Scientia Horticulturae 209 (2016) 79–85

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

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% O_2, balance N_2), Nitrogen (10–11% O_2 + 89–90% N_2), High Oxygen (85–90% O_2 + 10–15% N_2), Helium (75–80% He + 10–11% O_2, balance N_2) and Nitrous Oxide (75–80% N_2O + 10–11% O_2, balance N_2), 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.

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 O_2 levels combined with increased CO_2 levels, usually regulated by the respiration rate of the product and the permeability of the packaging film to these gases

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http://dx.doi.org/10.1016/j.scienta.2016.06.007
0304-4238/

(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 (N_2O), or high or low O_2 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 (C_2H_4) 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 O_2 by the noble gases. Competition for O_2 binding sites may reduce the activity of key enzymes involved in maturity and senescence process (Gorny and Agar, 1998).

N_2O 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 C_2H_4 production (Gouble et al., 1995; Rocculi et al., 2004, 2005; Palomer et al., 2005).

High levels of O_2 are associated with inhibition of the maturation and ripening process in several vegetables products (Escalona et al., 2006; Tomás-Callejas et al., 2011). N_2 enriched atmospheres have been reported as an alternative to traditional MAP for several vegetables (Liu and Xu, 2015; Xu et al., 2015). N_2 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, N_2, high O_2, He, and N_2O) 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, Darmstad, 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 O_2 permeability of 3000 mL m^{-2} day^{-1} and a CO_2 permeability of 9000 mL m^{-2} day^{-1} (Sealed Air, CRYOVAC, Chile).

The packaged leaves received one of six modified atmosphere treatments: Air control (0.03% CO_2 + 21% O_2, balance N_2), Argon (75–80% Ar + 10–11% O_2, balance N_2), Nitrogen (10–11% O_2 + 89–90% N_2), High Oxygen (85–90% O_2 + 10–15% N_2), Helium (75–80% He + 10–11% O_2, balance N_2) and Nitrous Oxide (75–80% N_2O + 10–11% O_2, balance N_2). 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 individuality 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 CO_2 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 (PoraPack Q, Waters, Milford MA, USA) using He as a carrier gas (Indura, Chile) at 50 psi. The GC was calibrated using standards of 1% CO_2 + 17% O_2, balance N_2 and 10% CO_2 + 5% O_2, balance N_2 (Indura, Chile). In case of high O_2 levels, a pure oxygen bottle was used to calibrate the GC. Injector, oven and detector temperatures were 200, 50 and 200 °C, respectively. CO_2, O_2 and N_2 percentages were measured by using this GC.

From the same container with leaves used before, C_2H_4 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 C_2H_4; (Indura, Chile) was used to calibrate the GC. C_2H_4 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 C_2H_4 kg^{-1} h^{-1}.

2.3. Gas content of packages

Evolution of CO_2 and O_2 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 O_2 and CO_2. N_2 concentration was determined by gas chromatography using the same equipment described for respiration rate determination section. To determine the con-

Table 1
Effect of non-conventional storage atmospheres on the respiration rate (mg CO_2 kg^{-1} h^{-1}) of arugula leaves stored at 5 °C up to 11 days.

Packagingatmosphere	Storage time (days)				
	0	2	5	8	11
[a]Air	[b,c] 67.0 ± 0.8 Ba	41.1 ± 1.2 ABb	33.8 ± 1.1 ABc	27.6 ± 1.3 Ac	21.8 ± 0.9 Ad
Ar	62.7 ± 1.9 BCa	41.1 ± 1.8 ABb	33.6 ± 1.8 ABc	26.3 ± 1.2 ABd	19.9 ± 1.0 Ae
N_2	58.2 ± 2.6 CDa	42.1 ± 1.6 ABb	36.3 ± 0.9 ABc	27.1 ± 0.7 Ad	21.1 ± 0.5 Ae
High O_2	35.5 ± 2.0 Ea	39.6 ± 2.1 Bab	31.2 ± 2.4 Bbc	25.5 ± 1.8 ABc	20.7 ± 1.4 Ac
He	53.3 ± 2.2 Da	46.8 ± 1.3 Ab	39.1 ± 1.2 Ac	28.5 ± 0.3 Ad	23.9 ± 0.5 Ad
N_2O	108.4 ± 4.4 Aa	46.4 ± 1.3 Ab	35.5 ± 1.5 ABc	20.8 ± 1.3 Bd	23.2 ± 0.6 Ad

