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Effect of Wash Water Temperature and Chlorination on Phenolic Metabolism and Browning of Stored Iceberg Lettuce Photosynthetic and Vascular Tissues

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Cut tissues from distinct anatomical locations in iceberg lettuce (*Lactuca sativa* L.) were subjected to washing in cold (4 °C) and warm (47 °C) water with or without chlorine to assess their propensity to discoloration during storage. Total protein (Bradford method) and phenolic (TPH; Folin−Ciocalteu method) contents and polyphenol oxidase (PPO; spectrophotometric method using catechol as a substrate), peroxidase (POD; guaiacol substrate), and phenylalanine ammonia-lyase (PAL; phenylalanine substrate) activities were determined in photosynthetic and vascular tissue from outer and inner leaves. Unprocessed photosynthetic and inner leaf tissues had significantly higher ($P < 0.05$) levels of protein and TPH and PPO and POD activities than vascular and outer leaf tissues. PAL activities (on a fresh weight basis) were similar in all tissues. Changes in browning (light reflectance measurement) and phenolic metabolism in all four tissue types were observed during aerobic storage at 5 °C over 10 days. PAL activity increased in all tissues after 1−2 days of storage and then gradually decreased. POD activity also increased steadily for the storage duration. Protein content and PPO activity remained constant. Edge browning (measured with a Minolta Chroma Meter) and TPH increased in all tissues, especially in outer vascular tissue. Cut photosynthetic and vascular tissues washed at 4 and 47 °C with and without 100 μg mL^{-1} chlorine for 3 min were analyzed during 7 days in storage at 5 °C. Enzyme activities and accumulation of phenolics in all tissues washed at 47 °C were significantly ($P < 0.05$) lower compared to controls or tissues washed at 4 °C. Chlorine had no additional effect at 47 °C but significantly ($P < 0.05$) reduced browning and accumulation of phenolics in lettuce washed at 4 °C. These results showed that inherent differences between tissues affect phenolic metabolism and browning in stored, fresh-cut lettuce.

KEYWORDS: Lettuce; *Lactuca sativa* L.; fresh-cut; phenolic metabolism; browning; phenylalanine ammonia-lyase; peroxidase; polyphenol oxidase

INTRODUCTION

Processing and distribution of leafy vegetables such as lettuce in a packaged, "fresh-cut" format has increased dramatically in recent years due to the convenience these products provide to end-users. Unfortunately, the damage inflicted to plant tissues by shredding or slicing accelerates reactions that lead to quality defects such as browning of cut edges, discoloration, and reduced turgidity (*1*). Other unit operations applied during processing, including washing, drying, packaging, and storage, also affect the extent and rate of physiological reactions and microbiological processes that influence the development of quality defects (*2*). A more thorough understanding of these effects could lead to improved processing schemes for quality retention.

Mechanical damage inflicted during cutting elicits wound responses that include ethylene synthesis, elevated respiration, oxidative browning, wound healing, secondary metabolite synthesis, and water loss (*3*, *4*). Prior research has shown that browning of wounded lettuce tissues is correlated with enhanced phenylalanine ammonia-lyase (PAL) activity (*5*). PAL-catalyzed deamination of L-phenylalanine to *trans*-cinnamic acid and ammonia is the first step in the phenylpropanoid pathway, a series of reactions that can, with the intervention of polyphenol oxidase (PPO), lead to the formation and accumulation of brown phenolic compounds (*6*). Peiser et al. (*5*) demonstrated that PAL-specific inhibitors reduce browning in excised lettuce midrib tissues. Ke and Saltveit (*7*) measured increases in PAL activity and soluble phenolic compounds in iceberg lettuce midrib tissue wounded by cutting or puncturing. Ke and Saltveit (*8*) have proposed that further oxidation of these compounds by PPO and/or peroxidase (POD) leads to the appearance of brown products.

Several treatments have been proposed to reduce browning and improve the quality of fresh-cut lettuce. Some success has been reported by dipping in vinegar or acetic acid solutions (10

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and 50 mL/L) for 5 s (*9*) or storage under modified atmosphere (5% O_2 plus 5% CO_2 in N_2) (*10*). Loaiza-Verlarde et al. (*11*) found that PAL activity, concentration of phenolic compounds, and browning of cut midrib iceberg lettuce tissues were reduced by mild heat shocks (45 °C for 120 s, 50 °C for 60 s, or 55 °C for 30 s) prior to storage. These authors postulated that heat represses wound-induced phenylpropanoid metabolism in favor of heat-shock protein synthesis. Suppression of browning by washing in warm (47 °C) chlorinated (100 μg/mL) water was also reported by Delaquis et al. (*12*, *13*). The latter studies also showed that washing at warm temperatures provides the additional benefit of improved destruction of microbial contaminants.

The heterogeneous nature of lettuce tissues may hinder assessment of physiological effects that result from processing. Lettuce leaves contain both photosynthetic and vascular tissues that vary in density from the outside to the inside of the head. Previous work indicates that the composition and physiological status of these tissues vary. Heimdal et al. (*14*) found that PPO activity in vascular (midrib) tissues of iceberg lettuce was one-seventh that of photosynthetic tissues on a fresh weight basis, even though specific PPO activities on a protein basis were comparable. Castañer et al. (*15*) noted differences in phenolic content and PPO activity between photosynthetic and midrib tissue in baby and romaine lettuces. Most research on wound-induced phenolic metabolism in iceberg lettuce has been carried out with rib or midrib tissue (*5*, *8*, *11*, *16*−*18*). Because commercial fresh-cut iceberg lettuce contains both photosynthetic and vascular tissue, information on the response of both tissues to processing and storage would be useful.

The present study on fresh-cut iceberg lettuce was conducted with three main objectives. The first was to determine whether phenolic metabolism in lettuce tissues varies with type and location within the head. The second objective was to assess changes in phenolic metabolism and browning in iceberg lettuce tissues during storage at 5 °C. The third objective was to determine the effect of wash water temperature and chlorination on these properties in photosynthetic and vascular tissues stored at 5 °C.

