

# Modified Atmosphere Packaged Cut Iceberg Lettuce: Effect of Temperature and O<sub>2</sub> Partial Pressure on Respiration and Quality

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The effect of oxygen and carbon dioxide partial pressure ( $p_{O_2}$  and  $p_{CO_2}$ , respectively) and temperature on respiratory patterns and quality of cut iceberg lettuce was examined in modified atmosphere (MA) packages. The changes in headspace  $p_{O_2}$  of packaged lettuce with time were related to void volume, storage temperature, mass, and decreasing respiration rate. Uptake of O<sub>2</sub> was modeled using a Michaelis–Menten-type equation. At 5 and 10 °C, maximum O<sub>2</sub> uptakes were 143 and 213  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$  and the  $p_{O_2}$  at half-maximal ( $k_{1/2}$ ) values were 0.26 and 0.19 kPa, respectively. On the basis of ethanol appearance in the headspace, the fermentation induction point was 0.3–0.4 kPa of O<sub>2</sub> at 5 °C and 0.6–0.8 kPa of O<sub>2</sub> at 10 °C. Acetaldehyde and ethyl acetate were detected at <0.4 kPa of O<sub>2</sub> at 5, 10, and 20 °C (1 kPa ~ 1% at STP). The extent of browning increased with headspace  $p_{O_2}$  but was not related to headspace  $p_{CO_2}$ .

**Keywords:** Modified atmosphere packaging; cut iceberg lettuce; color; respiratory patterns; volatile production

## INTRODUCTION

Sales of lightly processed produce (e.g., cut lettuce, cut carrots, or shredded cabbage) have increased dramatically in the past decade. During the cutting or shredding operations used in the preparation of these products, rupturing of plant cells induces physiological changes such as increased rates of respiration and phenolic metabolism (Bolin et al., 1977; Ohta and Sugawara, 1987). These, in turn, lead to flavor deterioration (McDonald et al., 1990), browning (Ballantyne et al., 1988; Heimdal et al., 1995), and a reduction in chlorophyll (Lipton and Harris, 1974; Zhuang et al., 1994).

Browning, the most common color defect found in cut iceberg lettuce, is caused by the oxidation of phenolic compounds in the presence of the enzyme polyphenol oxidase (Murata et al., 1995; Sharples et al., 1963). Polyphenol oxidase has a relatively low affinity for O<sub>2</sub>, with a reported  $k_m$  from 6 to 8 kPa (Burton, 1974). Modified atmosphere (MA) packaging can extend the shelf life of cut iceberg lettuce primarily by providing a sufficiently low O<sub>2</sub> partial pressure ( $p_{O_2}$ ) to retard browning. Krahn (1977) reported that browning of cut lettuce could be prevented by MA storage at <2% O<sub>2</sub> and 5–20% CO<sub>2</sub>, whereas Ballantyne et al. (1988) reported that undesirable odors and browning could be prevented in shredded lettuce stored in an MA of 1–3% O<sub>2</sub> and 5–6% CO<sub>2</sub>. McDonald et al. (1990) reported no discoloration or fermentation of cut lettuce when the CO<sub>2</sub> was <20% and O<sub>2</sub> was between 1 and 3%. However, further increases in CO<sub>2</sub> caused undesirable odors and flavors. Furthermore, McDonald et al. (1990) reported that browning, particularly around the leaf

edges, occurred at O<sub>2</sub> > 3%. In contrast, Bolin and Huxsoll (1991) reported undesirable flavors at ≤2% O<sub>2</sub> and ≥10% CO<sub>2</sub>. Specific values for the fermentation induction point have not been established for cut lettuce as a function of temperature.

The volatiles present in an MA package can influence the consumer's perception of that product (Song et al., 1997). The type and concentration of volatiles present in an MA package are generally a function of the product, partial pressures of O<sub>2</sub> ( $p_{O_2}$ ) and CO<sub>2</sub> ( $p_{CO_2}$ ), storage temperature, and time. There are few documented reports on the volatile patterns of cut lettuce during shelf life extension studies (Charron et al., 1996).

The objectives of this study were to (1) monitor changes in O<sub>2</sub>, CO<sub>2</sub>, and ethanol partial pressures and volatile compounds in MA-packaged cut iceberg lettuce stored under non-steady-state conditions at 5, 10, or 20 °C and (2) characterize the respiratory response, ethanol production, fermentation induction point, and color stability as a function of steady-state package  $p_{O_2}$  at 5 and 10 °C in the presence and absence of CO<sub>2</sub>.

## EXPERIMENTAL PROCEDURES

**Plant Material.** Fresh cut cv. Salinas lettuce (*Lactuca sativa* L.) was obtained from a local produce wholesaler within 1 h of being cut. In all experiments, the lettuce had been harvested up to several days earlier in the Salinas Valley, California. The wholesaler removed the outer leaves and cut the unwashed lettuce heads into ~2 × 1.55 cm pieces. Upon arrival at Michigan State University, the cut lettuce was sorted to remove pieces that were obviously damaged, weighed, and randomly assigned to treatments.