[a] Air, correspond to Air control; Ar: Argon; N_2: Nitrogen; High O_2: High Oxygen; He: Helium and N_2O: Nitrous Oxide packages.
[b] Values are means ± standard error of the mean (n = 3).
[c] Means followed by the same letter, uppercase and lowercase in column and row respectively, are not significantly different according to the Tukey test ($p \leq 0.05$).

centration of Ar, He and N_2O the following equation was used:

$$[Ar] \text{ or } [He] \text{ or } [N_2O] = 100 - ([O_2] + [CO_2] + [N_2])$$

2.4. Color

The color of the arugula leaves was measured using a tristimulus colorimeter (Minolta, CR-300, Japan), using an illuminant D65, an observation angle of 0°, and calibrated with a white standard.

CIELAB parameters were determined: L* (ranging from 0, black, to 100, white), and two color coordinates, a* (which takes positive values for reddish colors and negative values for greenish ones) and b* (positive for yellowish colors and negative for the bluish ones). From these coordinates, other color parameters were determined: the hue angle (hab) and the chroma (C*ab), as qualitative and quantitative attributes of color, respectively. Measurements were made on the adaxial surface of the leaf, using a background to prevent interference with the color. For each treatment, 24 leaves (8 per bag) were measures after 0, 2, 5, 8 and 11 days of storage at 5 °C.

2.5. Microbiological growth

Samples of 10 g of leaves from each repetition were transfer to sterile stomacher bags (BA 6141 CLR, Sewards, United Kingdom), 90 mL of sterile buffered peptone water (Merck, Darmstad, Germany) was added, and the contents were homogenized for 1 min in a stomacher (Easy Mix, AES Chemunex, France). When necessary, tenfold serial dilutions were prepared in sterile buffered peptone water. Aliquots of 1 mL were spread-plated onto count plates (Merck, Darmstad, Germany) of selective media.

Plates for psychrotrophic and mesophilic organisms were incubated at 5 °C for 7 days and 37 °C for 2 days, respectively. Enterobactericeae were plated in eosin methylene blue (Merck, Darmstad, Germany) and incubated at 37 °C for 2 days. Results were expressed as colon forming units per g of sample (cfu g^{-1}). All determinations were made in triplicate.

2.6. Sensory evaluation

Sensory evaluation was performed using a descriptive-quantitative analysis by a semi-trained sensory panel composed of 12 judges (20–45 years old). Samples were labeled with a three-digit code and presented using a completely randomized order. An unstructured linear 15 cm scale (where 0 means "absence of sensation" and 15 means "extremely high sensation") was used to evaluate appearance, color intensity, turgor and flavor.

2.7. Statistical analysis

The experimental design was completely randomized. A bag with 40 g of arugula leaves was considered the experimental unit. The residual data was subjected to normality and homogeneity tests of variance. Subsequently, variance (ANOVA) were performed, with 5% as the level at which significant differences were declared. When a significant difference between treatments was found, the means were compared by Tukey test ($p \leq 0.05$). Data were analyzed using the statistical program Infostat (Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

3.1. Respiration rate and ethylene emission

Higher respiration rates were observed immediately after processing (washing and packing), with values at time 0 ranging from 35.5 to 108.4 mg CO_2 kg^{-1} h^{-1} (Table 1). The lowest respiration rate was observed in leaves in the High Oxygen atmosphere, while the highest respiration rate was observed in leaves in the Nitrous Oxide atmosphere. As storage time increased, the respiration rate decreased progressively in all treatments.

