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Effect of initial oxygen concentration and film oxygen transmission rate on the quality of fresh-cut romaine lettuce

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Abstract: Modified atmosphere packaging (MAP) is widely used to maintain the quality of fresh-cut produce by matching the oxygen transmission rate (OTR) of the packaging film to the respiration rate of the packaged product. The effect of the interaction between film OTR and the initial headspace O_2 on quality of fresh-cut vegetables has not previously been reported. Romaine lettuce leaves were sliced, washed, dried and packaged with film OTRs of 8.0 and 16.6 pmol s^{-1} m^{-2} Pa^{-1}, and with initial headspace O_2 of 0, 1, 2.5, 10 and 21 kPa. Packages were hermetically sealed and stored at 5 °C for up to 14 days. For samples packaged in 16.6 OTR film, increasing the initial headspace O_2 concentration delayed O_2 depletion within the packages, hastened the onset and increased the intensity of discoloration, and inhibited the development of CO_2 injury, acetaldehyde and ethanol accumulation, off-odors and electrolyte leakage. With 8.0 OTR-packaged lettuce pieces, ≤1 kPa initial headspace O_2 treatments induced an essentially anaerobic environment within the packages and increased acetaldehyde and ethanol accumulation and off-odor development. Increasing the initial O_2 concentration above 1 kPa in 8.0 OTR packages transiently increased O_2 concentrations and reduced fermentative volatile production, off-odors, electrolyte leakage and CO_2 injury. Regardless of initial headspace O_2 concentration, all 16.6 OTR-packaged samples had severe discoloration after 14 days of storage. Quality was better maintained in 8.0 OTR-packaged lettuce pieces as the initial headspace O_2 concentration was increased. A 21 kPa initial O_2 treatment of 8.0 OTR-packaged lettuce maintained good quality throughout storage and had the best overall quality score.

Keywords: fresh-cut lettuce; gas composition; minimally processed; modified atmosphere; oxygen transmission rate; postharvest technology; respiration rate

INTRODUCTION

Fresh-cut produce industry has been rapidly growing in the past decade in response to an increased consumer demand for fresh and convenient food. However, the processing steps, such as cutting and peeling, involved in preparing the fresh-cut products also cause severe tissue damage, leading to rapid quality deterioration.[1] Modified atmosphere (MA) packaging is effective in prolonging the shelf-life of fresh-cut produce by decreasing oxygen and increasing carbon dioxide concentrations in the package.[2] The passive changes in package atmosphere are due to an interaction between respiration rate of the packaged commodity and the permeability characteristics of the film.[3,4] By matching film permeability for O_2 and CO_2 to the respiration rate of the packaged fresh-cut commodity, a beneficial MA can be established inside the package that will delay tissue senescence and extend shelf-life.[5,6] When film permeability is not appropriately matched to the produce respiration rate, O_2 may become depleted and CO_2 enriched within the package leading to anaerobic respiration and, consequently, to fermentative volatile accumulation and off-odor development or an inadequate control of O_2 (too high) may increase oxidative browning and senescence.[7,8] An initial package flushing with nitrogen or a low O_2–high CO_2 atmosphere can be used to establish steady-state MA more rapidly and to maintain shelf stability.[9] For fresh-cut lettuce, the additional benefit of gas flushing is to rapidly establish a low O_2 environment to control tissue browning.[10,11] Studies with whole commodities have shown that an initial gas flush can accelerate gas equilibration without altering the steady-state gas composition. No scientific study on the effect of initial O_2 has been done specifically with the fresh-cut produce, where the plant tissues have been mechanically damaged, and therefore wound response may apply. The objectives of this study were to investigate the effect of initial O_2 flushing and package oxygen transmission rate (OTR)

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on quality maintenance of packaged fresh-cut romaine lettuce and to evaluate the interaction, if any, between initial O_2 concentration and OTR.

MATERIALS AND METHODS

Produce processing and packaging

Romaine lettuce bunches (*Lactuca savita* L) were obtained from the cold storage room (5 °C) from a wholesale market and processed within 5 days after harvest in a clean processing room. Fresh romaine lettuce leaves were cut into approximately 2.5 cm × 2.5 cm slices, washed using an industrial size washer (Model MPW 800, Meyer Machine Co, San Antonio, TX, USA) in 100 mg ml^{-1} chlorine solution (NaOCl) adjusted to pH 6.5 with HCl for 1 min at 5 °C, and centrifuged using a salad centrifugal dryer (Model T-304, Meyer Machine Co) at 650 rev min^{-1} (∼111 g) for 2.5 min to remove excess water. Fresh-cut romaine lettuce pieces (170 g each) were flushed with 0, 1, 2.5, 10 or 21 kPa O_2, packaged in polypropylene bags (19 cm × 19 cm) with film OTRs of 8.0 and 16.6 pmol O_2 s^{-1} m^{-2} Pa^{-1} (see below), and stored at 5 °C for 14 days.

