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Effect of Different Washing Procedures on Phenolic Metabolism of Shredded, Packaged Iceberg Lettuce during Storage

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Different washing treatments applying chlorinated, ozonated, and tap water were examined for their effect on the phenolic metabolism of minimally processed iceberg lettuce (*Lactuca sativa* L.) during storage in consumer-sized bags at 4 °C for up to 9 days. To eliminate problems associated with raw material inhomogeneity, processing was conducted on a pilot-plant scale under operating conditions of industrial practice. Inherent product heterogeneity caused by diverse lettuce leaf tissues was compensated for by pooling large-sized samples, and frequent sampling ensured significant data about the activities of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD), as well as the contents of caffeic acid derivatives over storage time. In the homogeneous lettuce samples, specific responses caused by different washing procedures were detectable. PAL activity in the samples increased for up to 5−8 days of storage. Compared to tap and ozonated water, the use of chlorinated water (100−200 mg/L free chlorine) for washing trimmed heads or shredded lettuce significantly reduced PAL activity and the concomitant rise of 3,5-di-*O*-caffeoylquinic acid (isochlorogenic acid isomer) concentrations. The phenolic acids *O*-caffeoyltartaric (caftaric acid), di-*O*-caffeoyltartaric (chicoric acid), 5-*O*-caffeoylquinic (chlorogenic acid isomer), and *O*-caffeoylmalic were less influenced by different washing treatments. Individual contents either were constant or decreased during storage. Additionally, the novel finding of a further caffeic acid isomer, tentatively identified as *meso*-di-*O*-caffeoyltartaric acid, is reported. PPO and POD activities were less affected by different washing treatments and thus were less suitable physiological indicators of stress reactions triggered by alternative processing.

KEYWORDS: Iceberg lettuce; *Lactuca sativa* L.; fresh-cut; minimal processing; washing procedure; chlorine; ozone; phenolic metabolism; phenylalanine ammonia-lyase; polyphenol oxidase; peroxidase

INTRODUCTION

Fresh-cut produce has been one of the fastest growing commodities over the past 10 years, and there are no signs of this trend slowing (*1*). The increasing popularity of minimally processed vegetables (MPVs) such as packaged, fresh-cut lettuce has been attributed to the health benefits associated with fresh produce, as well as to the convenience these ready-to-eat products provide to the consumer (*2*, *3*).

Unit operations applied in commercial fresh-cut lettuce processing usually include trimming, coring, cutting, washing, centrifugation, and packaging (*4*). The objective of washing is to remove soil and other debris, reduce the microbial load, limit the development of browning, and lower the product temperature (*5*). Lettuce is commonly washed after shredding using chilled, chlorinated water at concentrations of 50−200 mg/L free chlorine often followed by a rinsing step applying tap water. However, as recent experiments under operation conditions of industrial practice have shown (*6*), prewashing trimmed iceberg lettuce heads in chlorinated or ozonated water prior to shredding and subsequent washing of the cut produce in tap water represents a useful alternative washing procedure. In particular, besides appropriate reduction of the initial microbial load, the consumption of free chlorine due to cellular leakage was reduced compared with that of conventional washing of shredded lettuce in chlorinated water (*6*). Furthermore, the amount of disinfectant byproducts in the wastewater was markedly reduced or even absent when ozonated water was used.

Vegetable processing, in particular the mechanical and physical stresses during cutting operations, creates a wound signal that elicits various physiological and biochemical responses in adjacent and distant tissues (*7*). Wound-induced changes include moisture loss, elevated respiration, production of ethylene, and the activation of the phenylpropanoid metabolism that results in the accumulation of phenolic compounds and subsequent tissue browning—a major cause of quality loss in minimally processed lettuce (*7*, *8*). Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5), the first enzyme unique to the biosynthesis of many phenolic compounds, is the bridge between the primary metabolite phenylalanine and secondary metabolism

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since it catalyzes the conversion of L-phenylalanine to *trans*-cinnamic acid, with the latter being the skeleton of all phenylpropanoids (*9*, *10*). In various studies with diverse lettuce (*Lactuca sativa* L.) cultivars, wounding has led to an increase in the synthesis and activity of PAL (*3*, *8*, *11*−*14*). Apart from flavonoids, caffeic acid derivatives were found to be the most predominant soluble phenolic compounds in lettuce. Either naturally occurring or synthesized after stress, their varying contents depend on the cultivar, as well as on the morphological tissue type along with its location within the lettuce head (*3*, *11*, *13*−*19*). The phenolic compounds may serve as natural substrates to oxidative enzymes such as polyphenol oxidase (PPO; EC 1.14.18.1) and peroxidase (POD; EC 1.11.1.7) to yield *o*-quinones which eventually polymerize in non-enzyme-catalyzed reactions, resulting in brown pigments (*20*, *21*). In this context, flavonol derivatives in iceberg lettuce play a minor role since their content is rather low compared with that of other lettuce cultivars (*13*, *17*, *18*, *22*). Due to their light-dependent biosynthesis, flavonols are not evenly distributed within the lettuce head but accumulate in the outer leaves, which are usually removed during commercial processing (*23*−*25*).

Most research on wound-induced phenolic metabolism in lettuce has been carried out on a laboratory scale with selected tissue types (white vascular/green photosynthetic/red colored) from distinct locations of the head, and data concerning PAL, PPO, POD, phenolic metabolites, or browning in minimally processed lettuce upon storage are not always consistent (*3*, *8*, *11*−*14*, *16*−*18*). The disagreements may be ascribed to the varying composition and physiological status of the lettuce leaf tissues, as well as to different cultivars, piece sizes, and cutting and storage conditions. In many studies, perforated and high-permeability film bags were used, or a controlled atmosphere with humidified air was applied (*3*, *11*−*14*, *16*−*17*). Up to now, there has been only little information about the influence of different washing treatments on phenolic metabolism in homogeneous, consumer-sized edible portions of fresh-cut iceberg lettuce stored in a modified atmosphere (*18*, *26*). However, in terms of further optimization of the production process and prolongation of shelf life, detailed knowledge concerning this interrelation is indispensable. Therefore, the object of the present work was to study the effect of different washing procedures applying chlorinated, ozonated, and tap water on the phenolic metabolism of minimally processed iceberg lettuce during refrigerated storage. Experiments were conducted on a pilot-plant scale under operation conditions of industrial practice to yield consumer-sized products and to eliminate problems associated with raw material inhomogeneity. Inherent product heterogeneity caused by diverse lettuce leaf tissues was compensated for by pooling large-sized samples, and frequent sampling ensured significant data throughout storage.

MATERIALS AND METHODS

Chemicals. 1,4-Dithioerythritol, hydrogen peroxide (H_2O_2) (30%, v/v), L-phenylalanine, sodium hypochlorite solution (≥13% active chlorine), and *trans*-cinnamic acid were purchased from Fluka (Buchs, Switzerland). Bovine serum albumin and 5-*O*-caffeoylquinic acid (chlorogenic acid, 5-CQA) were obtained from Roth (Karlsruhe, Germany) and polyvinylpolypyrrolidone (Poliplasdone XL-10, PVPP) was obtained from Hedinger (Stuttgart, Germany). All other chemicals were of analytical or HPLC grade and supplied by VWR (Darmstadt, Germany). Milli-Q system (Millipore, Bedford, MA) ultrapure water was used for analytical purposes.

Plant Material. Iceberg lettuce (*L. sativa* L. cv. Fortunas) grown under commercial conditions in Germany (Ilshofen) in 2002 was delivered by a local processor. After harvest (July and September), the lettuce was cooled overnight (<4 °C) at the local grower's facility, transported to Hohenheim University in a refrigerated truck, and kept in a dark cooling chamber (4 °C) until processing (within 24 h). Thus, lettuce heads were supplied and used for processing according to German commercial practices.

