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High oxygen atmospheres can induce russet spotting development in minimally processed iceberg lettuce

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ABSTRACT

High oxygen modified atmospheres have been suggested as an alternative preservation technique to classical low O_2 modified atmosphere packaging (MAP) for fresh-cut lettuce. The advantages of high O_2 storage would be a strong reduction of browning, avoidance of low O_2 levels linked to off-odors, and the inhibition of microbial growth. However, storage under high O_2 potentially could increase both production of ethylene and sensitivity to this hormone in lettuce tissue, leading to the development of quality problems linked to ethylene. In this study, different quality parameters (sensory quality, microbiological load, electrolyte leakage, volatile metabolites) were studied on fresh-cut iceberg lettuce stored under different gas conditions at 7 °C: (low O_2 MAP (3% O_2 compensated with N_2), atmospheric conditions, high O_2 MAP (50 or 90% O_2 compensated with N_2)). Furthermore, additional experiments using ethylene absorbers were performed in order to assess the link between high O_2 storage, ethylene accumulation, and russet spotting. There was no significant difference between storage conditions regarding growth of mesophilic bacteria and yeasts, electrolyte leakage, or ethanol production. On the other hand, high O_2 atmospheres reduced browning but promoted russet spotting development compared with low O_2 and atmospheric conditions. In further specific experiments the relationship between high O_2 storage, ethylene production, and russet spotting in fresh-cut iceberg lettuce were made evident.

1. Introduction

Equilibrium modified atmosphere packaging (EMAP) is used to extend the shelf-life of fresh-cut produce (Cameron, 2003). This method usually decreases O_2 and increases CO_2 concentrations within the package, to slow down the metabolic rate and reduce discoloration processes, prolonging the shelf-life of products (Bennik et al., 1996). In real practice, for the preservation of fresh-cut iceberg lettuce, low O_2 atmospheres (<1%) combined with 7–10% CO_2, and low temperature (Cantwell and Suslow, 2001) are used. However, under the dynamic temperature conditions of the supply chain, it is a challenge to maintain O_2 and CO_2 concentrations inside packages within the desired range. For instance, O_2 levels in the packages can get close to 0%, triggering fermentative volatiles production and off-odor development (Smyth et al., 1998).

Day (1996) suggested that high O_2 MAP (HO_2) can be used as an alternative to low O_2 MAP (LO_2), in order to prolong the shelf-life of ready-to-eat vegetables. The beneficial effect of HO_2 would be based in preventing anaerobic fermentation reactions, inhibiting enzymatic discoloration, and reducing microbial growth on fresh-cut vegetables (Day, 2001). Different studies have reported positive effects of HO_2 storage on the quality of minimally processed vegetables (Jacxsens et al., 2001; Allende et al., 2004; Escalona et al., 2007). The main drawback for the use of HO_2 at industrial scale is the fact that O_2 concentrations higher than 25% are explosive and special precautions have to be taken on the work floor (BCGA, 1998). Furthermore, Kader and Ben-Yehoshua (2000) reported that HO_2 storage increases ethylene production, and intensifies some of the effects of this plant hormone on lettuce heads, including physiological disorders such as russet spotting.

Russet spotting (RS) is a physiological disorder of lettuce characterized by brownish depressions in the surface of midrib tissue that

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affect visual quality of the product (Cantwell and Suslow, 2001). Although this disorder makes lettuce unmarketable (Cantwell and Suslow, 2001), Lopez-Galvez et al. (1996) determined that RS contributed less than browning to the loss of visual quality of fresh-cut lettuce salads. RS is linked with exposure to ethylene in storage atmosphere, even in very low levels (<1 μL/L). It has been suggested that HO_2 storage could promote the production of ethylene due to the role of O_2 as substrate of the enzyme 1-aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase) that converts ACC in ethylene (Oms-Oliu et al., 2008). Furthermore, in a study on the softening of bananas, Jiang and Joyce (2003) hypothesized that high O_2 could promote synthesis of new ethylene binding sites. Development of RS in lettuce stored under high oxygen atmospheres has been reported before in lettuce heads (Klaustermeyer and Morris, 1975), but not in minimally processed lettuce.

In this work, changes in different parameters of fresh-cut iceberg stored under low O_2 MAP, atmospheric conditions, and HO_2 MAP were compared. Furthermore, the effect of HO_2 MAP on ethylene accumulation and russet spotting development was assessed.