Film OTR analyses

The OTR values of the two films used to prepare the packages were 110 and 225 pmol s^{-1} m^{-2} Pa^{-1}, as determined by the film manufacturer (Packaging Concept Inc, Salinas, CA) at 23 °C, 1 atm using a MOCON apparatus according to an ASTM International procedure D3985-81.[12] At 5 °C in air, the film OTRs were 8.0 and 16.6 pmol s^{-1} m^{-2} Pa^{-1} (8.0 and 16.6 OTR films), as determined following an exponential decay method for determining OTRs through a plastic film in a static cell.[13] The slopes obtained from linear regressions of the test cell data (average of three samples) predicted the film OTRs. Gas transmission rates through plastic films calculated from test cell data using the exponential decay procedure compare favorably with values obtained by a MOCON apparatus.

Respiration rate analyses

Fresh-cut romaine lettuce samples (200 g each) were placed in sealed containers at 5 °C and treated with humidified 0, 1, 2.5, 10 or 21 kPa O_2 (balance N_2) at a flow rate of 20 ml min^{-1}. The CO_2 content of the outlet streams from samples was monitored every 6 h for 14 days using a gas chromatograph (HP 5890a, Hewlett-Packard Co, Rockville, MD) at 60 °C fitted with a Hayesep Q column (2.4 m × 3 mm) and a thermal conductivity detector. The O_2 and CO_2 concentrations within packages of fresh-cut romaine lettuce were measured using a gas analyzer system (Model Combi Check 9800-1, PBI Dansensor Co, Rinsted, Denmark). Evolved CO_2 values were used for calculating and reporting respiration rates while O_2 consumption values were used as a back check for verifying the overall validity of the measurements.

Volatile analyses

Ethanol and acetaldehyde concentrations in the headspace of packages containing fresh-cut romaine lettuce were determined using a gas chromatographic procedure. Gas samples (250 µl) were collected from packages of fresh-cut romaine lettuce using gas-tight glass syringes and were injected into a glass-lined splitless injection port of a gas chromatograph (model 6890N, Agilent Technologies, Rockville, MD) equipped with a flame ionization detector (FID). Volatiles were separated using a capillary column (DB-WAX, 30 m × 0.32 mm id, 1.0 µm coating thickness, J&W Scientific, Folsom, CA). The carrier gas was ultra-purified helium (99.9999%) at a flow velocity of 36 cm s^{-1}. The temperature program was isothermal for 5 min at 40 °C and then raised at 15 °C min^{-1} to 160 °C and held for 3 min. Injector and detector temperatures were 200 °C and 250 °C, respectively. Quantification was done using a relative calibration procedure using known concentrations of standards.

Electrolyte leakage

For electrical conductivity measurement, the procedure of Jiang *et al*[14] was followed with slight modification. Briefly, 50-g samples of fresh-cut romaine lettuce were randomly removed from the package after 0, 1, 5, 10 and 14 days in storage and immersed in 500-ml aliquots of distilled water for 30 min at 5 °C. Conductivity was measured using a conductivity meter (Model 135A, Orion Research Inc, Beverly, MA) by inserting a probe into the sample solutions. Total sample electrolyte levels were determined on the same sample after freeze (−20 °C for 24 h)/thawing, and electrolyte leakage was expressed as percentage of total electrolytes.[15]

Quality attributes

Sensory quality was evaluated by a panel of three trained judges. The samples were coded with three-digit numbers to mask the treatment identity in an effort to minimize test subjectivity and to ensure test accuracy. Off-odor was evaluated immediately after opening the packages and scored on a five-point scale where 0 = none; 1 = slight; 2 = moderate; 3 = strong; and 4 = severe.[7] A score of 3 was considered non-acceptable. Discoloration was evaluated using a five-point scale based on the degree of discoloration where 0 = none; 1 = beginning of discoloration as oval pinkish lesions on the edge of midrib; 2 = moderate browning on both sides of midrib; 3 = strong browning along the midrib; and 4 = severe browning along the midrib. High CO_2 (low O_2) injury also was scored on a five-point scale based on the degree of brown staining of the lettuce pieces where 0 = none; 1 = beginning of injury; 2 =< 1/4 surface area injured; 3 = 1/4 to 1/2 surface area injured; and 4 => 1/2 surface area injured. For both discoloration and CO_2 injury, results are expressed as an index score calculated by taking the sum of each score multiplied by the corresponding number of pieces in four replicate 170-g samples having that score, dividing by the total number of lettuce pieces evaluated, and then multiplying by 100.[16] Overall quality was evaluated after 14 days of storage using a nine-point hedonic scale where 9 = like extremely; 7 = like moderately; 5 = neither like nor dislike; 3 = dislike moderately; and 1 = dislike extremely;[17] a score of 6 was considered the limit of salability.[7,18]

Experimental design and statistical analysis

All aspects of the initial O_2 concentration–film OTR experiment was conducted using a completely randomized design and repeated: results from both experiments were similar and results from the second experiment are presented. Product quality and package atmospheres were measured immediately after packaging and after 0.25, 1, 3, 5, 10 and/or 14 days of storage with three replications. All quality evaluations were conducted in a temperature-controlled room at 5 °C to minimize the effect of temperature variations during testing. Data were analyzed as a three-factor linear model using the Proc Mixed procedure of SAS (SAS Institute, Cary, NC) with storage time, film OTR and initial O_2 flush as the factors. Any treatments where all the values were zero or had the same value (no variability) were omitted from the analysis.

When effect(s) were statistically significant, mean comparisons were done with Sidak-adjusted p-values at $\alpha = 0.05$. Unless stated otherwise, only significant effects are discussed.