MATERIALS AND METHODS

Preparation of Lettuce Tissues. Iceberg lettuce (*Lactuca sativa* L. type Salinas) grown in the United States (California or Arizona) was purchased from a local retailer and was kept at 1 °C until used (within 24 h). The heads were prepared for further processing by removing and discarding wrapper leaves and excision of the core with a stainless steel tube sharpened at one end. Each head was then weighed.

For some experiments the heads were separated into four tissue types: outer photosynthetic, outer vascular, inner photosynthetic, and inner vascular. Outermost leaves were removed from the head until approximately half of the whole weight was collected. Green or yellowish green tissues were separated from white (midrib) tissues with a sharp stainless steel knife. These samples constituted photosynthetic and vascular outer tissues, respectively. The remaining leaves were separated into inner photosynthetic and vascular tissues. Photosynthetic tissues from outer leaves were generally darker green than photosynthetic tissues from inner leaves. Experiments on wash treatments were conducted with photosynthetic and vascular tissues excised as described above but without regard to location within the head.

Changes in Phenolic Metabolism and Browning during Storage. Outer photosynthetic, outer vascular, inner photosynthetic, and inner vascular tissues were obtained as described above. Each lot was cut into squares (approximately 3 cm × 3 cm) using a sharp stainless steel knife and mixed thoroughly. The cut pieces were packed into six high-permeability film bags (35 cm × 25 cm, PD941, Cryovac, Mississauga, ON, Canada; oxygen transmission rate = 16544 mL of $O_2/m^2/24$ h) in 100 g lots. Excess air was gently expelled from the bags before they were sealed with a Swissvac MINOR 2 vacuum sealer (Luzern, Switzerland). The bags were stored flat on metal mesh shelves in a single layer at 5 ± 1 °C in the dark. One bag of each tissue type was removed after 0, 1, 2, 4, 7, and 10 days of storage for analysis of browning, protein and total phenolic contents, and PPO, POD, and PAL activities. Three replicate trials were conducted.

Effect of Wash Treatments on Stored Photosynthetic and Vascular Tissues. Several heads of lettuce were processed to obtain 2.2 kg each of photosynthetic and vascular tissues. The tissues were cut into 3 × 3 cm squares and mixed thoroughly. Tissues corresponding to each type were divided into four 400 g lots for wash treatments, and one 500 g lot was retained to serve as a nonwashed control. The control lot was divided into five 100 g amounts, and each was packed directly into PD941 film bags. The bags were stored flat on metal mesh shelves in a single layer at 5 ± 1 °C in the dark. One bag of each tissue type was analyzed for browning, protein and TPH contents, and PPO, POD, and PAL activities after 0, 1, 2, 4, and 7 days of storage.

The remaining 400 g lots of each tissue type were washed in stainless steel pails containing 7.5 L of water under the following conditions: 4 °C; 4 °C plus 100 μg mL^{-1} sodium hypochlorite (~95 μg mL^{-1} free chlorine, pH 9.1); 47 °C; and 47 °C plus 100 μg mL^{-1} sodium hypochlorite. Water temperature in the pails before and after washing varied by <1° C. Chlorinated water solutions were prepared with commercial grade sodium hypochlorite (5% NaOCl). Total and free chlorine concentrations were measured using a Hach (model CN-66; Loveland, CO) test kit. The lettuce was stirred constantly with a plastic spoon for 3 min and was removed to a colander to drain. Excess water was removed in a home salad spinner, and the lettuce was packed in 100 g amounts in PD941 film bags, which were stored as described above. One bag of each tissue type was removed after 1, 2, 4, and 7 days of storage at 5 °C for analyses of browning, protein and TPH contents, and PPO, POD, and PAL activities. Three replicate trials were conducted.

Analytical Procedures. *Browning.* The color (L^*, a^*, and b^*) of lettuce was measured using a Minolta Chroma Meter CR-200 (Osaka, Japan) calibrated to a standard white tile (Y-93.2, x-0.3165, y-0.3342). The illuminant was the CIE (Commission Internationale de l'Eclairage) D_{65}. Several randomly drawn leaves were stacked to measure color along the cut edges. Six measurements were made per bag. Surface color was also recorded for 12 lettuce pieces per bag. The hue angle ($H°$) was calculated as (*19*)

$$H° = \tan^{-1}(b^*/a^*) \qquad \text{when } a^* > 0 \text{ and } b^* \leq 0$$

and

$$H° = 180° + \tan^{-1}(b^*/a^*) \qquad \text{when } a^* < 0$$

The browning of tissue edges and surfaces was also assessed visually using the 1−5 hedonic scale of Peiser et al. (*24*), where 1 = none, 3 = moderate, and 5 = severe browning.

Total Phenolic Content. Samples were prepared by cutting the tissues into squares (approximately 1 cm × 1 cm) using a stainless steel knife. These were thoroughly mixed to minimize subsampling variation. Three, 25 g subsamples were prepared for three separate extraction procedures for total phenolic (TPH) content and PPO, POD, and PAL activities.

The procedure of Ke and Saltveit (*8*) was modified for extraction of phenolics. A 25 g subsample was placed into a semimicro blender jar (50−250 mL capacity) with 50 mL of chilled methanol. The mixture was blended for 60 s. The resultant homogenate was filtered through four layers of Miracloth (Calbiochem-Novabiochem Corp., La Jolla, CA) and was centrifuged at 15000*g* for 10 min at 4 °C. The supernatant was then filtered through Whatman No. 4 paper, and the clarified supernatant was kept on ice until analyzed (within 5 h).

TPH content was measured using a modified version of the Singleton and Rossi (*20*) method. Chlorogenic acid at several concentrations (0−100 mg/L) in 80% methanol was used as a standard. The assay procedure consisted of adding 0.2 mL of methanol extract or chlorogenic acid standard to a test tube, followed by 1.0 mL of diluted (1:30) Folin−Ciocalteu phenol reagent from Sigma Chemical Co. (St.

