximum at around 263 nm was observed. A small shoulder at around 320 nm was, however, observed in the extracts of 2,4-D treated disks.

Quantification of the HPLC analysis of the different caffeoyl derivatives clearly showed that the $CaCl_2$ treatment did not prevent phenylpropanoid biosynthesis, while both acetic acid and 2,4-D did prevent phenylpropanoid biosynthesis to different degrees (Table 3). These differences are illustrated in the HPLC chromatograms (at 320 nm) of the extracts of disks treated with the different browning inhibitors (Figure 5). No differences were observed between extracts of control and $CaCl_2$ treated disk. The biosynthesis of phenolic acid derivatives was largely reduced, but not suppressed, by the application of the auxin, 2,4-D.

The HPLC analyses at 265 nm also showed that other metabolites were present in the treated disks. These compounds were not detected in the control, but they were observed as traces in the $CaCl_2$ treated disks, in higher amounts in the 2,4-D treated disks and in much higher amounts in the acetic acid treated disks (Figure 6). These compounds have a maximum absorbance at around 267 nm and are highly polar as reveals by their very short retention times. These compounds were not extracted from aqueous extracts by ethyl acetate, but they were readily extracted with n-butanol. After acid hydrolysis, they were completely transformed into ethyl acetate extractable compounds with spectra showing absorption maxima at around 280 nm. The spectra were very similar to those of catechin and epicatechin, which have already been reported to be induced in iceberg lettuce during russet spot development (Ke and Saltveit, 1988). However, the compounds obtained by hydrolysis failed to co-chromatography with authentic

