



Metabolic activity, microbial growth and sensory quality of arugula leaves (*Eruca vesicaria* Mill.) stored under non-conventional modified atmosphere packaging



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ARTICLE INFO

Article history:

Received 24 January 2016

Received in revised form 26 May 2016

Accepted 10 June 2016

Available online 25 June 2016

Keywords:

Postharvest

MAP

Noble gases

Fresh cut

Respiratory rate

Sensory characteristics

ABSTRACT

Arugula compositional characteristics linked to vitamin content, polyphenols and other health beneficial compounds have increased consumer interest to include it in the diet. This leafy vegetable is very susceptible to yellowing and dehydration, reducing its shelf life period. This study evaluated the effects of non-conventional modified packaging atmospheres, i.e. Argon (75–80% Ar + 10–11% O₂, balance N₂), Nitrogen (10–11% O₂ + 89–90% N₂), High Oxygen (85–90% O₂ + 10–15% N₂), Helium (75–80% He + 10–11% O₂, balance N₂) and Nitrous Oxide (75–80% N₂O + 10–11% O₂, balance N₂), on metabolic activity, microbiological growth and sensory characteristics of fresh cut arugula (*Eruca vesicaria* Mill.) leaves. The High Oxygen atmospheres reduced the respiration rate until 5 days of storage, but there were no differences among treatments at the end of the storage period. There were no differences in ethylene emission among treatments at any of the evaluation dates. Non-conventional atmospheres had 0.5–1 log unit lower microbial growth counts than air packaging after 8 days of storage. Color and sensory characteristics were not affected by the storage conditions. The non-conventional atmospheres maintained some quality characteristics of arugula leaves, suggesting it could be an alternative to conventional modified atmosphere used to maintain arugula shelf life.

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1. Introduction

Arugula (*Eruca vesicaria* Mill.) belongs to the Brassicaceae family, and is an important component of the salad vegetable market (Pasini et al., 2011; Bell et al., 2015). Its distinctive flavor and its nutritional properties are responsible for growing consumer interest in including it in the diet. Arugula nutritional values are associated with the large concentration of glucosinolates, which are also responsible for its pungent aroma and flavor (Bennett et al., 2002; Kim et al., 2004). Poly glycosylated flavonoid compounds, ascorbic acid and other compounds are implicated in gastrointestinal tract and cardiovascular health benefits, as well as proven anti-carcinogenic effects (Lynn et al., 2006; Bjorkman et al., 2011; Traka and Mithen, 2011).

Similar to other leafy vegetables, arugula has several features including high metabolic activity and high surface to volume ratio, that make it very perishable and susceptible to quality losses. Yellowing due to chlorophyll degradation is the most serious postharvest alteration in arugula leaves. Dehydration reduces the shelf life considerably, especially when temperature and relative humidity during storage are not managed properly (Koukounaras et al., 2007, 2010; Løkke et al., 2012).

To preserve quality and achieve the potential shelf life of 12–15 days, arugula should be stored at 0 °C and 95–100% RH (Koukounaras et al., 2007). However, these conditions are difficult to achieve in commercial situations where products are usually stored at 5–10 °C (Nunes et al., 2009; Lundén et al., 2014). In order to reduce yellowing and restrict dehydration, arugula should be packed under modified atmosphere (MAP) conditions, to maintain quality and extend shelf life (Løkke et al., 2012).

Conventional MAP consists of reduced O₂ levels combined with increased CO₂ levels, usually regulated by the respiration rate of the product and the permeability of the packaging film to these gases

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(Sandhya, 2010; Vigneault et al., 2012). However, in order to generate more efficient MAP conditions, there has been much recent interest in non-conventional modified atmospheres (Artés et al., 2009). This includes replacing the original atmospheric gas composition with noble gases (e.g. He, Ar, or Xe), nitrous oxide (N₂O), or high or low O₂ concentrations that could help to maintain the quality of some fresh vegetables (Tomás-Callejas et al., 2011).

Although the mechanisms involved in these atmospheres are partially known or unknown, several studies show promising results, particularly in decreasing respiratory rate, ethylene (C₂H₄) production and microbial growth in some fresh cut products (Escalona et al., 2006; Robles et al., 2010; Pinela et al., 2016).

Noble gases such as Ar, Kr, and Xe can form clathrate hydrates around the solute molecules in some vegetables and fruits. In aqueous solution these compounds inhibit enzymatic reactions and reduce metabolic activity in the product due to hydrophobic hydration, particularly under pressures above the critical pressure point (Zhang et al., 2008; Wu et al., 2012; Lagnika et al., 2013). Another possible mechanism is competitive inhibition of molecular O₂ by the noble gases. Competition for O₂ binding sites may reduce the activity of key enzymes involved in maturity and senescence process (Gorny and Agar, 1998).

N₂O is highly soluble in vegetables cells, and can reversibly delay the processes associated with ripening and senescence, by affecting cytochrome C oxidase activity in the mitochondria (Sowa and Towill, 1991), and by extending the lag phase preceding the rise in C₂H₄ production (Gouble et al., 1995; Rocculi et al., 2004, 2005; Palomer et al., 2005).

High levels of O₂ are associated with inhibition of the maturation and ripening process in several vegetables products (Escalona et al., 2006; Tomás-Callejas et al., 2011). N₂ enriched atmospheres have been reported as an alternative to traditional MAP for several vegetables (Liu and Xu, 2015; Xu et al., 2015). N₂ is chemically stable and, like noble gases, can form clathrate hydrate or hydrate gas under appropriate temperature and pressure conditions (Disalvo et al., 2008; Xu et al., 2015). This slows down the physiological processes and extends shelf life.

