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Short postharvest storage under low relative humidity improves quality and shelf life of minimally processed baby spinach (*Spinacia oleracea* L.)

María S. Medina, Juan A. Tudela, Alicia Marín, Ana Allende, María I. Gil *

Research Group on Quality, Safety and Bioactivity of Plant Foods, Food Science and Technology Department, CEBAS-CSIC, P.O. Box 164, E-30100 Espinardo, Murcia, Spain

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ABSTRACT

The maintenance of relative humidity (RH) after harvest is not always possible and can influence the quality of the raw material for minimal processing. The objective of this study was to evaluate if short-term postharvest exposure to different RH conditions such as high (99%), medium (85%) and low (72%) influenced the quality and shelf life of minimally processed baby spinach. Weight loss, water content (WC), osmotic potential, electrolyte leakage, headspace gas composition, sensory evaluation, colour, texture and microbiological populations were evaluated before and after processing, as well as during shelf life. Baby spinach exposed to low RH conditions on the one hand significantly showed lower water content and higher osmotic potential and stiffness after exposure for 36 h at 15 °C when compared to high RH conditions. After processing, samples exposed to low RH were rehydrated and no differences in dehydration were observed among samples exposed to different RH conditions. However, the percentage of damaged leaves increased significantly from 7.5% to 12.5% due to the process, this percentage particularly increasing with increasing RH. On the other hand, processed baby spinach exposed to high RH had a higher respiration rate, higher percentage of leaf damage, and increased electrolyte leakage, causing a decrease in quality resulting in a shelf life 4 d shorter than baby spinach exposed to low RH. The observed changes were mainly linked to a significant postharvest breakage, which influenced the susceptibility to microbial colonization. Psychrophilic bacteria and *Pseudomonas* counts of samples exposed to high RH were 1 log higher than those exposed to low and medium RH. To minimize the impact of leaf damage, baby spinach should be processed at medium–low hydration levels. This study shows that controlled RH after harvest is critical as it can influence the microbiological population and the maintenance of acceptable visual quality.

1. Introduction

Water loss is a major cause of postharvest deterioration because it results not only in direct quantitative losses (loss of saleable weight), but also in losses in appearance (wilting and shrivelling) and textural quality (softening, flaccidity and loss of crispness) (Kader, 1992). It has been demonstrated that at a given temperature and rate of air movement, the rate of water loss from the commodity depends on the relative humidity (RH) (Kays, 1991). While temperature management is an accepted practice and the simplest and easiest way of delaying vegetable deterioration, maintenance of the recommended RH after harvest is infrequent (Paull, 1999).

Leafy vegetables are generally characterized as very perishable, with high respiration and water loss rates (Wang, 2003). If leafy vegetables are stored below optimum humidity levels (95–98% RH), transpiration increases and moisture is lost (Hardenburg et al., 1986). Shrivelling due to water loss can cause rapid quality deterioration of fresh young specialty greens (Cantwell et al., 1998; Wang, 2003). Agüero et al. (2011) reported a reduction in quality parameters of lettuce heads stored at low RH (70–72%) and highlighted the importance of protecting fresh vegetables from dehydration after harvest. The significance of moisture loss in determining the shelf life and quality of harvested plant organs is hardly surprising, since the organ severed from the parent plant cannot replenish water lost by transpiration (Ben-Yehoshua and Rodov, 2003). Not only loss of moisture but also excess water can place a harvested commodity in a stress situation and shorten its storage life. Surplus turgor pressure caused either by absorption of external water or by water redistribution within different fruit tissues may influence shelf life (Ben-Yehoshua and Rodov, 2003).

Minimally processed baby leaves are very popular with consumers for nutritional reasons and due to their convenience. Moreover, freshness is the main quality factor desired for leafy greens. Tenderness, cleanliness, and uniformity of green colour are also desirable (Wright, 2004). They must be handled carefully to

* Corresponding author. Tel.: +34 968 396 315; fax: +34 968 396 213.
E-mail address: migil@cebas.csic.es (M.I. Gil).

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avoid mechanical damage and water loss. As reported for whole leafy vegetables, unfavourable postharvest conditions before minimal processing can affect the quality of the raw product and shorten the shelf life. Additionally, minimal processing operations for baby leaves, such as washing, drying and packaging can contribute to leaf damage which accelerates a reduction in product shelf life (Brecht, 1995). For example, the 'processability', which is the ability to withstand these processing operations, can be affected by the tissue moisture content (Clarkson et al., 2003). Other variables that affect the shelf life of minimally processed leafy vegetables are time of the day of harvest, temperature, relative humidity (RH) and light conditions after harvest (Clarkson et al., 2005; Martínez-Sánchez et al., 2011). It has been reported that harvest of baby leaves following 'end of day' harvest practices increased shelf life by 164% when compared with "early morning" harvested material, although this is dependent on the species (Clarkson et al., 2005). Changes in leaf cell wall and turgor pressure contributed to a more processable leaf with extended postharvest shelf life. However, there is no available literature on quality characteristics affected by excess or reduced moisture content of leafy vegetables as raw material for minimal processing, except that of whole lettuce during storage (Agüero et al., 2008, 2011).