RESULTS

Respiration rate

Respiration rates, measured as evolved CO_2, increased upon processing, peaked at ~6 h, and then decreased gradually before stabilizing around 4 days after processing (data not shown). Lettuce pieces that were continuously flushed with 0, 1.0 and 2.5 kPa O_2 had lower respiration rates than those continuously flushed with 10 or 21 kPa.

Gas composition

In package atmospheres of fresh-cut romaine lettuce, the O_2 concentration decreased in a hyperbolic decay pattern, then increased in some treatments before reaching an apparent steady state after 1 to 10 days (Fig 1, top). Packages flushed with ≤2.5 kPa initial O_2 in 8.0 OTR packages exhibited a rapid depletion of O_2 to ~0 kPa within 1 day, while 10 and 21 kPa initial O_2 treatments reached essentially anaerobic conditions after 3 and 10 days, respectively. The O_2 concentration in 16.6 OTR packages flushed with ≤2.5 kPa initial O_2 decreased to a minimum value by day 3 before increasing ~1.5 kPa and stabilizing at ~10 days. The O_2 concentration in 16.6 OTR packages flushed with 10 or 21 kPa initial O_2 showed a hyperbolic decay before stabilizing around days 3 and 10, respectively.

The CO_2 concentration in both 8.0 and 16.6 OTR packages increased in a hyperbolic pattern during the first 5 to 10 days and changed little thereafter (Fig 1, bottom). The CO_2 concentration was generally higher in 8.0 OTR packages flushed with 0 or 1 kPa O_2 than with any other treatments. The CO_2 concentration reached or exceeded 10 kPa during storage in 8.0 OTR packages regardless of the initial O_2 treatment. In contrast, the CO_2 concentration did not exceed 7 kPa during storage in 16.6 OTR packages. After 5 days, the CO_2 concentration was lower in 16.6 OTR than in 8.0 OTR packages regardless of the initial O_2 treatment.

Volatile levels

The ethanol vapor concentration inside 8.0 and 16.6 OTR packages increased generally throughout storage (Fig 2, top). Ethanol accumulated more rapidly and to higher levels in 8.0 OTR packages flushed with 0 or 1 kPa O_2 than with any other treatment. Flushing both packages with increasing concentrations of O_2 correspondingly decreased ethanol accumulation. Ethanol accumulation in 16.6 OTR packages was less than half of that in 8.0 OTR packages flushed with ≤10 kPa O_2. 8.0 OTR packages flushed with 21 kPa O_2 had two to six times less ethanol accumulation during storage than that in the same packages flushed with lower O_2 concentrations and a similar accumulation to that with 16.6 OTR package treatments. Acetaldehyde gas essentially followed the same patterns of accumulation within the packages as ethanol vapors albeit at about a threefold lower magnitude (Fig 2, bottom). Acetaldehyde gas and ethanol vapor concentrations exceeded 1 Pa and 2 kPa, respectively, in 8.0 OTR packages flushed with 0 or 1 kPa O_2.

Electrolyte leakage

Electrolyte leakages of all samples decreased during the first day of storage, then remained stable thereafter or increased slightly at the end of storage (Fig 3). Throughout storage, samples flushed with 0 or 1 kPa O_2 had higher electrolyte leakage than those flushed with higher concentrations of O_2. While no significant differences in electrolyte leakage occurred among 2.5, 10 and 21 kPa O_2-flushed samples packaged with either film, leakage was generally higher in correspondingly treated 8.0 than in 16.6 OTR-packaged samples.

Discoloration and CO_2 injury

Barely detectable discoloration developed on 8.0 OTR-packaged samples flushed with <21 kPa O_2 whereas noticeable discoloration developed on samples flushed with 21 kPa initial O_2 treatment (Table 1). More severe discoloration occurred on samples packaged with 16.6 OTR films. Both the onset and the intensity of the discoloration in the 16.6 OTR-packaged samples were directly related to the concentration of O_2 in the flush treatments.