**CO<sub>2</sub> Production Rate in a Flow-through System.** The CO<sub>2</sub> production rate of 50-g samples of freshly cut iceberg lettuce (analyzed ~2 h after cutting) was measured at 5 °C in a flow-through system using CO<sub>2</sub>-scrubbed air. Inlet and outlet  $p_{CO_2}$  values of each 946-mL glass jar were measured over an 80-h period, and CO<sub>2</sub> production rates were calculated using the air flow rate of 25 mL·s<sup>-1</sup>. The experiment was conducted twice during April and May 1997, and 12 samples were

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**Table 1. Oxygen and CO<sub>2</sub> Permeabilities for the LDPE Packaging Film Used in These Experiments at 5, 10, and 20 °C**

temp (°C)	$P_{O_2} \cdot A \cdot \Delta x^{-1}{}^a$ (nmol·s <sup>-1</sup> ·kPa <sup>-1</sup> )	$P_{CO_2} \cdot A \cdot \Delta x^{-1}{}^b$ (nmol·s <sup>-1</sup> ·kPa <sup>-1</sup> )
5	0.4019	2.395
10	0.5445	4.596
20	0.9688	7.691

<sup>a</sup>  $P_{O_2}$  is the measured LDPE film permeability constant for O<sub>2</sub>;  $A$  = is the package area (916 cm<sup>2</sup>);  $\Delta x$  is the film thickness (53.6  $\mu$ m). <sup>b</sup>  $P_{CO_2}$  is the measured LDPE film permeability constant for CO<sub>2</sub>.

measured each time. The best fit line for CO<sub>2</sub> production versus time was determined using nonlinear regression analysis (version 4.10, SAS Institute, Cary, NC, 1998).

**MA Package Studies.** Cut lettuce was processed as described and sealed in packages constructed of low-density polyethylene (LDPE) film (Dow Chemical, Midland, MI) that was 53.6  $\mu$  thick and had a surface area of 916 cm<sup>2</sup>. Film permeabilities to O<sub>2</sub> and CO<sub>2</sub> were measured at 5, 10, and 20 °C (Table 1) as described by Joles et al. (1994). A septum made of silicone rubber on electrical insulation tape was attached to each package to facilitate extraction of headspace samples.

**Non-Steady-State MA Package Experiments.** To study changes under non-steady-state conditions, packages containing 50 ± 0.5, 150 ± 0.5, or 300 ± 0.5 g of cut lettuce were prepared and sealed without flushing and placed at 5, 10, or 20 °C for 10 days. There were six replicates per treatment. At each temperature, two control packages were included with an adequate number of pinholes in the LDPE film to maintain atmospheric  $p_{O_2}$  and  $p_{CO_2}$  inside the package but prevent significant water loss. In all packages,  $p_{O_2}$  and  $p_{CO_2}$  were measured daily, headspace ethanol partial pressure ( $p_{EtOH}$ ) was assessed every other day, and volatiles and void volume were measured on days 0 and 10. Void volume was determined by the measured dilution of a known amount of injected ethane (Talasila and Cameron, 1997). The experiment was conducted three times during March, April, and May 1997. Oxygen depletion, CO<sub>2</sub> and ethanol accumulation, and changes in void volume and volatile compounds were similar within treatments; data are presented from single packages because it was not possible to make all of the packages with the same initial void volume.

**Steady-State MA Package Experiments.** To study respiratory behavior under steady-state conditions, packages containing 50 ± 0.5, 60 ± 0.5, 70 ± 0.5, 80 ± 0.5, 100 ± 0.5, 120 ± 0.5, or 150 ± 0.5 g of cut iceberg lettuce were prepared and partially sealed with and without 15 g of CaO (98% purity; Aldrich, Milwaukee, WI) as a CO<sub>2</sub> scrubber (Joles et al., 1994) sealed in a 10 × 12 cm spun-bonded polyethylene pouch (Tyvek type 1059B, DuPont, Wilmington, DE). Before final sealing, the bags were flushed with N<sub>2</sub> to a  $p_{O_2}$  between 1 and 5 kPa to shorten the time to steady-state conditions and minimize exposure to elevated  $p_{O_2}$ . Six replicate packages at each weight were prepared with or without the CO<sub>2</sub> scrubber and placed at 5 or 10 °C. Once steady-state conditions were established, after 4 and 6 days at 10 or 5 °C, respectively,  $p_{O_2}$ ,  $p_{CO_2}$ , and  $p_{EtOH}$  were measured. Color was measured after 6 days of storage at 5 and 10 °C. Using the permeability values, packaging film area and thickness, product weight, and package steady-state  $p_{O_2}$  and  $p_{CO_2}$ , rates of O<sub>2</sub> uptake and CO<sub>2</sub> production were calculated based on Fick's law of gas diffusion (Beaudry et al., 1992). The respiratory quotient (RQ) was calculated from the ratio of CO<sub>2</sub> production rate to O<sub>2</sub> uptake rate. Aerobic RQ is typically near 1.0, and elevated RQ values have been related to the level of ethanol production (Joles et al., 1994). A Michaelis–Menten-type equation was used to describe O<sub>2</sub> uptake as a function of package  $p_{O_2}$  (Joles et al., 1994). Nonlinear regression analysis was carried out using respiration data to estimate values for  $k_{1/2}$  and maximum O<sub>2</sub> uptake at 5 and 10 °C in the presence and absence of CO<sub>2</sub> (version 4.10, SAS Institute, Cary, NC, 1998). This experiment was conducted three times during October and November

1997. The lettuce used in this experiment was obtained from a different region of the Salinas Valley from that used in the spring experiments.

