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Effect of non-conventional atmospheres and bio-based packaging on the quality and safety of *Listeria monocytogenes*-inoculated fresh-cut celery (*Apium graveolens* L.) during storage

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ABSTRACT

The increased consumption of fresh-cut celery has led to the need to explore packaging alternatives for fresh-cut celery that can meet consumer, market, and industry needs. In this study, the effect of bio-based packaging and non-conventional atmospheres on the quality and safety of chlorine-sanitized celery sticks stored at 7 °C was investigated. Two materials differing in permeability [a bio-based polyester (polylactic acid (PLA)) and a petroleum-based polyolefin (polypropylene/low density polyethylene (PP/PE)] and four initial gas compositions [air (A-PLA or A-PP/PE), 95 kPa O_2 + 5 kPa N_2 (O_2-PLA), 99 kPa N_2 + 1 kPa O_2 (N_2-PLA), and 6 kPa O_2 + 12 kPa CO_2 + 82 kPa N_2 (CO_2-PLA)] were evaluated. Changes in headspace composition, weight loss, surface and cut end color, texture, ethanol content, appearance, and growth of *Listeria monocytogenes* on inoculated celery sticks were assessed during 21 d of storage. Active MAP (CO_2-PLA) out-performed passive MAP (A-PLA) in maintaining celery stick quality but not safety. Conventional active MAP (CO_2-PLA) out-performed non-conventional active MAPs (O_2-PLA and N_2-PLA) in maintaining celery stick quality throughout storage, but O_2-PLA suppressed *L. monocytogenes* growth while CO_2-PLA promoted growth during the first 10 d of storage. PLA and PP/PE materials affected celery stick quality but not *Listeria* growth. This study shows that the initial gas composition and packaging material both impact the quality and safety of celery sticks. Overall, the combination PLA and 95 kPa O_2 proved most beneficial in maximizing both the safety and quality of celery sticks during one week of storage at 7 °C.

1. Introduction

Consumer demand for minimally processed fruits and vegetables has increased dramatically over the last ten years, with celery (*Apium graveolens* L.) being among the most desired fresh-cut products due to its taste, texture and low caloric content (United Fresh Foundation, 2010). Celery is also a good source of vitamins A and C (Rizzo and Muratore, 2009) as well as potassium and dietary fiber. However, damage and stress encountered by celery during processing can lead to changes in color (Viña and Chaves, 2003; Gómez and Artés, 2005; Viña et al., 2007; Rizzo and Muratore, 2009), pithiness development (Saltveit and Mangrich, 1996; Gómez and Artés, 2005), and loss of turgor (Prakash et al., 2000; Viña and Chaves, 2003; Viña et al., 2007).

Various strategies have been developed to better maintain celery freshness. Low temperature storage combined with high CO_2/low O_2 controlled atmospheres can extend the shelf life of celery by several days (Saltveit, 1997; Suslow and Cantwell, 2000; Gómez and Artés, 2004) as can passive modified atmosphere packaging (MAP) (Viña and Chaves, 2003; Gómez and Artés, 2005; Viña and Chaves, 2006; Viña et al., 2007; Rizzo and Muratore, 2009). However, no information has been published about the benefits from active MAP, in which the package atmosphere is established at the time of sealing, over passive MAP on the shelf life of fresh-cut celery. In addition, the effect of non-conventional active packaging atmospheres, including noble gases, superatmospheric oxygen,

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and nitrogen, on shelf life extension of fresh-cut produce has been explored for some products (Allende et al., 2004; Rocculi et al., 2005; Escalona et al., 2006; Artés et al., 2009), but not for celery.

Studies dealing with fresh-cut celery have commonly been carried out at 4–5 °C (Gómez and Artés, 2004, 2005; Rizzo and Muratore, 2009). However, a recent study revealed serious problems in maintaining the temperature in refrigeration equipment in food stores (Lundén et al., 2014), and surveys in the U.S. revealed that 20% of domestic and commercial refrigerators operate at temperatures around 10 °C (Jol et al., 2006). For this reason, studies on celery under real storage conditions during distribution and retail instead of optimal ones are necessary.

Several studies have focused on the microbiological safety of fresh-cut celery (Prakash et al., 2000; Lu et al., 2005; Kwak et al., 2011; Vandamm et al., 2013). However, very little information has been published on *Listeria monocytogenes* in celery, most likely because fresh produce has not been traditionally considered a special risk product (Aguado et al., 2004). Recently, *L. monocytogenes* was identified in ready-to-eat salads containing raw celery (Cordano and Jacquet, 2009) indicating that celery occasionally poses a risk to the consumers. Furthermore, from January to October 2010, ten cases of hospital-acquired listeriosis in Texas (USA), including five deaths, were traced to commercially diced celery that was used as an ingredient in chicken salad (Gaul et al., 2013).

Fresh-cut produce is commonly marketed in packages made from petroleum-based materials. However, environmental concerns surrounding these packages have created a need for more environmentally friendly materials (Tharanathan, 2003). Polylactic acid (PLA), a commercially produced bio-based plastic material, is one suitable alternative for packaging of fresh produce (Almenar et al., 2008, 2010; Joo et al., 2011; Forney et al., 2012). PLA-based packages are clear and highly appealing to consumers (Koutsimanis et al., 2012). Although this material has been previously evaluated for some types of whole and fresh-cut produce, its suitability for fresh-cut celery packaging has not yet been assessed. Based on the expanding market for fresh-cut celery, the objective of this study was to compare the effect of packaging material and initial gas composition on the quality and safety of celery sticks stored at 7 °C. One bio-based and one petroleum-based packaging film were compared using passive atmosphere packaging, while four different in-package gas compositions were evaluated using the bio-based material. Finally, various quality parameters were studied and safety was evaluated in terms of *L. monocytogenes* growth.

