Impact of modified atmospheres on the vitamin C content of salad-cut romaine and other lettuces

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Abstract

Modified atmosphere packaging (MAP) is a key technology for maintaining salad-cut lettuce quality, mainly by reducing cut-edge discoloration. However, the use of MAP may have detrimental consequences, and here we studied the impact of low-O_2 atmospheres (0.2 to 1%) alone or in combination with high-CO_2 atmospheres (3, 6, 9-10%) on vitamin C concentrations. Iceberg and Romaine lettuces were trimmed, cut manually into salad-size pieces, rinsed in chlorinated water (50 ppm sodium hypochlorite, pH 7 for 20 s), spun in a manual spinner, packaged in perforated plastic bags, and stored in in the dark in containers with flows of humidified air or controlled atmospheres for up to 12 days at 5°C. Green leaf lettuce was prepared as whole washed leaves and also stored under the same conditions. Pieces or leaves were evaluated for appearance (overall visual quality, discoloration, and decay) and composition (ascorbic acid and dehydroascorbic acid by HPLC). Initial total vitamin C concentrations in cut iceberg, cut romaine, and green leaf lettuce leaves were 10, 25-40, and 55 mg 100 g^{-1} fw, respectively. The rate of vitamin C loss varied, but, in all experiments, total ascorbic acid concentrations decreased more rapidly in pieces or leaves stored in CO_2-containing atmospheres than in air or low O_2, alone, and before the loss of marketable quality. Of six enzymes in the ascorbate-glutathione pathway that were assayed, only ascorbate peroxidase activities were lower in a 10% CO_2 atmosphere than in air-stored cut romaine lettuce. Reduced and total glutathione concentrations were substantially lower in the CO_2-stored romaine lettuce.

Keywords: ascorbic acid, dehydroascorbic acid, ascorbate-glutathione cycle, visual quality

INTRODUCTION

Vitamin C is a potent biological antioxidant. Antioxidants protect biological processes in plants and animals by slowing or preventing oxidation of living systems by reducing metal ions, quenching free radicals, and scavenging reactive oxygen species (Davey et al., 2000; Gest et al., 2013). Ascorbic acid (AA) and the partially oxidized form dehydroascorbic acid (DHA) are both active in the human body, and comprise the total ascorbate or vitamin C pool through regeneration of DHA to AA via the ascorbic acid-glutathione cycle (Smirnoff, 2000).

Fresh fruits and vegetables provide more than 90% of the vitamin C in the human diet (Vicente et al., 2009; Asensi-Fabado and Munné-Bosch, 2010). Total ascorbate levels tend to decline in fruits and vegetables after harvest, and the decline is largely controlled by storage temperature (Tay and Perera, 2004; Bergquist et al., 2006; Rickman et al., 2007).

Leafy greens such as spinach and lettuce stored at low temperature under modified atmospheres show significant declines in AA and total ascorbate (Gil et al., 1999; Hodges and Forney, 2003; Ermen et al., 2006). Salad-cut lettuce is packaged in modified atmosphere packaging (MAP) with low O_2 (0.1-0.8%) and elevated CO_2 (6-10%) concentrations, mainly to control cut-edge discoloration and achieve an extended shelf-life, but these atmospheres can result in off-odors, loss of crisp texture, and increased loss of nutrient quality compared with product without MAP (López-Gálvez et al., 1997; Rico et al., 2007). Although iceberg lettuce is not an important source of vitamin C, darker green romaine and leaf lettuces are good sources of this vitamin (USDA, 2015). To date, little research has been done to

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determine the primary cause of vitamin C loss under MAP conditions. Both O_2 and CO_2 concentrations are modified in MAP, but the use of controlled atmospheres (CA) allows the differentiation of the low-O_2 and high-CO_2 effects on vitamin C concentrations.

The main objectives of this research were to study the quality and vitamin C changes of salad-cut romaine and other lettuces stored in air, low-O_2-, and high-CO_2-containing CA. Glutathione concentrations and activities of several enzymes in the ascorbate-glutathione cycle were analyzed in one test, as well as the activity of L-galactono-1,4-lactone dehydrogenase (GLDH), the last enzyme of the AA synthesis pathway.