The differences among treatments remained until the 8th day of storage. At 2 and 5 days of storage, the leaves stored in High Oxygen had the lower respiratory activity than Helium storage, but on day 8, leaves stored in Nitrous Oxide, High Oxygen and Argon had the lowest respiration rates. After 11 days at 5 °C, respiration rates were 44–69% lower than initial rates (Table 1). However, there were no differences among treatments after 11 days of storage.

The initial ethylene emission rates were about 10 times higher than the values registered on subsequent days of analysis (Table 2). At time 0, the highest C_2H_4 emission was observed in leaves packaged in Nitrous Oxide, followed by the leaves stored in Nitrogen, Argon and Air. The lowest values were observed in the Helium and High Oxygen treatment. At later evaluation dates, no differences in ethylene emission rates were observed among the storage atmospheres (Table 2).

The high initial respiration and C_2H_4 emission rates are likely attributable to the stress resulting from the processing operation, has been noted by many other researches (e.g. Inestroza-Lizardo and Escalona, 2015; Silveira et al., 2015) Respiration of arugula leaves in the High Oxygen atmosphere remained relatively low and stable after processing. Contrary to our finding, Martínez-Hernández et al. (2013) reported that storage at 90% O_2 caused a strong increase in respiration rate in fresh-cut broccoli, starting from 3 days of storage at 5 °C. Char et al. (2012) found that arugula leaves stored at 5 °C for 8 days in low O_2 (5–6% O_2), or in Argon (65–70% Ar + 5–6% O_2, balance N_2), or in Helium (70–75% He + 5–6% O_2, balance N_2) did not have any differences in respiration rate.

On the contrary, Silveira et al. (2014) found that watercress stored in Low O_2 (89.7% N_2, 10.3% O_2), Argon (89.9% Ar, 10.1% O_2), Helium (90.1% He, 9.9% O_2) and Nitrous Oxide (89.3% N_2O, 10.7% O_2) atmosphere at 5° C during 6 days presented reduction on the respiration activity compared to the leaves stored in air condition. However, no substantial effect was observed on C_2H_4

Table 2
Effect of non-conventional storage atmospheres on the ethylene emission ($\mu L\, C_2H_4\, kg^{-1}\, h^{-1}$) of arugula leaves stored at 5 °C up to 11 days.

Packagingatmosphere	Storage time (days)				
	0	2	5	8	11
[a]Air	[b,c]1095.7 ± 16.2 Ba	159.2 ± 2.4 Ab	86.6 ± 3.0 Abc	75.6 ± 5.9 Ac	46.1 ± 2.9 Ac
Ar	1039.2 ± 66.3 Ba	146.4 ± 6.6 ABb	88.2 ± 1.0 Ab	45.0 ± 2.9 Abc	28.9 ± 1.5 Ae
N_2	1025.0 ± 40.1 Ba	144.8 ± 5.6 Ab	66.6 ± 3.0 Abc	41.5 ± 1.2 Ac	21.5 ± 1.0 Ac
High O_2	709.6 ± 24.7 Ca	114.7 ± 5.6 Ab	78.0± 9.0 Ab	49.9 ± 3.8 Ab	38.2 ± 1.0 Ab
He	780.0 ± 19.9 Ca	148.1 ± 3.6 Ab	84.8 ± 2.2 Abc	45.2 ± 1.2 Ac	32.4 ± 0.5 Ac
N_2O	1181.7 ± 62.8 Aa	159.6 ± 3.2 Ab	93.7 ± 7.7 Abc	44.9 ± 3.1 Ac	34.2 ± 0.6 Ac

[a] Air, correspond to Air control; Ar: Argon; N_2: Nitrogen; High O_2: High Oxygen; He: Helium and N_2O: Nitrous Oxide packages.
[b] Values are means ± standard error of the mean (n = 3).
[c] Means followed by the same letter, uppercase and lowercase in column and row respectively, are not significantly different according to the Tukey test ($p \leq 0.05$).

emission. Similarly to the reported by Kader and Ben-Yehoshua (2000), regarding to the use of high O_2 levels also in the non-conventional atmospheres, the metabolic response depends on the product considered, being observed increase, decrease or no effect. With respect to the effect on the respiration rate of the other tested gases, our results are also different from assayed product. These differences may be due to physical tissue composition that includes aspects related to the gases exchanged (intercellular spaces proportion, surface characteristics as composition etc.) (Zhang et al., 2015). Aspects that should be extensively studied in futures works.