2. Materials and methods

2.1. Materials

2.1.1. Celery

Celery (*A. graveolens* L.) was purchased from a local distributor (Stan Setas Produce, Lansing, MI, USA), transported under refrigeration to the MSU School of Packaging, maintained at 7 °C and used within 2 d of delivery. Celery was visually inspected, selected, and minimally processed with a stainless steel knife (Granton, Sheffield, UK) into 10 cm long sticks (~20 g each). After processing, the celery sticks were rinsed with cold tap water and stored inside a temperature controlled chamber at 7 °C overnight. The following day, the celery sticks were immersed in a commercial sanitizer containing 50 mg L^{-1} available chlorine at pH 7.5 (XY-12, Ecolab, St. Paul, MN, USA) for 1 min at 7 °C, centrifuged for 1 min using a batch spin dryer (SD50 LT, Heinzen Manufacturing Intl., CA, USA) and packaged. The celery sticks were not rinsed after sanitizing, following the current processing conditions of celery processors in the United States, meaning some residual chlorine could be present after processing. The sticks intended for *L. monocytogenes* inoculation were treated as described in Section 2.2.7.

2.1.2. Packaging materials

Two packaging films were used: A 51 μm-thick film composed of polypropylene (PP) and low density polyethylene (LDPE) (commonly used in fresh-cut produce packaging in the U.S.) and a 44-μm thick film made of polylactic acid (PLA) coated with high molecular weight PLA (EVLON EV-HS1, BI-AX International Inc., Wingham, ON, Canada). Codes used for the petroleum-based polyolefin and for the bio-based polyester from now on are PP/PE and PLA, respectively.

2.2. Methods

2.2.1. Packaging and storage

Both packaging films were formed into 20 cm length × 15 cm width bags using an impulse sealer (Ceratek, Sencorp Systems Inc., Hyannis, MA, USA). An amount of 10–12 celery sticks were placed in each bag (≈190 g per bag). Half of the PLA and PP/PE bags were sealed without atmosphere replacement for passive modified atmosphere packaging (MAP) (A-PLA and A-PP/PE). The air in the remaining PLA bags was replaced with 95 kPa O_2 + 5 kPa N_2 (O_2-PLA), 99 kPa N_2 + 1 kPa O_2 (N_2-PLA), or 6 kPa O_2 + 12 kPa CO_2 + 82 kPa N_2 (CO_2-PLA) using a Multivac flusher (Model A300/16, Sepp Haggenmüller KG, Wolfertschwenden, Germany) for the active MAP treatments. As a control, celery was packaged under the same conditions as in the passive MAP treatment, but the bottom of the bags was partially cut off, creating a non-modified atmosphere package (coded as open bags). All packages were stored at 7 °C until the day of testing.

2.2.2. Packaging material characterization

Oxygen, carbon dioxide, water vapor and ethanol transmission rates were assessed for PLA and PP/PE. Oxygen transmission rate (OTR) was determined at 23 °C and 0% RH according to the American Society for Testing Materials (ASTM) method D3985 (ASTM, 2005) using an OX-TRAN® Model 2/21 (MOCON, Minneapolis, MN, USA). Carbon dioxide transmission rate (CO_2TR) was measured using a PERMATRAN-C™ Model 4/41 (MOCON) under the same conditions. Water vapor transmission rate (WVTR) was determined at 23 °C and 100% RH according to ASTM method F1249 (ASTM, 2006) using a PERMATRAN-W™ Model 3/33 (MOCON). Permeation cells made from stainless steel were used to gravimetrically determine the ethanol transmission rate for PP/PE and PLA using a slightly modified ASTM method E96-80 (ASTM, 1980). All testing was conducted in triplicate.

In all cases, the permeability coefficients (kg m m^{-2} s^{-1} Pa^{-1}) were obtained as follows:

$$P = \frac{TR \times l}{\Delta p}$$

where TR is the transmission rate value (kg m^{-2} s^{-1}), l (m) is the film thickness, and Δp is the partial pressure differential across the film (Pa). The permselectivity coefficient (β; ratio of CO_2 to O_2 permeation) was also calculated.

2.2.3. Atmosphere composition

Progressive changes in CO_2, O_2, and N_2 were monitored in all packages using a gas chromatograph (Thermo Scientific Trace GC Ultra, Thermo Electron S.p.A., Rodano, Italy) equipped with a thermal conductivity detector and a Carboxen 1010 Plot capillary column, 30 m in length with a film thickness of 30 μm, and an internal diameter of 0.53 mm (Supelco, Bellefonte, PA, USA). Helium was used as a carrier gas (3 mL min^{-1}). Using a syringe (SGE, Austin, TX, USA), a 100-μL headspace sample was collected through a silicone septum attached to each package. Initial gas compositions were

determined to verify correct flushing by the Multivac machine. Subsequently, CO_2, O_2, and N_2 levels were monitored after 1, 3, 5, 7, 10, 14, and 21 d. New bags were used for each analysis day.

Ethanol concentration was determined after 3, 7, 14, and 21 d by injecting 1 mL headspace samples into a gas chromatograph equipped with a flame ionization detector (Hach Carle Series 100 AGC, Loveland, CO, USA) fitted with a 2 m long, 2 mm internal diameter stainless steel column packed with Chromosorb OV-103, 60/80 mesh (Altech Associates Inc., Deerfield, IL, USA). Ethanol content was expressed as microliters of ethanol per liter ($\mu L\,L^{-1}$).

2.2.4. Texture

A texture analyzer (TA-XT2i, Stable MicroSystem, Godalming, UK) equipped with a 50 kg-load cell was used to determine celery stick firmness and toughness. The assays were performed using a 3-point bending probe (constant span length of 4 cm acting perpendicularly to the celery), which was positioned horizontally with the outer convex surface upwards, centered on the supports, and the pressing force was applied vertically to the middle of the celery. Each stick was flexed at a constant speed of $2\,mm\,s^{-1}$ until failure. Firmness (N) was calculated as the measured force at 5 mm of the load-displacement curve. Toughness ($J\,m^{-3}$) was defined as the area below the load-time curve. For texture measurements, all sticks (≈10 per bag, ≈ 60 sticks per treatment) were analyzed.

2.2.5. Color

Celery color was determined after 0, 3, 7, 14, and 21 d of storage using a Minolta CR300 colorimeter (Osaka, Japan) calibrated with a standard white plate ($Y = 93.84$; $x = 0.3132$; $y = 0.3191$) and set with a C illuminant, 2° observer. Readings taken from both the outer convex surface and cut ends were expressed as L^*, a^* and b^* parameters. Hue angle ($h°$) was calculated using the a^* and b^* parameters ($h° = \tan^{-1}(b^*/a^*)$). For color measurements, all sticks (≈10 per bag, ≈60 sticks per treatment) were measured at two points on the surface, and at the two cut ends.

