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 ºC 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 colour, 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.

Keywords: *Nasturtium officinale*, fresh-cut watercress, conventional packaging, modified atmosphere packaging, refrigerated storage, postharvest quality

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 colour. Most conventional postharvest treatments can't 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 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 colour, 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 ºC 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 47885-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 ($DPPH^{\bullet}$) 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 ºC 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 ºC 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 colour 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 $L^*a^*b^*$ colour space values were registered through the computerized system using a colour data software "Spectra Magic Nx" (version CM-S100W 2.03.0006). Average values were considered to determine the colour 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 ºC; 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, wherein melezitose was used as internal standard for free sugars and tocol for tocopherols. The results were expressed in mg per kg of fresh weight.

Organic acids were analyzed by ultra fast liquid chromatography (UFLC) coupled to a photodiode array detector (PDA) according to Pereira et al. (2013). Briefly, fresh tissue (9 g) was ground and the grinding paste was subsequently extracted by stirring with 25 mL of meta-phosphoric acid (25 ºC at 150 rpm) for 45 min and subsequently filtered through Whatman No. 4 paper. Before analysis, samples were filtered through 0.2 μm nylon filters. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 nm or 245 nm (for ascorbic acid) with calibration curves obtained from commercial standards of each compound. The results were expressed in mg per kg of fresh weight.

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column. Procedures and equipment were described by Pereira et al. (2011). The identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.7. In vitro bioactive properties evaluation

Watercress extracts were prepared according to Pereira et al. (2011), using a mixture of methanol:water (80:20, v/v) as extraction solvent. Briefly, a fine dried powder (20 mesh; ~1 g) was stirred (150 rpm) with 50 mL of extraction solvent for 1 h at 25 ºC. The supernatant was filtered through Whatman No. 4 paper and the residue was re-extracted with an additional portion of solvent (50 mL). The combined extracts were then evaporated at 35 ºC under reduced pressure (Free Zone 4.5, Labconco; Kansas City, MO, USA), redissolved in the same solvent (final concentration 5 g L^{-1}) and successively diluted to different concentrations to evaluate their antioxidant activity and the total phenolic and flavonoid content.

Four different *in vitro* assays were performed to evaluate the hydromethanolic extracts antioxidant activity (Pinela et al., 2015): $DPPH^{\bullet}$ scavenging activity, reducing power (measured by ferricyanide Prussian blue assay), β-carotene blanching inhibition, and thiobarbituric acid reactive substances (TBARS) formation inhibition. Briefly, the $DPPH^{\bullet}$ scavenging activity and the reducing power assays were performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc; Winooski, VT, USA). The reduction of $DPPH^{\bullet}$ was determined by measuring the absorbance at 515 nm and calculated as a percentage of $DPPH^{\bullet}$ discolouration using the equation: $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_{DPPH} is the absorbance of the $DPPH^{\bullet}$ solution and A_S is the absorbance of the solution containing the sample extract. The reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm. The β-carotene bleaching inhibition was evaluated by measuring the capacity to neutralize linoleate free radicals, which was monitored at 470 nm in a Model 200 spectrophotometer (AnalytikJena, Jena, Germany), and calculated using the equation:

($A_{\beta T2}/A_{\beta T0}$) × 100, where $A_{\beta T2}$ is the absorbance of the emulsion after 2 h of incubation at 50 ºC and $A_{\beta T0}$ is the initial absorbance. The TBARS formation inhibition was evaluated in porcine (*Sus scrofa*) brain homogenates (brains were obtained from official slaughtered animals). The colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) complex formed during heating at 80 ºC for 20 min was measured at 532 nm, and the inhibition ratio calculated using the equation: [(A - B)/A] × 100%, where A and B correspond to the absorbance of the control and the sample solution, respectively. All results were expressed in EC_{50} values (g L^{-1}), *i.e.*, sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay. Trolox was used as positive control.

Total phenolic (Wolfe et al., 2003) and flavonoid (Jia et al., 1999) contents were quantified according to the authors in the hydromethanolic extracts concentrated at 0.625 or 1.25 g L^{-1} by reading the absorbance at 765 nm or 510 nm, respectively. The standard curves were calculated using gallic acid (for phenolics) and catechin (for flavonoids), and the results were respectively expressed as g of gallic acid equivalents (GAE) or catechin equivalents (CE) per kg of extract.