Table 1. Properties of Different Tissue Types in Unprocessed Iceberg Lettuce ($n = 6$)

characteristic	tissue type			
	outer photosynthetic	outer vascular	inner photosynthetic	inner vascular
proportional composition of head (% of total head wt)	32.8 a[a]	22.6 b	23.0 b	21.1 b
protein content (μg g^{-1})	1300 b	534 d	1933 a	866 c
total phenolics (μg g^{-1})	159 b	115 c	265 a	148 b
PPO activity (units g^{-1} of fwt)	397 b	90 d	630 a	161 c
PPO activity (units mg^{-1} of protein)	308 a	168 b	329 a	185 b
POD activity (units g^{-1} of fwt)	52 b	29 d	86 a	41 c
POD activity (units mg^{-1} of protein)	40 c	55 a	45 bc	49 ab
PAL activity (μmol of cinnamic acid g^{-1} h^{-1})	0.036 b	0.074 a	0.041 ab	0.047 ab
PAL activity (μmol of cinnamic acid mg^{-1} of protein h^{-1})	0.026 b	0.122 a	0.017 b	0.043 b

[a] Means within each row followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

Louis, MO), and 0.8 mL of 708 mM Na_2CO_3. The Na_2CO_3 solution was added within 5 min of adding the phenol reagent. The solution was vortexed and allowed to incubate for 60 min at room temperature before absorbance was measured at 765 nm with a UV−vis spectrophotometer (Beckman DU-600, Fullerton, CA).

HPLC Analysis of Phenolics. Concentrations of selected phenolic acids in stored lettuce tissues were determined by HPLC. For these samples, a 10 mL aliquot of clarified methanol supernatant from the TPH extraction was filtered through a 0.45 μm Acrodisc LC PVDF syringe filter (Pall Gelman Laboratory, Montreal, PQ, Canada) and stored at −25 °C until analyzed by HPLC. Phenolics in the filtered methanol extracts were analyzed using a Waters HPLC system (Mississauga, ON, Canada) with a Waters 990 photodiode array detector. A reverse-phase Supelcosil LC-18 column (25 cm × 2.1 mm i.d.) from Supelco, Inc. (Bellefonte, PA) maintained at 25 °C with an Aquapore RP-18 ODS 1.5 cm guard cartridge (Applied Biosystems, Inc., Foster City, CA) was used for separation. A 50 μL aliquot of sample extract or chlorogenic acid standard in 80% methanol (0−100 mg/L) was applied to the column. Methods from Tomás-Barberán et al. (*18*) and Mazza et al. (*21*) were modified to achieve satisfactory separation. A total solvent flow rate of 0.35 mL/min was employed with solvent A [5% (v/v) formic acid] and solvent B (100% methanol). The gradient used in proportions of solvent B was as follows: 0−5 min, 5%; 5−40 min, 50%; 40−43 min, 100%; 43−45 min, 5%; and 45−49 min, 5%. Four major peaks [caffeoyltartaric, chlorogenic (5-caffeoylquinic), dicaffeoyltartaric, and isochlorogenic (3,5-dicaffeoylquinic) acids] were identified according to the results of Tomás-Barberán et al. (*18*) and quantified as chlorogenic acid equivalents. Quantification was based on a standard curve prepared using chlorogenic acid.

PPO and POD Activities. PPO and POD activities were determined in extracts prepared using a modified version of the method of Flurkey and Jen (*22*). Lettuce tissues were cut into 1 × 1 cm squares using a stainless steel knife. A 25 g subsample was placed in a semimicro blender jar with 50 mL of chilled 0.05 M phosphate buffer (pH 6.2) and 3.75 g of PVPP. The mixture was blended for 60 s, filtered through four layers of Miracloth, and centrifuged at 15000*g* for 10 min at 4 °C. The supernatant was filtered through Whatman No. 4 paper, and the clarified supernatant was kept on ice until assayed (within 5 h).

PPO and POD activities were measured using procedures described by Flurkey and Jen (*22*), with some modifications. A 50 μL aliquot of extract was added to a cuvette containing 2.5 mL of 0.08 M catechol in 0.05 M phosphate buffer (pH 6.2) to assay for PPO activity. The Parafilm-covered cuvette was inverted three times, and absorbance (410 nm) was measured every 2 s for 40 s. The slopes of linear portions of resultant curves were calculated for the first 20 s of the assay. One unit of PPO activity was defined as a 0.1 absorbance unit change per minute at 410 nm.

A 0.25 mL aliquot of extract was added to a cuvette containing 2.5 mL of 0.4% (v/v) guaiacol and 0.04% H_2O_2 in 0.05 M phosphate buffer (pH 6.2) to assay for POD activity. Absorbance at 470 nm was monitored for 30 s. Slopes for the linear portion of each curve were calculated for the first 12 s of the assay. One unit of POD activity was defined as a 0.1 absorbance unit increase per minute at 470 nm.

PAL Activity. The extraction procedure of Ke and Saltveit (*23*) was employed with some modifications. Twenty-five grams of tissue cut into 1 cm × 1 cm squares was added to a semimicro blender jar with 50 mL of chilled 0.05 M borate buffer (pH 8.5) containing 5 mM β-mercaptoethanol and 3.75 g of PVPP. The mixture was blended for 60 s, filtered through four layers of Miracloth, and then centrifuged at 15000*g* for 10 min at 4 °C. The supernatant was filtered through Whatman No. 4 paper. A 2.5 mL aliquot of the clarified supernatant was passed through a PD-10 column (Amersham Pharmacia Biotech Inc. Canada, Baie D'Urfé, PQ, Canada) containing Sephadex G-25M to remove low molecular weight compounds. A 3.5 mL volume of eluent containing the enzyme was collected from the column and was kept on ice until assayed (within 5 h).