**Gas Analysis.** Headspace  $p_{O_2}$  and  $p_{CO_2}$  were determined by using an insulin-type syringe to withdraw duplicate 0.5-mL samples through the septum of each package. The samples were injected into an N<sub>2</sub> stream (2.5 mL·s<sup>-1</sup> flow rate) connected to an O<sub>2</sub> analyzer (S-3A/II with a calcia-zirconia electrochemical detection cell; Ametek Co., Thermo Instrument Analytical Division, Pittsburgh, PA) and an infrared CO<sub>2</sub> analyzer (ADC 225-Mk3; Analytical Development Co., Hertfordshire, U.K.) in series and expressed as kilopascals assuming standard atmospheric pressure (note that 1 kPa ~ 1% at STP).

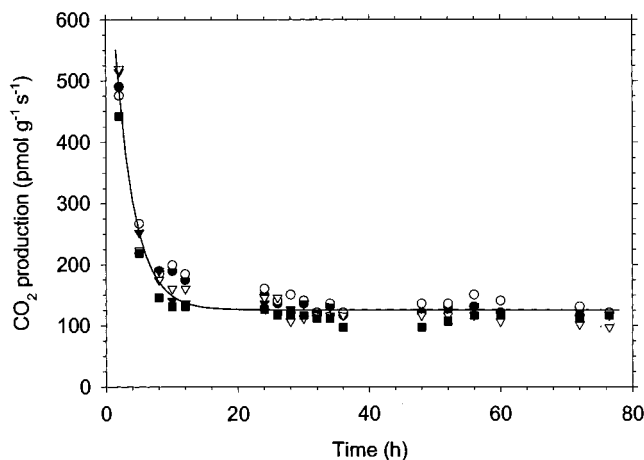
Headspace  $p_{EtOH}$  was determined using a glass gastight syringe (0.5-mL Gastight 1750; Hamilton Co., Reno, NV) to withdraw duplicate 0.2-mL samples from the headspace of each package. The samples were injected into a gas chromatograph (Carle series 100; Hach Co., Loveland, CO) equipped with a 70-cm packed column (Haysep 80/100; Alltech Associates, Deerfield, IL) and a flame ionization detector. Ethanol standards were prepared by creating a range of steady-state  $p_{EtOH}$  in 1-qt (0.9463-L) glass containers with gastight lids as follows: 50, 100, 250, 500, or 1000  $\mu$ L of pure ethanol was mixed in 200 mL of distilled water and equilibrated at 5 °C for at least 24 h. Headspace  $p_{EtOH}$  in each of these containers was determined using a gas chromatograph (GC). Note that headspace  $p_{EtOH}$  over 1000  $\mu$ L·L<sup>-1</sup> in solution is 2.1 Pa at 5 °C (Smyth et al., 1998).

**Volatile Analysis.** Volatile standards were prepared by placing 10  $\mu$ L of ethanol and 40  $\mu$ L of acetaldehyde (Sigma Chemical Co., St. Louis, MO) into a specially designed gastight 4.4-L glass volumetric flask fitted with a tapered ground glass stopper containing a gastight Mininert valve (Alltech Associates). The flask was sealed immediately after addition of the standard solutions, and the liquid was allowed to vaporize overnight at laboratory temperature (~22 °C) to provide known ethanol and acetaldehyde headspace partial pressures. The headspace volatiles in the standard flask were collected and analyzed in exactly the same manner as the volatiles in the MA-packaged lettuce, and the results were used to calculate the concentration of ethanol and acetaldehyde in the MA packages.

Headspace volatiles other than EtOH and acetaldehyde were collected and analyzed on days 0 and 10 from 300 ± 0.5 g MA packages stored at 5, 10, or 20 °C. A solid-phase microextraction (SPME) (Supelco Co., Bellefonte, PA) fiber coated with poly(dimethylsiloxane)–divinylbenzene (1 cm long, 65  $\mu$ m thick) was used to collect the headspace volatiles within each package (Song et al., 1997). Preliminary experiments were carried out to determine the time required for volatile adsorption in the headspace of the packaged lettuce. An adsorption time of 4 min was found to be most suitable for detection of short- to medium-chain alcohols and esters and was used for all sampling. Volatile collection was carried out at the temperature at which the packaged lettuce was stored.