2.8. Statistical analysis

Samples of the 10 replicates of each treatment were divided into three batches and independently analyzed in triplicate for each quality parameter. Data were expressed as mean±standard deviation. All statistical tests were performed at a 5% significance level using SPSS Statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

The differences among treatments were analyzed using one-way analysis of variance (ANOVA). The fulfilment the ANOVA requirements, specifically the normal

distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Furthermore, a linear discriminant analysis (LDA) was used to evaluate the effect of the different packaging atmospheres on the overall postharvest quality of watercress samples. A stepwise technique, using the Wilks' λ method with the usual probabilities of *F* (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination processes, where the inclusion of a new variable is preceded by ensuring that all variables selected previously remain significant (Maroco, 2003). With this approach, it is possible to determine which of the independent variables account most for the differences in the average score profiles of the different treatments. To verify the significance of canonical discriminant functions, the Wilks' λ test was applied. A leaving-one-out cross-validation procedure was carried out to assess the model performance.

3. Results and discussion

3.1. Headspace gas composition

The initial levels of N_2 and Ar inside the non-conventional packaging systems reached values of above 98 and 95%, respectively. After 7 d of refrigerated storage, the N_2 concentration inside the N_2-enriched MAP decreased approximately 23%, while this gas evolved within the Ar-enriched MAP (constituting 27% of the headspace composition). In both MAP, the final content of CO_2 and O_2 were less than 16 and 9% for N_2- and Ar-enriched MAP, respectively. The final headspace gas composition of air-packaged samples revealed comparable values of N_2 (~73%), while the percentages of CO_2 and

O_2 were approximately 15 and 12%, respectively. The observed changes can be attributed to the plant respiration and diffusion of gases through the LDPE film (Choi et al., 2014).

3.2. Physical and physicochemical parameters

The green colour is an important attribute for the perception of watercress freshness. Based on the one-way ANOVA *p*-values it is possible to conclude that the assayed packaging atmospheres induced significant changes on *L** and *a** colour values, TSS and pH; but had no effect on *b** value (**Table 1**). Thus, the tested packaging atmospheres did not induce watercress yellowing. The *a** value was only significantly different in samples packaged in Ar-enriched atmospheres, which reveal the highest values, corresponding to an increased redness. Samples stored under Ar-enriched atmospheres showed also the highest lightness (*L**) values, followed by N_2-enriched MAP, air-packaging and vacuum-packaging, which showed similar values to the non-stored control. A slight increase of lightness in watercress samples stored under different non-conventional MAP was also reported by Silveira et al. (2014), but no clear effect of the tested non-conventional gases on colour parameters was reported. Slight increases were also found in fresh-cut red chard (*Beta vulgaris* var. cycla) baby leaves as a general trend for all treatments during storage at 5 ºC for 8 d (Tomás-Callejas et al. 2011). Similarly, Char et al. (2012) also recorded an increase in lightness with the storage time (8 d at 5 ºC) in ready-to-eat arugula (*Eruca vesicaria* Mill.) salads, suggesting incipient yellowing of leaves.

The TSS content in plant tissues is mainly related to the presence of soluble sugars and also smaller amounts of organic acids, vitamins and proteins. **Table 1** shows that the highest TSS values were found in control samples (day 0) and N_2-enriched MAP

samples. On the other hand, a significant reduction in TSS index was verified in vacuum-packaged and Ar-enriched MAP samples, probably related with over-maturation or senescence. The utilization of free sugars in various metabolic processes could also cause the decrease of TSS contents.

A decrease in pH values was observed in packaged samples compared to non-stored control (**Table 1**). This acidification was more marked in N_2-enriched MAP samples, while air-packaging was the most appropriate treatment to maintain the initial pH value. These results could be related to the increase of organic acids, namely malic acid, and reduced glucose levels, probably due to fermentative processes.

3.3. Chemical composition

The nutritional value and the sugars profiles obtained for the different packaging atmospheres are presented in **Table 2**. As it can be concluded from the one-way ANOVA *p*-values, the assayed packaging atmospheres induced significant changes in all nutritional parameters and quantified sugars. The single component with similar values for all packaging atmospheres was the ash content. In general, the moisture content increased in packaged samples, especially in those under vacuum and Ar-enriched atmospheres (it should be noticed that before packaging the samples were sprayed with water). Non-conventional MAP was the best treatment to retain the protein levels, while N_2 and air atmospheres seem to be suitable choices to preserve fat content and energetic value along storage time.

