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Postharvest quality changes in fresh-cut watercress stored under conventional and inert gas-enriched modified atmosphere packaging

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ABSTRACT

The effect of modified atmosphere packaging (MAP) on the postharvest quality of fresh-cut watercress (*Nasturtium officinale* R. Br.) stored at 4 °C for 7 d was studied. A portion of watercress was immediately analyzed (non-stored control) and the remaining fresh material was stored packaged under atmospheres enriched with N_2, Ar, air, or vacuum. The analyzed parameters included color, total soluble solids, pH, macronutrients, the individual profiles of sugars, organic acids, tocopherols and fatty acids, and total phenolics and flavonoids. Furthermore, four *in vitro* assays were performed to evaluate the antioxidant activity. After assessing the effect on individual quality parameters, it was possible to conclude that air was the less efficient atmosphere in preserving quality attributes of the non-stored control samples during cold storage. In turn, Ar-enriched MAP was the most suitable choice to preserve the overall postharvest quality. The present study also highlighted the nutritional and antioxidant properties of watercress, as well as the interest of its inclusion in human diets.

1. Introduction

As a response to consumers' demand for fresh, healthy and easy-to-prepare food products, conjoint with consumer lifestyle changes, with little time to prepare a convenient meal and to have a balanced diet, a wide variety of minimally processed vegetables has been developed (Ramos et al., 2013). Among them, watercress (*Nasturtium officinale* R. Br.) stands out due to its consumption since ancient times. This perennial species of the Brassicaceae family grows in and around water and is highly appreciated in the Mediterranean cuisine, being eaten raw in salads, soups and other recipes (Carvalho and Morales, 2013). Apart from its interesting nutritional value (Manchali et al., 2012; Pereira et al., 2011), this vegetable has medicinal and therapeutic properties (Alwi et al., 2010; Casanova and Carballo, 2011; Freitas et al., 2013; Hecht et al., 1995; Manchali et al., 2012; Sadeghi et al., 2014), mainly due to its high content in bioactive molecules.

A limiting factor that reduces watercress consumption is its perishable nature, characterized by a reduced shelf-life after harvest of approximately seven days (Cruz et al., 2009; Silveira et al., 2014). The main symptoms of quality loss are surface dehydration, softening of tissues and loss of green color. Most conventional postharvest treatments can not control all parameters necessary to extend produce shelf-life, without compromising its quality (Pinela and Ferreira, 2015). Additionally, consumers are looking for safe food products that suffer minimal processing with high quality retention (Ramos et al., 2013). To satisfy these requirements, it is necessary to design appropriate and more sustainable postharvest treatments, aiming to preserve the quality and extend the shelf-life of fresh vegetables including watercress. For this reason, novel postharvest technologies are being investigated by the food industry, such as modified atmosphere packaging (MAP) combined with cold storage (Pinela and Ferreira, 2015).

MAP is an economical and effective technology that involves altering the air surrounding the product in the package to another composition. Using this method, the initial fresh state of the product may be prolonged by reducing the metabolic activity and chemical oxidation, thus retarding compositional changes associated with maturation and senescence, reducing microorganism growth and retaining all attributes that consumers consider as freshness markers (Murcia et al., 2009; Niemira and Fan, 2014). Recently, the use of non-conventional argon (Ar)- and nitrogen (N_2)-enriched atmospheres has gained a considerable interest (Artés et al., 2009; Char et al., 2012). Ar is biochemically active, probably due to its enhanced solubility in water, and appears to

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interfere with enzymatic oxygen receptor sites, thus reducing metabolic activity of the food product (Char et al., 2012). This gas has also been reported to reduce microbial growth and to improve quality of fresh produce (Jamie and Saltveit, 2002). Regarding N_2, it has a low solubility in water and other food constituents and does not support the growth of aerobic microbes, thereby inhibiting the growth of aerobic spoilage (Sandhya, 2010). When properly used, this technology may preserve and extend the quality of food, allowing a longer period for commercialisation. Even so, the application of MAP to a specific food product, such as watercress, requires further research.

A previous study demonstrated the effectiveness of non-conventional MAP in preserving some quality attributes of fresh-cut watercress (Silveira et al., 2014). However, no clear effect of the studied gases on color, total polyphenols, microbial growth, or sensory parameters was verified. In this study we explored and compared the effects of conventional and non-conventional MAP enriched with inert gases on quality parameters of fresh-cut watercress stored at 4 °C for 7 d.