2.2.6. Weight loss

After 21 d of storage, weight loss was determined for all celery stick treatments (6 bags/treatment) by subtracting the final weight from the initial weight with the results expressed as % weight loss.

2.2.7. Preparation of inoculum, inoculation of samples, and microbiological analysis

Stock cultures of three avirulent strains of *L. monocytogenes* (J22F, M3, J29H) were maintained at −80 °C and subcultured twice (24 h/37 °C) in 200 mL of trypticase soy broth (Difco, Becton Dickinson, Sparks, MD, USA) containing 0.6% (w/v) yeast extract (Difco, Becton Dickinson) (TSB-YE). Cultures were combined in equal volumes and diluted 1:100 in tap water (~15 °C) to contain 7.19 ± 0.13 log CFU/mL. The bacterial population in the final cocktail culture was confirmed by appropriately diluting in sterile 0.1% phosphate buffer solution and plating on Modified Oxford Agar (Neogen Corporation, Lansing, MI, USA) (MOX) plates. These plates were then incubated at 37 °C for 48 h prior to enumeration. The celery sticks were then inoculated by immersion for 30 min to obtain 4.35 ± 0.33 log CFU/g. After centrifugal drying and 18–22 h of storage at 4 °C, the celery sticks were immersed in 60 L of tap water containing $50\,mg\,L^{-1}$ available chlorine (XY-12, Ecolab, USA) for 1 min, centrifugally dried and then packaged in both PLA and PP/PE bags in a glove chamber (Labconco 50004 Fiberglass Glove Box, Kansas City, MO, USA) using the gas compositions described in Section 2.2. The chamber was used instead of the Multivac machine due to the special safety precautions required for handling the *L. monocytogenes*. Additional PLA-packaged, passive modified atmosphere samples were prepared using unsanitized inoculated fresh-cut celery (immersed in 60 L of tap water).

Following 0, 1, 3, 5, 7, 10, 14, and 21 d of storage at 7 °C, two celery stick samples (~50 g) from each package were diluted 1:4 in Difco Neutralizing Buffer (Becton Dickinson, Sparks, MD, USA) and homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 1 min. Samples were either appropriately diluted in sterile 0.1% phosphate buffer solution and plated on MOX, or filtered using 0.45 μm membrane filters (Millipore, Millipore Corporation, Billerica, MA) and placed on 60 mm diameter petri plates containing MOX for quantification of *L. monocytogenes* after 48 h of incubation at 37 °C.

2.2.8. Statistical analysis

All of the experiments were conducted in triplicate and data was expressed as the means ± standard errors. Three replications consisting of two bags each (a total of 6 bags) were analyzed per treatment on each day of testing for quality analysis. For *Listeria* analysis, one bag for each of the three replicates was used (a total of 3 bags). Data were subjected to statistical analysis using a statistical software package IBM SPSS version 19 (IBM Corporation Software Group, Somers, NY, USA). One-way analysis of variance (ANOVA) and Duncan multiple range tests at a significance level $P < 0.05$ were used, except for the microbiological and texture analyses, where comparison of means was conducted with the least significant difference (LSD) test ($P < 0.05$).

3. Results and discussion

3.1. Packaging material characterization

O_2, CO_2, water vapor and ethanol permeability coefficients of PLA and PP/PE are shown in Table 1. The materials had different permeabilities to the gases and vapors tested. The O_2 and CO_2 permeabilities of PLA were 4 and 5 times lower than those of the PP/PE, respectively. Given that a low permeability is necessary to reduce gas exchange and maintain the flushed gas inside package, PLA could be a viable bio-based material to maintain desired gas compositions in active MAP. The CO_2 and O_2 permeability coefficients of PLA were very similar to those reported for oriented PLA sheets ($33 \pm 8 \times 10^{-18}\,kg\,m\,m^{-2}\,s^{-1}\,Pa^{-1}$ and $4 \pm 0 \times 10^{-18}\,kg\,m\,m^{-2}\,s^{-1}\,Pa^{-1}$, respectively) by Joo et al. (2012). The ethanol permeability of PLA was lower than that of PP/PE. Information on the permeability of plastics to ethanol is very limited (Robertson, 2013), and no data on the permeability of PLA to ethanol has been found in the literature. The ethanol permeability coefficient of LDPE has been reported as $3.24 \times 10^{-17}\,kg\,m\,m^{-2}\,s^{-1}\,Pa^{-1}$ (Robertson, 2013). This value is

Table 1
Carbon dioxide, oxygen, water and ethanol permeability coefficients for PLA and PP/PE films.

Material	Permeability coefficient				β
	CO_2[a]	O_2[a]	H_2O[b]	Ethanol[a]	(P_{CO_2}/P_{O_2})
PLA	30.34 ± 9.07	5.67 ± 1.17	21.86 ± 3.22	20.01 ± 5.76	5.35
PP/PE	113.4 ± 13.8	28.35 ± 4.61	1.08 ± 0.24	110.11 ± 2.01	4.00

[a] $\times 10^{-18}\,kg\,m\,m^{-2}\,s^{-1}\,Pa^{-1}$.
[b] $\times 10^{-15}\,kg\,m\,m^{-2}\,s^{-1}\,Pa^{-1}$.