The nutritional value of watercress was already described. Pereira et al. (2011) reported lower protein content (~9.2 g kg^{-1}) and a slightly higher ash (~11.4 g kg^{-1}), fat (2 g kg^{-1}) and carbohydrate (45.1 g kg^{-1}) contents in watercress samples collected in the same region. Despite this, the energetic contribution (~983 kJ kg^{-1}) was similar to that

described in this study (~1023 kJ kg^{-1}) for non-stored control samples. Other work describes a higher protein (30 g kg^{-1}) and fat (10 g kg^{-1}) content, but much lower values for carbohydrates (3.5 g kg^{-1}) (Manchali et al., 2012). Nevertheless, the protein and fat contents described in this work for the non-stored control samples are very similar to those present in the USDA National Nutrient Database for Standard Reference (23 and 1 g kg^{-1}, respectively) (USDA, 2011). These small differences in the reported values may be explained by the use of different analytical procedures and slight differences in the phenological stage of watercress in the collection period, as well as variations on soil characteristics and annual climatic conditions of the locations where the samples were collected, which may affect the plant composition during the growing season (Nikolopoulou et al., 2007).

Fructose, glucose and sucrose were identified in watercress samples, being fructose the most abundant. Fructose, glucose and total sugars gave the highest values in control samples, while sucrose was higher in packaged samples, especially in those stored under conventional packaging systems. Among treatments, vacuum-packaging retained the highest levels of fructose, sucrose and total sugars. No significant statistical differences were found among treatments for glucose. The decrease of fructose and glucose contents in stored samples can be related to its use by the plant to produce the energy required to its metabolism, once reducing sugars are the main substrates in the respiration process. Similar results were found by Workneh et al. (2012) which reported lower levels of glucose and an increase in the sucrose content on tomatoes during the first 8 days of storage at 13 °C, after disinfection treatments and packaging in micro-perforated packages. Besides these sugars, Pereira et al. (2011) also reported the presence of trehalose and raffinose in wild samples of watercress; being sucrose the main sugar in those samples, followed by fructose and glucose. The total sugars content

of our non-stored control (~2034 mg kg^{-1}), analysed immediately after harvest, was similar to that described by other authors, *i.e.*, 2099 mg kg^{-1} (Pereira et al., 2011) and 2000 mg kg^{-1} (USDA, 2011).

Likewise, the organic acids content was also affected by the tested packaging atmospheres. Oxalic, malic, citric and fumaric acids were detected (**Table 2**), being oxalic acid the most abundant. Levels of this organic acid were higher in the non-stored control and samples stored under N_2-enriched atmospheres. Citric acid was also found in higher amounts in non-stored control samples. Regarding malic and fumaric acids, their values were higher in air-packaged samples. In general, air-packaging and N_2-enriched MAP increased the total organic acid levels while vacuum-packaging and Ar-enriched MAP showed lower values than the non-stored control.

Contrarily to some available results, reporting the presence of ascorbic acid in watercress (Cruz et al., 2008 and 2009; Gonçalves et al., 2009; Pereira et al., 2011), this vitamin was not detected in our samples or in those previously analyzed by Pereira et al. (2013).

The results for fatty acids composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the ratios of PUFA/SFA and n-6/n-3 are shown in **Table 3**. From the one-way ANOVA *p*-values, we can conclude that the assayed packaging atmospheres induced significant changes in the detected fatty acids and given ratios. Up to 23 fatty acids were detected in most of the samples, being palmitic (C16:0) and α-linolenic (C18:3n3) acids the most abundant, in agreement to Pereira et al. (2011). Palmitic acid gave lower values in stored samples, while α-linolenic acid increased under the same conditions. Other abundant fatty acids were linoleic (C18:2n6), stearic (C18:0), lignoceric (C24:0) and eicosadienoic (C20:2) acids. Both palmitoleic (C16:1) and nervonic acid (C24:1) were

only detected in samples submitted to refrigerated storage. SFA (~56.8%) levels were higher than MUFA (~1.03%) and PUFA (~42.2%) in control samples. All the assayed packaging atmospheres induced positive effects, namely an increase in MUFA and PUFA contents and PUFA/SFA ratios, and decreased SFA levels and n-6/n-3 ratios, as recommended for a "good nutritional quality" (Guil et al., 1996). N_2-packaged samples reveal the lowest SFA (~31.4%) content and the highest values of PUFA (~66.1%), as well as the highest PUFA/SFA ratios (~2.11) and lowest n-6/n-3 polyunsaturated fatty acid ratios (~0.19).