3.2. Gas content of packages

The O_2 concentration in the Helium package atmosphere remained stable for 2 days, and then increased slightly (Fig. 1a). In the Argon, Nitrogen and Nitrous Oxide treatments, the O_2 concentration remained almost constant below the concentration in the Air control packages throughout the storage period. In the High Oxygen treatment, the O_2 concentration decreased to less than half of the initial concentration by 5 days of storage, remained stable to day 8, and then declined to slightly below the concentration in the Air control packages by day 11. The O_2 concentration in the Air control packages remained constant, at ambient level, throughout the experiment (Fig. 1a).

The CO_2 concentration increased in all of the treatments, due to respiration of the arugula leaves (Fig. 1b). From the second day of storage until the end of the experiment the CO_2 concentration in all of the modified atmosphere packages remained ca. 3–4%. However, in the Air control treatment, the CO_2 concentration remained below 1%.

The O_2 concentrations in the modified atmosphere packages was similar to the recommendations for storage of leafy vegetables like celery, parsley, cilantro and spinach, but the CO_2 concentrations were lower than the recommended 5–10% for these vegetables (Gómez and Artés, 2005; Sandhya, 2010). This is likely due to the high CO_2 permeability of the film used in the experiment, which prevented retention of CO_2 inside the packages. A similar result was observed in the High Oxygen treatment, in which the O_2 concentration did not remain at the desired level.

The Ar, He and N_2O gases dissipated during the first 5 days of storage replaced by atmospheric N_2 (Fig. 1c). Progressive reduction of these gases during storage has been reported elsewhere, and was usually attributed the permeability of the plastic film, the size of the gas atoms, and the concentration gradients of those gases from the inside to the outside of the storage container which may be construed as limiting for the implementation of this technology (Tomás-Callejas et al., 2011; Char et al., 2012; Silveira et al., 2014).

3.3. Color

Color changes during storage are shown in Table 3. The lightness (L*) of the arugula leaves increased in all treatments during