Furthermore, to compare the phenolic composition of diverse unprocessed *L. sativa* L. varieties/cultivars, iceberg lettuce (var. *capitata*), romaine lettuce (var. *longifolia*), red-pigmented lettuce (var. *crispa* cv. Lollo Rosso), and the green Lollo Bionda cultivar were purchased at a local retail market and stored at 4 °C prior to analysis (within 24 h).

Processing. Experiments were conducted in July (series 1) and replicated in September (series 2) 2002 as previously described (*6*). Within a series, lots of ∼55 kg of iceberg lettuce (∼80 heads) of the same batch were processed in each of four lines (I−IV) with different experimental arrangement to apply prewashing and washing procedures. Lettuce heads were prepared for further processing by removing and discarding wrapper leaves and excision of the core with sharp stainless steel knives. Cored and trimmed lettuce heads were treated as follows: (I) shredding with subsequent washing in tap water (4 °C, 90 s); (II) shredding with subsequent washing in chlorinated tap water containing 100 mg/L free chlorine (4 °C, 90 s); (III) prewashing trimmed heads in chlorinated tap water containing 200 mg/L free chlorine (4 °C, 120 s) prior to shredding and subsequent washing in tap water (4 °C, 90 s); (IV) prewashing trimmed heads in ozonated water containing 1 mg/L O_3 (4 °C, 120 s) prior to shredding and subsequent washing in tap water (4 °C, 90 s).

Lettuce was shredded (width 6 mm) using a GS 10 multipurpose belt cutting machine (Kronen, Willstätt, Germany). Trimmed heads and shredded lettuce were placed in the longitudinal water current of a continuously operated flow-through GEWA 2600 washing machine (Kronen) with a 500 L filling volume.

The concentration of free chlorine in the tap water used for processing was <0.3 mg/L. Chlorinated water was prepared by adding sodium hypochlorite solution to tap water to obtain concentrations of 100 and 200 mg/L free chlorine. The pH of the washing solutions was adjusted to the pH of tap water (∼8.0) using hydrochloric acid (HCl). Ozonated water was supplied by an SWO 30 ozone generator system (Ozomatic, Baunatal, Germany).

Shredded lettuce was centrifuged (650 rpm, 35 s) prior to packaging using a Turbo K50-4 centrifuge (Kronen). To minimize product heterogeneity, processed lettuce was thoroughly pooled, blended, and subsequently packaged into film bags (Amcor Flexibles, Bristol, U.K.; 25 cm × 25 cm, 35 μm antimist coated oriented polypropylene, O_2 and CO_2 transmission rates at 23 °C and 0% RH of ∼900 and 4000 $cm^3\ m^{-2}\ day^{-1}\ atm^{-1}$, respectively) in 200 ± 2 g quantities at 4 °C. After heat sealing under ambient atmosphere using an R 25 packaging machine (Boss, Friedrichsdorf, Germany), the bags were stored in a dark cooling chamber at 4 °C and removed on each sampling date.

Headspace Analysis. Prior to further sample preparation, O_2 and CO_2 concentrations in the packages were monitored using a CheckMate 9900 O_2/CO_2 gas analyzer (PBI-Dansensor, Ringsted, Denmark) as described earlier (*6*).

Sample Preparation. For each sample, the contents of 5 bags per washing procedure per day were pooled, rapidly frozen by immersion in liquid nitrogen, and cut using a UM 12 universal machine (Stephan und Söhne, Hameln, Germany) equipped with a double-wall cryogenic vessel. Prior to freeze-drying using a Lyovac GT 4 (Steris, Hürth, Germany), aliquots of cut, frozen lettuce were stored at −80 °C in closed plastic containers. After lyophilization, dry weights were determined and the freeze-dried samples stored at −30 °C in closed plastic containers. Prior to sample analyses, aliquots of the lyophilizate were finely ground in a water-cooled A10 laboratory mill (Ika, Staufen, Germany) to warrant maximum homogeneity.

Extraction of Caffeic Acid Derivatives. The procedure of Hisaminato et al. (*27*) was modified for the extraction of caffeic acid derivatives. Ground lyophilizate aliquots (1 g) and ascorbic acid (250 mg) were homogenized with 100 mL of aqueous methanol (MeOH; 70%, v/v) for 2 min using an Ultra-Turrax (Ika). The homogenate was vacuum-filtered through Schleicher & Schuell no. 595 paper (Dassel, Germany), the filter residue was rinsed twice with 10 mL each of

aqueous MeOH (70%, v/v), and the combined filtrates were concentrated under reduced pressure (<40 °C) until complete evaporation of MeOH. The aqueous solution plus a subsequent water rinse (2 × 10 mL) was acidified with HCl to pH 2 and extracted three times with ethyl acetate (each 50 mL). The combined supernatants were dried over sodium sulfate and filtered through Schleicher & Schuell no. 595 1/2 paper. The organic solvent plus a subsequent 20 mL ethyl acetate rinse was evaporated to dryness (<40 °C), and the residue was dissolved in 5 mL of MeOH. An aliquot was membrane-filtered (0.45 μm, Whatman, Clifton, NJ) and used for high-performance liquid chromatography (HPLC).

To compare the caffeic acid derivative composition of different unprocessed lettuce varieties/cultivars, whole leaves of iceberg, romaine, Lollo Rosso, and Lollo Bionda lettuce were rinsed with tap water and manually cut into small pieces. Fresh sample aliquots (25 g) were immediately extracted as described above according to 1 g of lyophilizate.

HPLC Analysis of Caffeic Acid Derivatives. The separation of caffeic acid derivatives was performed using a series 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany) equipped with ChemStation software, a G1322A degasser, a G1312A binary gradient pump, a G1329/1330A thermoautosampler, a G1316A column oven, and a G1315A diode array detector (DAD). The column used was a 150 × 3.0 mm i.d. Synergi C18 4 μm Hydro-RP (Phenomenex, Torrance, CA), with a 4.0 × 2.0 mm i.d. C18 ODS guard column, operated at a temperature of 25 °C. The mobile phase consisted of 5% (v/v) formic acid in water (eluent A) and 5% (v/v) formic acid in methanol (eluent B). The gradient program was as follows: 12% B to 27% B (18 min), 27% B to 45% B (18 min), 45% B to 60% B (4 min), 60% B (3 min), 60% B to 12% B (3 min), 12% B (4 min). The injection volume for all samples was 3 μL. Chromatograms were recorded at 330 nm at a flow rate of 0.3 mL/min. Spectra were recorded from 200 to 450 nm (interval 0.5 nm).

Identification and Quantification of Caffeic Acid Derivatives. *Identification.* Caffeic acid derivatives were identified by means of their UV and mass spectrometric data and retention time and by comparison with previously published chromatograms of lettuce extracts. HPLC-DAD/MS^n analyses were performed with an HPLC system (Agilent, Waldbronn, Germany) identical to the system described above coupled on-line with an Esquire 3000+ ion trap mass spectrometer (Bruker, Bremen, Germany) fitted with an electrospray ionization (ESI) source. Negative ion mass spectra of the column eluate were recorded in the range m/z 50−1100. Nitrogen was used both as the drying gas at a flow rate of 9.0 L/min and as the nebulizing gas at a pressure of 40.0 psi. The nebulizer temperature was set at 365 °C. The capillary voltage was set to 4.00 kV. Collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.0 V. 5-CQA was used for the optimization of ionization parameters.

Quantification. Caffeic acid derivatives were quantified by external standard calibration with 5-CQA dissolved in MeOH. Two separate lyophilizate extracts per sample were analyzed.