MATERIALS AND METHODS

Lettuce preparation and storage

Lettuces (romaine hearts, heads of iceberg or green leaf, cultivars unknown) were obtained from commercial growers or from a produce wholesaler. The lettuces had been field trimmed and vacuum cooled, were 0 to 2 days from harvest, were transported in coolers and were stored at 0°C until used. Lettuce was prepared under clean conditions at 7.5°C, using middle leaves of the heads. The tops and bottoms were trimmed and leaves were manually cut into salad-size pieces (2.5×2.5 cm; salad-cut); for the experiment measuring enzymes, green leaf tissue was used after removing midribs. Pieces were washed in cold water containing 50 ppm NaOCl (pH 7.0) for 30 sec before drying in a salad spinner (Dynamic Salad Spinner, model 06140). About 150 g of salad-cut pieces were placed in perforated plastic bags at 5°C in the dark in chambers through which humidified air or CA flowed. The CA mixtures were prepared by mixing humidified air, N_2 and CO_2 and were maintained within ±5% of the stated O_2 and CO_2 concentrations. The CA conditions were selected to cover a range of modified atmospheres possible in salad bags. Three or four replicates from each atmosphere were evaluated and sampled every 3 or 4 days for quality and composition and every 6 days for enzymatic activity.

Visual quality determinations

Visual quality was scored on a 9 to 1 scale, where 9 = excellent, fresh appearance, 7 = good, 5 = fair, 3 = poor (useable but not saleable), and 1 = unusable. Intermediate numbers were assigned where appropriate, and a score of 6 was considered the limit of marketability. One visual quality score was given to an entire sample. Discoloration was scored on a 1 to 5 scale, where 1 = none, 2 = slight, 3 = moderate, 4 = moderately severe, and 5 = maximum or severe. Decay was scored on a 1 to 5 scale where 1 = no macroscopic decay, 2 = slight decay, but product salable, 3 = moderate, product useable but not salable, 4 = moderately severe, unusable, and 5 = severe.

Composition

To characterize lettuces, percentage dry weight was determined on 50 g leaf tissue that was oven dried at 70°C in a forced air oven for 3 days; chlorophyll, carotenoid, and sugar concentrations were determined by spectrophotometry.

For HPLC analysis of AA and DHA, determinations were performed using a Shimadzu system consisting of Shimadzu SIL-10AD VP autosampler (4°C), DGU-14A degasser, LC-10AD VP dual-piston pump, COT-10A VP column oven, SPD-M10A VP diode array detector, and CBM-10AW VP controller. All mobile phases were filtered and degassed prior to use. Vitamin C was measured as AA and DHA following the method of Tausz et al. (1996). For each replicate, a composite 4 g chopped sample was measured into a tube and 16 mL 2% oxalic acid was immediately added. Samples were held briefly at 0°C before extraction using an Ultra-Turrax T25 Basic (12,000 rpm) for 1 min. Duplicate 1.3 mL aliquots were transferred to microfuge tubes and frozen at -80°C until analysis. Tubes were removed from the freezer and allowed to thaw at room temperature before centrifuging at 14,000 rpm (4°C) for 15 min. Supernatants were filtered through Phenomenex 17 mm syringe filters (non-sterile 0.45 µm PVDF). A 0.6 mL aliquot was taken and reacted with 0.2 mL 30 mM OPDA in 5% methanol. Samples were mixed briefly and derivatized in the dark at room temperature for

35 min before loading in the HPLC autosampler (4°C). A 10 µL aliquot of each sample was injected and run on a Phenomenex Luna C18 column (15 cm×4.6 mm×5 µm) with C18 guard column (4×3 mm) using a mobile phase consisting of 50 mM KH_2PO_4 and 5 mM CTAB in 5% methanol (pH 4.59) at a flow rate of 1.2 mL min^{-1}. The column temperature was maintained at 40°C. Peaks were detected and confirmed based on elution time and wavelength (AA, 262 nm, DHA, 348 nm). Ascorbate concentrations were calculated using standard curves for AA and DHA.