The assayed treatments induced also significant changes in the tocopherols content (**Tables 3**), with the lowest values being recorded in control samples. α-Tocopherol was always the most abundant isoform, followed by γ-tocopherol and β-tocopherol, in accordance with the results previously reported by Pereira et al. (2011). Air-packaged samples reveal the highest content of α- and β-tocopherol, and total tocopherols content. The highest values for γ-tocopherol were shown by the vacuum-packaged samples. In general, non-conventional MAP was able to maintain similar levels of β- and γ-isoforms, comparatively with the non-stored control, whilst vacuum-packaging presented the closest ones regarding the total tocopherols content. According to the literature, stress conditions can induce an increase in the total tocopherol levels (Munné-Bosch, 2005; Yusuf et al., 2010). In these situations, there is an increase in tocopherol synthesis, followed by a net tocopherol loss. The initial enhanced tocopherol levels contribute to tissue protection by reducing reactive oxygen species levels and inhibiting lipid peroxidation, thus avoiding oxidative damage (Munné-Bosch, 2005). Additionally, α-tocopherol plays a major role in the alleviation of stress, and its levels change significantly under stress (such as that induced by the different packaging conditions), as a result of the altered expression of pathway-related genes, degradation and recycling

(Munné-Bosch, 2005). Thus, tocopherol increases verified in this work can be related to the assayed packaging atmosphere incapacity to maintain favourable preservation conditions to watercress. Nevertheless, the observed increase contributes to a higher bioactivity.

3.4. In vitro bioactive properties

Table 4 presents the values for antioxidant activity and total phenolic and flavonoid contents of the watercress extracts. From the one-way ANOVA *p*-values, it can be concluded that the assayed packaging atmospheres induced significant changes in these parameters. Non-stored control samples showed the highest DPPH$^{\bullet}$ scavenging activity, reducing power and β-carotene blanching inhibition capacity. Regarding the assayed packaging atmospheres, Ar-enriched MAP were the best treatment to preserve the DPPH$^{\bullet}$ scavenging activity and β-carotene blanching inhibition capacity, showing also an increase in the total phenolics content. In turn, samples under N_2-enriched MAP present lower lipid peroxidation inhibition capacity, despite the high levels of total phenolics and flavonoids. The TBARS formation inhibition capacity was improved by conventional packaging relatively to the non-stored control samples. However, air-packaging gave the highest EC_{50} values (corresponding to the lowest antioxidant activity) in the DPPH$^{\bullet}$ and reducing power assays.

Similar phenolic (96-97 g GAE kg^{-1} extract) and higher flavonoid (62-63 g CE kg^{-1} extract) contents were reported by Yazdanparast et al. (2008) and Bahramikia and Yazdanparast (2010) in hydro-ethanolic extracts of a watercress sample from Iran, as well as analogous TBARS inhibition properties (0.27 g L^{-1}). On the other hand, the study conducted by Pereira et al. (2011) reported lower values for phenolics (~50.42 g GAE kg^{-1} extract) but similar flavonoids content for the control samples (~35.17 g CE

kg^{-1} extract) in methanolic extracts. Regarding its antioxidant performance, assessed using the same *in vitro* methodologies, only the $DPPH^{\bullet}$ scavenging capacity gave better results (~0.13 g L^{-1}) in comparison with our hydromethanolic extracts (except for the N_2-packaged sample which showed a higher EC_{50} value in the TBARS assay). Regarding the effect of non-conventional MAP on the total polyphenols content of watercress, Silveira et al. (2014) reported initial contents of ~2 g GAE kg^{-1} extract (obtained after homogenizing 1 g of frozen watercress with 3 mL of methanol/water (4:1, v/v)), where the lowest value corresponded to the samples packaged in N_2-enriched atmospheres. They also found maintenance on polyphenol levels during 13 days of storage, with the same trend observed at the beginning of the experiment, and higher levels in air-packaged and Ar-enriched MAP samples. An antioxidant activity increase was verified in He and N_2O packaged samples at the end of the storage period. Tomás-Callejas et al. (2011) reported an increase of 61% in the polyphenol content of red chard stored under N_2-enriched MAP for 6 d and attributed this behavior to the wound-induced phenomenon in phenolic metabolism.