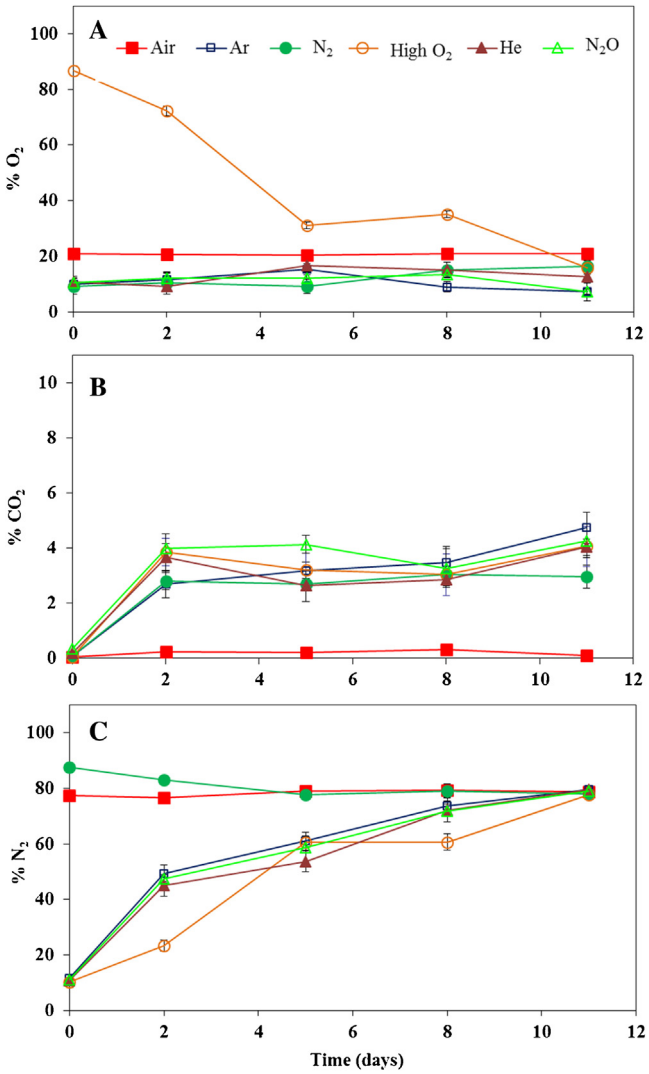

Fig. 1. Oxygen (a), carbon dioxide (b) and nitrogen (c) content in packages of arugula leaves stored at 5 °C up to 11 days. Vertical bars indicate the standard errors of the means (n = 3). Air, correspond to Air control; Ar: Argon; N_2: Nitrogen; High O_2: High Oxygen; He: Helium and N_2O: Nitrous Oxide packages.

storage showing that the leaves were clearer compared to initial values. This is similar to the findings using minimally processed chard packaged in modified atmospheres enriched with O_2, He, N_2 and N_2O after 8 days at 5 °C (Tomás-Callejas et al., 2011), and is likely attributable to chlorophyll degradation with increasing time in storage. Similarly, the chroma (C *) increased during storage i.e. from 22.5 to 26.8 at the start of the experiment, to 26.2 to 28.4 at the end of storage. Hue angle values were initially ca. 120.4–123.2, and

Table 3
Effect of non-conventional storage atmospheres on the L and Chroma of arugula leaves stored at 5 °C up to 11 days.

Packaging atmosphere	L			
	2 days	5 days	8 days	11 days
[a]Air	[b,c]43.4 ± 0.4 ABb	44.4 ± 0.1 Bab	45.6 ± 0.5 ABa	44.7 ± 0.6 Bab
Ar	43.9 ± 0.1 ABc	47.1 ± 0.9 Aa	46.4 ± 0.2 Aab	44.7 ± 1.0 Bbc
N_2	44.1 ± 0.4 ABb	45.7 ± 0.5 ABb	46.0 ± 0.5 Ab	48.4 ± 0.5 Aa
High O_2	42.4 ± 0.7 Bc	44.7 ± 0.5 ABb	43.3 ± 0.2 Bbc	47.4 ± 0.5 Aa
He	46.7 ± 0.2 Ab	44.8 ± 0.2 ABb	46.1 ± 1.0 Aab	47.8 ± 0.4 Aa
N_2O	44.3 ± 0.4 ABb	45.6 ± 0.3 ABb	45.9 ± 0.8 Ab	48.4 ± 0.9 Aa
Chroma				
Air	22.5 ± 1.0 Bb	27.2 ± 0.8 Aa	27.6 ± 0.7 ABa	28.3 ± 0.2 Aa
Ar	23.3 ± 1.0 Bc	29.3 ± 0.6 Aa	27.1 ± 0.5 Bab	26.2 ± 0.5 Ab
N_2	26.5 ± 0.8 Aa	28.9 ± 1.1 Aa	28.6 ± 0.8 ABa	27.0 ± 0.1 Aa
High O_2	22.9 ± 1.3 Bb	27.6 ± 0.2 Aa	26.9 ± 0.7 Ba	27.1 ± 0.7 Aa
He	26.8 ± 0.2 Ab	29.5 ± 0.4 Aa	30.0 ± 0.6 Aa	28.4 ± 0.5 Aa
N_2O	24.4 ± 0.6 Bc	28.5 ± 0.5 Aa	29.9 ± 0.7 Aa	27.9 ± 0.7 Aa

[a] Air, correspond to Air control; Ar: Argon; N_2: Nitrogen; High O_2: High Oxygen; He: Helium and N_2O: Nitrous Oxide packages.
[b] Values are means ± standard error of the mean (n = 24).
[c] Means followed by the same letter, uppercase and lowercase in column and row respectively, are not significantly different according to the Tukey test ($p \leq 0.05$).

remained practically stable throughout the storage period. After 11 days at 5 °C, values were ca. 120.4–121.6, with no differences among treatments (data not shown).

