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# Effect of non-conventional modified atmosphere packaging on fresh cut watercress (*Nasturtium officinale* R. Br.) quality

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## ARTICLE INFO

## ABSTRACT

In recent years, the minimally processed food industry has increased due to a consumer trend toward healthier eating. Among these products, watercress represents an interesting alternative due to its high content of functional compounds. The aim of this study was to investigate the effect of non-conventional modified atmosphere packaging (nitrogen (89.7% $N_2$, 10.3% $O_2$), argon (89.9% Ar, 10.1% $O_2$), helium (90.1% He, 9.9% $O_2$), nitric dioxide (89.3% $N_2O$, 10.7% $O_2$) and air (0.03% $CO_2$, 21% $O_2$)) on fresh-cut watercress leaves preserved for 13 days at 5 °C. The respiratory rate was reduced by the non-conventional atmosphere up to 3 days of storage, and no significant effects were observed on $C_2H_4$ production. In addition, mesophilic microbial growth was reduced up to 3 days of storage, and no effect was observed on psychrotrophic and Enterobactericeae counts. He and $N_2O$ atmospheres increased the antioxidant activity of watercress at the end of the storage period. Nevertheless, there was no clear effect of non-conventional gases on the color parameters, polyphenol contents and sensory parameters of fresh-cut watercress.

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## 1. Introduction

Watercress (*Nasturtium officinale* R. Br.) is a leafy vegetable of the *Brassicaceae* family that grows in and around water, and is highly appreciated due to its nutritional value. Watercress is considered a good source of essential vitamins, minerals and bioactive molecules that induce phase II enzymes that aid in the metabolism of xenobiotics, such as lutein, zeaxanthin, 7-methylsulfinylheptyl and 8-methylsulfinyloctyl isothiocyanates (Rose et al., 2000), that prevent carcinogenesis. Normally, fresh watercress leaves have a short shelf-life of approximately 7 days, which can be extended by managing storage conditions, namely the temperature and atmospheric composition. Recommended storage conditions are 0 °C and more than 95% RH, which conserves the leaves for 2–3 weeks (Hruschka and Wang, 1979).

Modified atmosphere packaging (MAP) is widely used to maximize the shelf-life of several fruit and vegetables. MAP is based on an increase in $CO_2$ and a decrease in $O_2$ concentrations, thus reducing metabolic activity. When properly used (taking into account

specific product requirements), it can effectively preserve the quality of fresh products (Sandhya, 2010). Therefore, watercress could benefit from the use of modified atmospheres, with recommended $CO_2$ levels above 7% and $O_2$ levels above 5% (Aharoni et al., 1989).

Recently, there has been great interest in the potential benefits of non-conventional MAP applications, a novel technology that replaces original atmospheric gas partial pressure with noble gases, such as helium (He), argon (Ar) or xenon (Xe), nitrous oxide ($N_2O$) or superatmospheric oxygen ($O_2$) (Artés et al., 2009). Non-conventional MAP has been successfully used to preserve fresh cut vegetables and fruit, although its commercial use requires further research.

Although the ability of the noble gases to be combined with other atoms is extremely limited, several studies have shown that they exert an effect on the metabolic activity of various vegetable products through unknown mechanisms. For example, Ar gas, which is a major component of the atmosphere inside packaging, reduces microbial growth and improves the quality retention of fresh produce such as broccoli, lettuce and arugula (Day, 1998; Jamie and Saltveit, 2002; Char et al., 2012). Ar is biochemically active due to its enhanced solubility in water compared to nitrogen ($N_2$), which is considered inert, and it also interferes with enzymatic oxygen receptor sites (Spencer, 1995). Therefore, an Ar-enriched atmosphere does not directly affect the metabolism of plant tissues by reducing the activity of enzymes, but rather, it enhances

the diffusion of gases such as $CO_2$ and ethylene ($C_2H_4$) from plant tissues because it is denser than the $N_2$ (Gorny and Agar, 1998).

Similarly, enriched He atmospheres increase $O_2$ diffusion, decreasing the concentration gradient between the inside and outside of the cell, which maintains ultra low $O_2$ concentrations, minimizing the risk of fermentation (Day, 1998). In addition, enriched He MAP reduced mesophilic bacteria counts on mizuna baby leaves, keeping it safe for consumers for 8 days at 5 °C (Robles et al., 2010). For enriched He and Ar atmospheres combined with $H_2O_2$, it has been reported that respiratory activity and microbial growth were reduced, color characteristics were retained and the bioactive compound content was increased in fresh cut arugula stored at 5 °C for 8 days (Char et al., 2012).

Another gas that has attracted research interest is $N_2O$, which is widely used in medicine and has a chemical structure similar to that of $CO_2$, providing advantageous physical properties, such as high solubility (Gouble et al., 1995). $N_2O$ partially inhibits respiration by affecting cytochrome oxidase C activity in the mitochondria, a phenomenon observed in isolated seeds, leaves or cell suspensions that decreases metabolism of the product and increases storage life (Sowa and Towill, 1991).

$N_2O$ gas inhibits ripening by extending the lag phase preceding the rise in ethylene, and it delays color change in pre-climacteric tomato, avocado and banana fruit (Gouble et al., 1995; Leshem and Wills, 1998; Palomer et al., 2005). The objective of this study was to evaluate the effect of non-conventional atmosphere packaging on the physiological and quality of fresh cut watercress during refrigerated storage.

## 2. Materials and methods

### 2.1. Plant material

Watercress (*Nastirtium officinale*) leaves were grown in a floating root hydroponic system for 30 days by Tango Hidrohuerta, a commercial grower located in Comuna de Calera de Tango (Región Metropolitana, Chile). The watercress leaves were hand-harvested using disinfected scissors. On the same day, the watercress leaves were transported at 7 °C in a thermal container to the Centro de Estudios Postcosecha (CEPOC) of Facultad de Ciencias Agronómicas, Universidad de Chile. The leaves were stored for 24 h at 5 °C and 95% RH in macro-perforated bags until further processing.