Enzyme Extraction. PAL was extracted as previously decribed (*28*) with some modifications. Ground lyophilizate aliquots (150 mg), PVPP (150 mg), and calcium chloride dihydrate (75 mg) were mixed with 10 mL of chilled 50 mM Tris−HCl buffer (TB) (pH 8.8) containing 1.5 mM 1,4-dithioerythritol. After the mixture was stirred for 60 min, the homogenate was filtered through Schleicher & Schuell no. 595 1/2 paper, and then centrifuged at 1500*g* for 10 min. A 3 mL aliquot of the clarified supernatant was applied to an Econo-Pac 10 DG desalting column (Bio-Rad, Hercules, CA) preequilibrated with 20 mL of 50 mM TB (pH 8.8), and subsequently eluted with 4 mL of 50 mM TB (pH 8.8). The collected column eluent was kept on ice until it was used to perform kinetic PAL assays.

PPO and POD were extracted as previously decribed (*29*) with some modifications. Ground lyophilizate aliquots (150 mg) and PVPP (150 mg) were mixed with 10 mL of chilled 50 mM citrate−phosphate buffer (PB) (pH 6.5). After the mixture was stirred for 60 min, the homogenate was filtered through Schleicher & Schuell no. 595 1/2 paper, and then centrifuged at 1500*g* for 10 min. The clarified supernatant was kept on ice until it was used to perform kinetic PPO and POD assays.

All extraction protocols were carried out at 4 °C and conducted in triplicate for each sample.

Kinetic Assays. PAL activity was assayed as previously decribed (*30*) with some modifications by HPLC determination of *trans*-cinnamic acid. The reaction mixture contained a 0.2 mL aliquot of enzymatic extract and 50 μL of 100 mM L-phenylalanine (as a PAL substrate) in 50 mM TB (pH 8.8). After incubation of the mixture at 37 °C for 30 min using a water bath, 0.75 mL of MeOH was added to stop the reaction. Control reaction mixtures contained boiled extracts. Aliquots of each reaction mixture were membrane-filtered (0.45 μm, Whatman) and analyzed by HPLC applying the same Hewlett-Packard HPLC system as described above. The column used was a 150 × 3.0 mm i.d. Aqua C18 3 μm 125 Å (Phenomenex) with a 4.0 × 2.0 mm i.d. C18 ODS guard column, operated at a temperature of 25 °C. Elution was performed isocratically using 0.5% (v/v) acetic acid in water and acetonitrile (50:50, v/v) as the mobile phase. The injection volume for all samples was 3 μL. *trans*-Cinnamic acid was detected at 276 nm at a flow rate of 0.3 mL/min and quantified by external standard calibration.

PPO activity was determined as previously decribed (*31*) with some modifications. The reaction mixture consisted of 1.5 mL of reaction buffer (RB) (2 mM sodium dodecyl sulfate (SDS) in 50 mM PB (pH 6.5)), 0.2 mL of 0.5 M L-proline in RB, and a 0.1 mL aliquot of enzyme extract previously diluted 1:5 (v/v) in 50 mM PB (pH 6.5). The reaction was started by the addition of 0.2 mL of 25 mM 4-methylcatechol in RB. After preincubation for 40 s, product formation was followed by measuring the accumulation of the pink proline−catechol adduct at 525 nm (ϵ = 1550 L M^{-1} cm^{-1}) every 15 s for 3 min. Slopes for the linear portion of each resultant curve were calculated for the first 30 s of the recording. Control reaction mixtures contained boiled extracts. SDS was added to activate latent PPO. Thus, total (active plus latent) soluble PPO activity was measured (*32*, *33*).

POD was determined as previously decribed (*34*) with some modifications. Kahn (*35*) and Kahn and Andrawis (*36*) demonstrated that tropolone plus H_2O_2 was both a POD substrate and a highly effective PPO inhibitor, and could therefore improve POD and PPO differentiation. A 0.2 mL aliquot of enzyme extract was added to 1.3 mL of 50 mM PB (pH 6.5) containing 12 mM tropolone and 3.3 mM H_2O_2. After preincubation for 40 s, product formation was followed by measuring the accumulation of the yellow product at 418 nm (ϵ = 2075 L M^{-1} cm^{-1}) every 15 s for 3 min. Slopes for the linear portion of each resultant curve were calculated for the first 30 s of the recording. Control reaction mixtures contained boiled extracts. No POD activity could be measured in the absence of H_2O_2 in the assay mixture.

For performing kinetic assays and protein content determination, each individual enzymatic extract was used separately and analyzed in duplicate (PAL) and triplicate (PPO, POD). Thus, the shown data represent the mean ± standard deviation of six (PAL) and nine (PPO, POD) values per sample.

The spectrophotometric assays for determining PPO and POD activities and protein content were recorded at 25 °C in a Cary 100 Conc spectrophotometer (Varian, Victoria Mulgrave, Australia) equipped with a Varian Cary temperature controller and Varian Cary Win UV 2.0 software.

Protein Determination. Protein content was determined according to the method of Bradford (*37*) using bovine serum albumin as a standard.

Data Analysis. Linear regression fittings were carried out by using SigmaPlot, version 8.0 (SPSS Science, Erkrath, Germany). Data were subjected to one way analysis of variance (ANOVA) using SigmaStat, version 2.03 (SPSS Science), for different washing procedures and separately for each series. In the case of significant differences, individual washing procedures were compared using the Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

Gaseous Atmosphere in the Packages. As described previously (*6*), lettuce processed in each line (I−IV) exhibited a similar response to the different washing procedures in relation to O_2 and CO_2 concentrations in the bags during storage.

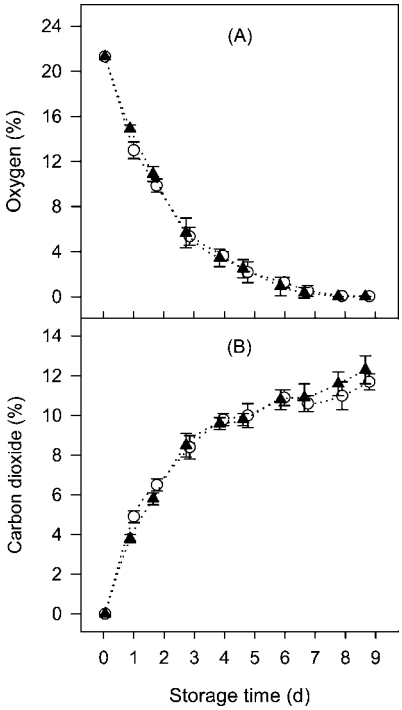


Figure 1. Changes in headspace O_2 (**A**) and CO_2 (**B**) levels in packages of shredded iceberg lettuce during storage at 4 °C: (○) processing line I, shredding with subsequent washing in tap water; (▲) line III, prewashing trimmed heads in chlorinated tap water prior to shredding and subsequent washing in tap water. Each data point represents the mean of 7–19 separate bags (± standard deviation) from series 1.

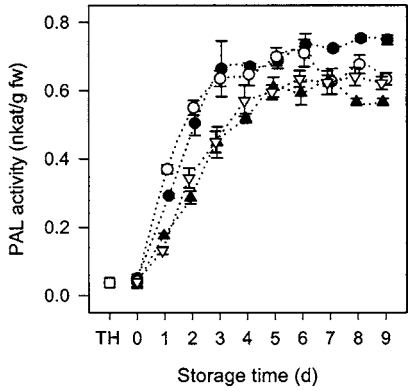


Figure 2. Changes in PAL activity in shredded, packaged iceberg lettuce during storage at 4 °C as affected by different washing procedures: (□) TH = trimmed lettuce heads prior to further processing; (○) line I, shredding with subsequent washing in tap water; (▽) line II, shredding with subsequent washing in chlorinated tap water; (▲) line III, prewashing trimmed heads in chlorinated tap water prior to shredding and subsequent washing in tap water; (●) line IV, prewashing trimmed heads in ozonated water prior to shredding and subsequent washing in tap water. Each data point represents the mean of six values ± standard deviation from series 1 (fw = fresh weight).