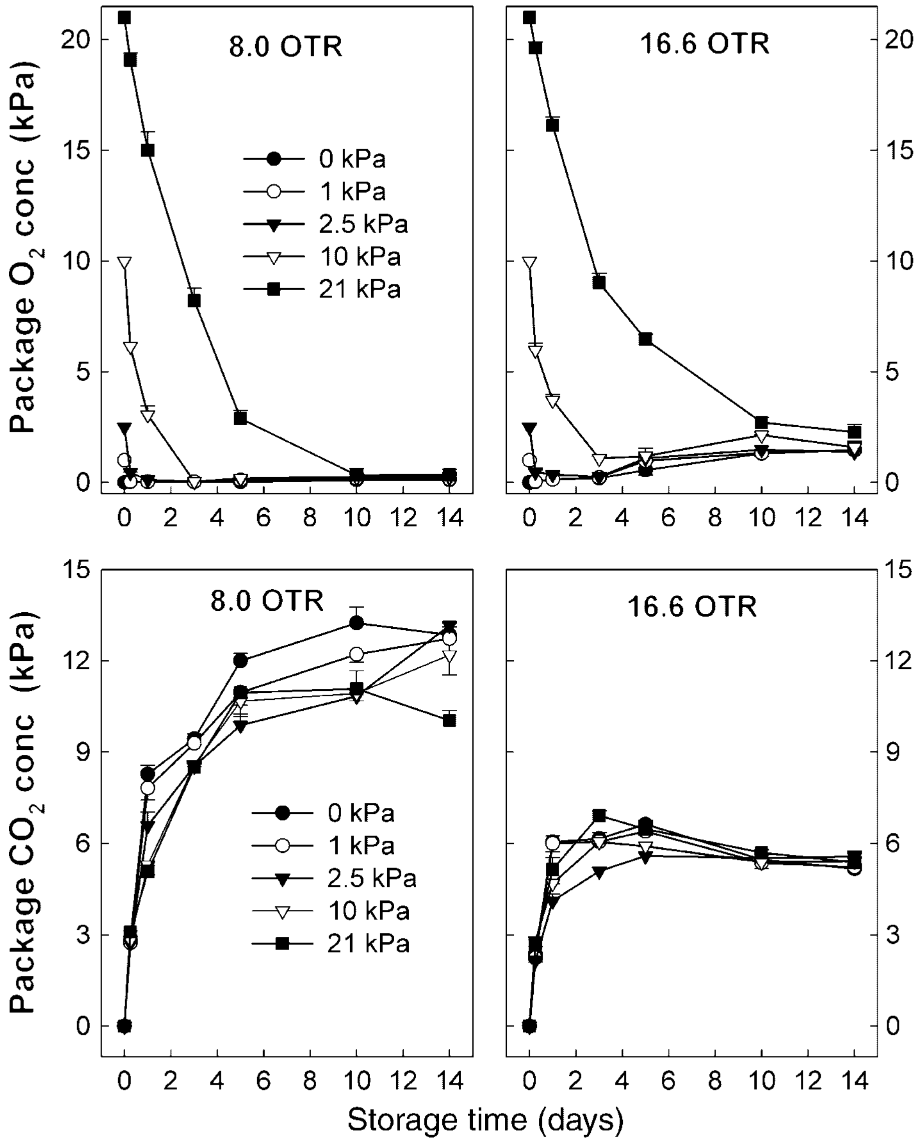

Figure 1. Effect of packaging film OTR and initial O_2 flushing on the O_2 and CO_2 concentrations within packages of fresh-cut romaine lettuce stored at 5 °C for 14 days. Fresh-cut romaine lettuce samples (170 g) were flushed with 0, 1, 2.5, 10 and 21 kPa O_2 (balance N_2), sealed in 19 cm × 19 cm packages prepared from polypropylene films having OTR of 8.0 and 16.6 pmol s^{-1} m^{-2} Pa^{-1} at 5 °C, and stored at 5 °C for up to 14 days. Each symbol is the mean of three replicate measurements; vertical lines represent SE. SE lines were not shown when masked by the symbol.

Carbon dioxide injury, as indicated by brown staining mainly on outer and immature leaf surfaces, occurred on 8.0 OTR-packaged samples by day 10 (Table 2). While generally not significant, there appeared to be an inverse relationship between the concentration of O_2 in the initial flush treatment and the degree of CO_2 injury of 8.0 OTR-packaged samples. Carbon dioxide injury was barely detectable or did not occur in 16.6 OTR-packaged samples flushed with ≥2.5 kPa O_2.

Sensory quality

Off-odor was first detected in packaged samples flushed with ≤2.5 kPa O_2 on day 1 (Fig 4). By day 10, off-odor was higher in 8.0 OTR-packaged samples flushed with ≤10 kPa O_2 than in any other treated samples. For both package types, flushing with 21 kPa O_2 delayed the onset and the intensity of off-odors compared with flushing with <10 kPa O_2. While not always significant, there appeared to be an inverse relationship between the intensity of off-odors and the concentration of O_2 in the initial flush treatment (Fig 1). At the end of storage, overall quality was higher in 8.0 OTR-packaged samples flushed with ≥2.5 kPa O_2 than with any other treatment (Fig 5). Overall quality of 16.6 OTR-packaged samples was low due to severe discoloration regardless of the O_2 concentration in the initial flush (Table 1, Fig 5). Overall quality was low in 8.0 OTR-packaged samples flushed with 0 or 1 kPa O_2 due primarily to the intensity of off-odors (Figs 4 and 5). A quality score >5 is considered acceptable and occurred only in 8.0 OTR-packaged samples flushed with ≥2.5 kPa O_2.

DISCUSSION

Fresh cutting produce leads to increased respiration and ethylene production rates, and may induce