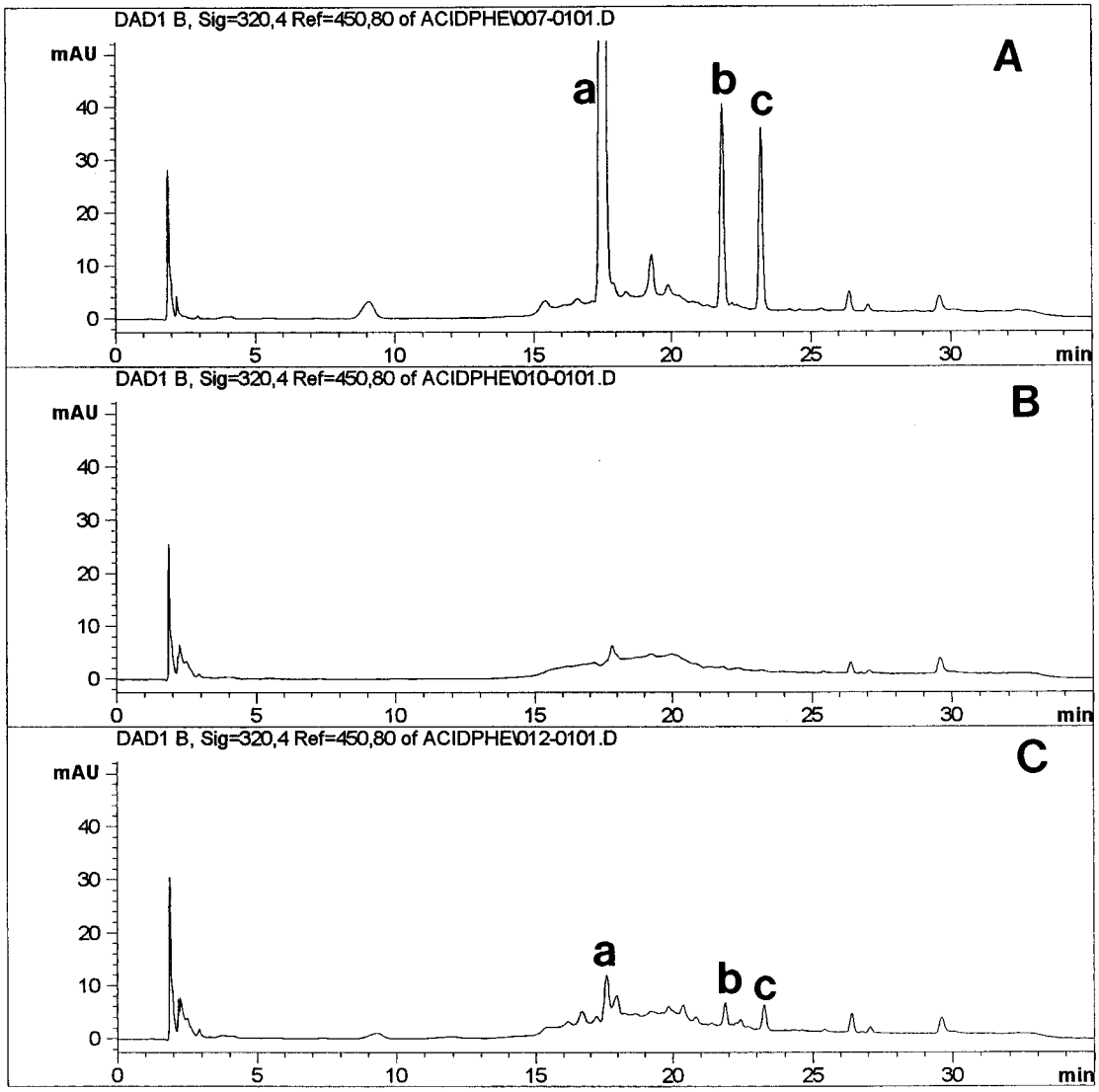

Figure 5. Effect of calcium chloride (A), acetic acid (B) and 2,4-D (C) treatments on the phenolic composition of lettuce stem disks (extracts obtained by procedure 1). Chromatograms recorded at 320 nm. (HPLC conditions: System 2 of Materials and Methods.) (a) Chlorogenic acid; (b) chicoric acid; (c) isochlorogenic acid.

markers of these flavonoids. Identification of the metabolites which accumulate after acetic acid treatment is still in progress.

The effect of different browning inhibitors on wound-induced PAL activity was also tested in disks stored for 3 days (Table 3). Soluble PAL activity decreased with the three treatments, but at different rates. PAL activity was completely inhibited in disks treated with acetic acid, but only inhibited around 60% compared to the control in disks treated with either $CaCl_2$ or 2,4-D.

The effect of acetic acid on PAL activity was measured in control stem disks in which PAL activity had been induced by excision and 3 days of storage. Disks were washed with 0.5 M acetic acid and blotted with paper, and PAL activity was determined. No PAL activity was detected in the homogenized, acetic acid washed disks extracted in borate buffer (pH 8.5). However, the extract from acetic acid treated disks had a pH of 6.0, while the extract from the control had a pH of 8.2. No PAL activity was detected even when the pH of the extract from acetic acid treated disks was adjusted to 8.5 by the addition of concentrated NaOH. This shows that the acetic acid treatment caused an irreversible inactivation of the enzyme.

DISCUSSION

Wounding iceberg lettuce stem tissue induces PAL activity and the synthesis and accumulation of soluble phenolic compounds (caffeic acid derivatives). Oxidation of these compounds to *o*-quinones by POP, and the polymerization of these quinones produces insoluble brown pigments. This browning process is inhibited by washing the wounded tissue with aqueous solutions of $CaCl_2$, 2,4-D, or acetic acid.

Calcium treatment decreased PAL activity to around 60% of the control, in agreement with previous reports (Ke and Saltveit, 1986), but it had only a slight effect on the accumulation of phenolic compounds, which were produced and accumulated to levels similar to those found in the control. The effect of $CaCl_2$ in preventing browning must, therefore, be at the level of PPO. This enzyme is membrane-bound (Mayer, 1987), and is present in a latent form in lettuce tissue (Chazarra et al., 1996). When wounding occurs, membranes are degraded, and the fatty acids released can activate the latent PPO (Mayer 1987). The role of calcium might be to preserve membrane structure, and thereby keep PPO in its latent form, or to prevent cellular decompartmentalization and the mixing of soluble phenolic