The method of Ke and Saltveit (*23*) was used to measure PAL activity. Column eluent (1.0 mL) or buffer solution was added to each of two cuvettes. A 0.12 mL aliquot of 0.05 M borate buffer (pH 8.5) was added to the first and 0.12 mL of 100 mM phenylalanine in 0.05 M borate buffer (pH 8.5) to the second. The cuvettes were inverted three times, placed in a 40 °C water bath for 5 min, and cooled in a room temperature water bath for 3 min before measurement of absorbance at 290 nm. The cuvettes were then incubated for 60 min at 40 °C and cooled at room temperature, and then absorbance was read anew. The change in absorbance at 290 nm (ΔA_{290}) after 60 min was calculated for all cuvettes. The overall ΔA_{290} for a sample was then calculated as

$$\Delta A_{290} \text{ sample with phenylalanine} - \Delta A_{290} \text{ sample with borate buffer}$$

A standard curve for *trans*-cinnamic acid at various concentrations (0−150 μM) in 0.05 M borate buffer (pH 8.5) was obtained at 290 nm. The standard curve was used to calculate the amount of cinnamic acid formed in the samples. One unit of PAL activity was defined as the formation of 1 μmol of cinnamic acid per hour.

Protein Content. Protein content was measured using the Bio-Rad protein reagent (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin (BSA) (Sigma Diagnostics, St. Louis, MO) at several concentrations (0−250 mg/L) as a standard. Extracts prepared for PPO, POD, and PAL activities were diluted four times in their respective buffers. A 15 μL volume of standard or diluted extract followed by 250 μL of diluted (1:5) Bio-Rad protein reagent was added to individual wells in 96-well flat-bottom EIA microplates (ICN Biomedicals Inc., Aurora, OH). The plates were incubated at room temperature for 30 min before reading of absorbances at 595 nm with a SpectraMax Plus 384 microplate reader (Molecular Devices Corp., Sunnyvale, CA).

Gas Analysis. Carbon dioxide and oxygen concentrations in lettuce bags were measured by gas chromatography as described by Moyls et al. (*24*). Headspace gas analyses were conducted on all bags from two replicate trials conducted with stored lettuce tissues of various types.

Statistical Analysis. Data were analyzed using the SAS (SAS Inc., Cary, NC, ver. 8.1) ANOVA procedure. Means were compared using Duncan's multiple-range test. For analyses of differences in cut tissue types during storage, factors were the tissue type, location, and storage time. To analyze data for the effect of wash treatment, factors were tissue type, storage time, and wash treatment.

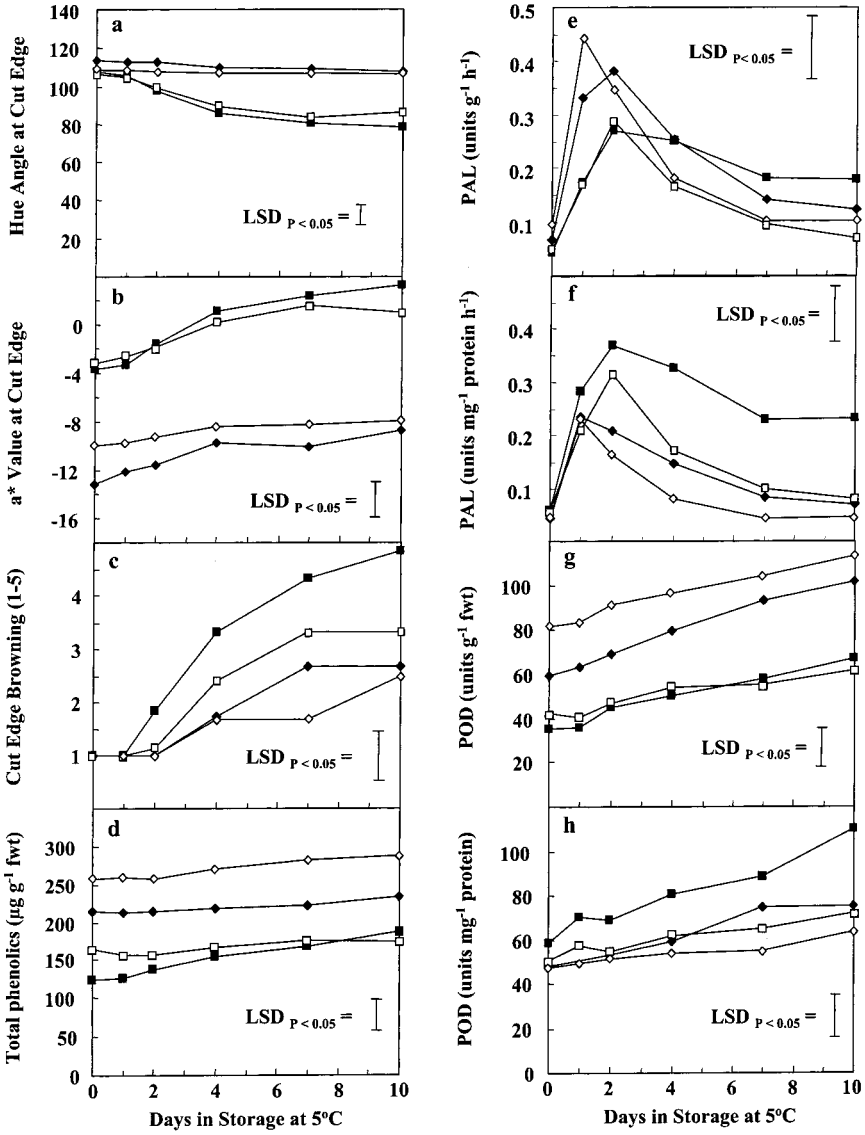

Figure 1. Edge hue angle (a), edge *a** value (b), edge browning rating (c), total phenolic content (d), PAL activity on fresh weight (e) and protein (f) bases, and POD activity on fresh weight (g) and protein (h) bases in cut photosynthetic and vascular iceberg lettuce tissues from inner and outer leaves during storage at 5 °C: (◆) outer photosynthetic tissue; (◇) inner photosynthetic tissue; (■) outer vascular tissue; (□) inner vascular tissue.