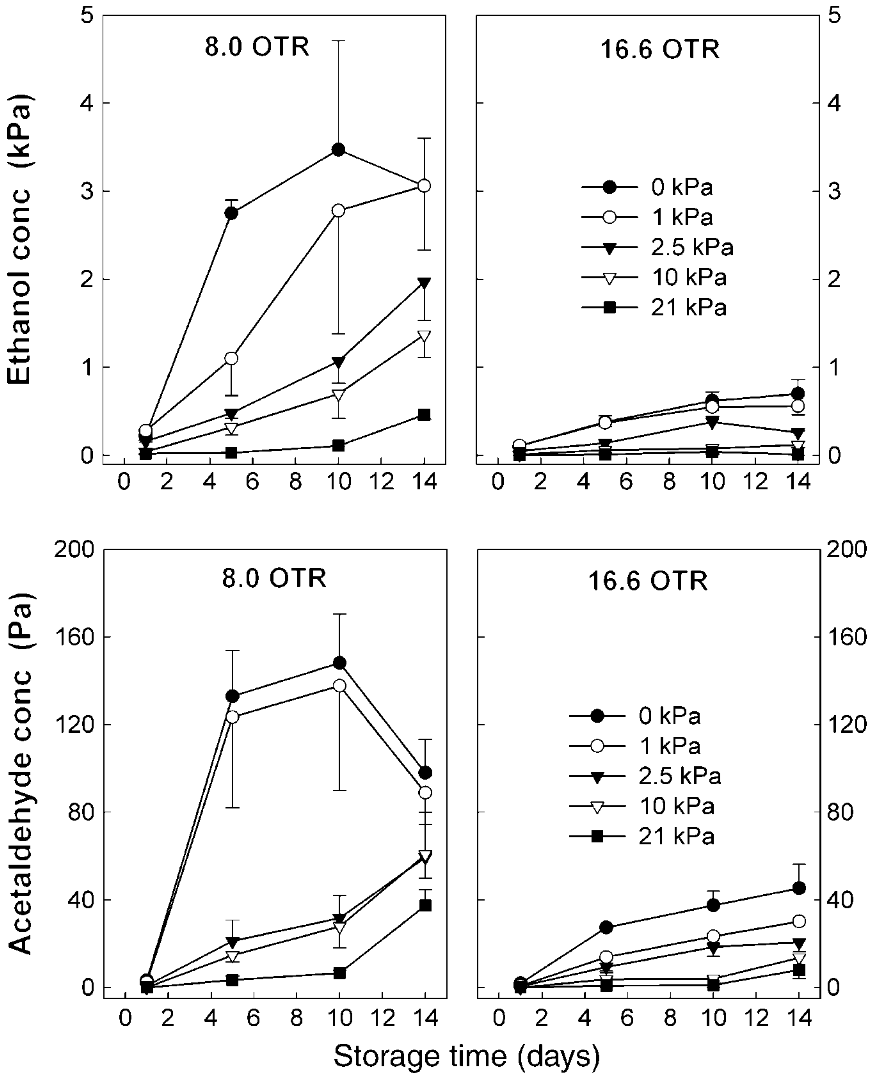

Figure 2. Effect of packaging film OTR and initial O_2 flushing on the ethanol vapor and acetaldehyde gas concentrations within packages of fresh-cut romaine lettuce stored at 5 °C for up to 14 days. Treatments are described in the legend to Fig 2. Each symbol is the mean of three replicate measurements; vertical lines represent SE. SE lines were not shown when masked by the symbol.

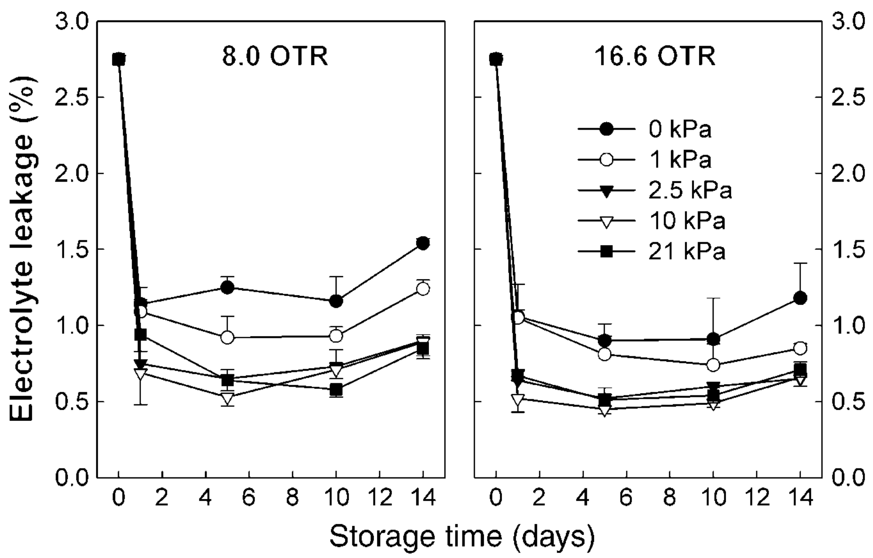

Figure 3. Effect of packaging film OTR and initial O_2 flushing on electrolyte leakage from packaged fresh-cut romaine lettuce stored at 5 °C for up to 14 days. Treatments are described in the legend to Fig 2. Electrolyte leakage is expressed as a percentage of total electrolytes leaked from 50-g samples during a 30-min incubation in 500-ml aliquots of distilled water at 5 °C. Each symbol is the mean of three replicate measurements; vertical lines represent SE. SE lines were not shown when masked by the symbol.