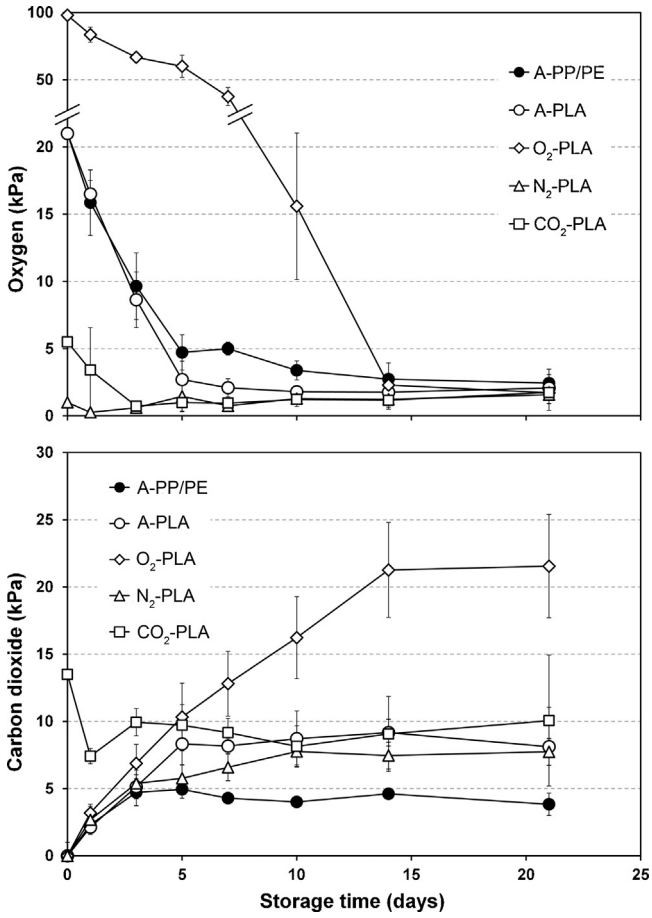

Fig. 1. Oxygen and carbon dioxide contents of celery stick packages for different materials and initial gas compositions stored at 7 °C. Error bars indicate standard deviations of the means.

lower than our value for PP/PE, which indicates differences in properties between different PE (i.e., additives, crystallinity, etc). The water vapor permeability coefficient of PP/PE was 20 times lower than that of PLA. Our PLA results are in agreement with those reported by Almenar and Auras (2010) under the same analysis conditions (11.98×10^{-15} kg m m^{-2} s^{-1} Pa^{-1}). The O_2, CO_2, and water permeability values of PP/PE were between those reported for LDPE and PP (Almenar and Auras, 2010).

3.2. Atmosphere composition

The steady state atmospheres achieved in the A-PLA, N_2-PLA, and CO_2-PLA bags ranged between 1–2 kPa O_2 and 8–10 kPa CO_2 (Fig. 1) with these values close to the atmospheres recommended by Gómez and Artés (2004) for shelf life extension of celery during refrigerated storage. The steady state atmospheres reached in the O_2-PLA bags was 2 kPa O_2 and 22 kPa CO_2 (Fig. 1). The steady state atmosphere was achieved faster in CO_2-PLA (3 d) than A-PLA and N_2-PLA bags (7 d). Levels of CO_2 and O_2 in the O_2-PLA bags stabilized after 14 d with the A-PP/PE bags reaching steady state after 10 d of storage (2.5 kPa O_2 and 4 kPa CO_2). Bags made from PLA equilibrated faster than those made from PP/PE due to the greater permselectivity of the PLA (5.35 and 4.00 for PLA and PP/PE, respectively) resulting from their different permeability to O_2 and CO_2 (Table 1). Therefore, PLA would be preferred over PP/PE for fresh-cut celery. Gas evolution in the A-PP/PE bags was very close to that obtained by Gómez and Artés (2005) but non-optimal when celery sticks were packaged in 25 μm LDPE bags and stored at 4 °C. These same authors found that the higher CO_2 (7 kPa) and lower O_2 (5 kPa) levels achieved in 35 μm OPP bags worked better to maintain fresh-cut celery quality. The relatively higher CO_2 concentration reached in the bags with high O_2 can be attributed to a more stable respiration rate of the celery since O_2 was not a limiting factor during the first 14 d of storage. However, an increased respiration rate due to tissue damage (Cantwell and Suslow, 2002; Martínez et al., 2005; Iqbal et al., 2008), microbial growth (Jacxsens et al., 2003; Artés-Hernandez et al., 2007; Silveira et al., 2008), or headspace volume reduction could be contributing to CO_2 accumulation for this treatment.

Throughout storage, the headspace for PP/PE bags yielded far lower ethanol levels compared to PLA bags age which is most likely due to the 5-fold greater ethanol permeability of the PP/PE film which favored the escape of ethanol from the package headspace (Table 1). Comparing the different in-package gas compositions, faster ethanol accumulation was observed in the CO_2-PLA and N_2-PLA bags due to their low level of O_2 (~1 kPa) (Table 2) which led to earlier hypoxia. Many vegetables generate ethanol through fermentative metabolism at O_2 levels <2 kPa. Since ethanol was not detected in the A-PLA and O_2-PLA bags until day 14, the packages remained sufficiently aerobic to minimize fermentation for at least a week ($P < 0.05$). The ethanol content in all PLA bags increased from week 2 to 3 with the greatest increase seen in O_2-PLA bags, likely due to the higher CO_2 content. High levels of CO_2 alone (>20 kPa CO_2) or in combination with low levels of O_2 (>20 kPa CO_2 and <2 kPa O_2) have been related to the accumulation of ethanol due to fermentation (Jacxsens et al., 2001). A sensory evaluation is needed to determine the impact of ethanol concentration on consumer acceptance of packaged celery sticks.

3.3. Weight loss

The weight of celery sticks in open bags decreased more than 30% after 21 d of storage at 7 °C (Table 2), showing the need for hermetically sealed containers during the postharvest period. Significantly greater ($P < 0.05$) weight loss was seen after

Table 2
Weight loss (%) and ethanol content (μL L^{-1}) for fresh-cut celery in packages differing in initial gas composition and packaging material during storage at 7 °C.

Packaging code	Storage time (days)				
	3	7	14	21	
	Ethanol	Ethanol	Ethanol	Ethanol	Weight loss
Open bags	–	–	–	–	30.99 ± 12.42
A-PP/PE	0.00 ± 0.00^{a*}	0.06 ± 0.11^{a}	0.32 ± 0.25^{a}	0.39 ± 0.28^{a}	0.73 ± 0.77
A-PLA	0.00 ± 0.00^{a}	0.73 ± 0.98^{ab}	12.92 ± 8.94^{ab}	16.34 ± 17.41^{ab}	4.50 ± 0.14
O_2-PLA	0.00 ± 0.00^{a}	0.13 ± 0.16^{a}	12.99 ± 6.80^{ab}	47.88 ± 22.89^{c}	4.67 ± 0.57
CO_2-PLA	8.15 ± 9.98^{b}	6.32 ± 5.42^{ab}	16.31 ± 11.81^{b}	31.97 ± 29.17^{bc}	4.53 ± 0.33
N_2-PLA	10.72 ± 6.25^{b}	7.66 ± 4.93^{b}	17.59 ± 13.55^{b}	19.99 ± 22.36^{abc}	4.87 ± 0.40

Different letters indicate significant differences ($P < 0.05$) between treatments for each time point.
* Each value represents the mean of three replicates ± standard deviation.