Total glutathione, glutathione (GSH), and oxidized glutathione (GSSG) were determined by the spectrophotometric method described by Hodges and Forney (2003). Determinations were made by following production of 5-thio-2-nitrobenzoic acid (TNB) from the action of glutathione reductase (GR) with NADPH at 412 nm. For enzyme activities, a sample of chopped leaf tissue was measured into a Falcon tube, frozen using liquid N_2 and held at -80°C until extraction and analysis. The extraction of crude enzyme for ascorbate peroxidase (APX; E.C. 1.11.1.11), dehydroascorbate reductase (DHAR; E.C. 1.8.5.1), monodehydroascorbate reductase (MDHAR; E.C. 1.6.5.4), and glutathione reductase (GR; E.C. 1.6.4.2) was performed according to Cakmak and Marschner (1992). Activity of DHAR was assayed according to Cakmak and Marschner (1992), while APX, MDHAR, and GR were assayed as described by Hodges and Forney (2003). L-Galactono-1,4-lactone dehydrogenase (GLDH; E.C. 1.3.2.3) extraction and assay were performed using the method of Hodges and Forney (2003). All assays were performed at room temperature. Protein concentrations of enzyme extracts were measured on a spectrophotometer using the BioRad Protein Assay Dye Reagent Concentrate (Bradford, 1976).

Statistics

Three or four replicates of all treatments were used in the experiments. Data were analyzed as means ± standard deviation or subjected to ANOVA (Sigmaplot 11.0) with mean separation by LSD at $P<0.05$.

RESULTS AND DISCUSSION

Although various appearance and compositional attributes were evaluated, only visual quality and vitamin C changes are reported here. Changes in visual quality were mostly due to increases in cut-edge discoloration (data not shown); decay was negligible. Temperature greatly affected visual quality changes in salad-cut iceberg (data not shown) and salad-cut romaine stored in air and CA conditions (Figure 1). The CA (1% O_2 + 9% CO_2) retarded quality loss at the three temperatures in both salad-cut iceberg and romaine lettuces. The vitamin C content of iceberg lettuce was low, and decreased from 10 to 6 and 3 mg 100 g^{-1} FW in air and CA, respectively (data not shown). Total vitamin C content in salad-cut romaine decreased from about 25 to 14 mg 100 g^{-1} FW in air, but decreased much more (to 5 mg 100 g^{-1} FW) under CA conditions. Both AA and DHA concentrations were affected by CA conditions (data not shown). Temperature over the range of 0 to 10°C had little impact on changes in vitamin C content, in contrast to the notable effect of the atmosphere. In another experiment, salad-cut romaine was stored at 5°C in 1% O_2 or air alone or with 10% CO_2 (Figure 2). The CO_2-containing atmospheres helped retard the loss of visual quality, mainly by retarding discoloration on the cut edges (data not shown). Total vitamin C decreased, but remained above 30 mg/100 g FW in product stored in low O_2 or air over 15 days. In contrast, vitamin C dropped to about 15 mg 100 g^{-1} FW in the CO_2-containing atmospheres by day 6. The visual quality results reported here are consistent with previous research (López-Gálvez et al., 1997; Rico et al., 2007).