RESULTS AND DISCUSSION

Properties of Photosynthetic and Vascular Tissues. Compositional analysis of fresh lettuce tissues revealed significant variation due to tissue type and location within the head (**Table 1**). Differences between photosynthetic and vascular tissues tended to be large, whereas differences between outer or inner locations within the lettuce head were less pronounced. Outer photosynthetic tissues accounted for a slightly higher proportion of the head fresh weight, whereas the other three tissue types were present in similar proportions. Protein and TPH contents were lower in vascular than in photosynthetic tissues and, in turn, lower in outer than in inner tissues (**Table 1**). Heimdal et al. (*14*) also reported higher protein contents in iceberg lettuce photosynthetic tissues. The phenolic content of baby romaine lettuce photosynthetic tissues was likewise found to exceed that in midrib tissues in a study by Castañer et al. (*15*). Differences between outer and inner leaves were not evaluated in these papers.

PPO and POD activities (on a fresh weight basis) were highest in inner photosynthetic tissues, followed by outer photosynthetic and inner and outer vascular tissues (**Table 1**). However, specific PPO activities on a protein basis were equal for inner and outer tissues, and specific POD activities were similar for all tissue types. Interpretations of enzyme activity in lettuce tissue may therefore vary depending on the units used to express activity. For this reason, the results presented in this work were given on both fresh weight and protein bases.

Because PPO activity has often been associated with chloroplasts (*25*), it was expected that photosynthetic tissues should have higher PPO activity. PPO activity in photosynthetic tissue was ~4 times higher than in vascular tissue on a fresh weight basis and 2 times higher on a protein basis (**Table 1**). Heimdal et al. (*14*) found that PPO activity based on fresh weight of iceberg lettuce photosynthetic tissue was ~7 times higher than in vascular tissue, and that activity was similar on the basis of protein content. Disparities may be ascribed to differences in lettuce type. Heimdal et al. (*14*) used an "Ithaca-type", whereas a "Salinas-type" lettuce was used in this study.

The properties of lettuce tissues were highly influenced by tissue type. These observations suggest that phenolic metabolism in photosynthetic and vascular tissues from inner and outer

Table 2. Significance for Effects of Tissue Type, Location within the Head, and Length of Storage Differences on Color Measurements, Visual Browning, and Selected Enzyme Activities in Packaged Iceberg Lettuce Stored at 5 °C[a]

	variable						
source	hue angle	*a** value	browning	total phenolics	PPO activity	POD activity	PAL activity
location	0.6689	0.0733	0.0009	<0.0001	<0.0001	0.0003	0.1362
tissue	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0023
location × tissue	0.0423	0.0027	0.0616	0.0022	0.0034	0.0021	0.2415
day	<0.0001	<0.0001	<0.0001	0.0066	0.9244	<0.0001	<0.0001
location × day	0.7630	0.4145	0.1284	0.8653	0.9416	0.7152	0.1418
tissue × day	<0.0001	0.1122	0.0003	0.9303	0.9933	0.6950	0.0009
location × tissue × day	0.9912	0.9974	0.5247	0.4664	0.8967	0.9992	0.7156

[a] Values are $P > F$ determined from three-way ANOVA.

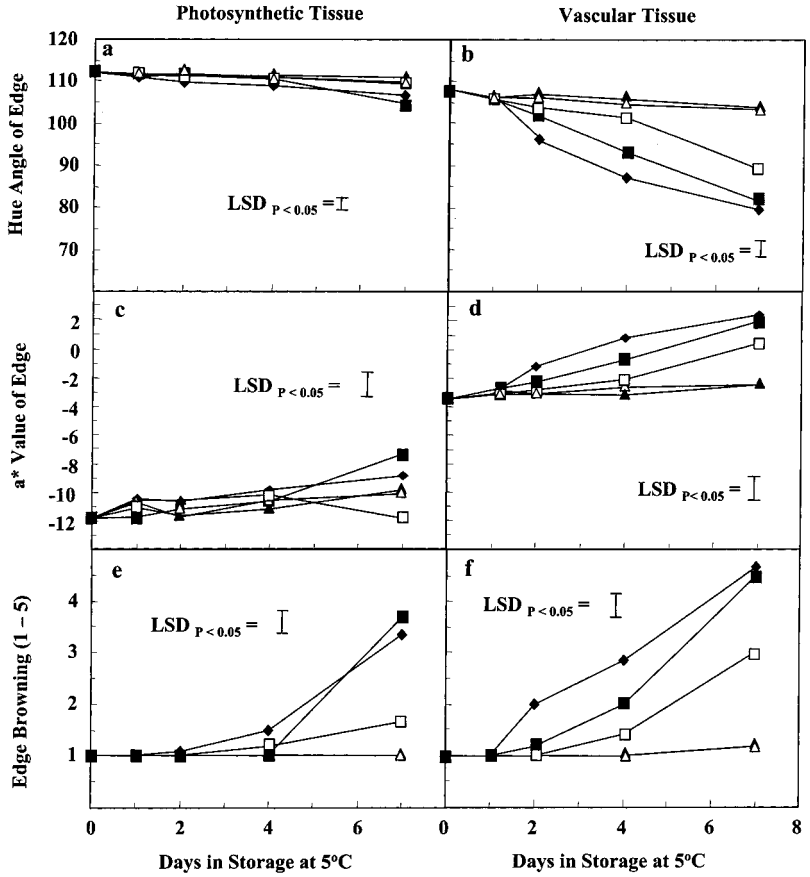

Figure 2. Edge hue angles (a, b), edge *a** values (c, d), and edge browning ratings (e, f) of cut photosynthetic and vascular iceberg lettuce tissue stored at 5 °C: (◆) unwashed control; (■) washed at 4 °C; (□) washed at 4 °C with 100 μg/mL sodium hypochlorite; (▲) washed at 47 °C; (△) washed at 47 °C with 100 μg/mL sodium hypochlorite.

leaves is equally important to understanding quality changes in packaged, cut lettuce.