The SPME then was placed into the glass-lined splitless injection port in the GC (HP-6890, Hewlett-Packard Co., Wilmington, DE), and the volatiles were desorbed from the fiber at 200 °C for 90 s. The volatiles were separated on a capillary column (HP-5, 25 m, 0.25-mm i.d., 0.34- $\mu$ m coating thickness). The column temperature was held isothermally at 40 °C for 1.0 min, raised at 55 °C/min to 250 °C, and held for 1 min. The GC/mass spectrometer (GC/MS) transfer line temperature was 220 °C. The carrier gas used was ultrapure helium (99.999%) at 1.5 mL/min.

Volatile detection was performed by time-of-flight mass spectrometry (TOFMS) using electron impact ionization (FCD-650, LECO Corp., St. Joseph, MI). Mass spectra were collected at 40 spectra/s over an  $m/z$  range of 40–300. The GC/MS data were analyzed using LECO deconvolution software (Pegasus II version 1.04, 1997). Identification of volatile components was confirmed by comparison of collected mass spectra with those of authenticated standards and spectra in the National Institute for Standards and Technology mass spectral library



**Figure 1.** Cut iceberg lettuce  $\text{CO}_2$  production rates measured at 5 °C in a flow-through system over a period of 80 h (lettuce was cut at time 0). The inlet air was scrubbed of  $\text{CO}_2$ , and the flow rate was  $25 \text{ mL}\cdot\text{s}^{-1}$ . Each symbol represents a separate replicate, and the line is best fit by nonlinear regression (see text).

(search version 1.5). The chromatograms presented are representative of those obtained from three separate experiments.

**Color Evaluation.** Color was assessed using a Minolta Chroma Meter (Model CR-100, Minolta Corp., Ramsey, NJ). The instrument was calibrated using a standard white reflector plate with a single layer of the LDPE film placed on its surface. Chromaticity was measured using  $L^*$  (light/dark),  $a^*$  (green/red), and  $b^*$  (yellow/blue) values. Ten random areas were measured through the packaging film, and mean values from replicate experiments were reported.

## RESULTS AND DISCUSSION

**$\text{CO}_2$  Production Rates in a Flow-through System.** At 5 °C, the  $\text{CO}_2$  production rate decreased exponentially with time (Figure 1). The data were fitted as follows:

$$\text{CO}_2 \text{ production} = 125.7 + [713.8 \exp(-0.345t)] \text{ pmol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$$

( $R^2 = 0.95$ ) where  $t$  is time (h). Units presented conform to those proposed by Banks et al. (1995). Production of  $\text{CO}_2$  dropped from  $\sim 470 \text{ pmol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$  at 1 h to  $\sim 125 \text{ pmol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$  after 24 h (Figure 1). There was little if any change in respiration after the first 24 h. A drop in respiration rate was reported for asparagus after harvest (Saltveit and Kasmire, 1985) and may have been a result of wound metabolism or depletion of storage reserves with time.

**Respiratory Changes in Unflushed MA-Packaged Cut Lettuce.** At 20 °C, ethanol was detected within the first 24 h of storage, while at 10 and 5 °C, ethanol was first detected after 2 and 4 days, respectively, in headspace in packages containing 300 g of lettuce (Figure 2). Theoretically, rates of  $\text{O}_2$  depletion and  $\text{CO}_2$  accumulation are affected by the void volume and temperature (Talasila and Cameron, 1997). On day 0, the void volume was 0.61 L (SD = 0.03 L), 0.40 L (SD = 0.04 L), and 0.25 L (SD = 0.03 L) in packages containing 300, 150, or 50 g of cut lettuce, respectively. At 5, 10, and 20 °C, the void volume increased in packages containing 300 or 150 g of cut lettuce over the 10-day storage period (Figure 2). Void volume increase was presumably a result of fermentation and correlated

well with measured increases in RQ and  $p_{\text{EtOH}}$ . The extent of fermentation and change in void volume was greatest at higher temperatures. Void volume did not change measurably in packages containing 50 g of lettuce at 5, 10, or 20 °C. Lettuce in these packages was not fermenting as indicated by the RQ values and the absence of ethanol. Ethanol was first detected at 0.4 kPa of  $\text{O}_2$  in all packages containing 300 or 150 g of cut lettuce. This value is a crude estimation of the fermentation induction point because headspace  $p_{\text{EtOH}}$  values were analyzed only every other day.