### 2.2. Sample preparation and treatment

Processing began by selecting the raw material and removing yellowing and damaged leaves. The leaves were then sanitized for 2 min in a sodium hypochlorite solution at 5 °C (100 mg L$^{-1}$), and the pH was adjusted to 6.5 using 2 N citric acid. Subsequently, the watercress leaves were rinsed in tapwater, drained on a stainless steel mesh for 3 min and spin-dried using a manual centrifuge for 2 min to eliminate excess water. Approximately 40 g of leaves were packaged in polypropylene (PP) bags (0.16 m × 0.22 m) with an $O_2$ permeability of 3000 mL m$^{-2}$ d$^{-1}$ and $CO_2$ permeability of 9000 mL m$^{-2}$ d$^{-1}$ at 23 °C (data provided by the supplier). The leaves were packaged in five different atmospheric conditions: air (0.03% $CO_2$, 21% $O_2$), nitrogen (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 nitric dioxide (89.3% $N_2O$, 10.7% $O_2$). The concentrations used were selected based on previous work in leaf products (Tomás-Callejas et al., 2011; Char et al., 2012).

$N_2$, Ar, He and $N_2O$ (99.99% purity) (Indura, Chile), were injected into the bags using a gas mixer just before heat sealing. In the case of leaves stored in air, 7 perforations of 0.5 mm were made in the bags. Three replicates of each treatment were analyzed after 1, 3, 6, 9 and 13 days of storage at 5 °C.

### 2.3. Respiration rate and $C_2H_4$ emission

Samples (200 g) were placed in 4 L plastic containers in a humidified atmosphere in which 94–96% of $N_2$, Ar, He or $N_2O$ were continuously injected at 5 °C for up to 6 days. The containers were closed for 1.5 h, and samples of 10 and 1 mL were collected from the headspace though a silicone septum using a plastic syringe to assess the respiration rate and $C_2H_4$ emission. Gas samples were analyzed using a gas chromatograph (GC) (Hewlett Packard 5890 Series II, USA) equipped with a thermal conductivity detector (Hewlett Packard, USA), with injector, oven and detector temperatures of 50, 50 and 200 °C, respectively. The carrier gas was He (Indura, Chile) at a pressure of 50 psi, and a commercial standard ($CO_2$ 10%) (Indura, Chile) was used. The respiration rate is expressed as mg $CO_2$ kg$^{-1}$ h$^{-1}$.

Ethylene was measured by injecting 1 mL sample into a gas chromatograph (Agilent Technologies 7820A, USA) equipped with a flame ionization detector and a 1.20 m × 3.18 mm column (Porapak QN 80/100, Norwalk, CT, USA) using He as the carrier at a flow rate of 60 mL min$^{-1}$. A commercial standard (0.5 ppm $C_2H_4$, Indura, Santiago, Chile) was used. $C_2H_4$ production is expressed as µL $C_2H_4$ kg$^{-1}$ h$^{-1}$. Three replicates were preformed for each treatment, and the samples were evaluated on days 1, 3 and 6.

### 2.4. Atmosphere composition

The $O_2$ and $CO_2$ concentrations inside the bags were monitored using a portable gas analyzer (Checkpoint, PBI Dansensor, Ringsted, Denmark) that was previously calibrated by sampling atmospheric air (0% $CO_2$ and 21% $O_2$). Gas samples were taken through a silicon septum affixed outside the bags. $O_2$ and $CO_2$ values are expressed as partial pressures. Simultaneously, 10 mL gas samples were withdrawn from the packages using a gas-tight syringe and analyzed in the same gas chromatograph used to determine the respiration rate. In this case $O_2$, $CO_2$ and $N_2$ concentration were determined. Ar and He concentrations in the bags were calculated using Eq. (1): [Ar] or [He] or [$N_2O$] = 100 − ([$O_2$] + [$CO_2$] + [$N_2$]), where the concentration values are expressed as a percentage (%).

### 2.5. Color measurement

Samples were placed on a black surface to reduce external interferences, and color was measured in the adaxial face of the leave using a compact tristimulus colorimeter (Minolta CR-300, Tokyo, Japan) equipped with a D65 illuminant source. The instrument was previously calibrated on a white plate ($Y$ = 92.6, $x$ = 0.3161, $y$ = 0.3325) at an observation angle of 0°. Data were collected on thirty randomly selected leaves. The values were expressed in the CIE Lab system parameters as lightness (L), hue angle (H*) and chroma (C*).

In addition, yellow coloration was evaluated using a scale with 5 categories, where 5 corresponded to dark green, 3 yellow green and 1 to yellow.

### 2.6. Microbiological growth

Standard enumeration methods were used to determine microbial growth. Three random samples of approximately 10 g of leaves were taken at each evaluation time, and homogenized in 90 mL of sterile buffered peptone water for 1 min in a sterile stomacher bags (Easy Mix, AES Chemunex, France). Serial dilutions were prepared in the same buffered solution for plating. Total aerobic mesophile and psychrotroph counts were assessed on plate count agar (PCA)

after 48 h or 7 days of incubation at 35 °C and 7 °C, respectively. *Enterobactericeae* enumeration was performed on violet red bile agar (VRBD) after 48 h incubation at 37 °C, and molds and yeast were assessed on potato dextrose agar acidified with 1% lactic acid after 7 days of incubation at 25 °C. All culture media were purchased from Merck (Darmstad, Germany).