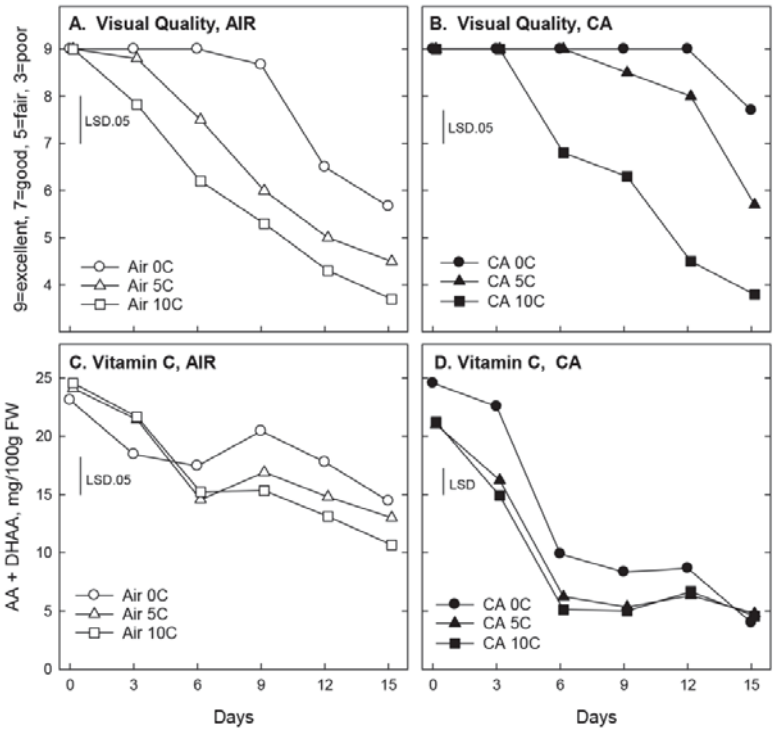

Figure 1. Visual quality and total vitamin C content of salad-cut romaine lettuce stored in air or CA (1% O_2 + 9% CO_2) at three temperatures. Data are means of three replicates with mean separation by LSD.05.

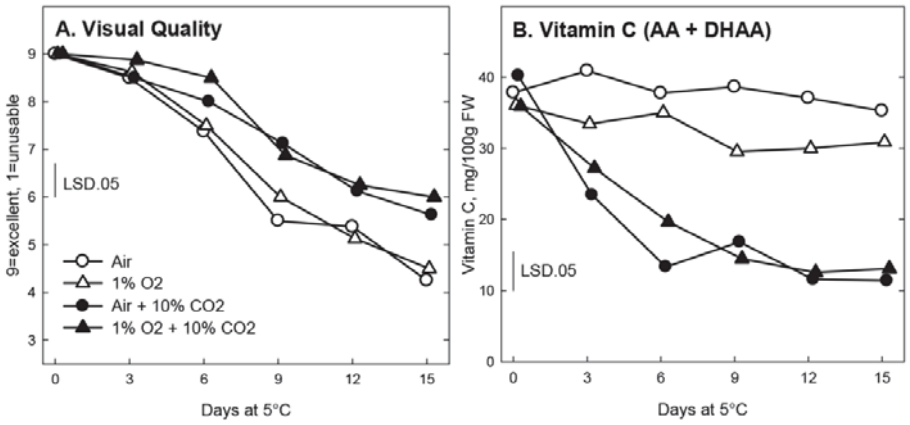

Figure 2. Visual quality and total vitamin C content of salad-cut romaine stored at 5°C in air and 1% O_2 alone or with 10% CO_2. Data are means of three replicates with mean separation by LSD.05.

Experiments were conducted on salad-cut romaine and whole leaves of green lettuce using atmospheres closer to those in commercial salad bags (Figure 3). An atmosphere of 0.2% O_2 was applied alone or with 3, 6 or 9% CO_2. Only the atmospheres with 6 or 9% CO_2 provided sufficient benefit to the salad-cut romaine (Figure 3A), while the visual quality of whole leaves was benefited only marginally by the CO_2-containing atmospheres (Figure 3C). All CO_2-containing atmospheres resulted in a significant decline in vitamin C compared with 0.2% O_2 alone (Figure 3B, D). While the pattern of vitamin C changes was similar, the

