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Modified atmosphere generated during storage under light conditions is the main factor responsible for the quality changes of baby spinach

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ABSTRACT

Minimally processed products are generally exposed to low temperature and uncontrolled light conditions during the supply chain. The positive effect of low temperature on quality of baby spinach is widely reported, but there is little information available about the light effect. The objective of this study was to obtain insight about the cause-effect of light exposure of baby spinach on leaf quality and senescence parameters. Minimally processed baby spinach was stored in passive Modified Atmosphere Packaging (MAP) and in Controlled Atmosphere (CA) under different light conditions. In passive MAP, three very different headspace gas compositions within the bags due to photosynthesis and respiration reactions were generated that strongly affected the quality characteristics. To isolate the light effect from the atmosphere composition influence, and thus understand the mechanisms causing the quality changes, baby spinach was stored under two CA of 0.5 kPa O_2 + 10 kPa CO_2 (low O_2 + high CO_2 levels) and air combined with 2 light conditions (continuous light and darkness). The changes observed under the different light conditions were mainly caused by the differences in gas composition. Under light, MAP with high O_2 partial pressure (pO_2) and low CO_2 partial pressure (pCO_2) was detrimental because of the growth of *Pseudomonas* spp. and the progress in tissue senescence due to oxidative stress, increasing cell damage, lipid peroxidation and chlorophyll degradation. Under darkness, MAP with low pO_2 and high pCO_2 was also detrimental because of the intense off-odour developments, the increase in pH and electrolyte leakage and the reduction in chlorophyll fluorescence. Our results showed that the modified atmosphere generated with exposure to the different light conditions affects the quality of baby spinach mainly because of the high pO_2 under light and high pCO_2 under darkness.

1. Introduction

Baby spinach constitutes an important source of fibres and bioactive compounds such as vitamin C, vitamin A and polyphenols in the diet (Gil et al., 1999; Pandrangi and LaBorde, 2004; Xie et al., 2013). As a minimally processed product, baby spinach is characterized by a limited shelf life due to product quality deterioration and off-odours development (Wang, 2003; Tudela et al., 2013). Shelf life of minimally processed baby spinach packaged in perforated bags is about 8 days at 8 °C (Kou et al., 2014) and under 7–10 kPa O_2 and 5–10 kPa CO_2 moderate benefit is offered to spinach by delaying yellowing (Suslow and Cantwell, 1998). To ensure the optimal product quality it is vital to maintain the recommended storage temperatures (Evans et al., 2007; Kou et al., 2015).

Minimally processed products, including baby spinach, are usually displayed under uncontrolled light condition through the supply chain (Glowacz et al., 2014). The influence of light on quality and shelf life of minimally processed vegetables is controversial since both positive and negative effects have been observed depending on the product (Lester et al., 2010; Costa et al., 2013; Gergoff Grozeff et al., 2013; Braidot et al., 2014; Glowacz et al., 2014). In general, light conditions in minimally processed vegetables affect some quality parameters during storage, promoting a decrease of leaf water content (Lester et al., 2010; Zhan et al., 2013; Glowacz et al., 2014; Xiao et al., 2014), and visual quality (Martínez-Sánchez et al., 2011; Xiao et al., 2014), preserving the cholorophyll content (Costa et al., 2013; Gergoff Grozeff et al., 2013; Zhan et al., 2013), promoting microbial internalization (Kroupitski et al., 2009) and increasing the content of bioactive compounds (Noichinda et al., 2007; Lester et al., 2010; Zhan et al.,

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2012; Gergoff Grozeff et al., 2013; Braidot et al., 2014). Moreover, exposure of the bags to different light conditions affects the headspace gas composition because under light leaf tissues continue the photosynthetic activity that depletes CO_2 and releases O_2 (Olarte et al., 2009; Martínez-Sánchez et al., 2011; Glowacz et al., 2014).

The effects of modified atmosphere packaging (MAP) on the quality maintenance of baby spinach have been extensively reviewed (Babic and Watada, 1996; Ko et al., 1996; McGill et al., 1996; Tudela et al., 2013). The major problem associated with minimally processed baby spinach is the strong off-odour development when stored under low O_2 and high CO_2 partial pressures (Tudela et al., 2013). No information is available on whether the quality changes observed when the product is stored under light are a direct consequence of the light exposure or the indirect consequence of the headspace gas composition generated in the bags. Hence, the objective of this study was to evaluate the effect of different light conditions and atmosphere composition on the quality of minimally processed baby spinach during storage. The quality parameters evaluated were related to leaf quality (sensory evaluation, water content, leaf colour and microbiological quality) and leaf senescence (cell morphology, lipid peroxidation, electrolyte leakage, chlorophyll fluorescence, chlorophyll content, and pH changes).

2. Materials and methods

2.1. Plant material and growing conditions

Baby spinach (*Spinacia oleracea* L.) was cultivated under commercial conditions in fields near Pulpí (Almería, Spain) by Primaflor S.L. Two different spinach varieties were evaluated because of the short period of time that each variety covers in the winter season. Sowing was performed directly on elevated beds using a plant density of 700 plants m^{-2}. Plant material (20 kg) was mechanically harvested on January 16th 2014 (trial 1) and March 7th 2014 (trial 2). The growing cycle was 57 days and 52 days for trial 1 and trial 2, respectively.