## 2.7. Total polyphenol contents

Total polyphenol contents were determined from the extract obtained after homogenizing 1 g of frozen watercress leaves with 3 mL of methanol/water solution (4:1, v/v) in an Ultraturrax (Janke and Kunkel, Ika-Labortechnik, Germany) at $24,000 \times g$ for 1 min. Samples were placed on an ice bed in the dark and homogenized with a vortex for 0, 30 min and 1 h. The mixture was then centrifuged (Hermle Z 326 K, Hermle Labortechnink, Wehingen, Germany) at $10,000 \times g$ for 15 min at 4 °C. The amount of total phenolic compounds was determined using 0.5 mL of the Folin-Ciocalteu reagent:water solution (1:1, v/v) after vortexing for 15–20 s. After 3 min, 1 mL of saturated sodium carbonate (75 g/L) and 1 mL of distilled water was added. The reaction mixture was incubated in the dark for 1 h, and its absorption was measured at 765 nm. The results are expressed as gallic acid equivalents (GA equiv.) in $mg\,g^{-1}$ (fw) (Singleton and Rossi, 1965).

## 2.8. Total antioxidant capability

The antioxidant capability of watercress leaves was evaluated using the Ferric-reducing antioxidant power assay (FRAP), following the method proposed by Benzie and Strain (1996), with some modifications. For the extract preparation, 0.5 g of each replicate were crushed to a fine powder with liquid nitrogen. Then, 4.5 mL of an ethanol water solution (1:1, v/v) was added, and the mixture was homogenized in an Ultraturrax and centrifuged at $10,000 \times g$ for 30 min at 4 °C. FRAP reagent was prepared from 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ + 16 mL $C_2H_4O_2$ per liter, pH 3.6), TPTZ (2, 4, 6-tripyridyl-s-triazine) solution (10 mM in 40 mM HCl) and ferric chloride ($FeCl_3 \cdot 6H_2O$) solution (20 mM in distilled water) in a proportion of 10:1:1 (v/v). Extracted samples (40 μL) were collected in discardable cuvettes, and FRAP reagents (900 μL) were added before measuring absorbance using a spectrophotometer UV–vis (T 70, PG Instruments Ltd., Leicester, United Kingdom) at 593 nm. The calibration curve was obtained using Trolox as a standard, and the results are expressed as Trolox equivalents (T equiv.) in $mg\,g^{-1}$ (fw).

## 2.9. Sensory evaluation

Sensory evaluation was performed by a quantitative descriptive analysis using a semi-trained panel of 12 judges (aged 20–40) and a non-structured scale of 15 cm. Subsamples of three replicates from each treatment were evaluated on each determination day to evaluate the appearance, color and turgency.

## 2.10. Statistical analysis

Data were processed by analysis of variance (ANOVA) and are reported as the mean ± standard error of three replicates. The Infostat, version 2012 (Universidad Nacional de Córdoba, Argentina) software package was used for this analysis. Significant differences between treatments were analyzed using Tukey's test ($p \leq 0.05$).

**Table 1**
Effect of non conventional atmospheres ($N_2$, Ar, He and $N_2O$) and air storage on respiration rate ($mg\,CO_2\,kg^{-1}\,h^{-1}$) of fresh cut watercress at 5 °C during 6 days.

| Treatment | Storage time (days) | | |
|---|---|---|---|
| Raw material at harvest 66.24 ± 0.78 | | | |
| | 1 | 3 | 6 |
| Air | [a,b]74.5 ± 7.93 Aa | 39.1 ± 5.31 Ab | 34.6 ± 0.78 Ab |
| $N_2$ | 25.7 ± 6.65 Ca | 24.7 ± 3.15 Ba | 26.3 ± 0.63 Ca |
| Ar | 29.9 ± 1.48 BCa | 26.9 ± 2.75 Ba | 25.5 ± 0.18 Ca |
| He | 35.3 ± 1.73 Ba | 22.5 ± 1.01 Ba | 27.4 ± 1.33 BCa |
| $N_2O$ | 25.4 ± 0.95 Ca | 26.6 ± 1.03 Ba | 19.8 ± 1.08 Ca |

[a] Mean value ± standard deviation error of the means ($n = 3$).
[b] Means followed by different letters, uppercase and lowercase for columns and rows respectively, are statistically different according to Tukey test at $p \leq 0.05$.

**Table 2**
Effect of non conventional atmospheres ($N_2$, Ar, He and $N_2O$) and air storage on ethylene emission ($\mu L\,C_2H_4\,kg^{-1}\,h^{-1}$) of fresh cut watercress at 5 °C during 6 days.

| Treatment | Storage time (days) | | |
|---|---|---|---|
| | 1 | 3 | 6 |
| Air | [a,b]3.6 ± 0.65 Aa | 2.7 ± 0.19 Ab | 0.9 ± 0.08 Cc |
| $N_2$ | 1.8 ± 0.67 Ba | 2.3 ± 0.82 Ba | 1.8 ± 0.77 Aa |
| Ar | 1.9 ± 0.48 Bab | 2.1 ± 0.63 BCa | 1.5 ± 0.26 Ab |
| He | 2.1 ± 0.56 Ba | 2.3 ± 0.31 Ba | 1.3 ± 0.62 Ab |
| $N_2O$ | 1.3 ± 0.65 Ca | 1.7 ± 0.28 Ca | 1.4 ± 0.81 Aa |

[a] Mean value ± standard deviation error of the means ($n = 3$).
[b] Means followed by different letters, uppercase and lowercase for columns and rows respectively, are statistically different according to Tukey test at $p \leq 0.05$.

## 3. Results and discussion

### 3.1. Respiration rate and ethylene ($C_2H_4$) emission

Watercress leaves stored in air showed an initial $CO_2$ emission of 74.5 $mg\,kg^{-1}\,h^{-1}$, which is over twice the value recorded for leaves packed in the absence of conventional gases (Table 1). Nevertheless, after 3 days, the respiration rate of this treatment decreased by more than half, but it still remained higher than the leaves packaged without conventional gases. Moreover, watercress packed in $N_2$, Ar, He and $N_2O$ displayed a constant respiration rate, with an average value of 26.0 $CO_2\,mg\,kg^{-1}\,h^{-1}$ throughout the storage period, and there were no significant differences among the treatment groups.