3.5. Overall postharvest quality assessment using LDA

After evaluating the effects on individual quality parameters, a LDA was applied to obtain a complete perspective about the effects of MAP on the overall postharvest quality of fresh-cut watercress. The basic purpose of this discriminant analysis was finding the packaging atmosphere (categorical dependent variable) that maintained physicochemical, nutritional and antioxidant profiles (set of quantitative independent variables) of the non-stored control samples throughout the storage time. The significant independent variables were selected following the stepwise method of the LDA,

according to the Wilks' λ test. Only variables with a statistically significant classification performance ($p < 0.05$) were kept in the analysis.

The discriminant model selected 4 significant functions, which included 100.0% of the observed variance. The graph representation (**Figure 1**) of the three first functions (function 1: 67.7%, function 2: 19.0%, function 3: 8.0%) showed the complete individualization of markers corresponding to each packaging atmosphere. Function 1, mainly correlated with C18:3 and β-carotene bleaching inhibition, as deduced from the canonical discriminant functions standardized coefficients, separated primarily air-packaged and N_2-enriched MAP samples, which were also placed far from the non-stored control samples through function 2 (more correlated to capric acid (C10:0) and C18:3). Function 3, more powerfully correlated with TBARS formation inhibition and MUFA content, clarified that air-packaged samples were those with the most dissimilar chemical and antioxidant profiles, in comparison to the non-stored control samples. The complete individualization of the four packaging atmospheres (100.0% of the assayed species for the originally grouped cases as well as for the cross-validated cases) indicates their lack of effectiveness in maintaining the original quality attributes of the non-stored watercress samples during refrigerated storage.

4. Conclusions

None of the tested packaging atmospheres induced watercress yellowing. In general, sucrose levels increased with refrigerated storage, especially in samples under conventional packaging; while vacuum-packaging preserved the highest levels of fructose and total sugars. Tocopherols also increased in stored samples, being the highest levels of α-, β- and total tocopherols recorded in air-packaged samples, which may have been caused by stress conditions. All packaged samples showed higher

MUFA and PUFA contents and PUFA/SFA ratios and lower SFA levels and n-6/n-3 ratios. Furthermore, Ar-enriched MAP was the best option to preserve the $DPPH^{\bullet}$ scavenging activity and β-carotene blanching inhibition capacity, as well as to increase the total phenolic content. Based on the LDA, it was possible to conclude that air-packaged samples were those that presented the most dissimilar profiles from the non-stored control samples, followed by those stored under N_2-enriched MAP. In turn, Ar-enriched MAP was the most suitable choice to preserve the overall postharvest quality of fresh-cut watercress. The present study also highlights the nutritional and health-promoting composition of these wild watercress samples, as well as the interest of its inclusion in traditional and modern diets. Despite these findings, further studies are of interest to evaluate other quality attributes as well as physiological parameters.

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Figure captions

Figure 1. Biplot of object scores (packaging atmospheres) and component loadings (evaluated quality parameters).

Table 1 Colour parameters, TSS (%) and pH values for fresh-cut watercress samples stored under conventional and inert gas-enriched MAP.[1]

	Harvest (day 0)	7 d of storage at 4 °C				*p*-values		
	Non-stored control	Conventional packaging		Non-conventional MAP		Homoscedasticity[2]	Normal distribution[3]	One-way ANOVA[4]
		Air	Vacuum	N_2	Ar			
*L**	39 ± 3^{c}	45 ± 2^{b}	41 ± 2^{c}	46 ± 3^{ab}	49 ± 4^{a}	0.t002	0.390	<0.001
*a**	-17 ± 2^{b}	-18 ± 1^{b}	-17 ± 2^{b}	-17 ± 1^{b}	-14 ± 2^{a}	0.720	0.033	<0.001
*b**	27 ± 2	31 ± 4	27 ± 3	30 ± 3	31 ± 5	0.204	0.016	0.050
TSS	3.4 ± 0.1^{a}	3.2 ± 0.1^{b}	2.9 ± 0.1^{c}	3.5 ± 0.1^{a}	3.0 ± 0.1^{c}	0.253	0.011	<0.001
pH	5.49 ± 0.04^{a}	5.40 ± 0.05^{b}	5.36 ± 0.04^{c}	5.23 ± 0.03^{d}	5.32 ± 0.04^{c}	0.575	0.484	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among packaging systems was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one packaging atmosphere differs from the others (in this case multiple comparison tests were performed). For each storage condition, means within a row with different letters differ significantly ($p<0.05$).
*L** (lightness ↔ darkness); *a** (redness ↔ greenness); *b** (yellowness ↔ blueness). TSS - total soluble solids.

