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

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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.

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

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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)