Therefore, changes in headspace O_2 and CO_2 levels of lines I and III from series 1 are shown as examples in **Figure 1**. Since O_2 was consumed and CO_2 was produced by respiration, their concentrations showed inverse course within 9 days of storage. Whereas the decline in O_2 was slightly faster in series 1 than in series 2, the CO_2 increase was nearly identical in both series. Depending on treatment, only slightly different gaseous atmospheres, especially within storage days 1−3, were detected. It is assumed that the differences are more likely caused by processing times and packaging than by individual treatments. Low O_2 levels are deemed favorable for the supression of enzymatic browning and can be achieved passively, as in our study, by respiration of living tissues, or actively by injection of specific gas mixtures into film bags. Since a decrease in quality is primarily based on visual appearance, a controlled or modified storage atmosphere with low O_2 (∼3−5%) and higher CO_2 levels (∼5−10%) prolonged the shelf life of cut iceberg lettuce by reducing the rate of visual deterioration and browning (*8*, *26*).

PAL. In trimmed lettuce heads (TH) and lettuce immediately after processing (day 0), PAL activities expressed on a protein and fresh weight basis were significantly lower compared to those in processed lettuce during storage (days 1−9). PAL activities during storage expressed on a fresh weight basis from series 1 are shown in **Figure 2**. The increase in wound-induced PAL activity and maximal levels were more pronounced in series 1 than in series 2 (data not shown). In both series, the increase was linear up to storage days 2 and 3 and reached a maximum after ∼5−7 (for lines II and III; chlorinated wash water) and ∼6−8 days (lines I and IV). At the same time as the oxygen content dropped below 1% (**Figure 1**), PAL activity was virtually inhibited and further PAL levels were constant or even decreased slightly until the end of storage. This was in accordance with the results of Ke and Saltveit (*38*), who observed reduced PAL activity in iceberg lettuce midrib segments exposed to 1.5% O_2 at 5 °C compared with segments stored in air. Furthermore, Siriphanich and Kader (*29*) reported gradually increasing PAL activity in lettuce tissue exposed to air plus 15% CO_2 at 0 °C for 12 days, whereas in lettuce tissue kept solely in air, PAL activity nearly remained unchanged. In the present study, protein contents during storage were slightly higher in series 1 than in series 2 but nearly constant within a series (data not shown).

In earlier studies (*3*, *8*, *11*, *13*, *14*, *39*) with excised lettuce segments under cooled (5 °C), aerobic conditions, the maximum of wound-induced PAL activity was already reached after 1−4 days, and activity declined to a basic level during further storage. The decrease may have resulted from the de novo synthesis of a PAL-inactivating factor described previously (*40*). Low temperatures may have a stronger effect on the formation rate of the inactivating factor than the rate of enzyme synthesis (*10*). When lettuce pieces were stored at elevated storage temperatures (>5 °C), the PAL maximum was shifted toward earlier storage days (*8*, *13*, *39*). Delayed PAL activity maxima (days 7 and 8) in our study may be caused by the deeper storage temperature (4 °C). In addition, PAL activity was found to be dependent on the lettuce cultivar and degree of wounding or rather on the size of the pieces (*8*, *11*). López-Gálvez et al. (*8*) observed an increasing maximum of wound-induced PAL activity with increasing severity of wounding.

In the present study, the storage temperature (4 °C) and piece size (6 mm slitting width) were held constant. Since differences in corresponding O_2/CO_2 levels within the packages of lettuce processed in lines I−IV were insignificant (**Figure 1**), variations of PAL activity within a series were ascribed to different washing procedures. The influences of chlorinated water on PAL activity applied both in a single washing step of shredded lettuce (line III) and in a prewashing step of trimmed lettuce heads (line III) were similar. Generally, compared to those obtained with a single washing in tap water (line I) or a prewashing with ozonated water (line IV), PAL activities in lettuce were significantly lower when chlorine was used. Due to chlorine, the slope of the linear PAL activity evolution (**Table 2**) was

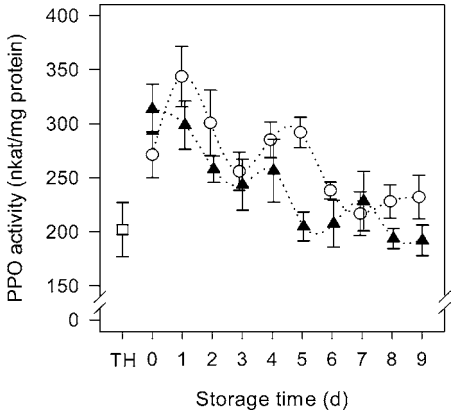


Figure 3. Changes in PPO activity in shredded, packaged iceberg lettuce during storage at 4 °C as affected by different washing procedures: (□) TH = trimmed lettuce heads prior to further processing; (○) line I, shredding with subsequent washing in tap water; (▲) line III, prewashing trimmed heads in chlorinated tap water prior to shredding and subsequent washing in tap water. Each data point represents the mean of nine values ± standard deviation from series 1.

significantly lower, especially during the early days of storage. In vascular tissue Fukumoto et al. (*3*) found that PAL activity was slightly reduced during the first 2 days of storage by washing iceberg lettuce in cooled, chlorinated water. However, due to added chlorine, the authors noted slightly enhanced PAL activity in photosynthetic tissue compared to lettuce merely washed in chilled, chlorine-free water.

PPO and POD. With increasing storage time, total (active plus latent) soluble PPO in lettuce processed in all lines (I−IV) tended toward lower activities. The PPO activities were slightly higher in series 1 than in series 2 (data not shown). Since specific effects caused by individual washing procedures were not noticeable, changes in PPO activity expressed on a protein basis of lines I and III from series 1 are shown as examples (**Figure 3**). Protein contents did not change significantly during storage (data not shown).

These findings are in accordance with those of Heimdahl et al. (*26*), who determined a slight decrease in PPO activity in three of four cultivars of shredded, modified atmosphere packaged iceberg lettuce during storage at 5 °C. The present study also supports the results of Siriphanich and Kader (*29*), who found inhibition of PPO by high CO_2 concentrations. When iceberg lettuce segments were stored under conditions preventing CO_2 accumulation, total PPO activity (active plus latent) remained constant, indicating no de novo synthesis due to initial wounding (*14*). Under similar conditions, according to Ke and Saltveit (*11*), Hisaminato et al. (*27*), and Fukumoto et al. (*3*), no significant changes in PPO activity in iceberg lettuce midrib segments during refrigerated storage were observed. In the latter study marked differences in activities between photosynthetic and vascular tissue were reported, whereas differences between outer or inner locations within a lettuce head were less pronounced. On a fresh matter basis, PPO and POD activities were highest in inner photosynthetic tissues, followed by outer photosynthetic and inner and outer vascular tissues. 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 (*3*).