Although there were some differences in color among the treatments, it is important to emphasize that numerical values that differ by less than three units are not perceptible by the human eye (Tomás-Callejas et al., 2011). The results obtained in this experiment suggest that the non-conventional modified atmospheres evaluated had no substantial effect on color maintenance. This agrees with similar work by Ansah et al. (2015) and Pinela et al. (2016), although the results can vary depending on the product being evaluated (Zhang et al., 2015).

3.4. Microbiological growth

Counts of aerobic mesophilic organisms were not initially affected by the different storage atmospheres (Fig. 2a). After 2 days at 5 °C, the counts ranged from 2.9 to 4.1 log cfu g^{-1}, but there were no significant differences among treatments. At 5 and 8 days, the leaves stored in non-conventional atmospheres had counts 0.5–1.0 log units lower than the counts for leaves stored in the Air control. At 11 days of storage, there were no significant differences among treatments, when counts were 5.2–6.4 log cfu g^{-1} (Fig. 2a).

Initially, there were no differences among storage atmospheres in counts of psychrotrophic bacteria, which ranged from 4.8 to 5.3 log cfu g^{-1} (Fig. 2b). The most noticeable differences were observed at the end of the experiment. At 11 days of storage, the leaves stored in Nitrous Oxide and Helium had lower counts than the leaves stored in the Air control.

There were no significant differences among treatments in the initial counts of the Enterobacteriaceae (Fig. 2c). After 5 and 8 days of storage, the Air control atmosphere had 1 log cfu g^{-1} more than the other treatments. However, this difference did not persist to the end of the storage period, when all treatments had counts of ca. 5 log cfu g^{-1}, with no differences among treatments.

The results of this work suggest that the non-conventional atmospheres, especially Nitrogen and Helium, inhibited growth of spoilage microorganisms at 5 °C. This effect was maintained until 8 days of storage in the case of mesophilic and Enterobacteriaceae organisms, and until 11 days of storage in the case of psychrotrophic organisms (Table 3). Although it has been shown by other workers that MAP does not effectively control microbiological growth by itself, its use as a complementary tool to reduce microbial deterioration as has been evaluated in several products, with varying results. The most widely studied non-conventional atmosphere is