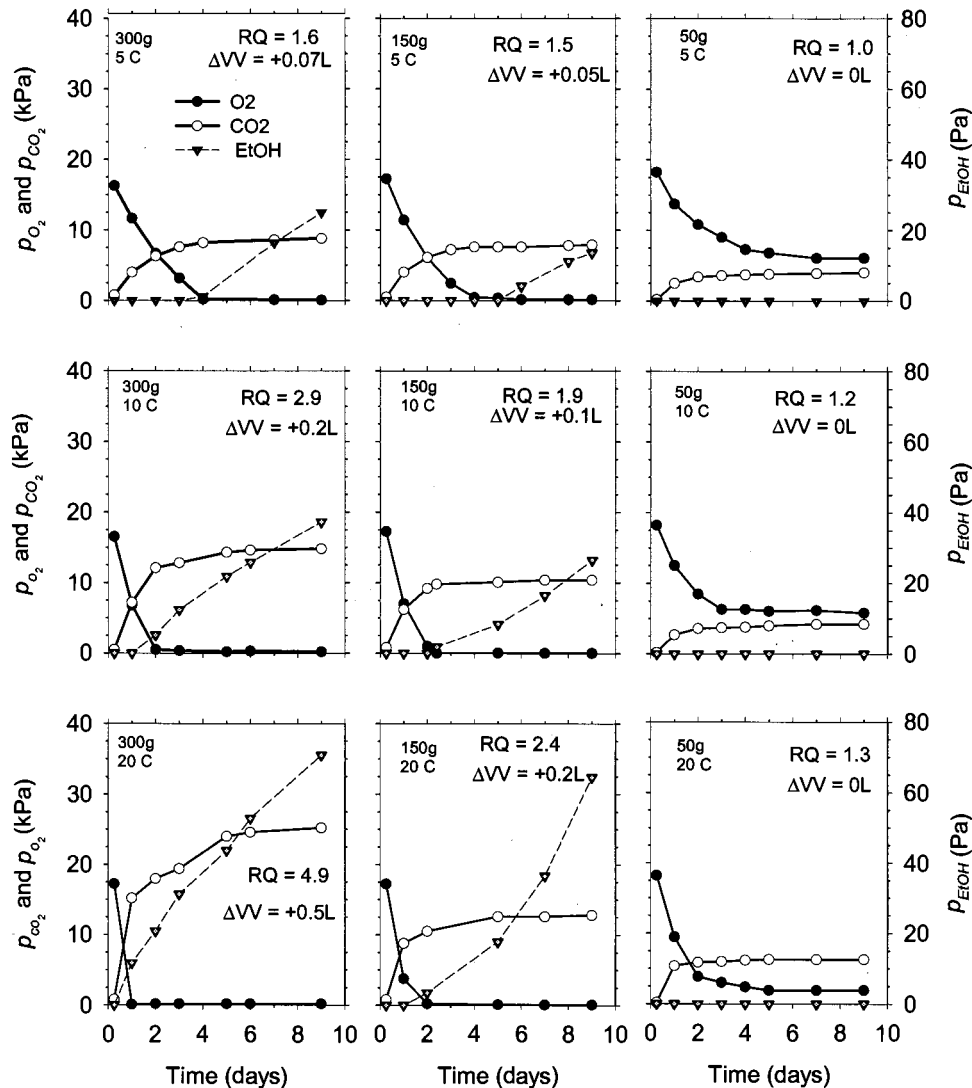
Volatiles present in the MA-packaged lettuce changed during the 10-day storage period, principally with fermentation. Several long-chain hydrocarbons were detected in the freshly packed lettuce by GC/MS (Figure 3A). These hydrocarbons were detected also in an empty LDPE bag and therefore were not derived from the lettuce per se. Long-chain hydrocarbons have very high human olfactory detection thresholds (Devos et al., 1990), so we assume that these levels would not contribute to the flavor characteristics of the lightly processed lettuce. Devos et al. (1990) reported that the human olfactory threshold levels for ethanol, acetaldehyde, and ethyl acetate were  $\sim 30$  ( $\sim 3 \text{ Pa}$ ),  $\sim 0.2$ , and  $\sim 2.6 \mu\text{L}\cdot\text{L}^{-1}$ , respectively. Ethanol and acetaldehyde were detected at concentrations above the human olfactory threshold levels in packages held at 5, 10, or 20 °C after 10 days of storage. Furthermore, the concentrations of these objectionable volatiles increased with increasing temperature (Figure 3B–D). The unlabeled peaks present in Figure 3B,C were primarily long-chain hydrocarbons according to the mass spectral library.

Dimethyl disulfide was found in all of the lettuce packages stored at 10 °C but not in those stored at 5 or 20 °C (Figure 3B–D; Table 2). The smell of dimethyl disulfide was easily detected in the packages, although no formal tests were conducted. Dimethyl disulfide is highly undesirable because of its putrid aroma (Lindsay et al., 1986) and its low human olfactory threshold detection level ( $12 \text{ nL}\cdot\text{L}^{-1}$ ) (Buttery et al., 1976; Lindsay and Rippe, 1986). Dimethyl disulfide has been identified in packages containing cabbage at 30 °C (Chin and Lindsay, 1993), cauliflower at 30 °C (Wallbank and Wheatley, 1976), and broccoli at 0 °C (Pentima et al., 1995), 7.5 and 10 °C (Hansen et al., 1992), and 15 °C (Forney et al., 1991). It seems unlikely that dimethyl disulfide was a product of the lettuce, because it was detected only at 10 °C. Dimethyl disulfide could have been a product of microorganisms with an optimum growing temperature of 10 °C.

Several short-chain methylated alcohols and esters were identified in MA packages held at 20 °C for 10 days (Figure 3D; Table 2). At this stage, the lettuce was inedible because of severe fermentation (tissue ethanol > 1 wt %) and bacterial proliferation.