In the present study with homogeneous samples comprising all types of leaf tissue, soluble specific POD activities in lettuce processed in each line (I−IV) virtually remained constant during storage. In trimmed lettuce heads, 21 ± 1 and 19 ± 1 nkat/mg protein were determined in series 1 and 2, respectively. On the basis of fresh weight, a slight increase of POD activity during storage in all processed samples compared to trimmed lettuce heads (33 ± 2 and 36 ± 1 nkat in series 1 and 2, respectively) was detected (data not shown). Ke and Saltveit (*11*) and Cantos et al. (*14*) noted that wounding led to increased POD activity in midrib tissue during storage, probably due to a de novo synthesis of peroxidase isoenzymes. The observed induction of peroxidase isoenzymes may be related to lignification processes for cell wall repair after tissue wounding (*14*). In the present work, specific effects caused by individual washing procedures were not detected, supporting the findings of Fukumoto et al. (*3*), who found POD activity to be rather influenced by the wash water temperature (4 and 47 °C) than by the chlorine concentration (0 and 100 mg/L).

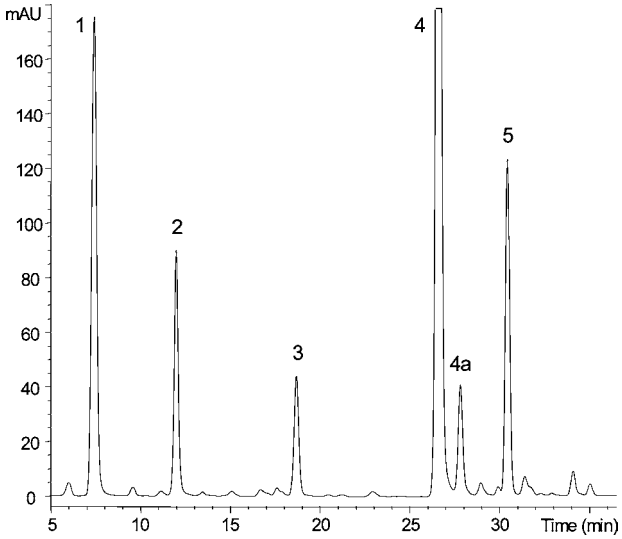
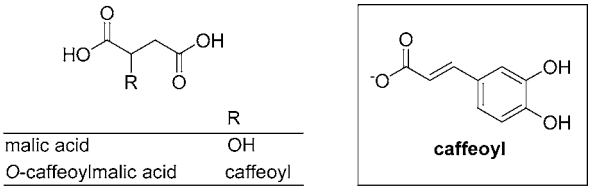


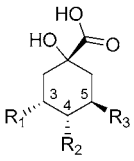
Figure 4. Separation of caffeic acid derivatives in iceberg lettuce extracts by high-performance liquid chromatography (330 nm). Peak assignment: (**1**) *O*-caffeoyltartaric acid (caftaric acid, CTA), (**2**) 5-*O*-caffeoylquinic acid (chlorogenic acid, 5-CQA), (**3**) *O*-caffeoylmalic acid (CMA), (**4**) di-*O*-caffeoyltartaric acid (chicoric acid, diCTA), (**4a**) di-*O*-caffeoyltartaric acid (tentatively identified as the *meso*-form, mdiCTA), (**5**) di-*O*-caffeoylquinic acid (isochlorogenic acid, tentatively identified as the 3,5-isomer, 3,5-diCQA).

Caffeic Acid Derivatives. *Identification*. The separation of caffeic acid derivatives in iceberg lettuce extracts by HPLC and their chemical structures are shown in **Figures 4** and **5**, respectively. Corresponding characteristic UV and mass spectrometric data of the separated compounds obtained by means of HPLC-DAD/ESI(−)-MS^n are given in **Table 1**.

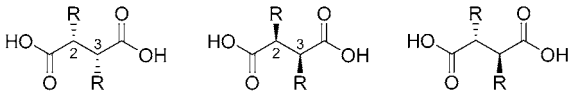
In previous studies (*3*, *14*−*19*, *27*), the major caffeic acid derivatives in lettuce extracts were assigned to caffeoyltartaric acid (caftaric acid, CTA), 5-*O*-caffeoylquinic acid (chlorogenic acid, 5-CQA), dicaffeoyltartaric acid (chicoric acid, diCTA), and dicaffeoylquinic acid (isochlorgenic acid, diCQA). In extracts of stored iceberg lettuce showing severe ethylene-induced browning ("russet spotting"), Tomás-Barberán et al. (*16*) identified several quinic acid derivatives (3-, 4-, and 5-caffeoylquinic acid and 3,4-, 3,5-, and 4,5-dicaffeoylquinic acid) on the basis of cochromatography with green coffee bean extracts. According to the same author, stored (3 days), wounded or ethylene-exposed iceberg lettuce tissue without russet spotting lesions only contained 5-CQA and 3,5-diCQA. In the present work, compounds **1**, **2**, and **5** (**Figure 4**) were assigned to CTA, 5-CQA, and diCQA (tentatively the 3,5-isomer (3,5-diCQA)), respectively. Analyses of the minor peaks between **4a** and **5**



	R
malic acid	OH
O-caffeoylmalic acid	caffeoyl



	R_1	R_2	R_3
quinic acid	OH	OH	OH
5-*O*-caffeoylquinic acid	OH	OH	caffeoyl
3,5-di-*O*-caffeoylquinic acid	caffeoyl	OH	caffeoyl



	$R_{(I)}$	$R_{(II)}$
(2*R*,3*R*)-tartaric acid (**a**)	OH	OH
(2*S*,3*S*)-tartaric acid (**b**)	OH	OH
meso-tartaric acid (**c**)	OH	OH
O-caffeoyltartaric acid	OH	caffeoyl
di-*O*-caffeoyltartaric acid	caffeoyl	caffeoyl

Figure 5. Structures of malic, quinic, and tartaric acid and their respective esters with caffeic acid occurring in iceberg lettuce.

(**Figure 4**) revealed pseudomolecular ions of *m*/*z* 515, indicating the presence of two further diCQA isomers. However, separation of these compounds was partly incomplete.

With respect to compounds **3** and **4** (**Figure 4**), conflicting data were published. Whereas Winter and Herrmann (*15*) and DuPont et al. (*22*) reported the occurrence of caffeoylmalic acid (CMA) in different lettuce cultivars, CMA has not been identified in other studies dealing with lettuce phenolics (*16*, *17*, *27*, *41*, *42*). While DuPont et al. (*22*) reported the separation of caffeoylmalate, caffeoylquinate, dicaffeoylquinate, caffeoylmalate, and dicaffeoylmalate in lettuce extracts by HPLC, they mentioned neither caffeoyltartrate nor dicaffeoyltartrate. Since the presence of dicaffeoylmalate has not yet been reported in lettuce, it is very likely that caffeoyltartrate and dicaffeoyltartrate might have been erroneously referred to as caffeoylmalate and dicaffeoylmalate previously (*22*). In the present study based on HPLC-DAD/ESI(−)-MS^n analysis (**Table 1**), peak **3** was unequivocally assigned to CMA. Furthermore, the major caffeic acid derivative (peak **4**, **Figure 4**) was identified as diCTA (chicoric acid), which is in agreement with the results of several previous studies (*3*, *15*, *19*, *27*, *42*). Since the UV and mass spectrometric data of compounds **4** and **4a** (**Figure 4**) were identical, the occurrence of two diCTA isomers was obvious. In view of the stereochemical properties of tartaric acid, two enantiomeric forms of diCTA ((2*R*,3*R*)- and (2*S*,3*S*)-di-*O*-caffeoyltartaric acid), as well as the achiral *meso*-isomer, may occur (see **Figure 5**). Whereas chromatographic resolution of the enantiomers seemed to be unlikely under the conditions applied, their separation from the *meso*-di-*O*-caffeoyltartaric acid (mdiCTA) appeared to be feasible. In conclusion, peaks **4** and **4a** in the present study were tentatively identified as the (2*S*,3*S*)-/(2*R*,3*R*)- and (*R*,*S*)-*meso*-forms (diCTA and mdiCTA), respectively. Wöldecke and Herrmann (*43*) isolated D-(+)-dicaffeoyltartaric acid from *Cichorium endivia* L., and in chicory (*Cichorium intybus* L.), D-chicoric acid was identified as (2*S*,3*S*)-(+)-diCTA (*44*). Additionally, the occurrence of the *meso*-tartaric acid ester of *p*-coumaric acid in spinach was reported (*15*).