Table 2 Proximate composition (g kg^{-1}), energetic value (kJ kg^{-1}), individual sugars (mg kg^{-1}) and organic acids (mg kg^{-1}) for fresh-cut watercress samples stored under conventional and inert gas-enriched MAP.[1]

	Harvest (day 0)	7 d of storage at 4 °C				*p*-values		
	Non-stored control	Conventional packaging		Non-conventional MAP		Homoscedasticity[2]	Normal distribution[3]	One-way ANOVA[4]
		Air	Vacuum	N_2	Ar			
Moisture	931 ± 10^{b}	941 ± 10^{ab}	945 ± 10^{a}	940 ± 10^{ab}	943 ± 10^{a}	0.860	0.346	0.001
Ash	9.4 ± 0.9	8.8 ± 0.4	8.7 ± 0.7	8.7 ± 0.7	8.9 ± 0.8	0.027	0.465	0.299
Proteins	22.4 ± 0.7^{a}	19.4 ± 0.6^{c}	19.8 ± 0.7^{c}	21.4 ± 0.9^{ab}	21.3 ± 0.7^{b}	0.697	0.496	<0.001
Fat	1.43 ± 0.08^{a}	1.3 ± 0.1^{ab}	1.2 ± 0.1^{b}	1.41 ± 0.08^{a}	1.27 ± 0.09^{b}	0.607	0.272	<0.001
Carbohydrates	35.6 ± 0.9^{a}	30 ± 1^{b}	25 ± 1^{d}	28 ± 1^{c}	26 ± 1^{d}	0.954	0.001	<0.001
Energy	1023 ± 15^{a}	869 ± 28^{b}	799 ± 18^{d}	877 ± 20^{b}	841 ± 17^{c}	0.651	<0.001	<0.001
Fructose	1104 ± 31^{a}	681 ± 28^{c}	977 ± 28^{b}	628 ± 99^{cd}	584 ± 42^{d}	<0.001	<0.001	<0.001
Glucose	696 ± 20^{a}	320 ± 41^{b}	347 ± 79^{b}	349 ± 25^{b}	324 ± 34^{b}	0.006	<0.001	<0.001
Sucrose	233 ± 51^{c}	446 ± 37^{a}	495 ± 57^{a}	380 ± 58^{b}	440 ± 30^{ab}	0.113	0.008	<0.001
Total sugars	2034 ± 31^{a}	1447 ± 38^{c}	1818 ± 56^{b}	1357 ± 159^{c}	1347 ± 46^{c}	0.001	0.001	<0.001
Oxalic acid	7541 ± 244^{a}	6319 ± 219^{b}	5507 ± 260^{c}	7262 ± 215^{a}	6442 ± 322^{b}	0.465	0.189	<0.001
Malic acid	730 ± 17^{d}	2892 ± 143^{a}	2109 ± 133^{b}	1988 ± 193^{bc}	1849 ± 77^{c}	0.002	0.001	<0.001
Citric acid	1089 ± 39^{a}	1000 ± 60^{b}	718 ± 52^{c}	974 ± 78^{b}	624 ± 77^{d}	0.179	0.001	<0.001
Fumaric acid	3.5 ± 0.2^{d}	15.0 ± 0.8^{a}	8.7 ± 0.7^{b}	6.2 ± 0.7^{c}	7.0 ± 0.5^{c}	0.027	<0.001	<0.001
Total organic acids	9364 ± 216^{b}	10226 ± 365^{a}	8343 ± 209^{d}	10230 ± 19^{a}	8922 ± 387^{c}	0.407	0.044	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among packaging systems was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one packaging atmosphere differs from the others (in this case multiple comparison tests were performed). For each storage condition, means within a row with different letters differ significantly ($p<0.05$).