The initial high respiration detected in leaves stored under air could be related to the stress generated during minimal processing operations, as the raw material showed a lower respiration rate (66.2 $mg\,CO_2\,kg^{-1}\,h^{-1}$) at the same time of evaluation (data not shown). This behavior was not observed on watercress packaged in non-conventional atmospheres, likely due to the lower $O_2$ level.

This initial stress effect has also been reported in different products and in fresh cut watercress stored at 5 °C (Hinojosa et al., 2013). Moreover, non-conventional modified atmosphere packaging decreased the initial rise in respiration and enabled the maintenance of low respiration for 6 days. A similar behavior was observed for $C_2H_4$ emission, where the highest initial value corresponded also to the watercress stored in air. However, after 3 and 6 days of storage, no differences were observed among treatment groups (Table 2).

The concentrations of the gases studied in this work were always kept at high levels (>80%) using daily injections, which could affect watercress metabolic activity. However, the increase in Ar, He and $N_2O$ concentrations also modified the $O_2$ and $CO_2$ levels, so changes in the metabolic activity of watercress could also be related to the reduction of $O_2$ and $CO_2$.

The reduced metabolic effect in fresh cut-products stored under non-conventional atmospheres has previously been reported in several different products. For example, a decrease in the respiration rate and $C_2H_4$ emission was observed in fresh-cut apple in response to treatment with high pressure Ar (150 MPa, 10 min). Contrary to our findings, the positive effect on metabolism was not delayed when Ar flux was applied for 10 min (Wu et al., 2012).

Regarding fresh cut leafy vegetables, our laboratory previously demonstrated that fresh cut arugula packaged in non-conventional modified atmospheres Ar (65–70% Ar + 5–6% $O_2$ balanced with $N_2$), He (70–75% He + 5–6% $O_2$ balanced with $N_2$) or $N_2$ (94–95% $N_2$ + 5–6% $O_2$) showed a progressive reduction in the respiration rate and $C_2H_4$ emission at 5 °C for 8 days (Char et al., 2012).

These effects on respiration and $C_2H_4$ emission may be because when noble gases dissolve in water, hydrophobic hydration occurs and enzymatic reactions are inhibited, resulting in restrained vegetable metabolism (Zhang et al., 2008).
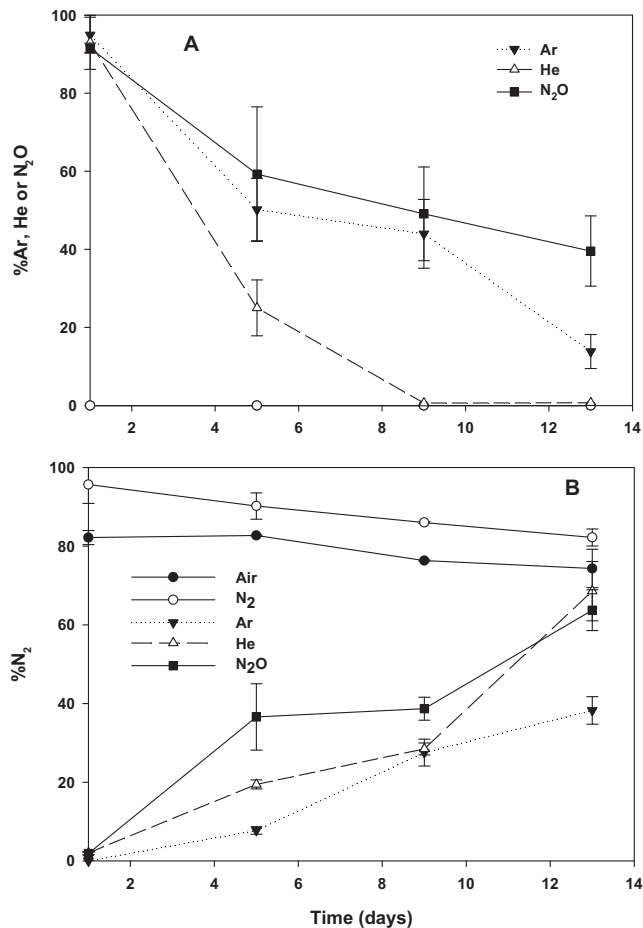
**Fig. 1.** Packaging gas composition of fresh cut watercress stored at 5 °C during 13 days expressed as percentage (%). (A) Argon (Ar); helium (He); and nitrous oxide ($N_2O$) and (B) nitrogen ($N_2$). Vertical bars indicate the standard error of the means ($n = 3$).



**Fig. 2.** Carbon dioxide and oxygen evolution inside fresh cut watercress packaging stored at 5 °C during 13 days expressed as percentage. (A) Carbon dioxide ($CO_2$) and (B) oxygen ($O_2$). Vertical bars indicate the standard error of the means ($n = 3$).

### 3.2. Gas concentration inside the bags

The initial levels of Ar, He and $N_2O$ inside the bags reached values of above 90% (Fig. 1A) and decreased during the storage period. The decline was particularly marked in the case of the He treatment, as the concentration dropped to 0% after 9 days of storage, which was much faster than the other gases. Ar and $N_2O$ concentrations decreased gradually, reaching final values of 13.8 and 39.6%, respectively.

The faster loss of He partial pressure observed in this study was also reported in fresh-cut red chard (Tomás-Callejas et al., 2011). The authors attributed this effect to the high gas permeability of the film, the small molecular size of the atom (0.005 nm atomic radius) and the high difference in He partial pressures inside and outside the packaging.