Recently, there has apparently been some controversy about the presence of diCQA in fresh lettuce leaf tissues (*17*, *18*, *41*, *42*). In red-pigmented lettuce (cv. Lollo Rosso), 3,5-diCQA was identified as the main caffeic acid derivative and found in large amounts, even in tissues that had not been subjected to wounding or ethylene stress, whereas diCTA was determined as a minor component (*17*). In contrast to Ferreres et al. (*17*), Caldwell (*41*) found significant levels of diCQA in Lollo Rosso, iceberg, and romaine lettuce only after wounding. When whole leaves of different *L. sativa* L. varieties/cultivars (Lollo Rosso, Lollo Bionda, iceberg, and romaine lettuce) were analyzed in the present study, HPLC profiles similar to those given in other papers (*15*−*17*, *27*, *41*) were obtained (data not shown). In all lettuce samples, the caffeic acid derivatives shown in **Table 1** were detected. In Lollo Rosso and Lollo Bionda extracts, 5-CQA, CMA, and diCTA were the major compounds, with

Table 2. Levels of PAL Activity and Increase in 3,5-DiCQA Concentrations of Shredded, Packaged Iceberg Lettuce during Storage at 4 °C as Affected by Different Washing Procedures

	series 1			series 2		
processing line[a]	slope[b] (nkat/day)	R^2 [c]	increase[d] in 3,5-diCQA (%)	slope[b] (nkat/day)	R^2 [c]	increase[d] in 3,5-diCQA (%)
I	0.25	0.98	385	0.09	0.99	229
II	0.16	0.96	223	0.08	0.94	96
III	0.13	1.00	230	0.08	0.99	110
IV	0.22	1.00	367	0.11	1.00	351

[a] (I) shredding with subsequent washing in tap water; (II) shredding with subsequent washing in chlorinated tap water; (III) prewashing of trimmed heads in chlorinated tap water prior to shredding and subsequent washing in tap water; (IV) prewashing of trimmed heads in ozonated water prior to shredding and subsequent washing in tap water. [b] The slope was determined by linear regression fitting of experimental data expressed on a fresh weight basis from day 0 to day 2. [c] Coefficient of determination for the linear regression fitting. [d] Increase between day 0 and day 9.

Table 1. UV Spectral Data and Characteristic Ions of Major Caffeic Acid Derivatives Extracted from Iceberg Lettuce

peak no.	compound (abbreviation)	HPLC-DAD λ_{max} (nm)	$[M - H]^-$ *m*/*z*	HPLC/ESI(−)–MS^2 and MS^3 *m*/*z*
1	*O*-caffeoyltartaric acid (CTA)	329, 306sh, 250	311	179, 149, 135, 131, 103, 87
2	5-*O*-caffeoylquinic acid (5-CQA)	326, 304sh, 252	353	191, 173, 127
3	*O*-caffeoylmalic acid (CMA)	329, 305sh, 250	295	179, 135, 133, 115
4	di-*O*-caffeoyltartaric acid (diCTA)	330, 306sh, 250	473	311, 293, 219, 179, 149, 135
4a	*meso*-di-*O*-caffeoyltartaric acid[a] (mdiCTA)	330, 306sh, 245	473	311, 293, 219, 179, 149, 135
5	3,5-di-*O*-caffeoylquinic acid[b] (3,5-diCQA)	328, 306sh, 252	515	353, 191, 179, 135

[a] Tentatively identified as the *meso*-form. [b] Tentatively identified as the 3,5-derivative.

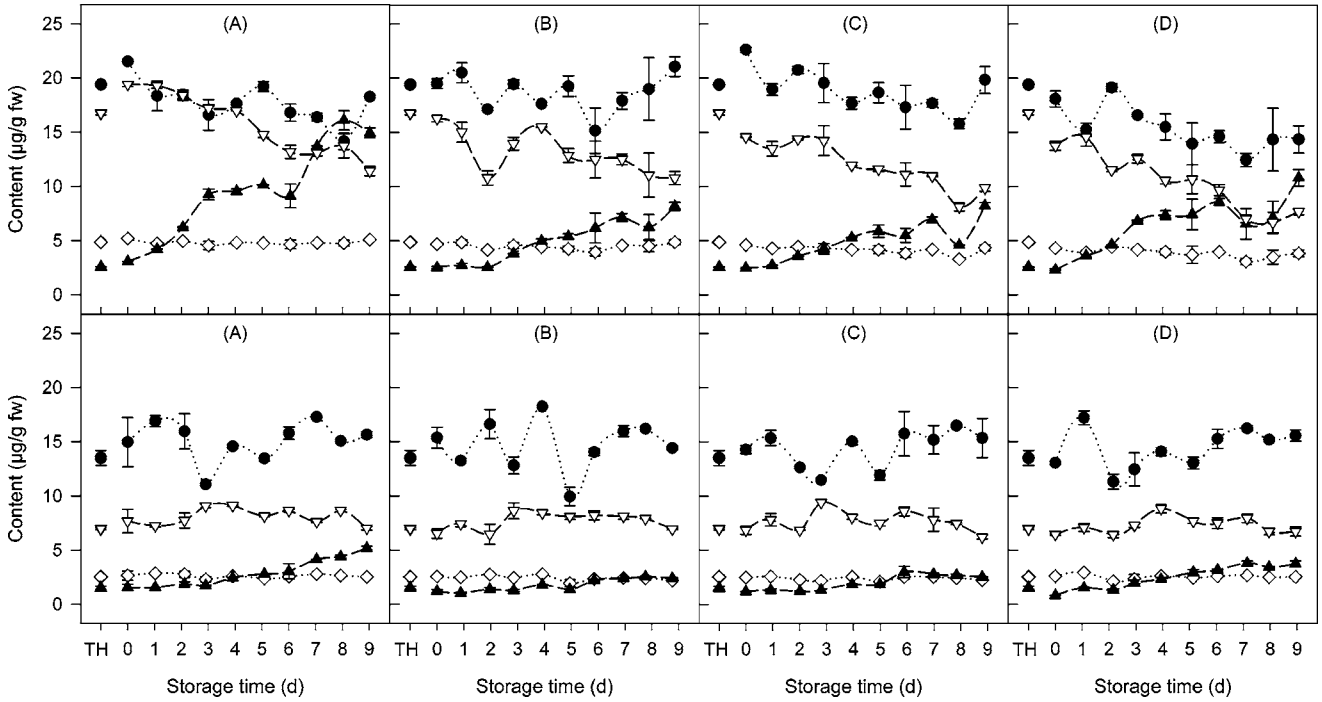


Figure 6. Effect of different washing procedures on selected caffeic acid derivatives in shredded, packaged iceberg lettuce during storage at 4 °C (upper line, series 1; lower line, series 2): TH = trimmed lettuce heads prior to further processing; (**A**) line I, shredding with subsequent washing in tap water; (**B**) line II, shredding with subsequent washing in chlorinated tap water; (**C**) line III, prewashing trimmed heads in chlorinated tap water prior to shredding and subsequent washing in tap water; (**D**) line IV, prewashing trimmed heads in ozonated water prior to shredding and subsequent washing in tap water; (●) CTA; (▽) 5-CQA; (▲) 3,5-diCQA; (◇) CMA. Each data point represents the mean of two values ± standard deviation (fw = fresh weight).

the latter being predominant, whereas significantly lower amounts of CTA and 3,5-diCQA were found (<6% of total caffeic acid derivatives). In contrast to our findings, CMA has not been identified by Caldwell (*41*) and Fererres et al. (*17*) in Lollo Rosso. In accordance with other reports (*3*, *15*, *19*, *27*), diCTA also was the predominant caffeic acid derivative in iceberg and romaine lettuce in the present study, whereas CMA and 3,5-diCQA were found in the lowest concentrations (<7% of total caffeic acid derivatives). Generally, the relative amounts of the different caffeoyl esters in iceberg and romaine lettuce were lower compared to those of the Lollo cultivars.