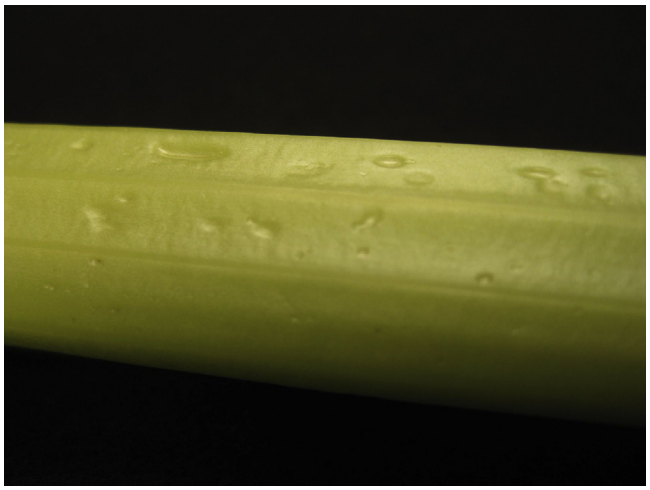

Fig. 2. Celery stick pitting from O_2-PLA bags after 21 d of storage at 7 °C.

21 d of storage using PLA (~4%) compared to PP/PE bags (<1%) (Table 2). The higher weight loss of the celery sticks in the PLA bags was due to the higher water vapor permeability coefficient of PLA ($21.86 \pm 3.22 \times 10^{-15}$ kg m m^{-2} s^{-1} Pa^{-1}) compared to PP/PE ($1.08 \pm 0.24 \times 10^{-15}$ kg m m^{-2} s^{-1} Pa^{-1}). The initial atmosphere did not affect celery weight loss since similar weights were obtained for all PLA-packaged celery at the end of the storage.

Celery packaged in PLA bags exhibited a >4% weight loss after 21 d, however, this was less than the maximum 10% water loss considered as a limit of acceptance for celery (Robinson et al., 1975). Therefore, celery sticks in PLA bags would likely remain acceptable for at least 21 d with PLA performing similarly to other plastics. Viña and Chaves (2003) reported a weight loss of 6.2% for minimally processed celery packaged in polyvinylchloride film-covered polystyrene trays after 28 d of storage at 10 °C. Assuming a linear relationship between weight loss and time, our results for celery sticks stored in PLA at 7 °C are very similar to those of Viña and Chaves (2003) after 21 d, although their water vapor transmission rate for polyvinyl chloride (4.63×10^{-7} kg m^{-2} s^{-1}) was 2.8 times lower than that of our PLA ($13.08 \pm 1.92 \times 10^{-7}$ kg m^{-2} s^{-1}).

3.4. Color

The surface color (hue angle) of celery sticks was affected by both the packaging material and gas composition (Table 3). At the end of storage, celery packaged in A-PLA was greener (higher $h°$) than that packaged in A-PP/PE ($P < 0.05$) with the former maintaining the green color of celery throughout storage. The difference was likely due to the higher CO_2 level in A-PLA bags (Fig. 1). In agreement, steady state CO_2 and O_2 concentrations of 5–7 kPa and 9–6 kPa, respectively, have been shown to preserve the external color of celery (Gómez and Artés, 2005). Among headspace gas compositions, the CO_2-PLA bags maintained the green color of celery better than the other treatments ($P < 0.05$) up to d 14, after which all samples were visibly decayed. In contrast, celery sticks packaged in O_2-PLA showed a more intense yellowing compared to the other treatments ($P < 0.05$). In addition, celery sticks packaged in the O_2-PLA bags developed numerous small pits on the surface that became increasingly evident over time (Fig. 2). The pitting development could be due to the high oxygen, but CO_2 accumulation could be also affecting, so further research needs to be carried out to clarify the origin of this disease. Surface lightness was affected by in-package gas composition, but not the packaging material with celery sticks packaged in CO_2-PLA bags exhibiting the lowest L^* value during the first week of storage ($P < 0.05$) (Table 3).

Table 3
Lightness (L^*) and hue ($h°$) values for surface and cut ends of the celery sticks packaged in different materials and initial gas compositions during storage at 7 °C.