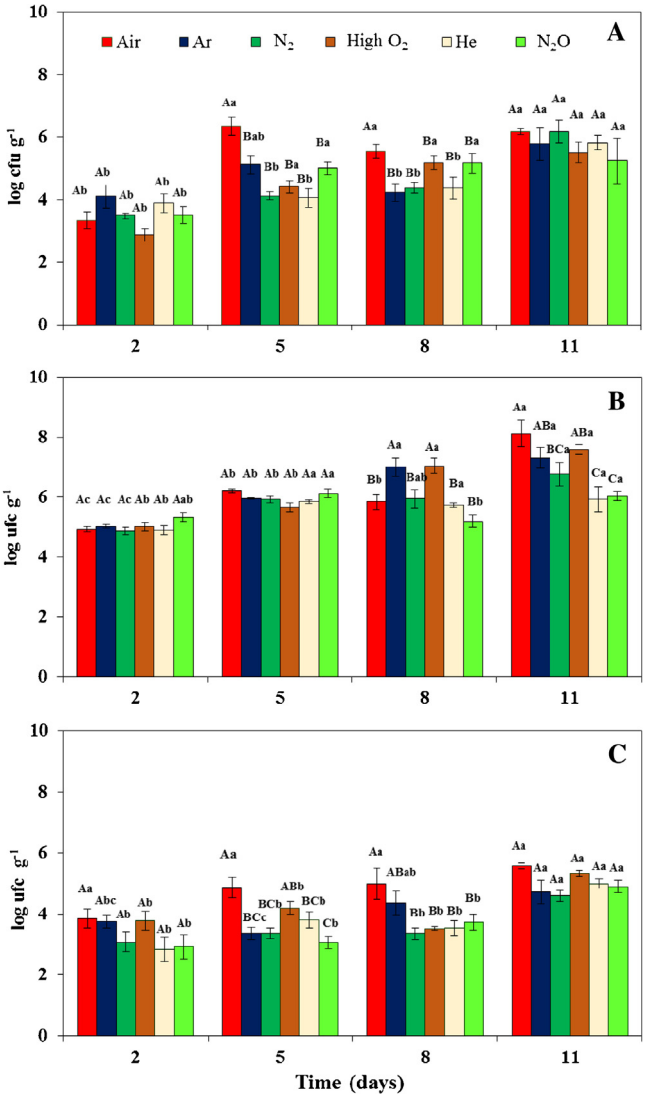

Fig. 2. Microbial growth from macerated arugula leaves stored at 5 °C for up to 11 days. Mesophilic (a), psychrotrophic (b) and enterobactericeae (c) growth. Vertical bars represent standard errors of the means (n = 3). Air, correspond to Air control; Ar: Argon; N_2: Nitrogen; High O_2: High Oxygen; He: Helium and N_2O: Nitrous Oxide packages. Means followed by different letters, uppercase and lowercase for time and treatment respectively, are statistically different according to Tukey test ($p \leq 0.05$).

Table 4
Effect of non-conventional storage atmospheres on the judged appearance of arugula leaves stored at 5 °C up to 11 days.

Packaging atmosphere	Appearance			
	2 days	5 days	8 days	11 days
[a]Air	[b,c] 12.6 ± 0.4 Aa	11.9 ± 0.4 Aa	9.1 ± 0.6 Aab	6.6 ± 0.7 Cb
Ar	11.8 ± 0.7 Aa	11.8 ± 0.8 Aa	11.6 ± 0.8 Aa	10.2 ± 0.7 Aa
N_2	10.2 ± 0.5 Aa	11.5 ± 0.9 Aa	9.6 ± 0.5 Aa	9.6 ± 0.9 ABa
High O_2	10.5 ± 0.8 Aa	11.1 ± 0.3 Aa	11.6 ± 0.6 Aa	10.8 ± 0.6 Aa
He	10.0 ± 0.9 Aa	10.1 ± 0.7 Aa	11.8 ± 0.6 Aa	9.2 ± 0.6 ABa
N_2O	11.5 ± 0.7 Aa	10.9 ± 0.7 Aa	10.6 ± 0.9 Aa	8.9 ± 0.5 Ba

[a] Air, correspond to Air control; Ar: Argon; N_2: Nitrogen; High O_2: High Oxygen; He: Helium and N_2O: Nitrous Oxide packages.
[b] Values are means ± standard error of the mean (n = 12).
[c] Means followed by the same letter, uppercase and lowercase in column and row respectively, are not significantly different according to the Tukey test ($p \leq 0.05$).

enriched O_2, which has been found to damage DNA and nucleoproteins (Moradas-Ferreira et al., 1996; Wszelaki and Mitcham, 2000). However, the reported effects of these atmospheres differ widely, depending on the product studied.