$N_2O$ fluctuation is likely related to its large molecular size and high solubility (77%) in vegetables cells (Gouble et al., 1995).

Moreover, the air-packaged leaves maintained constant $N_2$ levels throughout the storage period (over 80%), while the $N_2$ concentration in the bags packaged in this gas remained constant for 13 days in storage, with average values of 90%, because this concentration is similar to concentrations of $N_2$ present in the surrounding atmosphere. For the remaining treatments, a steady increase was observed over 5 days of storage, reaching final values of 38.6, 8.6 and 63.72% for Ar, He and $N_2O$ packaged leaves, respectively (Fig. 1B).

The changes in $CO_2$ and $O_2$ levels are displayed in Fig. 2A and B, respectively. In the $N_2$, Ar and He bags, $CO_2$ increased gradually, reaching storage values of approximately 4.3 and 4.8% at the end of the study. However, the leaves packaged in $N_2O$ atmosphere also displayed increased $CO_2$, but the final values only reached a maximum of 1.8% at the end of storage.

It is worth noting that $CO_2$ levels in non-conventional modified atmosphere packaged watercress remained lower than the values recommended by Aharoni et al. (1989), who argued that the levels of this gas must be greater than 7% to reduce the metabolic activity of the product. Our findings suggest that non-conventional atmosphere packaging influences the metabolism of plant tissues by both direct action and by competing with $O_2$.
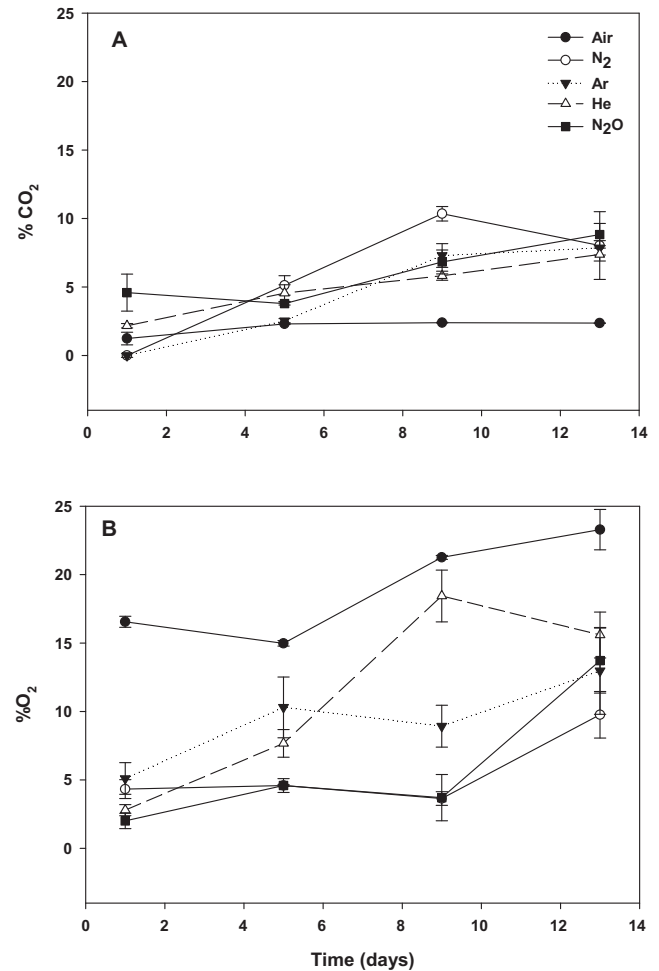
In most of the non-conventional packaging groups, $O_2$ levels were approximately 5% and above 0.5%, which is the theoretical limit for aerobic respiration at low temperature, throughout the entire storage period (Saltveit, 2003). According to Aharoni et al. (1989), the $O_2$ level should not be less than 5% in modified atmosphere conditions.

### 3.3. Color

Changes in color parameters are shown in Table 3. Throughout the storage period, a slight increase in lightness (L*) of leaves stored under different non-conventional atmospheres, with the exceptions of $N_2O$ and air, was observed, changing from an initial average value of 44 to a final average value of 49.

The Chroma (C*) values were slightly low for air and Ar packaged leaves at the beginning, even though there was no difference among treatments after 13 days of storage at 5 °C. In contrast, Hue angle was the highest at the beginning of the experiment, and the values decreased over time for all treatments, indicating leaf yellowing.

For visual analysis, using the color scale, none of the treatments was categorized below 3 throughout the storage, indicating an acceptable color from the point of view of the consumer.

Based on our results, there was no clear effect of non-conventional modified atmosphere packaging on color parameters, and the color of treated leaves did not improve compared to those stored in air. In contrast, arugula color parameters were substantially affected by non-conventional atmospheres (Char et al., 2012). A positive effect on the color of red chard packaged in He and $N_2$ active MAP (100 kPa initial gas partial pressure) maintained at 5 °C up to 8 days was also reported (Tomás-Callejas et al., 2011). Regardless, the direct effect of these gases on leaf color is unclear, especially because several other studies indicate that low $O_2$ and high $CO_2$ levels preserved the green color of various green vegetables for a longer time than air atmospheres by retarding chlorophyll degradation (Gómez and Artés, 2005; Fonseca et al., 2005; Tsouvaltzis et al., 2008).