Influence of the Washing Procedure and Storage. The contents of selected soluble caffeic acid derivatives in homogeneous lettuce samples as affected by the washing treatment during storage over 9 days are shown in **Figure 6** (upper line, series 1; lower line, series 2). In series 1, individual concentrations were generally higher and changes more pronounced. Whereas in series 1 the contents of 5-CQA and CTA in lettuce of all processing lines (I−IV) slightly decreased during storage, nearly constant values were found in series 2. The courses of both diCTA isomers strictly paralleled the course of CTA (not shown in **Figure 6**). The total contents of diCTA plus mdiCTA in processed lettuce during storage (days 0−9) ranged from 55 to 47 μg/g of fresh weight and from 50 to 48 μg/g of fresh weight in series 1 and 2, respectively. In accordance with earlier studies (*3*, *15*, *27*), diCTA was found to be the predominant caffeic acid derivative at any time during storage. CMA contents were constant over time irrespective of the washing procedure or storage. Changes of the phenolic composition mainly concerned caffeoylquinic acid derivatives (5-CQA and 3,5-diCQA), while the contents of caffeoyltartaric derivatives (CTA, diCTA, and mdiCTA) were less affected by minimal processing and storage than those of CQA derivatives. This was consistent with previously published data on several lettuce types (*3*, *14*, *16*, *18*).

Relating to the washing procedure, in the present work washing in chlorinated water resulted in reduced contents of caffeic acid derivatives. As demonstrated in **Table 2**, the rise of 3,5-diCQA in lettuce washed in water containing hypochlorite (lines II and III) was less pronounced compared to that in lines I and IV. Contact of chlorinated water with lettuce cut edges (line II) exerted the greatest inhibitory effect on the 3,5-diCQA formation. Also by washing merely trimmed lettuce heads in chlorinated water, 3,5-diCQA synthesis was strongly inhibited. These findings are supported by Fukumoto et al. (*3*), who detected lower 3,5-diCQA levels in stored cut iceberg lettuce washed with chlorinated water (100 mg/L, 4 °C) compared to lettuce treated without chlorination. In earlier reports, the accumulation of several phenolics in lettuce midribs paralleled the induction of PAL activity (*14*, *17*). However, using homogeneous lettuce samples, this effect was only confirmed for 3,5-diCQA. Its increase from day 0 to day 9 was in good correlation with the early (days 0−2) wound-induced PAL activity (**Table 2**).

With respect to the impact of the storage atmosphere on lettuce phenolics, Ferreres et al. (*17*) found that the content of 5-CQA in green tissue of red-pigmented lettuce (cv. Lollo Rosso) dropped markedly (−57.8%) during aerobic storage at 5 °C for 14 days, while its concentration in white tissue increased. Moreover, in white and green leaf tissue of Lollo Rosso stored in air for 7 days, the contents of CTA, 5-CQA, and 3,5-diCQA rose (*18*). In contrast to the aerobic storage, a modified atmosphere packaging with 2.5% O_2 and 14% CO_2 suppressed the formation (*18*). Concordant with previous findings (*18*, *29*), the effect of low O_2 and high CO_2 atmospheres

obviously suppressed the phenylpropanoid metabolism as demonstrated in the present study.

The results of the present study demonstrate that, due to alternative washing procedures, differences in individual attributes of the phenolic metabolism of shredded, packaged iceberg lettuce such as PAL activity and 3,5-diCQA content, respectively, were detectable. Furthermore, the visual quality of the stored lettuce samples was better when chlorinated water was used instead of tap and ozonated water (*6*). Consequently, good correlation exists among inhibition of wound-induced PAL activity, minimization of 3,5-diCQA accumulation, and reduced browning in shredded iceberg lettuce during storage.

ABBREVIATIONS USED

CTA,*O*-caffeoyltartaric acid (caftaric acid); 5-CQA, 5-*O*-caffeoylquinic acid (chlorogenic acid); CMA, *O*-caffeoylmalic acid; diCTA, di-*O*-caffeoyltartaric acid (chicoric acid); mdiCTA, *meso*-di-*O*-caffeoyltartaric acid; 3,5-diCQA, 3,5-di-*O*-caffeoylquinic acid (isochlorogenic acid isomer); DAD, diode array detector; ESI, electrospray ionization; fw, fresh weight; HCl, hydrochloric acid; HPLC, high-performance liquid chromatography; MeOH, methanol; PB, citrate−phosphate buffer; PVPP, polyvinylpolypyrrolidone; RB, reaction buffer; SDS, sodium dodecyl sulfate; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediole; TB, Tris−HCl buffer; tropolone, 2-hydroxy-2,4,6-cycloheptatrien-1-one.

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LITERATURE CITED

(1) Garrett, E. H. Fresh-cut produce: Tracks and Trends. In *Fresh-cut fruits and vegetables. Science, technology, and market*; Lamikanra, O., Ed.; CRC Press: Boca Raton, FL, 2002; pp 1−10.

(2) Alzamora, S. M.; López-Malo, A.; Tapia, M. S. Overview. In *Minimally processed fruits and vegetables: Fundamental aspects and applications*; Alzamora, S. M., Tapia, M. S., López-Malo, A., Eds.; Aspen Publishers: Gaithersburg, MD, 2000; pp 1−9.

(3) Fukumoto, L. R.; Toivonen, P. M. A.; Delaquis, P. J. Effect of wash water temperature and chlorination on phenolic metabolism and browning of stored iceberg lettuce photosynthetic and vascular tissues. *J. Agric. Food Chem.* **2002**, *50*, 4503−4511.

(4) King, A. D.; Magnuson, J. A.; Török, T.; Goodman, N. Microbial flora and storage quality of partially processes lettuce. *J. Food Sci.* **1991**, *56*, 459−461.

(5) Simons, L. K.; Sanguansri, P. Advances in the washing of minimally processed vegetables. *Food Aust.* **1997**, *49*, 75−80.

(6) Baur, S.; Klaiber, R.; Hammes, W. P.; Carle, C. Sensory and microbiological quality of shredded, packaged iceberg lettuce as affected by pre-washing procedures with chlorinated and ozonated water. *Innovative Food Sci. Emerging Technol.* **2004**, *5*, 45−55.

(7) Saltveit, M. E. Physical and physiological changes in minimally processed fruits and vegetables. In *Phytochemistry of fruit and vegetables*; Tomás-Barberán, F. A., Robins, R. J., Eds.; Clarendon Press: Oxford, England, 1997; pp 205−220.

(8) López-Gálvez, G.; Saltveit, M.; Cantwell, M. Wound-induced phenylalanine ammonia lyase activity: factors affecting ist induction and correlation with the quality of minimal processed lettuces. *Postharvest Biol. Technol.* **1996**, *9*, 223−233.

(9) Jones, D. H. Phenylalanine ammonia-lyase: Regulation of its induction, and its role in plant development. *Phytochemistry* **1984**, *23*, 1349−1359.