absolute concentrations in green leaves were double those of salad-cut romaine. Although vitamin C has been measured in romaine lettuce in several studies (Martínez-Sánchez et al., 2011; Zhan et al., 2012), there are no comparable data to those presented here, studying high-CO_2 atmospheres separately from low-O_2 atmospheres. Bartoli et al. (2006) suggested that reduced respiration rates during growth of leafy greens contributed to lower ascorbic acid synthesis. During storage, respiration rates of cut lettuce would be lower in 1% O_2 than in air, but there were no differences in ascorbic acid concentrations (Figure 2). A 0.2% O_2 atmosphere would be expected to reduce respiration rates significantly (Figure 3); high CO_2-containing atmospheres may further reduce the respiration rates, but not at 3% CO_2 (Kader et al., 1989; Brandenburg and Zagory, 2009). In both salad-cut romaine and green leaf lettuce, there were significant differences in ascorbic acid concentrations between the 0.2% O_2 atmosphere alone or when combined with 3% CO_2. Therefore, differences in respiration rates do not help explain the observed differences in vitamin C concentrations in relation to CO_2-containing atmospheres.

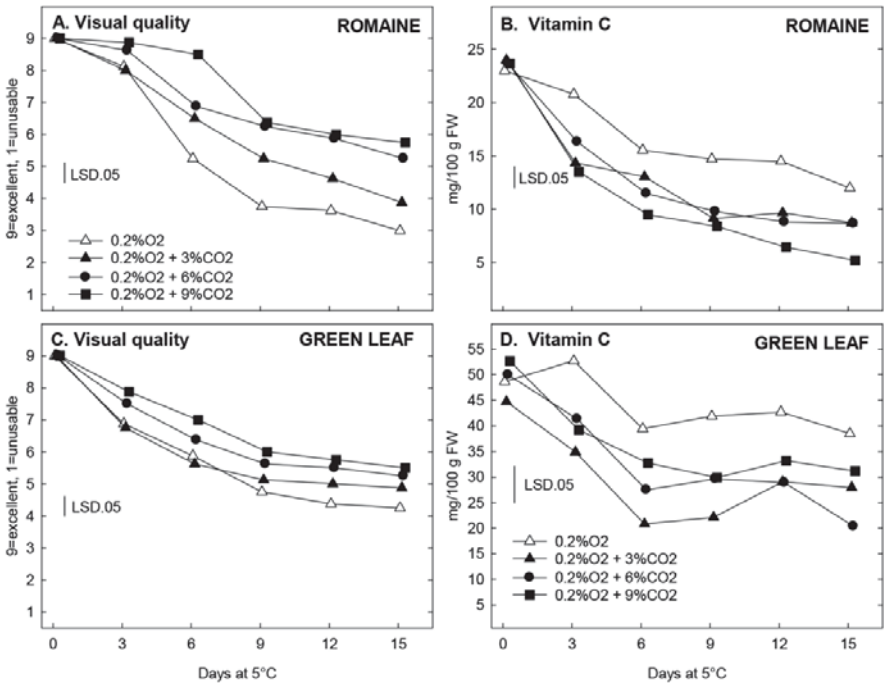

Figure 3. Visual quality and vitamin C (AA + DHA) concentrations of salad-cut romaine and individual leaves of green leaf lettuce stored at 5°C in 0.2% O_2 alone or with 3, 6, or 9% CO_2. Data are means of three replicates with mean separation by LSD.05.

In a final experiment, salad-cut romaine was stored at 5°C in air or CA (0.4% O_2 + 9% CO_2). Overall visual quality was maintained under CA (Figure 4A). The vitamin C content again was lower in the CA atmosphere than in air (Figure 4B), although the difference was less than in previous experiments. Activity of GLDH, responsible for the conversion of the precursor L-galactono-1,4-lactone to AA (Smirnoff, 2000), did not differ in product in air or CA (data not shown), supporting the idea that synthesis of vitamin C was not affected by the CA. Oxidized ascorbate is regenerated via the ascorbate-glutathione pathway. The activities of DHAR, MDHAR, and GR (Figure 4D-F) were not different in air- or CA-stored romaine. APX activity, however, was lower in CA-stored product (Figure 4C). The concentrations of reduced, oxidized, and total glutathione were also significantly lower in CA-stored romaine lettuce (Figure 4G-I). While most enzymes in the ascorbate-glutathione cycle were apparently not affected by CA conditions, the decrease in glutathione is consistent with reduced ability to regenerate AA from partially oxidized MDHA and DHA (Smirnoff, 2000;

Foyer and Noctor, 2011).