2. Materials and methods

2.1. Standards and reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47,885-U), other individual fatty acid isomers, tocopherols (α-, β-, and γ-isoforms), sugars (D(−)-fructose, D(+)-glucose anhydrous, D(+)-melezitose hydrate, D(+)-sucrose), organic acids (citric, malic, oxalic and fumaric acids), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and catechin standards were purchased from Sigma (St. Louis, MO, USA). Racemic tocol, 50 g L^{-1}, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH•)as obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (Millipore, model A10, Billerica, MA, USA).

2.2. Sampling and samples preparation

Watercress (*Nasturtium officinale* R. Br.) is claimed to have nutritional and healing properties when gathered in the proper season and phenological stage (Carvalho, 2010; Carvalho and Morales, 2013). Therefore, wild specimens were gathered in February 2014 in a local stream in the Bragança region (Trás-os-Montes, North-eastern Portugal), considering local consumers' sites, criteria and preferences. Subsequently, healthy and undamaged aerial parts (stalk and leaves) were selected, rinsed in tap water and drained to eliminate excess water. A portion of watercress was immediately analyzed (non-stored control), and the remaining fresh material was subjected to the treatments described below and analyzed in the end of the storage period. A voucher specimen was deposited in the Herbarium of the School of Agriculture of Bragança.

2.3. Samples packaging and storage

Approximately 20 g of watercress were placed in 11.5 cm × 15 cm sterilized packages made of low-density polyethylene film (black LDPE resin, thickness of 63 μm, the O_2 transmission rate was 7.99×10^{-7} L m^{-2} s^{-1} at 25 °C and standard pressure and the CO_2 transmission rate was 2.91×10^{-6} L m^{-2} s^{-1} at the same temperature and pressure conditions (VWR, Lisbon, Portugal); the headspace volume inside the packages was approximately 0.5 L) and packaged under four different atmospheres: (1) atmospheric air (control in passive MAP); (2) vacuum (no atmosphere); (3) N_2-enriched atmosphere; and (4) Ar-enriched atmosphere. Briefly, air-packaging consisted of sealing without eliminating the air in the package (20.8% O_2 and <0.1% CO_2) and vacuum-packaging was performed by eliminating the air with a domestic vacuum-packaging machine. For non-conventional MAP, the headspace air in the packages was first eliminated and then the target gas (100% N_2 or Ar) was injected.

A total of 40 packages were prepared, 10 for each treatment, and stored in the dark at 4 °C for 7 d.

2.4. Headspace gas analysis

The O_2, CO_2, and N_2 concentrations inside the packages were monitored using a portable gas analyzer (model Oxybaby 6.0, WITT, Denmark) previously calibrated by sampling atmospheric air. Ar concentration in the packages was calculated according to the equation: $100-([O_2]+[CO_2]+[N_2])$. Values were expressed as a percentage. Measurements were performed after packaging and at the end of the storage period.

2.5. Physical and physicochemical analysis

For color measurement, samples were placed on a black surface to reduce external interferences and data were collected on nine randomly selected leaves (adaxial surface) with a colorimeter (model CR-400; Konica Minolta Sensing Inc., Japan) previously calibrated using the standard white plate. Using illuminant C and the diaphragm opening of 8 mm, the CIE $L^*a^*b^*$ color space values were registered through the computerized system using a color data software "Spectra Magic Nx" (version CM-S100W 2.03.0006). Average values were considered to determine the color coordinates, where L^* represents lightness, a^* represents chromaticity on a green (−) to red (+) axis, and b^* represents chromaticity on a blue (−) to yellow (+) axis.

For total soluble solids (TSS) and pH determination, fresh tissue was ground and the grinding paste was subsequently filtered through Whatman No. 4 paper. The TSS content in the squeezed juice was measured with a digital hand refractometer (model HI 96801, Hanna Instruments, Woonsocket, RI, USA) and expressed as percentage (%). The pH was measured with a digital pH-meter (model pH 211, Hanna Instruments, Woonsocket, RI, USA) in the same juice.

2.6. Chemical composition analysis

Samples were analysed for moisture, proteins, fat, ash and carbohydrates using the AOAC procedures (AOAC, 2005). Briefly, the crude protein content (N × 6.25) was estimated by the macro-Kjeldahl method, using an automatic distillation and titration unit (model UDK152; VELP Scientifica, Italy); the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C; and total carbohydrates were calculated by difference. The results were expressed as g per kg of fresh weight. The total energy was calculated according to the equation: $4 \times (m_{proteins} + m_{carbohydrates}) + 9 \times (m_{fats})$ and further converted to kJ per kg of fresh weight.

Free sugars and tocopherols were determined by high performance liquid chromatography (HPLC) coupled to a refraction index detector (RI) or to a fluorescence detector (FP-2020; Jasco), respectively. Procedures and equipment were previously described by Pereira et al. (2011). The identification was made by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method,