Analysis location	Packaging code	Storage time (d)											
		0		3		7		14		21			
		L^*	$h°$	L^*	$h°$	L^*	$h°$	L^*	$h°$	L^*	$h°$		
Surface	Open bags	57.8 ± 4.9	118.2 ± 1.4	58.0 ± 6.9^{a*}	117.5 ± 1.9^{ab}	57.5 ± 4.7^{a}	118.1 ± 1.2^{b}	59.7 ± 5.3^{ns}	117.4 ± 2.3^{ab}	60.6 ± 5.4^{ns}	116.9 ± 2.4^{b}		
	A-PP/PE	57.8 ± 4.9	118.2 ± 1.4	60.6 ± 5.1^{b}	117.0 ± 2.6^{a}	59.4 ± 6.2^{ab}	117.8 ± 1.4^{b}	60.5 ± 5.6^{ns}	117.4 ± 3.2^{ab}	60.2 ± 4.8^{ns}	116.1 ± 3.2^{a}		
	A-PLA	57.8 ± 4.9	118.2 ± 1.4	59.0 ± 6.1^{ab}	117.8 ± 1.4^{bc}	59.3 ± 5.0^{ab}	118.0 ± 1.4^{b}	60.8 ± 5.9^{ns}	117.7 ± 2.2^{b}	60.5 ± 5.0^{ns}	117.3 ± 2.5^{b}		
	O_2-PLA	57.8 ± 4.9	118.2 ± 1.4	59.1 ± 5.3^{ab}	117.9 ± 1.5^{bc}	60.9 ± 5.7^{b}	117.3 ± 1.7^{a}	60.6 ± 5.7^{ns}	116.8 ± 2.1^{a}	60.6 ± 4.9^{ns}	115.7 ± 2.9^{a}		
	CO_2-PLA	57.8 ± 4.9	118.2 ± 1.4	57.8 ± 5.9^{a}	118.9 ± 1.9^{d}	58.8 ± 5.7^{a}	118.8 ± 1.4^{c}	59.9 ± 6.3^{ns}	118.4 ± 2.0^{c}	60.0 ± 6.3^{ns}	117.3 ± 2.1^{b}		
	N_2-PLA	57.8 ± 4.9	118.2 ± 1.4	58.7 ± 6.3^{ab}	118.3 ± 1.4^{c}	59.0 ± 5.9^{a}	118.3 ± 1.7^{b}	60.8 ± 5.7^{ns}	117.2 ± 2.9^{ab}	59.2 ± 5.3^{ns}	117.4 ± 1.9^{b}		
Cut ends	Open bags	56.3 ± 4.9	115.8 ± 1.5	60.4 ± 5.3^{b}	111.3 ± 4.0^{a}	63.8 ± 4.7^{c}	111.1 ± 4.4^{a}	63.7 ± 4.5^{c}	110.0 ± 4.1^{a}	64.88 ± 5.5^{d}	106.0 ± 4.7^{a}		
	A-PP/PE	56.3 ± 4.9	115.8 ± 1.5	59.1 ± 5.2^{ab}	113.0 ± 2.6^{b}	59.4 ± 5.6^{a}	111.9 ± 3.8^{ab}	59.7 ± 5.9^{a}	111.3 ± 3.7^{b}	60.3 ± 5.5^{a}	107.6 ± 5.6^{b}		
	A-PLA	56.3 ± 4.9	115.8 ± 1.5	59.1 ± 6.2^{ab}	113.7 ± 3.0^{b}	61.9 ± 5.9^{b}	113.4 ± 3.0^{c}	62.4 ± 5.8^{b}	112.9 ± 3.2^{c}	61.9 ± 6.2^{abc}	111.7 ± 3.2^{d}		
	O_2-PLA	56.3 ± 4.9	115.8 ± 1.5	60.6 ± 5.8^{b}	113.4 ± 2.6^{b}	61.8 ± 5.1^{b}	112.4 ± 3.1^{bc}	62.4 ± 5.9^{b}	110.5 ± 3.0^{ab}	61.3 ± 7.0^{ab}	110.0 ± 4.3^{c}		
	CO_2-PLA	56.3 ± 4.9	115.8 ± 1.5	60.6 ± 5.6^{b}	115.5 ± 2.7^{c}	61.5 ± 5.7^{b}	115.5 ± 2.5^{c}	61.7 ± 5.9^{ab}	114.3 ± 2.9^{d}	63.5 ± 5.5^{cd}	112.9 ± 3.4^{d}		
	N_2-PLA	56.3 ± 4.9	115.8 ± 1.5	57.8 ± 7.5^{a}	115.3 ± 2.5^{c}	61.6 ± 5.6^{b}	114.8 ± 2.8^{c}	61.9 ± 6.2^{ab}	113.6 ± 3.0^{cd}	62.5 ± 5.0^{bc}	112.4 ± 3.2^{d}		

Different letters indicate significant differences ($P < 0.05$) between treatments for each time point and analysis location.
* Each value represents the mean of three replicates ± standard deviation.

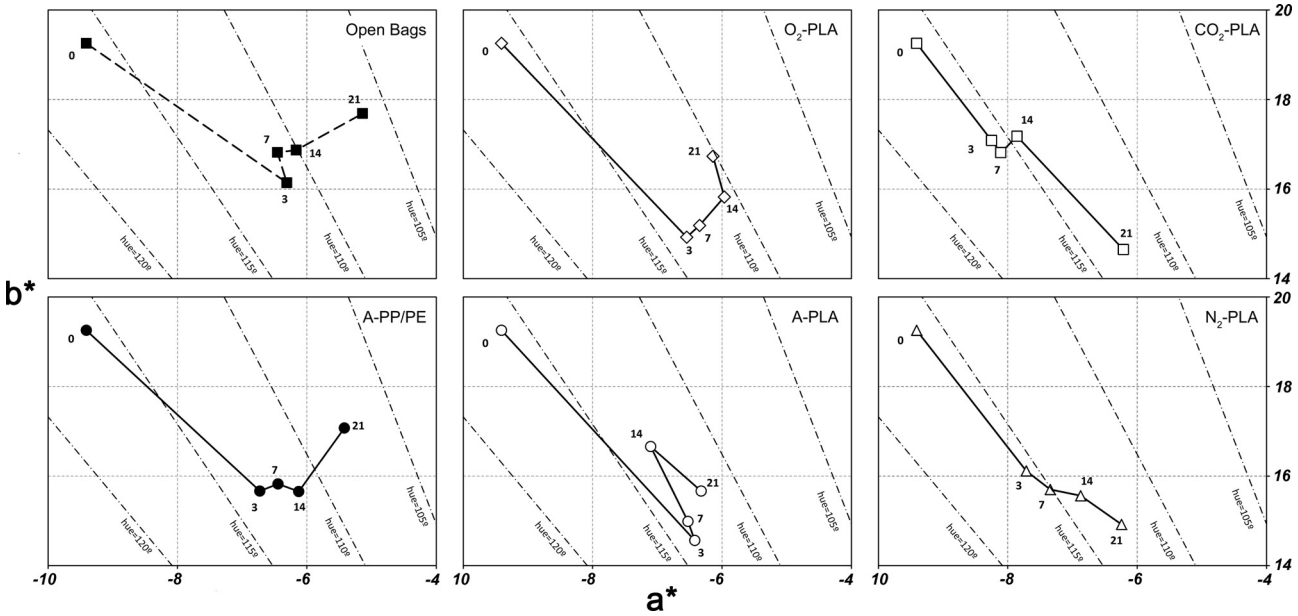

Fig. 3. Effect of initial gas composition and packaging material on the color (a^*, b^* and hue) evolution of the cut surfaces of celery sticks. Numbers (0, 3, 7, 14 and 21) indicate storage time in days at 7 °C. Transversal dotted lines indicate different guide hue angles.