Although the use of non-conventional atmosphere has been studied, the behavior observed depends on the product tested; and there is little knowledge of the mechanisms involved in the control processes. Therefore, the objective of the present work was to evaluate the effect of non-conventional MAP (i.e. Ar, N₂, high O₂, He, and N₂O) on the metabolic behavior and the sensory and microbiological quality of fresh-cut arugula.

2. Materials and methods

2.1. Plant material, preparation and treatments

The arugula (*Eruca vesicaria* Mill) used in this study was from a spring-summer commercial greenhouse crop grown in Calera de Tango (Región Metropolitana, Chile). Manual harvest using disinfected scissors was done after 30 days of growth, when the leaves had reached a length of about 10 cm. The harvested leaves were placed in perforated bags, transported under refrigerated conditions to the “Centro de Estudios Postcosecha” (CEPOC, Universidad de Chile), and held at 5 °C and 92% RH for 24 h prior to testing.

Working in a controlled temperature room at 8 °C, the raw material was sorted to remove any leaves with pathological and/or physiological defects. The leaves were washed twice, i.e. first in tap water for 1 min and then for 3 min in a sodium hypochlorite solution [100 mg L⁻¹ NaOCl (Clorox, Chile), pH adjusted to 6 with citric acid (Merck, Darmstadt, Germany)]. The washed leaves were rinsed with tap water, allowed to drain for 3 min on a stainless steel mesh and then centrifuged with a manual centrifuge (Ilko, Chile). The

temperature of the washing and disinfection solutions was 5 °C. Samples of about 40 g of prepared arugula leaves were placed in polypropylene bags with an O₂ permeability of 3000 mL m⁻² day⁻¹ and a CO₂ permeability of 9000 mL m⁻² day⁻¹ (Sealed Air, CRY-OVAC, Chile).

The packaged leaves received one of six modified atmosphere treatments: Air control (0.03% CO₂ + 21% O₂, balance N₂), Argon (75–80% Ar + 10–11% O₂, balance N₂), Nitrogen (10–11% O₂ + 89–90% N₂), High Oxygen (85–90% O₂ + 10–15% N₂), Helium (75–80% He + 10–11% O₂, balance N₂) and Nitrous Oxide (75–80% N₂O + 10–11% O₂, balance N₂). Gases to establish these atmospheres were provided from a gas mixer panel (CEPOC, Chile), using gases of 99.99% purity (Indura, Chile). The packages of leaves of 40 g per bag were flushed with the gas mixtures before being heat sealed. For the Air control treatment, seven holes of about 0.5 mm diameter distributed evenly on both sides were made in the heat sealed bag. All treatments were stored for 11 days at 5 °C. Three replicate bags were prepared for each treatment. Evaluations were made after 2, 5, 8 and 11 days of storage.

2.2. Respiration rate and ethylene emission

Respiration rate was determined using about 140 g leaves in closed 4 L plastic containers of each of the six atmosphere mixtures, which were stored at 5 °C during the course of the experiment. The initial gas mixtures were individually flushed into each container at the beginning of the experiment. Later, the containers were closed until the end of the experiment. On the evaluation day, at least two atmosphere samples were collected through the septum in each container during one hour, using a plastic syringe of 10 mL. The respiration rate was determined from the difference between two consecutive measurements. The results were expressed as mg CO₂ kg⁻¹ h⁻¹.

To assess the concentrations of the gases in the container, gas samples were injected into a gas chromatograph (GC, Hewlett Packard, 5890 series II, California, USA) provided with a thermal conductivity detector and a packed column (Porapak Q, Waters, Milford MA, USA) using He as a carrier gas (Indura, Chile) at 50 psi. The GC was calibrated using standards of 1% CO₂ + 17% O₂, balance N₂ and 10% CO₂ + 5% O₂, balance N₂ (Indura, Chile). In case of high O₂ levels, a pure oxygen bottle was used to calibrate the GC. Injector, oven and detector temperatures were 200, 50 and 200 °C, respectively. CO₂, O₂ and N₂ percentages were measured by using this GC.

From the same container with leaves used before, C₂H₄ was determined by injecting 1 mL gas samples into a GC (Agilent Technologies 7820A, CG System, USA) with a flame ionizer detector and Porapak column QN 80–100 mesh, 1.20 m × 3.18 mm (Norwalk, Connecticut, USA). The carrier gas was He and the flow rate was 60 mL min⁻¹. A commercial standard of 0.5 ppm C₂H₄; (Indura, Chile) was used to calibrate the GC. C₂H₄ emission was determined from the difference between two consecutive measurements on days 0, 2, 5, 8 and 11 at 5 °C. The results were expressed as μL C₂H₄ kg⁻¹ h⁻¹.

2.3. Gas content of packages

Evolution of CO₂ and O₂ gases into the plastic bags was determined with a manual gas analyzer (Checkpoint, PBI Dansensor, Ringsted, Denmark) which was tested with atmospheric air and same standards mentioned before. Samples were taken through a silicone septum affixed on the outside of the bags. Values were expressed as percentage of O₂ and CO₂. N₂ concentration was determined by gas chromatography using the same equipment described for respiration rate determination section. To determine the con-

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