**Respiratory and Color Changes in Nitrogen-Flushed, MA-Packaged Cut Iceberg Lettuce.** There was no significant effect of headspace  $p_{\text{CO}_2}$  on  $\text{O}_2$  uptake at either 5 or 10 °C ( $p < 0.05$ ) (Figure 4A). Consequently, the  $\text{O}_2$  uptake data obtained in the presence and absence of  $\text{CO}_2$  were combined and fitted to a Michaelis–Menten-type equation at each temperature. The  $k_{1/2}$  values were 0.26 and 0.19 kPa and maximum oxygen uptake rates were 143 and 213  $\text{pmol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$  at 5 and 10 °C, respectively. On the basis of these maximum rates, the estimated  $Q_{10}$  between 5 and 10 °C was  $\sim 2.2$ .





**Figure 2.** Changes in headspace partial pressure of O<sub>2</sub> ( $p_{O_2}$ ), CO<sub>2</sub> ( $p_{CO_2}$ ), and ethanol ( $p_{EtOH}$ ) with time in MA packages containing 300 ± 0.5, 150 ± 0.5, or 50 ± 0.5 g of cut iceberg lettuce stored at 5, 10, or 20 °C. RQ, respiratory quotient; ΔVV, change in void volume between days 0 and 10. Note: 1 kPa ~ 1% gas in the headspace.

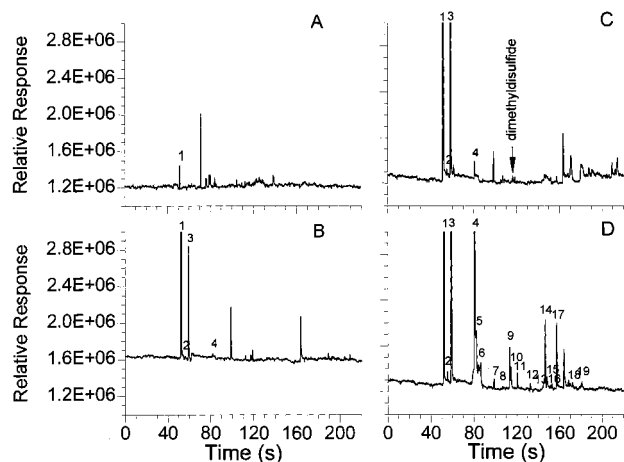
Maximum CO<sub>2</sub> production rates of ~90 and ~155 pmol·g<sup>-1</sup>·s<sup>-1</sup> were estimated at 5 and 10 °C, respectively (Figure 4B). In the flow-through system, CO<sub>2</sub> production rates had been ~120 pmol·g<sup>-1</sup>·s<sup>-1</sup> at 5 °C (Figure 1). The difference in respiratory behavior may be due to seasonal effects on lettuce physiology, since the experiments were conducted on lettuce harvested in the spring (flow-through) and fall (steady-state).

At 5 °C, the RQ was ~0.7 at  $p_{O_2} > 2$  kPa and gradually increased to 1 at  $p_{O_2} < 1$  kPa. Similar trends in RQ were observed in MA packages stored at 10 °C, although the extent of the increase was greater at 10 °C than at 5 °C below the fermentation induction point (Figure 5). Beaudry et al. (1992) and Talasila et al. (1994) found that the fermentative RQ breakpoint was sharply defined for MA-packaged blueberries and broccoli, respectively. For lettuce, RQ increased gradually with decreasing  $p_{O_2}$ , which made it difficult to define a clear lower O<sub>2</sub> limit based on RQ breakpoint alone.

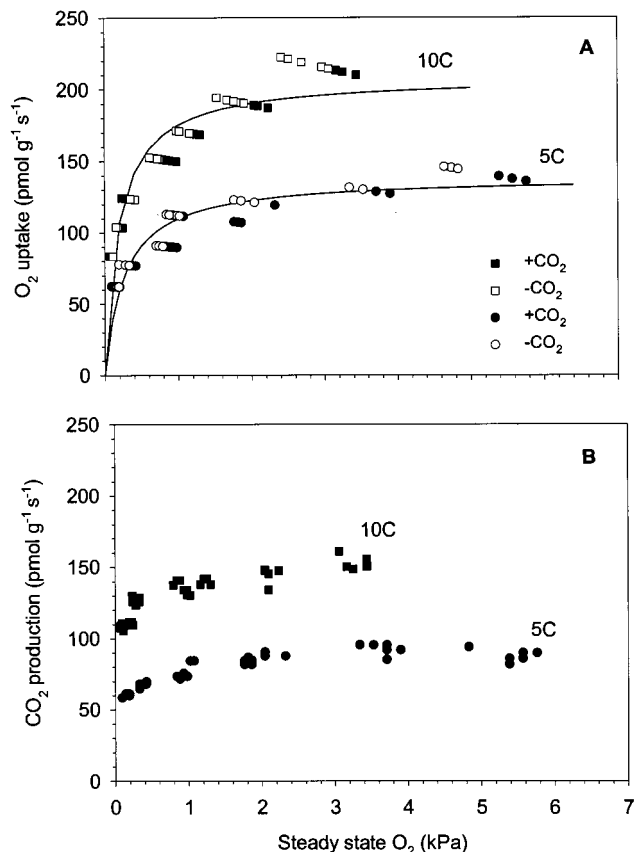
At 5 °C, ethanol was first detected at  $p_{O_2}$  values between 0.3 and 0.5 kPa, whereas at 10 °C, the ethanol induction point occurred between 0.6 and 0.8 kPa of O<sub>2</sub> (Figure 5). We have noted previously that the fermentation induction point increased with temperature for blueberries and raspberries (Beaudry et al., 1992; Joles