**Table 3**
Effect of non conventional atmospheres ($N_2$, Ar, He and $N_2O$) and air storage on color parameters (lightness, $L^*$; chroma, $C^*$ and hue angle, Hab) of fresh cut watercress at 5 °C during 13 days.

| Treatment $L^*$ | Storage time (days) 1 | 3 | 6 | 9 | 13 |
|---|---|---|---|---|---|
| Air | [a,b] 44.4 ± 0.3 Ec | 47.2 ± 0.4 Ab | 47.8 ± 0.3 ABab | 47.2 ± 0.5 Ab | 49.5 ± 0.4 Aa |
| $N_2$ | 45.1 ± 0.4 Cc | 45.7 ± 0.4 Abc | 47.6 ± 1.1 ABab | 46.9 ± 0.5 Aabc | 48.6 ± 0.6 Aa |
| Ar | 44.7 ± 0.3 Dc | 45.8 ± 0.9 Abc | 46.2 ± 0.3 Cabc | 47.3 ± 0.4 Aab | 47.6 ± 0.3 Aba |
| He | 45.3 ± 0.5 Ba | 47.1 ± 0.4 Aa | 48.2 ± 0.8 Aa | 48.1 ± 0.4 Aa | 47.4 ± 0.4 ABa |
| $N_2O$ | 46.2 ± 0.4 Aa | 47.4 ± 0.4 Aa | 46.8 ± 0.6 BCa | 47.5 ± 0.5 Aa | 45.3 ± 0.5 Ba |
| $C^*$ | | | | | |
| Air | 26.9 ± 0.2 Ac | 28.6 ± 0.2 Abc | 30.1 ± 0.4 ABCab | 30.1 ± 0.3 Aab | 31.1 ± 0.2 Aa |
| $N_2$ | 29.5 ± 0.2 Aa | 28.5 ± 0.2 Aa | 30.5 ± 0.3 Aa | 29.6 ± 0.2 Aa | 30.6 ± 0.2 Aa |
| Ar | 27.1 ± 0.2 Ac | 28.9 ± 0.3 Ab | 29.1 ± 0.3 Cb | 29.6 ± 0.3 Aab | 30.7 ± 0.2 Aa |
| He | 29.1 ± 0.3 Ac | 30.4 ± 0.2 Ab | 30.4 ± 0.2 Cb | 30.5 ± 0.3 Aab | 30.1 ± 0.3 Aa |
| $N_2O$ | 29.9 ± 0.4 Aa | 30.2 ± 0.4 Aa | 29.3 ± 0.2 ABa | 30.3 ± 0.3 Aa | 29.8 ± 0.2 Aa |
| Hab | | | | | |
| Air | 127.1 ± 2.1 Aa | 124.1 ± 7.6 Ab | 124.2 ± 7.5 Ab | 124.3 ± 6.7 Ab | 122.4 ± 9.3 Ac |
| $N_2$ | 125.7 ± 9.6 Ba | 125.3 ± 2.3 Aa | 124.1 ± 4.2 Aab | 124.8 ± 9.1 Aab | 123.1 ± 2.7 Ab |
| Ar | 126.4 ± 4.3 Ca | 125.3 ± 5.3 Ab | 125.1 ± 2.1 Ab | 124.8 ± 5.7 Ab | 123.2 ± 7.8 Ac |
| He | 125.4 ± 2.5 Da | 124.1 ± 5.1 Aa | 123.9 ± 1.6 Aa | 123.8 ± 3.6 Aa | 123.2 ± 3.8 Aa |
| $N_2O$ | 124.9 ± 4.4 Ea | 124.7 ± 2.9 Aa | 124.9 ± 3.6 Aa | 123.9 ± 3.5 Aa | 123.7 ± 3.3 Aa |

[a] Mean value ± standard deviation error of the means ($n = 3$).
[b] Means followed by different letters, uppercase and lowercase for columns and rows respectively, are statistically different according to Tukey test at $p \leq 0.05$.

### 3.4. Microbial growth

Contrary to expectations, non-conventional atmosphere packaging did not substantially suppress microbial growth (Fig. 3). Although $N_2$ and Ar packaging delayed mesophilic growth for 3 days in storage at 5 °C, no significant differences were found among the treatments, and the counts reached values above 8 log units, surpassing the limit of 6 cfu $g^{-1}$ established by Chilean legislation (Fig. 3A).

No differences were found in the prevalence of psychrotrophic microorganism counts among the treatment groups at any evaluation time (Fig. 3B). The initial counts were proximal to 5 log units, and after 13 days of storage, the counts were above 8 log cfu $g^{-1}$.

*Enterobacteriaceae* showed a similar behavior to that of psychrotrophic microorganisms, with an initial average load of approximately 4.5 log cfu $g^{-1}$ and an average value of 7 log cfu $g^{-1}$ at the end of cold storage (Fig. 3C).

Our findings are similar to other reports regarding non-conventional atmospheres on microbial growth responses. According to Koseki and Itoh (2002), aerobic mesophilic counts on fresh cut lettuce and cabbage stored for 5 days at 5 °C under $N_2$ enriched MAP did not differ from passive MAP.

Tomás-Callejas et al. (2011) did not observe significant reductions in aerobic mesophilic and *Enterobacteriaceae* growth on fresh cut red chard disinfected with 100 mg $L^{-1}$ NaClO and packaged in He, $N_2$ and $N_2O$ enriched MAPs compared to passive MAP. In this work, the authors also mentioned that the He enriched atmosphere favored psychrotrophic bacteria growth.

Moreover, Wu et al. (2012) reported no differences in aerobic mesophilic, psychrotrophic, molds and yeast growth on apple wedges flushed with Ar gas for 10 min compared to air packaging (Wu et al., 2012). However, reductions of these microbial groups were reported when Ar was applied at high pressure (150 MPa), as the counts were below 5 log cfu $g^{-1}$ after 14 days of storage at 4 °C.

In addition, inconsistent results were reported for fresh-cut arugula packaged in non-conventional atmospheres similar to those tested in this work, as enrichment with He and $N_2$ combined with $H_2O_2$ as sanitizer showed less psychrotrophic growth after 8 days at 5 °C. Moreover, an enriched He atmosphere combined with $H_2O_2$ or NaClO maintained the lowest aerobic mesophilic and *Enterobacteriaceae* growth (Char et al., 2012).