Baby spinach (*Spinacia oleracea* L.) has a large surface area and is characterized by high respiration and transpiration rates (Wang, 2003). Major problems in baby spinach during postharvest are tissue decay and development of off-odours (Allende et al., 2004). We hypothesized that baby leaves with a reduced water content could maintain their quality because they are less susceptible to damage during processing and to spoilage during storage when compared to leaves with higher moisture content. Thus, the objective of this study was to determine any differences in the quality and shelf life of baby spinach exposed to different RH (72%, 85% and 99% RH as low, medium and high RH, respectively) for 36 h at 15 °C, as short-term postharvest exposure, before processing. Baby spinach was selected for this study because this is a highly perishable product due to water loss. Quality parameters such as sensory evaluation, texture (maximum force, stiffness, elasticity and plasticity), electrolyte leakage, osmotic potential and microbiological quality were evaluated before and after processing and, during storage for up to 12 d at 7 °C.

2. Materials and methods

2.1. Plant material and sample preparation

Spinach (*Spinacia oleracea* L.) was cultivated under commercial conditions in Pulpí (Almería, Spain) by Primaflor S.L. The soil had a particle size distribution of 40% sand, 36% silt and 24% clay, classifying this soil texture as loam (USDA textural soil classification, 1987). Sowing was performed directly on elevated beds using a plant density of 700 plants m^{-2}. The harvest was carried out mechanically in three different dates (29th November 2010, 24th January 2011 and 25th May 2011). Eleven kg of plant material were sampled at each harvest day. After harvest, baby spinach leaves were transported (127 km) to the CEBAS-CSIC laboratory (Murcia, Spain). On arrival, leaves with defects such as bruising or discolouration were removed by hand. Three batches of approx. 3.5 kg were separated and stored, each one in a controlled RH chamber, for 36 h at 15 °C. The chambers used were hermetic chambers with control of temperature, RH, O_2, CO_2 and ethylene (Tecnidex, Paterna, Valencia, Spain). The RH conditions were created using ultrasonic humidifiers. These chambers were connected to a computer to register ambient parameters variations. Three different RH conditions were established: 72%, 85% and 99% as low, medium and high RH. Air free of ethylene was pumped through them.

Each batch was weighed before and immediately after removal from the chambers and weight loss (%) was calculated from these values. Water content (WC) of the samples before and after exposure to different RH was determined as described by Agüero et al. (2011).

2.2. Processing, packaging and storage conditions

Each batch was divided in three sub-batches of approx. 1.1 kg. Each sub-batch was independently processed in an isolated and cleaned minimal processing room at 15 °C (Takeuchi and Frank, 2000). Each sub-batch was washed for 60 s in tap water (1:10 proportion, w/v). Excess of water was removed by spinning for 1 min at 440 rpm in an automatic salad spinner (K-50, Kronen GmbH, Kehl am Rhein, Germany). Samples of 100 g were mechanically packed in a vertical packaging machine (Etna 280-X model, Ulma, Oñati, Spain) using a polypropylene (PP) film (Amcor Flexibles, Bristol, UK), 35 μm with O_2 permeance of 529 mL O_2 m^{-2} d^{-1} atm^{-1}, CO_2 permeance of 1981 mL CO_2 m^{-2} d^{-1} atm^{-1} and H_2O permeance of 8.41 g H_2O m^{-2} d^{-1} the for at 7 °C and 97% RH. Package size was 230 mm × 310 mm. Passive modified atmosphere packaging (MAP) was created by the respiration rate of the product and the film permeability characteristics as packages were sealed under air conditions. Packages were stored in darkness for 12 d at 7 °C.

Sensory evaluation, texture, electrolyte leakage and microbiological quality were evaluated before and after processing (0 d) and during storage (2, 6, 9, 12 d) whereas water content and osmotic potential were evaluated only before and after processing. After washing, the wash water was also characterized by the physicochemical parameters such as pH (Crison Basic 20^{+}, Barcelona, Spain), chemical oxygen demand (COD) (Spectroquant, Merck Darmstadt, Germany) and conductivity (Crison, 35 Crison instruments, Barcelona, Spain). The pH ranged from 7.96 to 8.19, COD was <25 mg/L and the conductivity ranged from 452.5 to 588.5 μS cm^{-1}.

2.3. Osmotic potential

To determine the osmotic potential (ψ_s), 10 g of leaves were frozen at −20 °C for 24 h and centrifuged at 2800 × *g* for 15 min (Centronic centrifuge, J.P. Selecta, Barcelona, Spain). Samples were filtered through double cheesecloth and 1 mL of filtrate was centrifuged at 11,925 × *g* for 10 min (Sigma microcentrifuge, 1-13, Biotech, Melsungen, Germany). Finally, the supernatant was filtered through a 0.45 μm polyvinylidene fluoride (PVDF) filter and the ψ_s was measured at 25 ± 1 °C with a microosmometer (Roebling 13DR, Löser Messtechnik, Berlin, Germany). The ψ_s was calculated according to Van't Hoff equation: $\psi_s = -RTc_s$, where $R = 8.31$ (m^3 Pa mol^{-1} K^{-1}), T = temperature (°K) and c_s = osmolarity (Osm m^{-3}).

2.4. Sensory evaluation

Baby spinach from three packages were combined and examined by a five member trained panel at the beginning of the experiment (before and after processing) and during the storage period. The evaluations were carried out immediately after samples were removed from storage. The code (3 digits) samples were presented to the judges to make independent evaluations. Panel members were requested to assess spinach quality in terms of off-odours, dehydration, overall visual quality and damaged leaf percentage. Off-odours and dehydration were evaluated using a 5 point scale where 5 = severe, 3 = moderate and 1 = none. Visual quality was evaluated considering freshness, appearance, colour uniformity, and brightness following a 9 point rating scale where 9 = excellent, 7 = good, 5 = fair (limit of consumer acceptability),