Changes in Stored Lettuce Tissues. Changes in phenolic metabolism and browning of different tissue types were assessed during storage at 5 °C. Oxygen content in the bags remained between 17 and 20% (v/v), and carbon dioxide content was <1% throughout the 10-day storage period, conditions that must be considered aerobic. The initial properties of different tissue types illustrated in **Figure 1** paralleled those obtained in the previous experiment (see **Table 1**). Edge color measurements ($H°$) were significantly higher and *a** values were lower in photosynthetic (outer and inner) than in vascular (outer and inner) tissues. Higher *a** values reflected the relative absence of green pigment in vascular tissue.

Some tissue properties were substantially altered during storage (**Figure 1**; **Table 2**). $H°$ of edges (**Figure 1a**) decreased toward 90°, indicating a shift to yellow color. The value of *a** (**Figure 1b**) increased, suggesting a shift from green ($-a$*) to red ($+a$*). Smaller changes in $H°$, *a**, and visual ratings for photosynthetic tissue indicated that color changes occurred more slowly than in vascular tissue. Vascular tissue, particularly from the outside of the head, developed brown discoloration at cut edges more quickly than did other tissue types (**Figure 1c**). Visual ratings of browning paralleled changes in *a** values and were inversely related to $H°$ for vascular tissue. In contrast, there was no clear association between instrumental color measurements and visual browning ratings for photosynthetic tissues. Interference due to chlorophyll may have affected Chroma Meter readings for photosynthetic tissue.

Protein content and PPO activity did not change significantly ($P < 0.05$) with storage (data not shown). Ke and Saltveit (*7*) also found that PPO activity did not change in stored, wounded

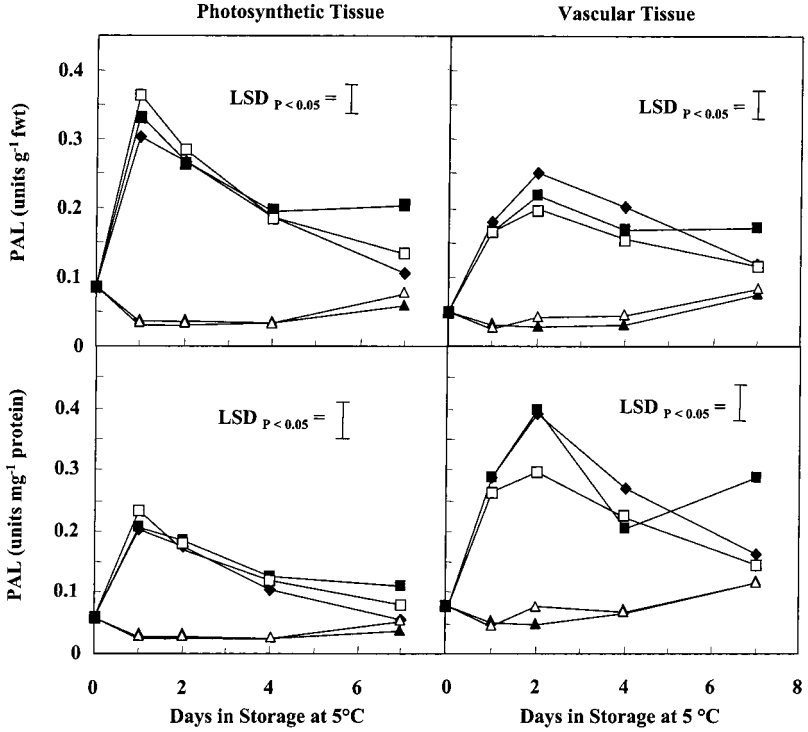

Figure 3. Phenylalanine ammonia-lyase (PAL) activity in photosynthetic and vascular iceberg lettuce tissues stored at 5 °C after washing at two temperatures: (◆) unwashed control; (■) washed at 4 °C; (□) washed at 4 °C with 100 μg/mL sodium hypochlorite; (▲) washed at 47 °C; (△) washed at 47 °C with 100 μg/mL sodium hypochlorite.

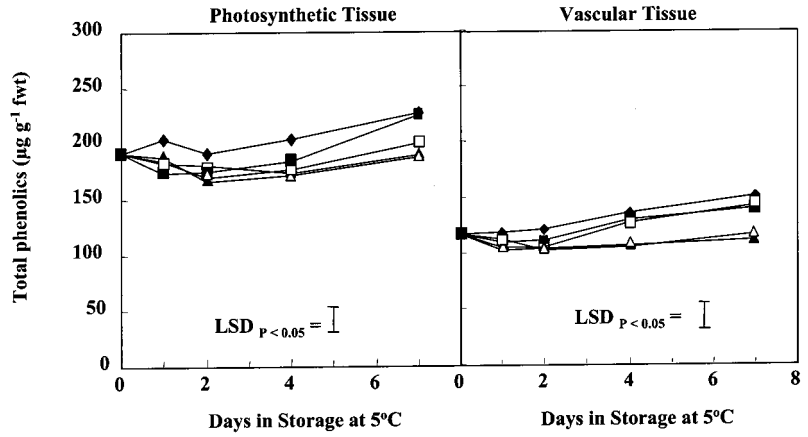

Figure 4. Total phenolic content of cut photosynthetic and vascular iceberg lettuce tissue stored at 5 °C after washing at two temperatures: (◆) unwashed control; (■) washed at 4 °C; (□) washed at 4 °C with 100 μg/mL sodium hypochlorite; (▲) washed at 47 °C; (△) washed at 47 °C with 100 μg/mL sodium hypochlorite.