Table 3 Fatty acids (relative percentage) and tocopherols (mg kg^{-1}) for fresh-cut watercress samples stored under conventional and inert gas-enriched MAP.[1]

	Harvest (day 0)	7 d of storage at 4 °C				*p*-values		
	Non-stored control	Conventional packaging		Non-conventional MAP		Homoscedasticity[2]	Normal distribution[3]	One-way ANOVA[4]
		Air	Vacuum	N_2	Ar			
C6:0	0.014 ± 0.002^{d}	0.097 ± 0.002^{a}	0.030 ± 0.002^{c}	0.039 ± 0.003^{b}	0.040 ± 0.005^{b}	0.003	<0.001	<0.001
C8:0	0.048 ± 0.005^{c}	0.130 ± 0.001^{a}	0.062 ± 0.004^{b}	0.044 ± 0.003^{c}	0.066 ± 0.005^{b}	0.004	<0.001	<0.001
C10:0	0.063 ± 0.005^{c}	0.373 ± 0.005^{a}	0.068 ± 0.004^{c}	0.060 ± 0.004^{c}	0.09 ± 0.01^{b}	0.001	<0.001	<0.001
C12:0	0.225 ± 0.004^{b}	0.317 ± 0.004^{a}	0.17 ± 0.01^{c}	0.115 ± 0.007^{e}	0.154 ± 0.005^{d}	0.031	<0.001	<0.001
C14:0	1.7 ± 0.1^{b}	1.5 ± 0.1^{c}	1.22 ± 0.05^{e}	1.35 ± 0.05^{d}	1.89 ± 0.05^{a}	0.640	0.150	<0.001
C15:0	0.38 ± 0.04^{c}	0.41 ± 0.01^{bc}	0.46 ± 0.02^{b}	0.43 ± 0.04^{bc}	0.59 ± 0.03^{a}	0.048	0.004	<0.001
C16:0	45 ± 1^{a}	29 ± 1^{c}	25.5 ± 0.4^{d}	22.9 ± 0.5^{e}	32.2 ± 0.5^{b}	0.143	<0.001	<0.001
C16:1	nd	0.17 ± 0.01^{d}	0.37 ± 0.03^{a}	0.30 ± 0.01^{c}	0.33 ± 0.04^{b}	<0.001	<0.001	<0.001
C17:0	0.89 ± 0.04^{a}	0.38 ± 0.03^{d}	0.75 ± 0.04^{b}	0.32 ± 0.01^{e}	0.57 ± 0.04^{c}	0.105	<0.001	<0.001
C18:0	4.1 ± 0.4^{a}	4.4 ± 0.1^{a}	3.1 ± 0.3^{b}	2.9 ± 0.2^{b}	4.17 ± 0.1^{a}	0.088	0.020	<0.001
C18:1n9	0.61 ± 0.05^{d}	1.47 ± 0.01^{b}	1.43 ± 0.05^{b}	1.19 ± 0.05^{c}	1.6 ± 0.2^{a}	0.036	<0.001	<0.001
C18:2n6	8.4 ± 0.5^{c}	10.3 ± 0.1^{a}	9.3 ± 0.3^{b}	9.0 ± 0.4^{bc}	9.1 ± 0.2^{b}	0.068	0.041	<0.001
C18:3n3	27 ± 1^{e}	43 ± 1^{c}	48.4 ± 0.5^{b}	54.6 ± 0.4^{a}	38.2 ± 0.5^{d}	0.636	0.002	<0.001
C20:0	0.25 ± 0.04^{d}	0.50 ± 0.01^{b}	0.61 ± 0.04^{a}	0.38 ± 0.02^{c}	0.57 ± 0.05^{a}	0.022	0.081	<0.001
C20:1	0.42 ± 0.05^{a}	0.18 ± 0.01^{b}	0.12 ± 0.01^{c}	0.09 ± 0.01^{c}	0.19 ± 0.01^{b}	<0.001	<0.001	<0.001
C20:2	2.3 ± 0.3^{a}	1.2 ± 0.1^{c}	2.5 ± 0.2^{a}	1.0 ± 0.1^{c}	1.7 ± 0.1^{b}	0.015	0.002	<0.001
C20:4n6	1.2 ± 0.2^{a}	0.53 ± 0.02^{c}	0.56 ± 0.05^{c}	0.19 ± 0.02^{d}	1.02 ± 0.05^{b}	<0.001	0.006	<0.001
C20:3n3+C21:0	1.0 ± 0.1^{a}	0.68 ± 0.03^{c}	0.76 ± 0.05^{bc}	0.72 ± 0.05^{c}	0.84 ± 0.05^{b}	0.066	0.002	<0.001
C20:5n3	0.3 ± 0.1^{b}	0.2 ± 0.1^{b}	0.9 ± 0.1^{a}	0.13 ± 0.01^{b}	1.0 ± 0.1^{a}	0.001	<0.001	<0.001