The color of cut celery stick ends was also affected by packaging material and initial gas composition (Table 3). Comparing the different materials, the cut celery stick ends in the A-PLA bags maintained a higher hue value (greener color) than those in A-PP/PE bags throughout storage. This was probably caused by the higher CO_2 content in the A-PLA bags. The effectiveness of low O_2 and high CO_2 levels in preserving the green color of the cut ends of fresh-cut celery in OPP bags at 4 °C (6 kPa O_2 and 7 kPa CO_2 at steady state) was reported by Gómez and Artés (2005). However, initially high O_2 levels caused the degradation of the green color and promoted yellowing in the cut ends. In contrast, initially high O_2 levels (95 kPa) reduced discoloration of chicory endive (Jacxsens et al., 2001). CO_2-PLA and N_2-PLA maintained a higher hue during storage, frequently associated to the maintenance of a greener color. The L^* value for the cut ends of celery rapidly increased during the first 7 d of storage and then stabilized thereafter (Table 3). Celery sticks stored at 10 °C in passive modified atmospheres have reportedly exhibited similar behavior (Viña and Chaves, 2003) which is likely related to increased hydration of the cut ends. Discoloration caused by reversible surface dehydration is common in fresh-cut produce including carrots (Tatsumi et al., 1991; Cisneros-Zevallos et al., 1995) and peaches (Gorny et al., 1998; González-Buesa et al., 2011). Although discoloration of cut ends due to lignin accumulation has been reported in many fresh-cut products, this does not seem to be the reason of the discoloration of the celery cut ends according to Viña and Chaves (2003). Polymerized phenol could be the cause of discoloration. Headspace gas composition had no effect on the L^* value for celery cut ends, but packaging film did have an effect (Table 3). PP/PE bags better maintained lightness of the celery cut ends after 7 and 14 d of storage. Cut end lightness was associated with a lower level of dehydration compared to the PLA bags, with this higher L^* due to the greater water vapor permeability of PLA (Table 1).

Fig. 3 shows the change in the a^* and b^* color coordinates and of the hue angle in the cut ends of celery sticks during storage, providing additional information about the change in color. The color evolution of the cut ends of the celery sticks in the A-PP/PE bags was close to that of the cut ends of the celery sticks in the open bags, indicating that the CO_2 accumulated inside the A-PP/PE was not enough to avoid color degradation. Comparing the different initial gas compositions, the ends of the celery sticks in CO_2-PLA bags showed more stable a^* and b^* coordinates, especially during the first 14 d of storage, indicating a more well maintained color to the initial one. However, O_2-PLA and A-PLA showed a large change in the a^* and b^* values during the first 7 d, indicating major changes in the color of the celery cut ends. The N_2-PLA bags after 3 and 7 d maintained intermediate values, indicating a less severe color evolution.

These results show that a high initial concentration of CO_2 in the headspace can help maintain the initial green color of the surface and cut ends of celery sticks while a high O_2 concentration promotes yellowing and other quality defects. While PLA can better maintain the green color of both cut and uncut celery, dehydration can become an issue.

3.5. Texture

Fig. 4a shows the evolution of firmness or loss of turgor of unpackaged and packaged celery sticks during 21 d at 7 °C, measured as the force at a displacement of 5 mm in the 3 bending point rig. Using open bags, celery stick firmness decreased from 56.2 ± 10.6 N to 18.4 ± 10.9 N during 21 d at 7 °C. This change in texture is likely due to water loss and pectin degradation as suggested by Viña and Chaves (2003). In contrast, both PLA and PP/PE bags maintained celery firmness during 14 d of storage. At day 21, PLA-packaged celery softened due to the relatively high water vapor permeability of PLA while celery sticks packaged in PP/PE maintained their turgor ($P < 0.05$) (Table 1). The different initial gas compositions did not affect the turgor of the celery sticks throughout the storage. This was due to the gas compositions having no effect on the weight loss of the celery sticks. Unlike firmness, the celery toughness was affected by the initial gas composition, but not the packaging material (Fig. 4b). Celery sticks maintained a slightly tougher texture in high O_2 compared to other gas mixtures during storage ($P < 0.05$). This was most likely due to maintenance of the celery respiration rate at high O_2 levels, which has the potential to increase lignin production and hardening of the celery fibers. Viña and Chaves (2003) reported lignification of fibers and/or xylem vessels in fresh cut celery during storage in passive modified atmospheres (>10 kPa O_2 and <4 kPa CO_2) with

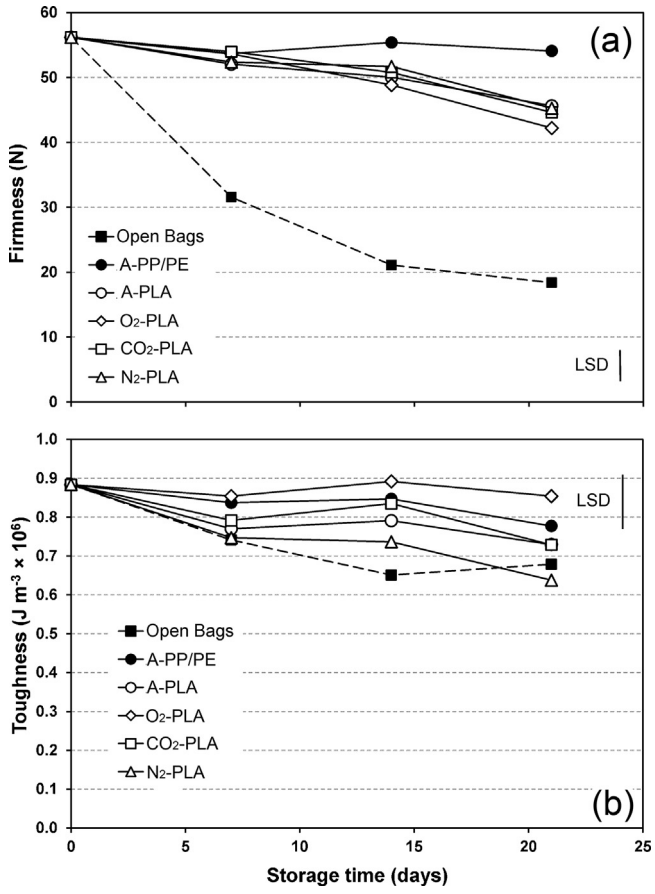

Fig. 4. Firmness (a) and toughness (b) of celery sticks packaged in different materials and initial gas compositions during storage at 7 °C.