Table 1. Discoloration index score of fresh-cut romaine lettuce stored for 1, 5, 10 or 14 days at 5 °C

	Discoloration index score[a]							
	Storage time in 8.0 OTR packages (days)				Storage time in 16.6 OTR packages (days)			
Initial O_2 flush treatment (kPa)	1	5	10	14	1	5	10	14
0	0	0	0	0	0	0	0.32 cd[b] y[c]	0.48 b x
1	0	0	0	0.01 d z	0	0	0.4 bc y	0.51 b x
2.5	0	0	0	0.01 d z	0	0.06 b z	0.54 b y	0.65 b x
10	0	0	0	0.02 d z	0	0.23 a y	0.71 a x	0.89 a x
21	0	0.12 b z	0.17 d z	0.18 c z	0	0.28 a y	0.84 a x	0.93 a x

[a] Discoloration was evaluated using a five-point scale based on the degree of discoloration where 0 = none; 1 = beginning of discoloration as oval pinkish lesions on the edge of midrib; 2 = moderate browning on both sides of midrib; 3 = strong browning along the midrib; and 4 = severe browning along the midrib. Results are expressed as an index score calculated by taking the sum of each score multiplied by the corresponding number of pieces in four replicate 170-g samples having that score, dividing by the total number of lettuce pieces evaluated, and then multiplying by 100.[16]

[b] Treatment means within time (columns) with different a, b, c, d letters are different at $\alpha = 0.05$ using Sidak-adjusted p-values.

[c] Time means within treatment (rows) with different x, y, z letters are different at $\alpha = 0.05$ using Sidak-adjusted p-values.

Table 2. Carbon dioxide injury index score of fresh-cut romaine lettuce stored for 1, 5, 10 or 14 days at 5 °C

	CO_2 injury index score[a]							
	Storage time in 8.0 OTR packages (days)				Storage time in 16.6 OTR packages (days)			
Initial O_2 flush treatment (kPa)	1	5	10	14	1	5	10	14
0	0	0.08[b]	0.13	0.14	0	0.05	0.05	0.09
1	0	0.02	0.07	0.10	0	0	0.04	0.05
2.5	0	0	0.06	0.07	0	0	0	0.02
10	0	0	0.06	0.05	0	0	0	0
21	0	0	0.02	0.02	0	0	0	0

[a] Carbon dioxide injury was scored on a five-point scale based on the degree of brown staining of the lettuce pieces where 0 = none; 1 = beginning of injury; 2 =< 1/4 surface area injured; 3 = 1/4 to 1/2 surface area injured; and 4 => 1/2 surface area injured. Results are presented as an index score calculated as described in footnote 1 of Table 1.

[b] Non-zero values were not significantly different from one another at $\alpha = 0.05$ using Sidak-adjusted p-values.

oxidative tissue browning.[8,19] The respiration rate of fresh-cut iceberg lettuce has been reported to decrease within 12 h after processing and to change little therafter.[8] In the present study, the respiration rate followed the same pattern of change after processing but took longer (~4 days) to stabilize. Continuously flushing lettuce samples with atmospheres containing $\leq$2.5 kPa O_2 inhibited respiration rates compared with samples continuously flushed with 10 or 21 kPa O_2, which is consistent with the earlier finding that controlled atmosphere storage inhibited respiration in fresh-cut produce.[20]

For fresh-cut lettuce, an optimum MA is considered to be 1–3 kPa O_2 and 5–10 kPa CO_2 and an initial flush with low O_2–high CO_2 atmosphere is known to enhance the establishment of optimal MA conditions.[7,9,21] In our experiments, the O_2 concentration in packages of fresh-cut lettuce were lower than those reported in other laboratory studies,[9,21] but was similar to those present in many commercial salad packages.[7] Apparent steady-state concentrations of CO_2 were higher and took longer to establish in 8.0 than in 16.6 OTR packages. Relatively stable CO_2 concentrations >10 kPa were reached after 5 days of storage in 8.0 OTR packages while CO_2 concentrations <7 kPa were approached within 3 days of storage in 16.6 OTR packages. The latter finding was similar to that reported by Heimdal *et al*[11] for packaged shredded lettuce where CO_2 stabilized at ~7 kPa after 2 days of storage at 5 °C. Samples packaged with 16.6 OTR film and flushed with 2.5 kPa O_2 had the lowest CO_2 accumulation until day 5 among all treatments and did not exceed 6 kPa CO_2 throughout storage (Fig 1). This result showed that flushing with 2.5 kPa O_2 may be near optimal for inhibiting fresh-cut lettuce respiration in the early stages of storage and for inhibiting ethanol and acetaldehyde production (Fig 2) when using relatively high OTR packaging. However, if the O_2 concentration in the packages becomes depleted, as in 8.0 OTR packaged samples (Fig 1), anaerobic respiration may be induced and lead to the accumulation of fermentative volatiles particularly ethanol and acetaldehyde (Fig 2).[8,10]

Electrolyte leakage is generally considered as an indirect measure of plant cell membrane damage, and has been used to predict vigor, deterioration, damage of seeds and chilling injury of fruits and vegetables.[14,22] Loss of membrane integrity due to damage of fruits and vegetables resulted in an increase in ion leakage rate.[14] Hong *et al*[15] reported that a rapid increase in electrolyte leakage from sliced tomatoes might have been due to a change in membrane fluidity caused by cutting-induced oxidative stress. Increased electrolyte leakage after fresh-cut processing may be due to induced leakage from cut and otherwise wounded tissues, while decreased leakage that occurred shortly after processing may be due to recovery of damaged cell membranes and absorption (or adsorption) of