midrib tissue. However, Cantos et al. (*26*) observed some increase in PPO activity during storage at 5 °C when protease inhibitors were used in extraction procedures. Browning in wounded lettuce has been more closely associated with PAL-initiated synthesis of phenylpropanoid compounds and their subsequent oxidation by PPO (*7*). In the present study, PAL activity increased and then gradually decreased to initial levels during storage (**Figure 1e**,**f**). PAL activity reached a maximum after 1 day in photosynthetic tissue and after 2 days in vascular tissue. This result mirrors the earlier findings of Ke and Saltveit (*7*) and Cantos et al. (*26*). However, specific PAL activity in vascular tissue was higher than in photosynthetic tissue, whereas the reverse was true when enzyme activity was based on fresh weight. The interaction of tissue type and storage time was significant ($P < 0.05$) for PAL activity on a fresh weight basis. Increased PAL activity was expected to lead to increases in total phenolic content for all tissue types. Surprisingly, phenolic levels significantly increased only in outer vascular tissue (**Figure 1d**). The greater increase in total phenolics in outer vascular tissue is a likely explanation for the tendency to faster browning.

POD activity increased with storage time for all tissues on a specific activity and fresh weight basis (**Figure 1g**,**h**). Ke and Saltveit (*7*) and Cantos et al. (*26*) noted that wounding led to increased POD activity in midrib tissue during storage. Ke and Saltveit (*7*) associated increased POD activity with cell wall lignification in response to wounding, because POD is involved in the lignin-specific pathway.

Inherent morphological differences in the four tissue types suggest caution should be maintained in the interpretation of phenolic metabolism in storage. For example, PAL activity in photosynthetic tissue on a fresh weight basis was much higher than in vascular tissue. However, single pieces of vascular tissue

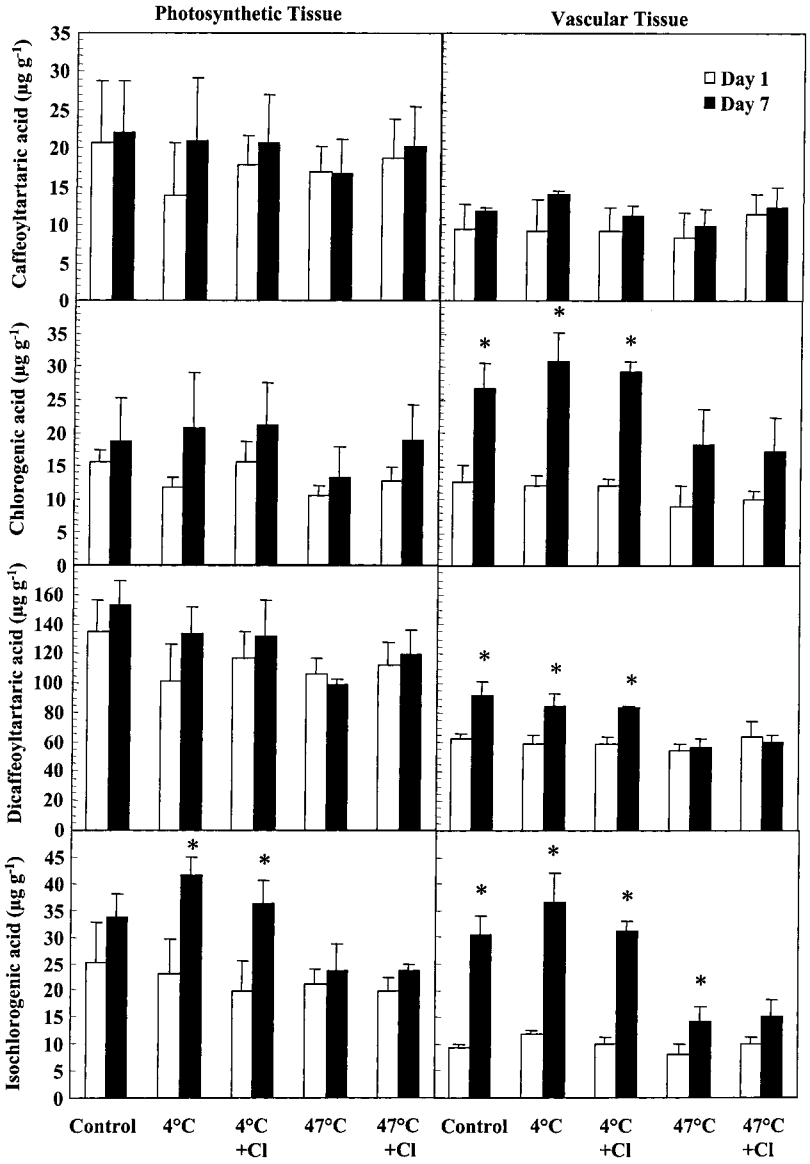

Figure 5. Concentrations of selected phenolic acids in cut photosynthetic and vascular iceberg lettuce tissues stored at 5 °C. Vertical bars represent one standard deviation. Means for columns labeled with * are significantly different ($P < 0.05$).

had a larger total volume and cut surface area than photosynthetic tissue because the former is considerably thicker. Random pieces (*30*) of each tissue type were weighed in one replicate trial. The average weights of the pieces ± standard deviations were 1.05 ± 0.49 g for outer photosynthetic, 2.22 ± 0.78 g for outer vascular, 1.38 ± 0.81 g for inner photosynthetic, and 2.15 ± 0.89 g for inner vascular. On a per piece basis, vascular tissue would therefore have similar or higher PAL activity than photosynthetic tissue.

Effect of Wash Treatments on Stored Photosynthetic and Vascular Tissues. All physiological characteristics were significantly ($P < 0.05$) affected by tissue type (**Figures 2**−**4** and **6**). Trends were similar to those described above for stored tissues. **Figure 2** shows the changes in $H°$, a^*, and visual ratings for browning of edges in stored photosynthetic and vascular tissues. Edge browning was strongly inhibited by washing at 47 °C with and without chlorine. Washing in water at 4 °C reduced browning slightly, but this effect was signifcantly ($P < 0.05$) enhanced by chlorination (**Figure 2e**,**f**). Browning was always more pronounced in vascular than in photosynthetic tissues.