### 3.5. Total polyphenol contents

Changes in the total polyphenol contents of fresh-cut watercress stored for 13 days at 5 °C are shown in Fig. 4. The initial total polyphenol content reached an average value of approximately 2 mg $g^{-1}$ f.w., where the lowest value corresponded to the leaves packaged in $N_2$. Polyphenol levels remained constant during 13 days of storage, with the same trend observed at the beginning of the experiment and higher levels in the leaves packaged in air, Ar and $N_2O$.

There have been several reports concerning the effect of non-conventional atmospheres, especially noble gases, on vegetables functional compounds. Fresh-cut red chard enriched with He, $O_2$ and $N_2$ MAPs had values of 93, 80 and 61%, respectively more than in conventional atmospheres, after 6 days of storage (Tomás-Callejas et al., 2011). However, at the end of storage, these treatments did not differ from the leaves packaged in MAP. Moreover, the reduction in total polyphenol values as a result of processing were restored, or even increased, by certain non-conventional packaging treatments, such as the enriched He atmosphere combined

with $H_2O_2$. After 8 days of storage, the arugula leaves treated with NaClO and enriched with both He and $N_2$ attained the highest polyphenol contents (Char et al., 2012). Considering the existing reports and the results obtained in this work, we cannot infer an effect of non-conventional atmospheres on polyphenol contents.

### 3.6. Antioxidant activity

As shown in Fig. 5, the highest initial antioxidant activity values corresponded to the watercress in air, followed by those stored in $N_2O$ conditions. The antioxidant activity increased until 3 days of storage, and a decline was observed in all treatments during storage. On day 13, the highest values corresponded to the leaves kept in enriched He and $N_2O$ atmospheres.

The total antioxidant activity of vegetables depends on many bioactive phytochemicals, such as flavonoids, phenolic acids, amino acids, ascorbic acid, tocopherols and pigments (Oms-Oliu et al., 2008; Murcia et al., 2009), and the relative weight of each compound depends on the product under consideration. Several authors have stated that phenolic compounds present higher antioxidant activity than vitamins and carotenoids (Podsedek, 2007; Murcia et al., 2009).

In the case of watercress, phenolic compounds may not be responsible for its antioxidant activity because treatments with greater phenolic compound content were not necessarily those with the highest antioxidant activity. Thus, the higher values observed after 13 days in He and $N_2O$ packaging watercress may be associated with higher vitamin C contents due to the lower $O_2$ and $CO_2$ levels, as both are involved in its oxidation (Gil et al., 1999; Ahn et al., 2005; Murcia et al., 2009). $CO_2$ stimulated oxidation by increasing ascorbate peroxidase activity or the inhibiting monodehydroascorbate and/or dehydroascorbate reductase with NADP/NADPH and glutathione as electron donors, which also increased dehydroascorbic acid levels (Murcia et al., 2009).

Among the studies that have evaluated the effect of non-conventional atmospheres on functional compounds, Char et al. (2012) reported a similar trend on fresh-cut arugula, where an initial increase in total antioxidant capability was observed and a positive effect of enriched He and $N_2$ atmospheres combined with NaClO maintained high levels (26 and 20%, respectively) (Char et al., 2012). However, in fresh-cut red chard, although there was a progressive loss of vitamin C during storage, the non-conventional atmospheres delayed vitamin C loss, which is one component of the antioxidant capacity of vegetable products (Tomás-Callejas et al., 2011).

### 3.7. Sensory evaluation

The different packaged atmospheres did not affect the initial appearance of watercress. However, during the storage period, a significant negative effect on this parameter was observed in $N_2O$ storage. This effect was not evident after 13 days of storage, where no differences were observed among treatments. However, the treatments were evaluated as acceptable for consumption by the panelists (data not shown).

No differences were detected in turgor for up to 6 days of storage at 5 °C (data not shown). In addition, there was no change in flavor in any treatment group or period of time (data not shown).
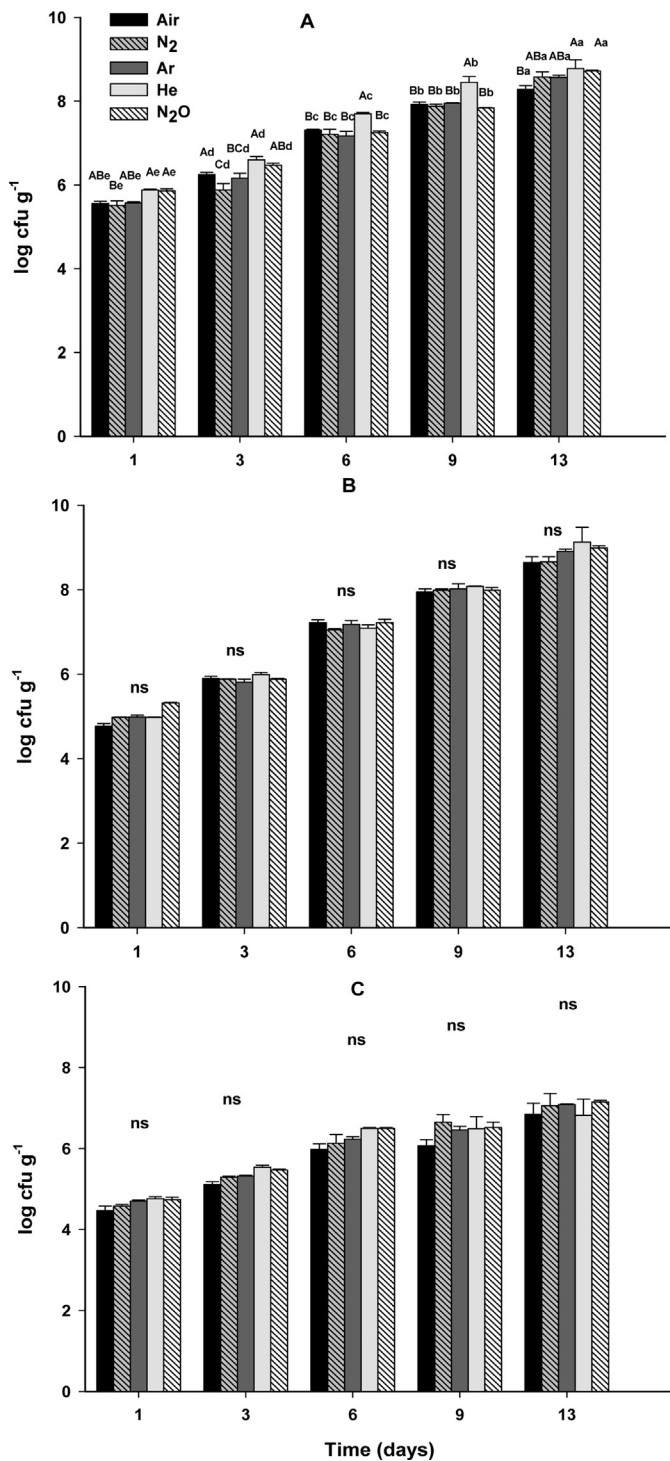
Fig. 3. Microbial growth of fresh cut watercress stored in air or in non-conventional atmospheres at 5 °C during 13 days. (A) Mesophilic (B) psychrotrophic and (C) *Enterobactericeae* growth. Vertical bars represent standard error of the means (n = 3). Means followed by different letters, uppercase and lowercase for time and treatment respectively, are statistically differences according to Tukey test at p ≤ 0.05; ns: not significant.