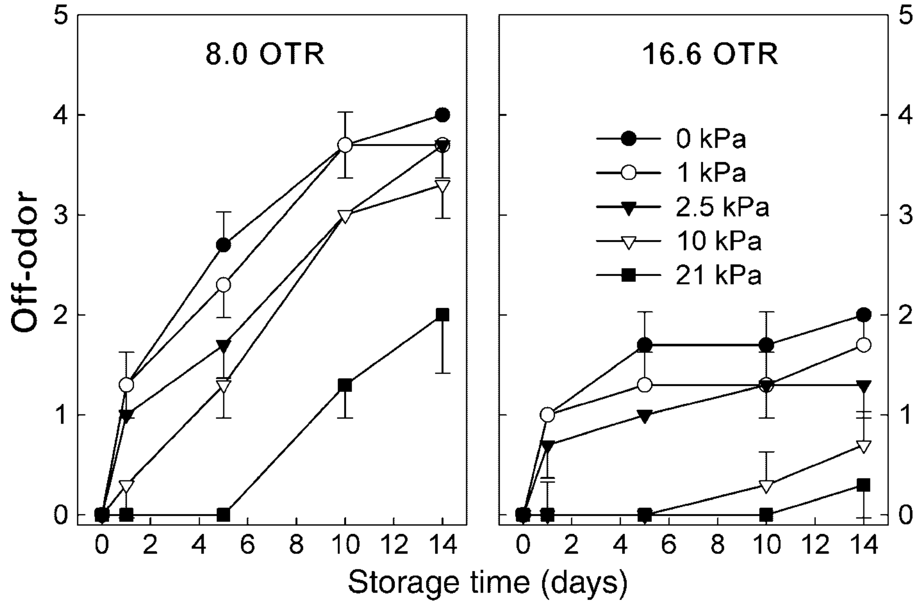

Figure 4. Effect of packaging film OTR and initial O_2 flushing on off-odor development within packages of fresh-cut romaine lettuce stored at 5 °C for up to 14 days. Treatments are described in the legend to Fig 2. Off-odor was scored by three trained panelists using a 0 to 4 hedonic scale where 0 = none; 1 = slight; 2 = moderate; 3 = strong; and 4 = severe. Each symbol is the mean of three replicate measurements; vertical lines represent SE. SE lines were not shown when masked by the symbol.

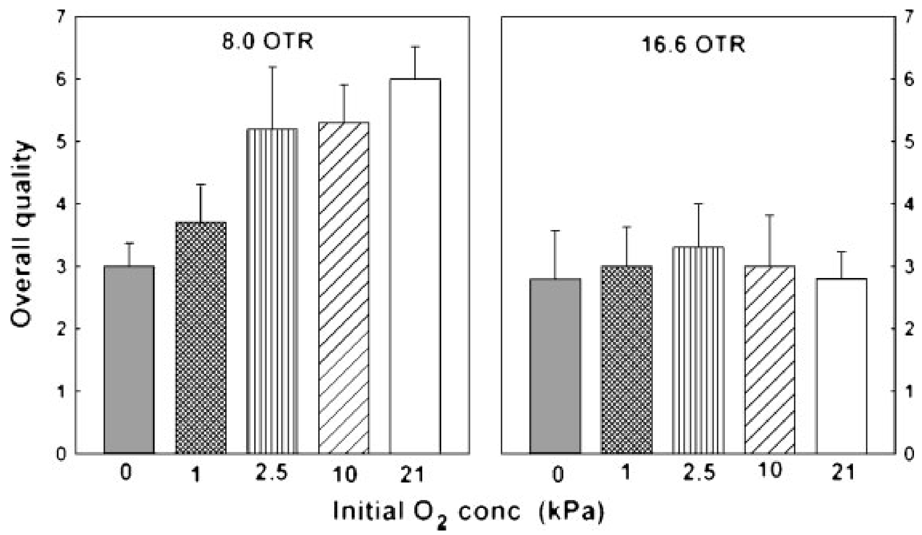

Figure 5. Effect of packaging film OTR and initial O_2 flushing on overall quality of packaged fresh-cut romaine lettuce stored at 5 °C for up to 14 days. Treatments are described in the legend to Fig 2. Overall quality was scored by three trained panelists using a 1 to 9 hedonic scale where 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; and 9 = like extremely. Each symbol is the mean of three replicate measurements; vertical lines represent SE.

electrolytes released during processing. The latter assumption is consistent with our finding that a 21 kPa O_2 treatment for 8 to 12 h before packaging fresh-cut lettuce decreased electrolyte leakage relative to lettuce pieces packaged immediately after processing and flushed with 1.5 kPa O_2.[23] In the present study, $\geq$2.5 kPa O_2 flush treatments decreased electrolyte leakage in both packages relative to flushes with 0 or 1 kPa O_2. Thus, $\geq$2.5 kPa O_2 treatment may be required after processing to aid wound recovery. However, prolonged exposure of lettuce pieces to above optimal O_2 concentrations would increase respiratory activity and oxidative browning, and decrease shelf stability.