Changes in PAL activity (**Figure 3**) and TPH content (**Figure 4**) showed that washing at 47 °C both with and without chlorine greatly reduced PAL activity but that differences in the accumulation of phenolic compounds were small. Reduced browning in tissues subjected to the 47 °C wash treatments was likely derived from this effect. Changes in individual phenolic compounds were monitored by HPLC analysis of methanol extracts after 1 and 7 days of storage. **Figure 5** shows that chlorogenic and isochlorogenic acid contents increased noticeably after 7 days in vascular tissue not washed at 47 °C. Dicaffeoyltartaric acid increased to a lesser extent. Tomás-Barberán et al. (*18*) reported similar trends in wounded midrib tissue after 3 days of storage at 5 or 10 °C. In contrast to vascular tissue, only isochlorogenic acid increased significantly after 7 days in photosynthetic tissue that was not washed at 47 °C. Wash treatments at 47 °C also delayed increases in POD activity (**Figure 6**). Reduction of browning in the warm water wash may, in part, be related to lower POD activity in treated tissues. However, the association of POD activity and lettuce browning has been reported to have both positive correlation (*7*) and no correlation (*26*, *27*).

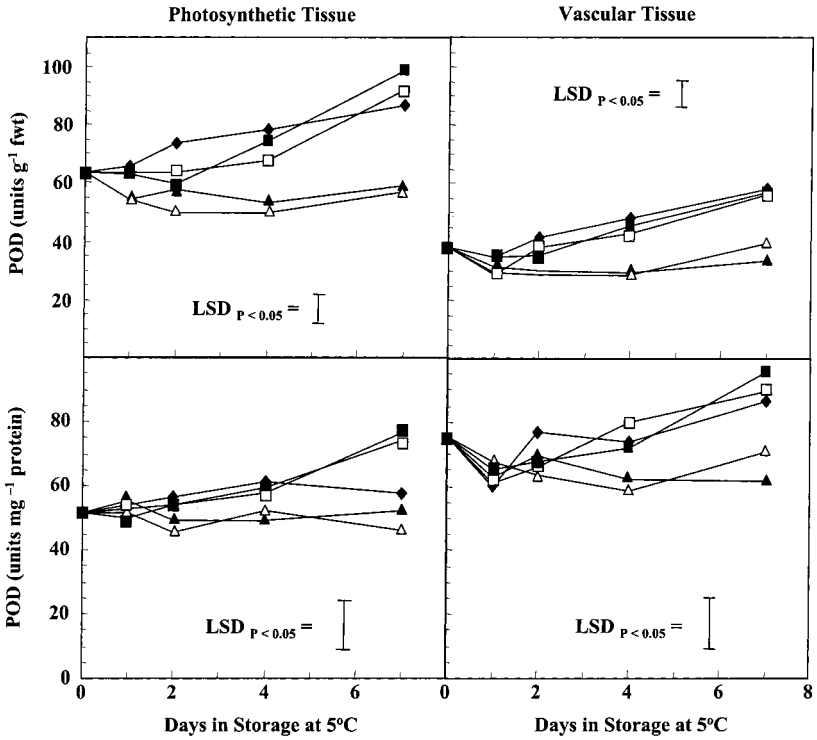

Figure 6. Peroxidase activity in fresh-cut photosynthetic and vascular iceberg lettuce tissues stored at 5 °C after washing at two temperatures: (◆) unwashed control; (■) washed at 4 °C; (□) washed at 4 °C with 100 μg/mL sodium hypochlorite; (▲) washed at 47 °C; (△) washed at 47 °C with 100 μg/mL sodium hypochlorite.

Although responses to heat treatment are generally similar to those reported by Loaiza-Verlarde et al. (*11*), the differences between photosynthetic and vascular tissues highlight apparent contradictions. Higher levels of phenolics were found in photosynthetic tissue, yet edge browning was less intense. Photosynthetic tissues are known to have antioxidant protectant systems due to the highly oxidative nature of some photosynthetic reactions (*28*). Protective enzymes in photosynthetic tissue include a complex of which POD is an important component; POD is known to recycle ascorbate (*28*). Ascorbate can act as an antioxidant and is commonly used in dips to delay cut edge browning in fruits and vegetables (*29*). Photosynthetic tissue had much higher levels of POD activity than vascular tissue. Therefore, despite the greater potential for browning of phenolics in photosynthetic tissue than vascular tissue, the actual level of browning may be lower due to antioxidant protectant systems.

This study provides information that may explain observations derived from previous research. Delaquis et al. (*12*, *13*) reported that washing lettuce in chlorinated water at 47 °C delayed browning. Differences in PAL activity and TPH content serve to explain these observations. Delaquis et al. (*12*) also reported that treated lettuce washed at 47 °C was perceived to be sweeter than lettuce washed at 4 °C. Many phenolic compounds have an astringent or bitter taste (*30*). The effect of washing at warm temperatures on the sensory quality of lettuce may therefore be related to alterations in phenylpropanoid metabolism. In this study, chlorine had no effect on browning of lettuce washed at 47 °C.

In conclusion, it is clear that phenolic metabolism varies in photosynthetic and vascular tissues from inner and outer leaves. Differences between tissues were maintained during storage, and browning of cut edges consequently varied among tissues. The order of browning potential was as follows: outer vascular > inner vascular > outer photosynthetic > inner photosynthetic tissues. Washing in warm water led to similar reductions in browning of both vascular and photosynthetic tissues. This suggests that both types of tissues responded in the same way to the treatment. However, because vascular tissue had a greater propensity for discoloration, the effects of mild heat on this tissue were greater in absolute magnitude. However, a contradiction was noted in that photosynthetic tissue exhibited less browning in storage despite containing more phenolics and similar PPO activity as vascular tissue. The comparatively lower level of browning in photosynthetic tissue should be investigated further as this may provide additional insight on endogenous mechanisms that control edge browning.

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