2. Materials and methods

2.1. Minimal processing

Iceberg lettuce (*Lactuca sativa* L.) heads were obtained from a fresh-cut produce processing company (ALLGRO bvba, Sint-Lievens-Houtem, Belgium). After reception in the laboratory, lettuce heads were kept refrigerated in the cold room at 4 °C before processing. After eliminating the soiled and decayed external leaves, lettuce heads were cut in half to determine their maturity stage based in a 5-point compactness scale (Gil et al., 2010). Heads with a compactness level of 2–4 were used for the experiments. The central stem was then discarded and the lettuce was cut into pieces of approximately 3 cm × 3 cm by means of stainless steel knives in aseptic conditions and kept in plastic bags at 7 °C. For washing, 1 kg of untreated fresh-cut lettuce was immersed into 10 L of tap water in a plastic bucket for 1 min and gently agitated by hand. The product was then rinsed in another bucket with clean water and finally was de-watered by centrifuging for 1 min using a manually operated centrifuge. All the processing steps were performed at 7 °C.

2.2. Packaging and storage

After washing and centrifuging, 50 g of lettuce were immediately sealed in bags of suitable packaging film (14.7 cm × 14.7 cm). Bags were stored in all cases in the dark and at 7 °C, a temperature that represents the storage temperature of these products at the retail level (Jacxsens et al., 2002). The following four storage conditions were used.

Low O_2 MAP (LO_2): This MAP consisted of lettuce packaged initially at approximately 3% of O_2 balanced by N_2, using OPP film with a permeability of 700 mL O_2/m^2 d atm measured at 7 °C and 90% RH (Amcor flexibles, Ledbury, UK). The initial gas concentration was obtained using a gas mixer (WITT KM 100-4 MEM, Witt-Gasetechnik, Witten, Germany) and flushing the adequate gas mixture in the packages.

Air packaging (AIR): This condition was obtained by packaging lettuce in perforated bags made of high barrier film (HB; permeability 2 mL O_2/m^2 d atm measured at 7 °C and 90% RH) (Euralpack, Wommelgen, Belgium).

HO_2 50% ($HO_2$50): consisted of lettuce packaged in HB film bags with an initial O_2 concentration of 50%, balanced by N_2. The atmosphere was introduced within the bags directly from a cylinder with pure O_2 by using a flow meter (Multivac Sepp Haggenmüller, Model C 300, Wolferschwenden, Germany).

HO_2 90% ($HO_2$90): consisted of lettuce packaged in HB film bags with an initial O_2 concentration of 90%, balanced by N_2. These bags were filled as described for $HO_2$50.

High barrier film was used for HO_2 treatments in order to reduce the decline in oxygen concentration inside the packages provoked by the permeability of the film and the respiration of the product.

2.3. Quality analysis

Samples were taken after 0, 2, 5 and 6 days of storage. On each day of analysis three bags from each treatment were taken from the cold room and treated as replicates.

2.3.1. Headspace gas analysis

O_2 and carbon dioxide concentrations were measured in 3 bags for each treatment and each sampling time. Measurements were performed by means of a gas analyzer (Checkmate 9900, PBI Dansensor, Ringsted, Denmark). The gas analyzer was equipped with a solid-state zirconia ion-selective electrode for O_2 determination. To measure CO_2, the gas analyzer used an IR sensor with IR source and dual wavelength filter.

2.3.2. Electrolyte leakage

The electrolyte leakage of fresh-cut iceberg lettuce was measured immediately after treatment and during storage to determine possible tissue deterioration. Tissue electrolyte leakage was determined by measuring changes in electrical conductivity (EC) of demineralized water after immersing lettuce samples (10 ± 0.1 g in 100 mL) for 30 min (t0.5), using a conductivity meter (CONSORT K511, Turnhout, Belgium). Total tissue electrical conductivity (TEC) was determined by measuring electrical conductivity after lettuce samples were kept frozen at −20 °C for 24 h and afterwards thawed (Hong et al., 2000). Relative electrolyte leakage (REC) was expressed as percentage of total electrolytes released after 30 min (Eq. (1)).

$$\text{REC}\ (\%) = ((\text{conductivity}\ \ \text{t0.5h} - \text{conductivity distilled water}) / \text{conductivity}\ \ \text{t24}) \times 100 \quad (1)$$

2.3.3. Microbial analysis

Regarding microbial ecology, the levels of aerobic mesophilic microorganisms and yeasts were determined as described in Lopez-Galvez et al. (2013).

2.3.4. Sensory quality

A panel of 3 trained members performed the sensory evaluation of the treatments on anonymous samples at the different sampling dates. As in Allende et al. (2008), overall visual quality was indicated in a hedonic scale from 1 to 9, 1 being the lowest quality value, 9 the highest quality value, and 5 the limit of acceptance. In these evaluations, russet spotting was studied separately, that is, it was not taken into account when giving a visual quality score. This was done in order to determine the potential improvement of the visual quality due to the use of HO_2 treatments in absence of RS. Quality deterioration parameters (browning, dehydration, off-odors) were evaluated in a 1–5 scale 1 being the better quality score (no defects), 5 the lowest quality value, and 3 the limit of acceptance. Finally, it was indicated if the sample was accepted or rejected by the panelist. In this first set of experiments it was only the presence or absence of RS was evaluated and the degree of development of the