(10) Creasy, L. L. The role of enzyme inactivation in the regulation of synthetic pathways: A case history. *Physiol. Plant.* **1987**, *71*, 389−392.

(11) Ke, D.; Saltveit, M. E., Jr. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiol. Plant.* **1989**, *76*, 412−418.

(12) Couture, R.; Cantwell, M. I.; Ke, D.; Saltveit, M. E., Jr. Physiological attributes related to quality attributes and storage life of minimally processed lettuce. *HortScience* **1993**, *28*, 723−725.

(13) Castañer, M.; Gil, M. I.; Ruíz, M. V.; Artés, F. Browning susceptibility of minimally processed baby and romaine lettuces. *Eur. Food Res. Technol.* **1999**, *209*, 52−56.

(14) Cantos, E.; Espín J. C.; Tomás-Barberán, F. A. Effect of wounding on phenolic enzymes in six minimally processed lettuce cultivars upon storage. *J. Agric. Food Chem.* **2001**, *49*, 322−330.

(15) Winter, M.; Herrmann, K. Esters and glucosides of hydroxycinnamic acids in vegetables. *J. Agric. Food Chem.* **1986**, *34*, 616−620.

(16) Tomás-Barberán, F. A.; Loaiza-Velarde, J. G.; Bonfanti, A.; Saltveit, M. E. Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. *J. Am. Soc. Hortic. Sci.* **1997**, *122*, 399−404.

(17) Ferreres, F.; Gil, M. I.; Castañer, M.; Tomás-Barberán, F. A. Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. *J. Agric. Food Chem.* **1997**, *45*, 4249−4254.

(18) Gil, M. I.; Castaner, M.; Ferreres, F.; Artés, F.; Tomás-Barberán, F. A. Modified-atmosphere packaging of minimally processed "Lollo Rosso" (*Lactuca sativa*)—Phenolic metabolites and quality changes. *Z. Lebensm.-Unters.-Forsch. A* **1998**, *206*, 350−354.

(19) Romani, A.; Pinelli, P.; Galdardi, C.; Sani, G.; Cimato, A.; Heimler, D. Polyphenols in greenhouse and open-air-grown lettuce. *Food Chem.* **2002**, *79*, 337−342.

(20) Yoruk, R.; Marshall, M. R. Physicochemical properties and function of plant polyphenol oxidase: A review. *J. Food Biochem.* **2003**, *27*, 361−422.

(21) Richard-Forget, F. C.; Gauillard, F. A. Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* cv. Williams) polyphenol oxidase and peroxidase: A possible involment of peroxidase in enzymatic browning. *J. Agric. Food Chem.* **1997**, *45*, 2472−2476.

(22) DuPont, M. S.; Mondin, Z.; Williamson, G.; Price, K. R. Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *J. Agric. Food Chem.* **2000**, *48*, 3957−3964.

(23) Wöldecke, M.; Herrmann, K. Flavonole und Flavone der Gemüsearten: IV. Flavonole und Flavone des Kopfsalates und der Endivien. *Z. Lebensm.-Unters.-Forsch.* **1974**, *156*, 153−157.

(24) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitive analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−596.

(25) Hohl, U.; Neubert, B.; Pforte, H.; Schonhof, I.; Böhm, H. Flavonoid concentrations in the inner leaves of head lettuce genotypes. *Eur. Food Res. Technol.* **2001**, *213*, 205−211.

(26) Heimdal, H.; Falk-Kühn, B.; Poll, L.; Larsen, L. M. Biochemical changes and sensory quality of shredded and MA-packaged iceberg lettuce. *J. Food Sci.* **1995**, *60*, 1265−1268, 1276.

(27) Hisaminato, H.; Murata, M.; Homma, S. Relationship between the enzymatic browning and phenylalanine ammonia-lyase activity of cut lettuce, and the prevention of browning by inhibitors of polyphenol biosynthesis. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1016−1021.

(28) Babic, I.; Amiot, M. J.; Nguyen-The, C.; Aubert, S. Changes in phenolic content in fresh ready-to-use shredded carrots during storage. *J. Food Sci.* **1992**, *58*, 351−356.
(29) Siriphanich, J.; Kader A. A. Effects of CO_2 on total phenolics, phenylalanine ammonia lyase, and polyphenol oxidase in lettuce tissue. *J. Am. Soc. Hortic. Sci.* **1985**, *110*, 249−253.
(30) Mitra, A.; Mayer, M. J.; Mellon, F. A.; Michael, A. J.; Narbad, A.; Parr, A. J.; Waldron, K. W.; Walton, N. J. 4-Hydroxycinnamoyl-CoA hydratase/ lyase, an enzyme of phenylpropanoid cleavage from *Pseudomonas*, causes formation of C_6-C_1 acid and alcohol glucose conjugates when expressed in hairy roots of *Datura stramonium* L. *Planta* **2002**, *215*, 79−89.
(31) Nüsslein, B.; Kurzmann, M.; Bauer, R.; Kreis, W. Enzymatic degradation of chicoric acid in *Echinacea purpurea* preparations. *J. Nat. Prod.* **2000**, *63*, 1615−1618.
(32) Moore, B. M.; Flurkey, W. H. Sodium dodecyl sulfate activation of a plant polyphenoloxidase. *J. Biol. Chem.* **1990**, *265*, 4982−4988.
(33) Espín, J. C.; Wichers, H. J. Activation of a latent mushroom (*Agaricus bisporus*) tyrosinase isoform by sodium dodecyl sulfate (SDS). Kinetic properties of the SDS-activated isoform. *J. Agric. Food Chem.* **1999**, *47*, 3518−3525.
(34) Kahn, V.; Andrawis, A. Tropolone is a substrate for horseradish peroxidase. *Phytochemistry* **1985**, *24*, 909−913.
(35) Kahn, V. Tropolone—a compound that can aid in differentiating between tyrosinase and peroxidase. *Phytochemistry* **1985**, *24*, 915−920.
(36) Kahn, V.; Andrawis, A. Inhibition of mushroom tyrosinase by tropolone. *Phytochemistry* **1985**, *24*, 905−908.
(37) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(38) Ke, D.; Saltveit, M. E. Regulation of russet spotting, phenolic metabolism, and IAA oxidase by low oxygen in iceberg lettuce. *J. Am. Soc. Hortic. Sci.* **1989**, *114*, 638−642.
(39) Loaiza-Velarde, J. G.; Saltveit, M. E. Heat shocks applied either before or after wounding reduce browning of lettuce leaf tissue. *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 227−234.
(40) Ritenour, M. A.; M.; Saltveit, M. E. Identification of a phenylalanine ammonia-lyase inactivating factor in harvested head lettuce (*Lactuca sativa*). *Physiol. Plant.* **1996**, *97*, 327−331.
(41) Caldwell, C. R. Alkylperoxyl radical scavenging activity of red leaf lettuce. *J. Agric. Food Chem.* **2003**, *51*, 4589−4595.
(42) Bennett, M.; Gallagher, M.; Fagg, J.; Bestwick, C.; Paul, T.; Beale, M.; Mansfield, J. The hypersensitive reaction, membrane damage and accumulation of autofluorescent phenolics in lettuce cells challenged by *Bremia lactucae*. *Plant J.* **1996**, *9*, 851−865.
(43) Wöldecke, M.; Herrmann, K. D-(+)-Dicaffeoyl-Weinsäure aus Endivien (*Cichorium endiva* L.). *Z. Naturforsch.* **1974**, *29c*, 360−361.
(44) Scarpati, M.; Oriente, G. Chicoric acid (dicaffeyltartaric acid). Isolation from chicory (*Chicorium intybus*) and synthesis. *Tetrahedron* **1958**, *4*, 43−48.

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