2.2. Processing, packaging and storage conditions

After harvest, baby spinach leaves were transported (150 km) under refrigerated conditions in polystyrene boxes to the CEBAS-CSIC laboratory (Murcia, Spain). Then, baby leaves were kept for 24 h at 4 °C and 70% relative humidity (RH) in darkness. After that, baby leaves were processed at 4 °C as described by Garrido et al. (2015). Samples were washed in a cold (4 °C) chlorine solution (NaOCl 100 mg L^{-1}) adjusted to pH 6.5 with citric acid, drained and then rinsed with tap water. The excess of water was removed by spinning in an automatic salad spinner (K-50, Kronen GmbH, Kehl am Rhein, Germany)

The study of the light exposure was carried out in two different trials: Modified Atmosphere Packaging (MAP) (trial 1) and Controlled Atmospheres (CA) (trial 2). In the first trial, the objective was to evaluate the effect of different light conditions on the visual quality of baby spinach during storage in MAP. Samples of 100 g were mechanically packed in a vertical packaging machine (Etna 280-X model, Ulma, Oñati, Spain) using a 35 μm polypropylene (PP) film (Amcor Flexibles, Bristol, UK) with O_2 permeance of 2.629 E^{-12} mol m^{-2} s^{-1} Pa^{-1}, CO_2 permeance of 9.84 E^{-12} mol m^{-2} s^{-1} Pa^{-1} and H_2O permeance of 5.408 E^{-6} mol m^{-2} d^{-1} at 7 °C and 97% RH. Package size was 230 mm × 310 mm. A passive modified atmosphere was created by the respiration rate of the product and the film permeability characteristics as bags were sealed under ambient air conditions. Bags were randomly separated into three groups and stored in shelves in a cold room for 12 days at 7 °C under 3 light conditions: continuous light, photoperiod 12 h and continuous darkness. Shelves with the bags subjected to darkness condition were covered with black polyethylene plastic film, avoiding light exposure. In the second trial, the objective was to investigate if the responsible factor for the quality changes was the light or the modified atmosphere generated. For this, samples of 300 g were stored in 5 L glass jars connected to a flow-through board system providing different CAs at a constant flow rate. A total of 32 jars were randomly separated into two batches of 16 jars connected to air (21 kPa O_2 + 0.04 kPa CO_2) and 16 jars to CA (0.5 kPa O_2 + 10 kPa CO_2). These atmospheres were humidified by bubbling through a previous connected flask containing 100 mL of water with a constant flow rate of 40 and 10 mL/min for air and CA, respectively. Under each atmosphere composition, half of the jars were stored in continuous light and half of them in darkness. Jars subjected to darkness were covered with black polyethylene plastic film to avoid light exposure. The jars were placed in a cold room and stored for 10 days at 7 °C. In both trials, samples under light conditions were exposed to conditions similar to those of retail sale with a total radiation of 5 ± 1 μmol m^{-2} s^{-1} obtained from fluorescent lights (Philips Ibérica S.A., Madrid, Spain) of 58 W each. The storage conditions were named as: Light + Air, Light + CA, Darkness + Air and Darkness + CA.

2.3. Headspace gas composition and respiration rate

Headspace gas composition within the bags and the atmosphere composition within the jars were monitored daily with an O_2 analyser with a ceramic oxide-zirconia electrochemical detection cell (CG-1000, Ametek, Thermox Instruments Co., Pittsburgh, PA, USA) and an infrared CO_2 detector (Via 510, Horiba Instruments Co., Irvine, CA, USA). Four replicates were used for each storage condition and sampling date. Partial pressures of O_2 (pO_2) and CO_2 (pCO_2) were expressed in kPa. Respiration rate was measured in the same bags used in trial 1 by means of the permeable system as described by Luna et al. (2013) with some modifications. Changes in O_2 in the bag headspace from the initial 20.9% were monitored as a function of time and fitted to a curve to determine a respiration rate function as proposed by Lee et al. (1996). Respiration rate was calculated when the headspace O_2 concentration within the bags stored at 7 °C reached a level of 17 kPa.

2.4. Leaf quality parameters: sensory evaluation, water content, leaf colour and microbiological quality

Sensory quality was evaluated by a five member trained panel (Tudela et al., 2013). About 250 g of baby spinach from 4 individual replicates were mixed together in the same tray for each storage condition and sampling date. Sensory quality was evaluated at 0, 10, and 12 days in MAP and at 0, 7, and 10 days in CA. Visual quality was evaluated using a 1–9 hedonic scale where 9 = excellent, 5 = limit of marketability and 1 = inedible. Off-odour development was evaluated in a 5 point scale where 5 = severe, 3 = moderate and 1 = none.

Leaf water content (WC) was analysed as described by Viacava et al. (2010) with some modifications. Fifteen samples from 3 individual replicates (5 samples per replicate) were measured per storage condition and sampling date. For WC determination, leaves were weighed to obtain fresh mass (FM). Samples were dried for 48 h in a forced air oven at 65 °C until constant weight to determine the dried mass (DM). Values of FM and DM were used to calculate WC according to the equation (Viacava et al., 2010):

$$WC(\%) = \frac{FM - DM}{FM} \times 100$$