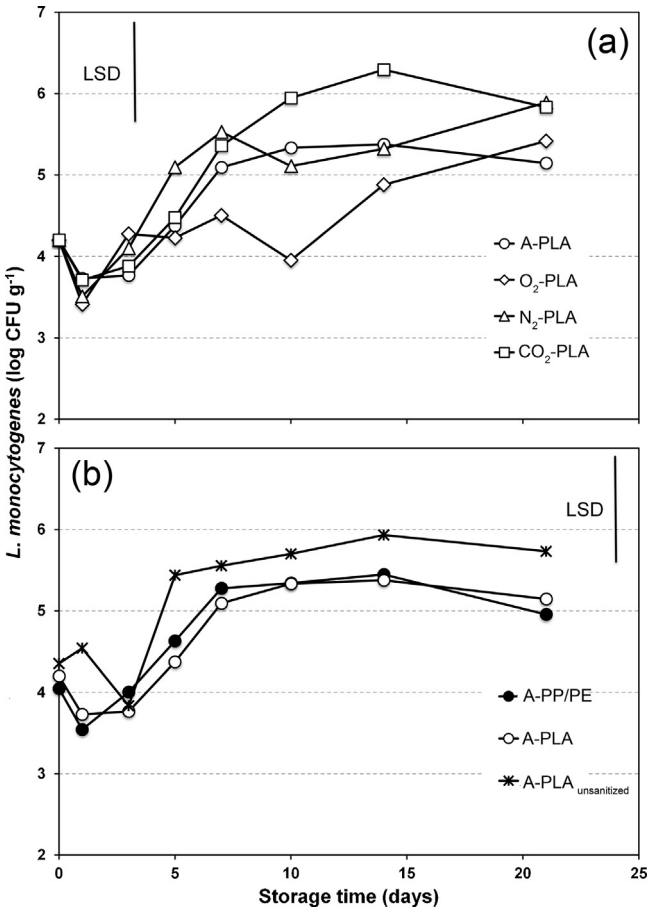

Fig. 5. *Listeria monocytogenes* populations on sanitized and unsanitized celery sticks packaged in different materials and initial gas compositions during storage at 7 °C ((a) compares initial headspace gas compositions and (b) compares materials as well as sanitation).

similar results obtained for asparagus during exposure to 21 kPa O_2 (Everson et al., 1992). Another possible explanation is damage of tissues by the high O_2 exposure during storage.

3.6. *L. monocytogenes*

Growth of *L. monocytogenes* on celery during 21 d of storage at 7 °C was impacted by initial headspace gas composition (Fig. 5a). While O_2-PLA bags showed no increase in *Listeria* populations during the first 10 d of storage ($P < 0.05$), CO_2-PLA, N_2-PLA, and A-PLA supported growth. Amanatidou et al. (1999) reported only a slight reduction in *Listeria* growth on agar plates that were exposed to 90 kPa O_2 at 8 °C. In other work, *L. monocytogenes* growth in fresh, processed, mixed salads (endive, curly endive, radicchio, lollo rosso and lollo bionda) was not affected by an initial oxygen level of 95 kPa during storage at 4 °C (Allende et al., 2002). Suppression of *Listeria* growth is most likely only visible above 4 °C. In fact, *Listeria* populations in fresh-cut celery stored at multiple temperatures in highly permeable bags and containers (<2 kPa CO_2 and ≈ 18 kPa O_2 at steady state) decreased at 4 °C due to the low temperature but not the gas composition (Vandamm et al., 2013).

After 10 and 14 d of storage at 7 °C, *Listeria* populations in the CO_2-PLA bags were slightly higher than those in the N_2-PLA and A-PLA bags, and significantly higher ($P < 0.05$) than those in the O_2-PLA bags (Fig. 5). Enhanced growth of *Listeria* at higher CO_2 levels has been previously reported for other types of produce including cut chicory endive (Bennik et al., 1996), shredded cabbage (Kallander et al., 1991), and shredded lettuce (Francis and O'Beirne, 2001). In contrast, an initial in-package gas composition of 5 kPa O_2 + 5 kPa CO_2 suppressed *Listeria* growth in shredded iceberg lettuce (Carrasco et al., 2008). These observed differences in *Listeria* behavior in packaged fresh-cut produce stored at low temperatures can be partially explained by the type of produce, modified atmosphere, inherent microflora and *Listeria* strain variations (Caleb et al., 2013).

At day 21, no differences ($P > 0.05$) were found between the *Listeria* populations on celery for the different packaging methods, likely due to similarly low O_2 levels in all packages. The O_2-PLA bags were the only packages able to maintain the initial *Listeria* populations during the first 10 d of storage, but no longer, due to the shift to anaerobic conditions. This can be attributed to the lack of competing aerobic microflora that occurred when O_2 was depleted. According to Farber et al. (1998), decreased growth of *L. monocytogenes* on cabbage packaged using a highly permeable film could be partially explained by the competing microflora such as lactic acid bacteria.

No differences ($P > 0.05$) were found between the *Listeria* populations on celery sticks packaged in different materials and between sanitized and unsanitized celery throughout storage (Fig. 5b), even though the CO_2 level was higher and the O_2 level lower for the PLA bags.

4. Conclusions

This study shows that the initial headspace gas composition and packaging material can significantly affect the quality and safety of celery sticks during the marketable period. Active MAP (CO_2-PLA) out-performed passive MAP (A-PLA) in maintaining

celery stick quality but not safety. Conventional active MAP (CO_2-PLA) out-performed non-conventional active MAP (O_2-PLA and N_2-PLA) in maintaining the quality of the celery sticks. Non-conventional active MAP with 95 kPa O_2 suppressed the growth of *L. monocytogenes*, while a high concentration of CO_2 promoted growth during the first 10 d of storage. When comparing PLA and PP/PE as a packaging material, both impacted the quality of celery to varying degrees, but not *Listeria* growth. PLA allowed quicker development of gas compositions that better maintained the green color of the celery surface and cut ends, but increased cut end dehydration and weight loss. However, this weight loss was well within tolerable limits with celery turgor also unaffected during two weeks of storage. PLA retained ethanol that was released into the package headspace while PP/PE favored its escape from the package. A sensory evaluation would be needed to determine the impact of ethanol concentration on the consumer acceptance of the packaged celery sticks. The combination PLA and an initial gas composition of 95 kPa O_2 would be a viable packaging option when storing celery for up to one week due to the few quality changes and maintained *Listeria* populations. However, during longer storage, celery exposed to this initial gas composition showed more visual and textural defects along with high levels of ethanol.

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