Discoloration as cut-edge browning (pink) is the major cause of quality loss in fresh-cut lettuce. Smyth *et al*[8] reported that color retention of lettuce pieces was excellent at 0.3 to 0.5 kPa O_2 at 5 °C. Lipton[21] reported that >7.5 kPa CO_2 concentrations effectively retarded and even prevented darkening of the cut stem surfaces regardless of O_2 concentration. In the present study, browning was essentially prevented in 8.0 OTR-packaged, <21 kPa O_2-flushed samples that had accumulated >10 kPa CO_2 and had $\leq$0.3 kPa O_2 by day 10 of storage at 5 °C (Table 1, Fig 1). The browning that occurred during storage of 8.0 OTR-packaged samples flushed with 21 kPa O_2 (Table 1) apparently resulted from above optimal O_2 and sub-optimal CO_2 concentrations that were present during the first few days of storage. The intensity of this discoloration was low and had little impact on overall quality.

Cut-edge browning intensity was affected by film OTR and the O_2 concentration of the initial flush. Unlike 8.0 OTR-packaged samples, severe browning occurred in 16.6 OTR-packaged samples even when flushed with 0 kPa O_2. The discoloration was probably due to the above optimal O_2 and/or sub-optimal CO_2 concentrations needed for browning control[8,21] that occurred in the 16.6 OTR packages during storage (Fig 1). The discoloration in 16.6 OTR-packaged samples was considered significant enough to adversely affect overall product quality.

High (5 to 15 kPa) CO_2 concentrations can cause the development of brown stain in romaine lettuce.[21,24] Brown stain is a typical symptom of CO_2 injury and it appears first on the midrib surfaces as a variable-sized sunken or yellowed area, which can later become more delineated and brown.[16] In the present study, brown stain was observed in all 8.0 OTR-packaged samples where CO_2 accumulated to >10 kPa during storage and in 16.6 OTR-packaged samples flushed with ≤ 2.5 kPa O_2 where CO_2 concentrations remained <7 kPa during storage. Brown stain in 16.6 OTR-packaged samples was prevented when the packages were flushed with 10 or 21 kPa O_2 (Table 1) even though such treatments resulted in similar CO_2 accumulation patterns as when 16.6 OTR packages were flushed with lower O_2 concentrations (Fig 1, bottom). Since the ≥ 10, but not the ≤ 2.5, kPa O_2 flushes also prevented O_2 depletion during the first few d storage in 16.6 OTR packages (Fig 1, top), we suggest that the intensity of CO_2-induced brown staining is affected by the O_2 as well as the CO_2 concentration patterns in the packages during storage. This assumed interaction of O_2 and CO_2 in the development of brown stain in lettuce is consistent with the finding of Lipton[21] that brown stain was absent in high O_2–7.5 kPa CO_2 atmospheres, but developed in 7.5 kPa CO_2 atmospheres containing 0.5–2.0 kPa O_2.

Fresh-cut lettuce is known to develop undesirable off-odors under low O_2 and elevated CO_2 atmospheres.[8,24] In this study, the patterns of off-odor development (Fig 4) correlated with ethanol and acetaldehyde accumulation patterns (Fig 2). There was also a strong relationship between package atmospheric conditions and off-odor development. Fresh-cut lettuce packaged with 8.0 OTR film developed stronger off-odors and more modified atmospheres than corresponding samples packaged with 16.6 OTR film.

Overall quality was evaluated to determine whether samples were acceptable at the end of storage. Overall quality was influenced by both the initial O_2 flush and film OTR. As previously reported, high CO_2 maintained good visual quality in fresh-cut lettuce.[10] Samples packaged with 8.0 OTR film and flushed with ≥ 2.5 kPa O_2 had better quality than lower O_2 flush and/or higher OTR packaging treatments because of the greater suppression of discoloration and lesser enhancements of CO_2 injury and off-odor development. This is somewhat inconsistent with the finding of Ballantyne *et al*[9] that flushing with low O_2–high CO_2 atmospheres maintained shelf stability of fresh-cut lettuce and with the suggestion of Smyth *et al*[8] to flush with <1 kPa O_2 when packaging fresh-cut iceberg lettuce. Our data showed that 8.0 OTR-packaged fresh-cut lettuce flushed with <1 kPa O_2 resulted in strong off-odor development, severe CO_2 injury, and an unacceptable overall quality score. However, 8.0 OTR-packaged samples flushed with 21 kPa O_2 had an overall quality score of 6, which is considered acceptable, at the end of 14 days of storage at 5 °C. Therefore, flushing with low O_2 or low O_2–high CO_2 atmospheres is not always optimal for maintaining quality and shelf stability. Adjusting the initial O_2 flush to match the OTR of the packaging film can provide a practical means for processors to minimize quality loss of fresh-cut lettuce particularly when packaging under sub-optimal OTR conditions.

CONCLUSION

Film OTR and O_2 flushing during packaging affected package atmospheres, and consequently affected the fermentative volatile production, off-odor development, electrolyte leakage, discoloration, CO_2 injury and the overall quality of fresh-cut romaine lettuce. Discoloration had the greatest effect on the overall quality of fresh-cut lettuce in the present study. Lettuce pieces packaged with 16.6 OTR film had severe discoloration even when flushed with 0 kPa O_2 during packaging. Discoloration was effectively controlled by packaging lettuce pieces with 8.0 OTR film and flushing with ≥ 2.5 kPa O_2. Fresh-cut romaine lettuce packaged with 8.0 OTR film and flushed with 21 kPa O_2 maintained good quality with a score of 6 on a 9-point scale during storage for 14 days at 5 °C.

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