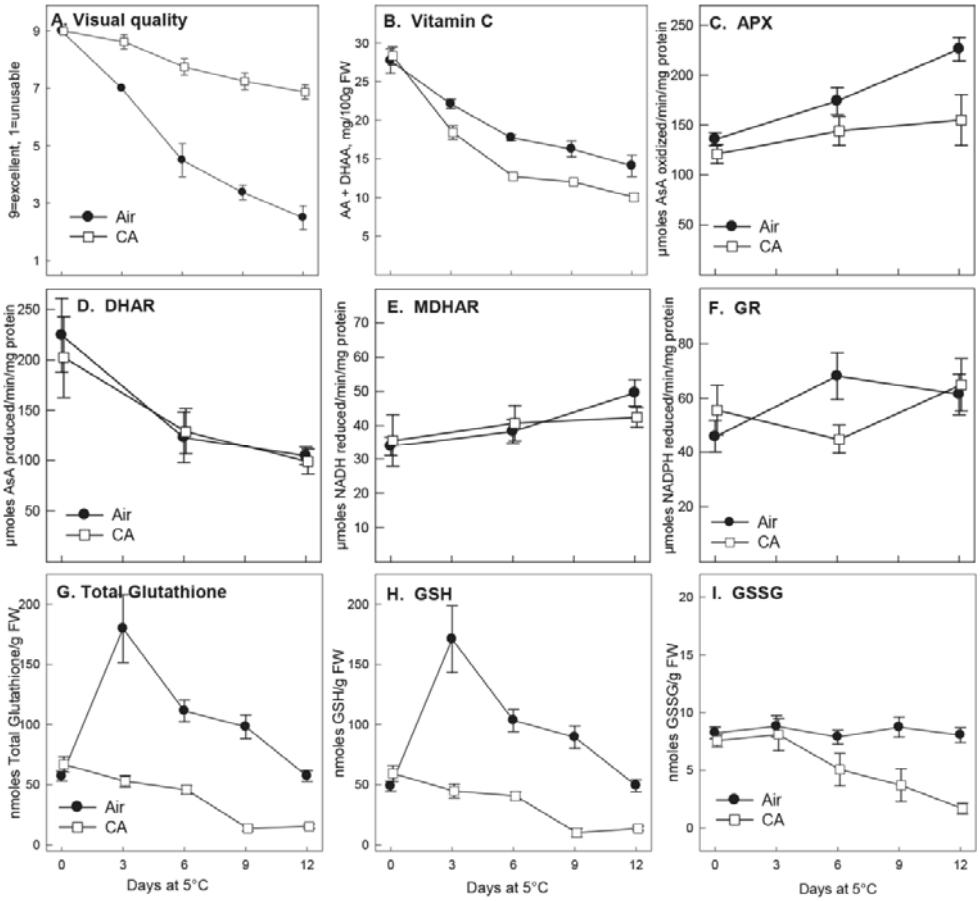

Figure 4. Visual quality (A), vitamin C content (B), activity of the enzymes APX (C), DHAR (D), MDHAR (E), and GR (F), and concentrations of total (G), reduced (H) and oxidized (I) glutathione of salad-cut romaine lettuce stored in air or CA (0.4% O_2 + 9% CO_2) at 5°C. Data are means of three replicates ± standard deviation.

CONCLUSIONS

Although modified atmospheres are important to maintain the visual quality of salad-cut lettuces, they detrimentally impacted their nutritional value. CA experiments clearly demonstrated that high CO_2 concentrations lead to a more rapid vitamin C loss than atmospheres without CO_2. Atmospheres with CO_2 as low as 3% still affected ascorbic acid concentrations. While enzymes in the ascorbate-glutathione cycle were not affected by CA conditions, there was a substantial decrease in glutathione concentrations, consistent with reduced regeneration of ascorbic acid. It is important to search for preservation conditions that maintain nutritional quality as well as visual appearance in value-added lettuce types that contain significant concentrations of vitamin C.

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