et al., 1994). After 4 days at 10 °C,  $p_{EtOH}$  increased to ~9 Pa, whereas at 5 °C,  $p_{EtOH}$  increased to ~4 Pa for packages having the lowest  $p_{O_2}$  after 6 days (Figure 5). Headspace  $p_{EtOH}$  was a function of storage time (Figure 2). There was no significant effect of  $p_{CO_2}$  on the pattern of ethanol accumulation or the fermentation induction point (Figure 5).

There was a slight rise in O<sub>2</sub> uptake whereas CO<sub>2</sub> production was largely constant with increasing  $p_{O_2}$  (Figure 4), which was reflected by a negative slope in RQ versus  $p_{O_2}$  (Figure 5). This phenomenon could be explained if there was a significantly greater contribution of phenolic oxidation to total oxidation (net O<sub>2</sub> uptake) at higher  $p_{O_2}$ . Phenolic oxidation readily occurs in cut lettuce (Ballantyne et al., 1988; Krahn, 1977; McDonald et al., 1990), has a reported  $k_m$  between 6 and 8 kPa (Burton, 1974), and involves O<sub>2</sub> uptake without the production of CO<sub>2</sub>. In contrast, respiratory metabolism involves O<sub>2</sub> uptake accompanied by the production of CO<sub>2</sub> and has a  $k_m$  in the tissue of <0.1 kPa. On this basis, phenolic oxidation would make a greater contribution at higher  $p_{O_2}$ , although the magnitude of O<sub>2</sub> uptake by phenolic oxidation in iceberg lettuce has not been reported to our knowledge. This area would be interesting for further research.



**Figure 3.** Volatile compounds present in MA packages containing  $300 \pm 0.5$  g of cut iceberg lettuce (A) 30 min after sealing, (B) after 10 days of storage at  $5^\circ\text{C}$ , steady-state partial pressure of  $\text{O}_2$  ( $p_{\text{O}_2}$ ) of 0.2 kPa, (C) after 10 days of storage at  $10^\circ\text{C}$ , steady-state ( $p_{\text{O}_2}$ ) of 0.1 kPa, and (D) after 10 days of storage at  $20^\circ\text{C}$ , steady-state partial pressure of  $\text{O}_2$  ( $p_{\text{O}_2}$ ) of 0.1 kPa. The volatiles were sampled using SPME and identified by GC/TOFMS. The peak numbers correspond to the compounds listed in Table 2.



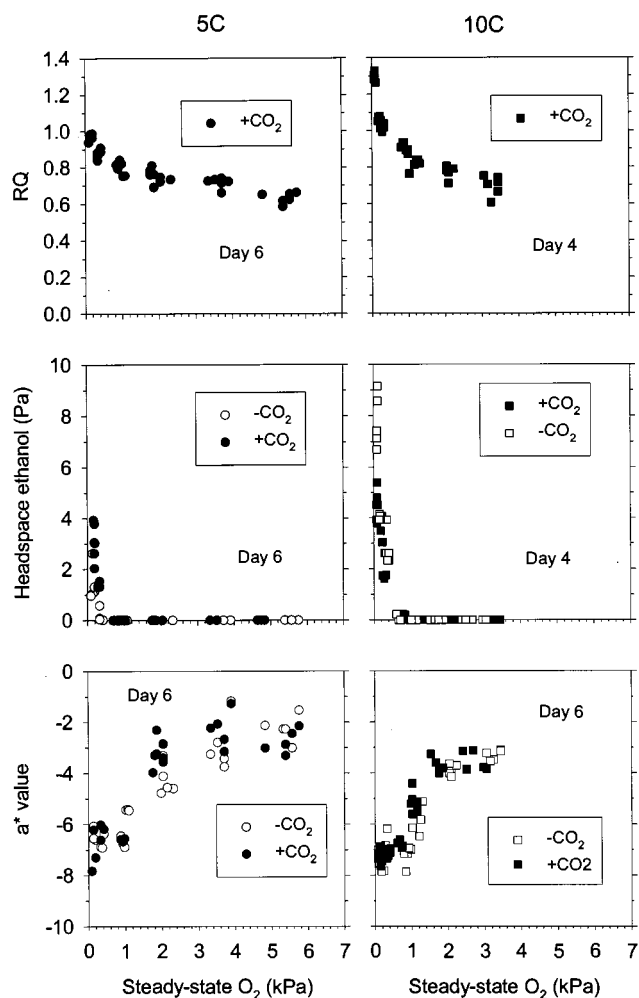
**Figure 4.**  $\text{O}_2$  uptake and  $\text{CO}_2$  production rates of cut lettuce as a function of steady-state partial pressure of  $\text{O}_2$  ( $p_{\text{O}_2}$ ) at 5 and  $10^\circ\text{C}$ . Cut iceberg lettuce (50–150 g) was sealed in  $\text{N}_2$ -flushed packages, surface area =  $916\text{ cm}^2$  and made of  $53.6\text{ }\mu\text{m}$  thick LDPE film. A  $\text{CO}_2$  scrubber (15 g of  $\text{CaO}$ ) was placed in half the packages. Oxygen uptake and  $\text{CO}_2$  production rates were calculated from steady-state  $\text{O}_2$  and  $\text{CO}_2$  partial pressures and film permeabilities.