Related to the effect of the non-conventional atmospheres on sensory parameters of fresh-cut products, Wu et al. (2012) reported that apple wedges treated with high pressure Ar had higher scores in all sensory attributes assayed (color, odor, taste and overall preference), except on firmness, compared to the control after 12 days of storage at 4 °C.

Fresh cut red chard treated with different non-conventional atmospheres showed a moderate decrease in overall sensory quality parameters. There were no
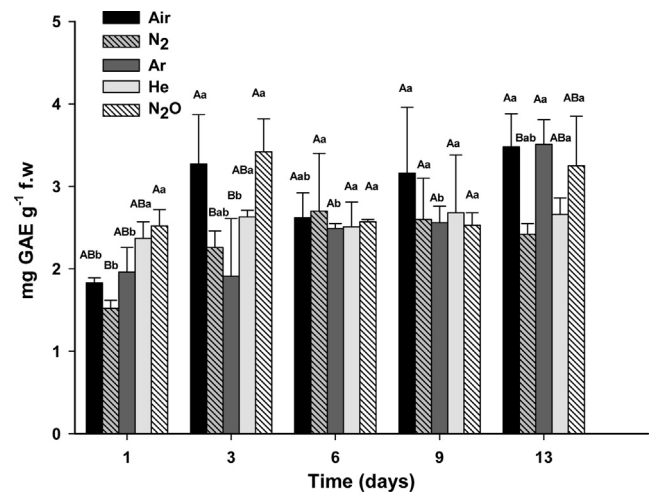


Fig. 4. Total antioxidant capacity of fresh cut watercress stored in air or in non-conventional atmospheres at 5 °C during 13 days. Vertical bars represent standard error of the means (n = 3). Means followed by different letters, uppercase and lowercase for time and treatment respectively, are statistically different according to Tukey test at p ≤ 0.05.
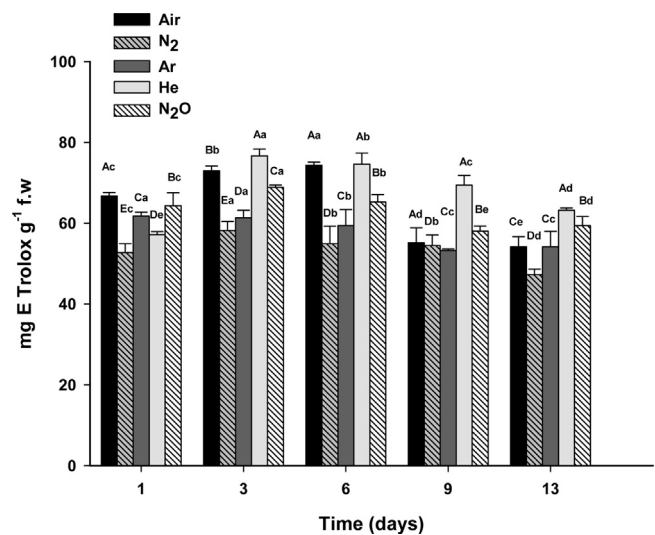


Fig. 5. Total polyphenols contents of fresh cut watercress stored in air or in non-conventional atmospheres at 5 °C during 13 days. Vertical bars represent standard error of the means (n = 3). Means followed by different letters, uppercase for time and lowercase to treatment, are statistically different according to Tukey test at p ≤ 0.05.

differences among treatments (including the control in passive MAP) after 8 days at 5 °C and they were scored at the limit of usability (Tomás-Callejas et al., 2011).

## 4. Conclusions

Watercress packaged in non-conventional atmospheres presented lower respiration rates and $C_2H_4$ emission compared to air packaging due to a synergistic effect between the low $O_2$ concentration and the non-conventional gases present inside the packages. Moreover, there was no clear effect of non-conventional atmospheres on microbial growth.

Antioxidant activity increased in He and $N_2O$ atmospheres at the end of the storage period, while no significant effects were observed on polyphenol contents, color or sensorial parameters. Our results suggest that non-conventional atmospheres, combined with other technologies that ensure low microbial counts at the beginning of the storage, could be used for watercress leaves.

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