High Oxygen does not always inhibit microbial growth, e.g. bacterial growth was not controlled in fresh-cut butter head lettuce stored in 75% O_2 (Escalona et al., 2007); 50 or 90% O_2 (balance N_2) did not control mesophilic and yeast growth (López-Gálvez et al., 2015). In atmosphere of 70% O_2 + 15% CO_2 (balance N_2) reduced spoilage and growth of pathogenic bacteria inoculated onto shredded cabbage stored at 5 °C (Lee et al., 2011). Similarly, 100% O_2 inhibited growth of mesophilic organisms and Enterobacteriaceae on fresh-cut baby red chard leaves (Tomás-Callejas et al., 2011) and O_2 at about 95%, inhibited growth of Listeria monocytogenes inoculated onto fresh-cut celery stored at 7 °C for 21 days (González-Buesa et al., 2014). Conversely, the growth of mesophilic bacteria was stimulated on fresh-cut baby tatsoi leaves (100% O_2) and in fresh-cut broccoli (90% O_2; Tomás-Callejas et al., 2011; Martínez-Hernández et al., 2013, respectively).

N_2 enriched atmospheres also had variable effects depending on the evaluated product. On fresh-cut lettuce and cabbage stored at 90–98% N_2, the mesophilic and psychrotrophic bacterial counts did not differ from counts in passive MAP after 5 days at 5 °C (Koseki and Itoh, 2002). Enriched N_2 atmospheres partially controlled microbial growth on fresh-cut watercress, i.e. mesophilic growth was controlled during the first 3 days of storage but the psychrotrophic and Enterobacteriaceae groups were not controlled (Silveira et al., 2014).

Neither of the noble gas-modified atmospheres, i.e. Helium and Argon, had strong effects on microbial growth. This contrasts with other work, in which high concentrations of these gases (80–90%) controlled the growth of various microbial groups in fresh-cut mizuna leaves (Robles et al., 2010) and fresh-cut Fuji apple (Wu et al., 2012), but were ineffective or stimulated microbial growth on fresh-cut baby red chard leaves (Tomás-Callejas et al., 2011) and fresh-cut watercress (Silveira et al., 2014).

3.5. Sensory evaluation

There were no differences among treatments in the appearance of arugula leaves during the first 8 days of storage at 5 °C (Table 4). After 11 days at 5 °C, arugula leaves stored in the Air control had the lowest appearance scores, although they were still within the range of consumer acceptability. Leaves in the remaining five treatments had higher appearance scores than those stored in Air control. However, Argon and High oxygen storage had a higher score (10.2 and 10.8, respectively).

Initial color intensity scores ranged from 10.6 to 12.5, while at the end of the storage the values were from 6.9 to 10.3. There were no significant differences in color intensity scores among treatments (data not shown). There were no significant differences in perceived turgor among treatments. The initial values ranged from 11.2 to 11.5 and the final values were from 8.8 to 10.6 (data not shown).

Overall, the sensory quality of arugula leaves stored in the five unconventional storage atmospheres at 5 °C for 11 days was as good as for leaves stored in the Air control, and in fact their appearance scores were better. This is consistent with the instrumental evaluations, i.e. no major effects attributable to the atmospheres were found. This agrees with previous studies on leafy vegetables, in which no substantial effects of unconventional atmosphere on sensory quality were reported (Tomás-Callejas et al., 2011; Silveira et al., 2014).

The dehydration and turgor are important defects in leafy vegetables during storage but no differences were found in this study because all treatments were packaged in bag with high humidity (Siomos and Koukounaras, 2007).

4. Conclusion

Non-conventional atmospheres, especially the High Oxygen atmosphere, maintained the quality of arugula better than the Air control for up to 8 days of storage. Growth of Enterobacteriaceae and mesophilic organisms and respiration activity were reduced. However, maintaining the high O_2 levels within the packaging was a limiting factor, given the characteristics of the plastics used. A more appropriate plastic film is needed to enable further the study and adoption of this technology. However, the use of a protected film packages allow to maintain a good visual appearance of the leaves compared with other studies in ambient air with low humidity.

Acknowledgments

The authors are grateful to CONICYT-CHILE (FONDECYT Project Postdoctorado N° 3130363) for financial support and also to the Red Temática Cyted Hortyfresco (113RT0480) for technical collaboration.

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