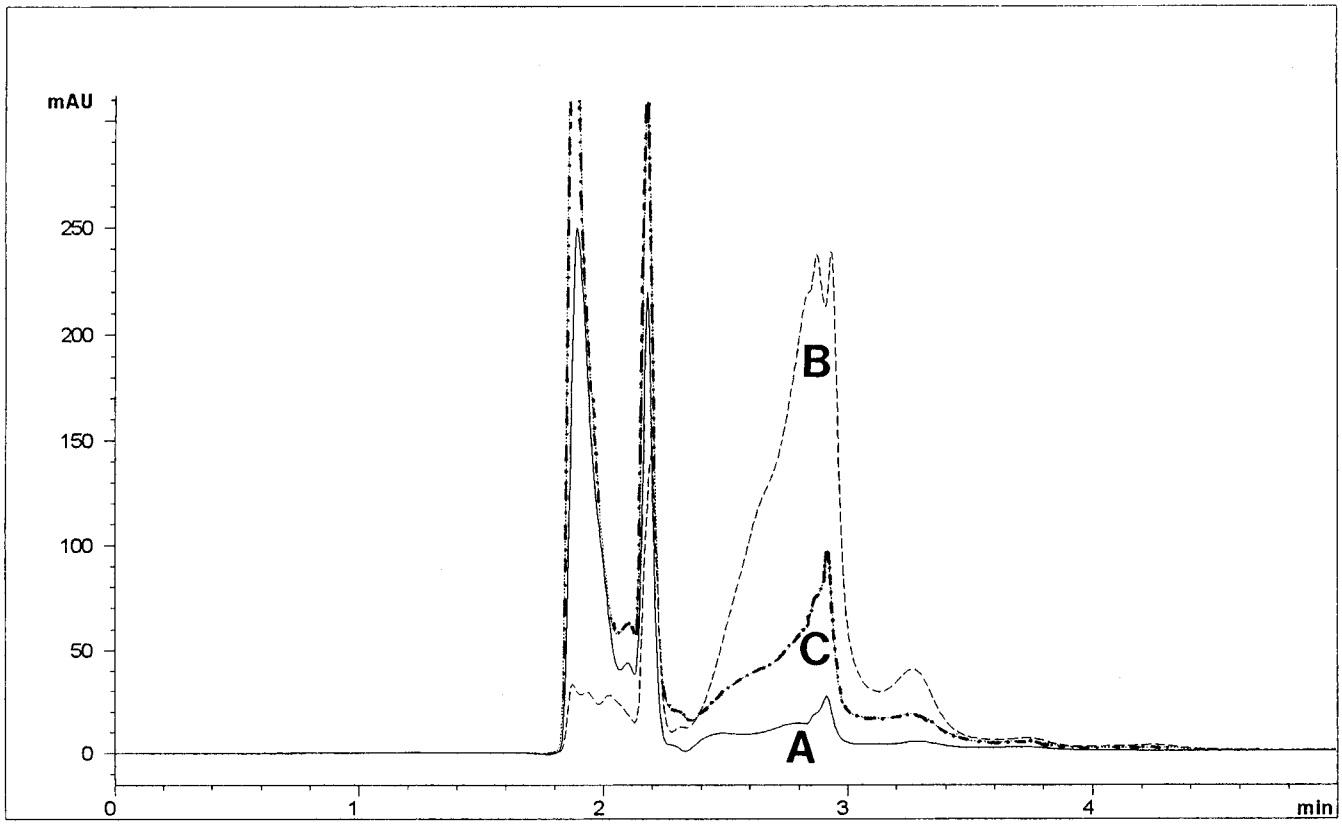

Figure 6. Detail of the first 5 min of the HPLC chromatograms of Figure 5, but in this case at 265 nm. (A) Calcium chloride; (B) acetic acid; (C) 2,4-D.

substrates, which are vacuolar, and membrane-bound PPO. In addition, PPO could be directly inhibited by $CaCl_2$.

The effect of 2,4-D on wound-induced phenolic metabolism appears to be different from calcium, although it also reduces PAL activity to 60% of the control. Since 2,4-D greatly reduce the biosynthesis of phenolic compounds, it may prevent browning by decreasing the supply of polyphenols available to PPO. Since PAL activity is not completely inhibited, the lack of phenolic biosynthesis can be due either to an early effect in the shikimate pathway, before the biosynthesis of phenylalanine, or it could act at the level of the enzymes responsible for the transformation of the product of PAL, cinnamic acid, into the caffeic acid derivatives. However, the accumulation of cinnamic acid or other related compounds was not observed. The hypothesis that 2,4-D acts at the level of the shikimic acid pathway or earlier appears most likely.

The effect of acetic acid is clear. It completely suppresses PAL activity and inhibits phenolic biosynthesis. Since the substrates are not synthesized, they are not available for PPO and browning does not occur. It is not clear, however, if the metabolites which accumulate in acetic acid treated disks (Figure 6) are biosynthesized "de novo" or if they are degradation products of polymeric polyphenols produced by the effect of acetic acid. Further research is needed in order to clarify this point. The effect of browning inhibition by acetic acid and other acids had been suggested to be due to the decrease in pH which decreases the activity of PPO. However, here we show that, in addition of this possible role, acetic acid has a direct effect on phenolic metabolism. It inhibits PAL activity and thereby prevents the formation of caffeic acid derivatives, while inducing the accumulation of other metabolites.

ACKNOWLEDGMENT

The authors thank Dr. M. J. Amiot (INRA, Avignon, France) for providing an authentic sample of cyrarin and Dr. M. N. Clifford (Surrey, U.K.) for useful discussion to phenolic compounds identification.

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Received for review July 3, 1996. Accepted November 13, 1996.[⊗] The authors are grateful to the Spanish CICYT for financial support (ALI-95-0001), M.I.G. to the CSIC for a fellowship (Contrato Temporal de Personal Investigador), and M.C. to the Fundacion Cultural CAM for a fellowship.

JF960478F

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1997.