Loss of green color was measured by an increase in  $a^*$  (Hunter, 1975), which indicated a change in tissue color from green to red. There was no observable tissue discoloration in packages when  $a^* < -6$  (data not

**Table 2.** Volatile Compounds in MA-Packaged Cut Iceberg Lettuce ( $0.1\text{ kPa}$  of  $\text{O}_2$ ) Stored at  $22^\circ\text{C}$  for 10 Days Sampled Using SPME and Identified by Using GC/TOFMS

peak <sup>a</sup>	volatile compd	peak	volatile compd
1	carbon dioxide	11	2-methylpropanoic acid ethyl ester
2	acetaldehyde	12	butanoic acid ethyl ester
3	ethanol	13	3-methyl-1-pentanol
4	ethyl acetate	14	3-methylbutanoic acid ethyl ester
5	acetic acid	15	2-methyl-2-propanol
6	2-methyl-1-propanol	16	3-methylbutyl acetate
7	2,2,3,3-tetramethylbutane	17	1,3,5,7-cyclooctatetraene
8	propanoic acid ethyl ester	18	2-pentylfuran
9	3-methyl-1-butanol	19	hexanoic acid ethyl ester
10	2-methyl-1-butanol		

<sup>a</sup>The peak numbers correspond to the volatile compounds shown in Figure 3D.



**Figure 5.** Influence of steady-state package oxygen partial pressure ( $p_{\text{O}_2}$ ) on RQ, headspace ethanol partial pressure samples, and color stability in the presence and absence of  $\text{CO}_2$  at 5 and  $10^\circ\text{C}$ . Cut iceberg lettuce samples (50–150 g) were sealed in  $\text{N}_2$ -flushed packages, surface area =  $916\text{ cm}^2$  and made of  $53.6\text{ }\mu\text{m}$  thick LDPE film. Color stability data were collected after 6 days. RQ and ethanol partial pressures were measured after 4 days at  $10^\circ\text{C}$  and after 6 days at  $5^\circ\text{C}$ .

shown). There were no differences in color stability between packages stored at the same temperature for 6 days in the presence or absence of  $\text{CO}_2$  (Figure 5). Green color retention was best in packages having  $p_{\text{O}_2}$

< 1 kPa on day 6 (Figure 5). There was a sharp increase in oxidation at  $p_{O_2} > 1$  kPa (Figure 5). If oxidation is a continuous process, these data suggest that there may be a threshold for visual detection of the oxidative products. This hypothesis would be reasonable if continued oxidation resulted in increasing polymerization and, hence, longer, more visible chain lengths of phenolic compounds.

In previous studies, recommended  $p_{O_2}$  consistently has been higher than 1 kPa (Ballantyne et al., 1988; Bolin and Huxsoll, 1991; McDonald et al., 1990). Our data, based on tests conducted in MA packages, suggest that  $p_{O_2}$  should be <1 kPa at 5–10 °C. At 5 °C, color retention was excellent and fermentation was limited at  $p_{O_2}$  of 0.3–0.5 kPa. However, the tolerance to low  $p_{O_2}$  could change with growing season, growing location, cultivar, or other factors. We have measured  $p_{O_2}$  in commercial packages to be consistently <1 kPa and detected significant ethanol accumulation, indicating low-oxygen injury (Cameron et al., 1995). Thus, there are definite risks associated with targeting low  $p_{O_2}$  in practice. Temperature abuse also can contribute to induction of fermentation. On the basis of the measured  $Q_{10}$  value of 2.26, a targeted  $p_{O_2}$  of 0.5 kPa at 5 °C would fall to ~0.4 and ~0.2 kPa of  $O_2$  at 10 and 20 °C, respectively. Both of these  $p_{O_2}$  values would result in product fermentation. Similarly, a targeted  $p_{O_2}$  of 0.75 kPa at 10 °C would rise to ~1 kPa at 5 °C and fall to 0.3 kPa at 20 °C. In this package combination, browning would occur at 5 °C, whereas fermentation would occur at 10 and 20 °C. These predictions highlight the importance of strict temperature control during distribution and handling. Cut lettuce produces a minimum of volatiles and undesirable odors even during the early stages of fermentation (Figure 3). This permits an extra degree of latitude during commercial distribution because the initiation of fermentation does not necessarily cause the product to be unsalable, particularly since most salads are served with strong dressings. Still, the discerning consumer will recognize the difference in quality.

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