C22:0	1.3 ± 0.1^{a}	0.9 ± 0.1^{b}	0.90 ± 0.05^{b}	0.85 ± 0.03^{b}	1.26 ± 0.05^{a}	0.006	<0.001	<0.001
C22:2	1.6 ± 0.1^{a}	0.57 ± 0.01^{b}	0.18 ± 0.01^{d}	0.34 ± 0.03^{c}	0.53 ± 0.05^{b}	<0.001	<0.001	<0.001
C24:0	2.8 ± 0.2^{a}	2.6 ± 0.1^{a}	1.7 ± 0.1^{b}	2.0 ± 0.1^{b}	2.7 ± 0.2^{a}	0.368	0.065	<0.001
C24:1	nd	0.8 ± 0.1^{c}	0.93 ± 0.05^{bc}	1.02 ± 0.05^{b}	1.21 ± 0.05^{a}	<0.001	<0.001	<0.001
SFA	57 ± 1^{a}	40 ± 1^{c}	34.6 ± 0.5^{d}	31.4 ± 0.3^{e}	44 ± 1^{b}	0.218	<0.001	<0.001
MUFA	1.0 ± 0.1^{d}	2.6 ± 0.2^{c}	2.85 ± 0.05^{b}	2.59 ± 0.05^{c}	3.3 ± 0.1^{a}	0.002	<0.001	<0.001
PUFA	42 ± 1^{e}	57 ± 1^{c}	63 ± 1^{b}	66 ± 1^{a}	52 ± 1^{d}	0.280	<0.001	<0.001
PUFA/SFA	0.74 ± 0.02^{e}	1.41 ± 0.05^{c}	1.81 ± 0.04^{b}	2.11 ± 0.03^{a}	1.18 ± 0.03^{d}	0.163	0.003	<0.001
n-6/n-3	0.47 ± 0.03^{a}	0.28 ± 0.01^{c}	0.25 ± 0.01^{d}	0.19 ± 0.01^{e}	0.31 ± 0.01^{b}	0.001	<0.001	<0.001
α-tocopherol	5.0 ± 0.3^{d}	12.4 ± 0.8^{a}	7.9 ± 0.1^{c}	11.2 ± 0.8^{b}	10.7 ± 0.1^{b}	<0.001	0.001	<0.001
β-tocopherol	0.11 ± 0.01^{b}	0.18 ± 0.02^{a}	0.10 ± 0.01^{bc}	0.09 ± 0.01^{c}	0.09 ± 0.01^{c}	0.073	<0.001	<0.001
γ-tocopherol	0.38 ± 0.03^{d}	0.63 ± 0.04^{b}	0.70 ± 0.05^{a}	0.51 ± 0.05^{c}	0.43 ± 0.03^{d}	0.306	0.017	<0.001
Total tocopherols	5.5 ± 0.4^{d}	13.2 ± 0.8^{a}	8.7 ± 0.1^{c}	11.8 ± 0.8^{b}	11.2 ± 0.1^{b}	<0.001	0.001	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among packaging systems was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one storage atmosphere differs from the others (in this case multiple comparison tests were performed). For each storage condition, means within a row with different letters differ significantly ($p<0.05$).

Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9); Linoleic acid (C18:2n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); *cis*-11-Eicosenoic acid (C20:1); *cis*-11,14-Eicosadienoic acid (C20:2); Arachidonic acid (C20:4n6); *cis*-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); *cis*-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3); Behenic acid (C22:0); *cis*-13,16-Docosadienoic acid (C22:2); Lignoceric acid (C24:0); Nervonic acid (C24:1). SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids. nd - not detected.

Table 4 Antioxidant activity (EC_{50} values, g L^{-1}) and total phenolics (g GAE kg^{-1} extract) and flavonoids (g CE kg^{-1} extract) for fresh-cut watercress samples stored under conventional and inert gas-enriched MAP.[1]

	Harvest (day 0)	7 d of storage at 4 °C				*p*-values		
	Non-stored control	Conventional packaging		Non-conventional MAP		Homoscedasticity[2]	Normal distribution[3]	One-way ANOVA[4]
		Air	Vacuum	N_2	Ar			
DPPH$^{\bullet}$ scavenging activity	0.49 ± 0.01^{e}	0.81 ± 0.01^{a}	0.65 ± 0.01^{c}	0.67 ± 0.02^{b}	0.61 ± 0.02^{d}	0.001	0.002	<0.001
Reducing power	0.38 ± 0.01^{e}	0.48 ± 0.01^{a}	0.43 ± 0.01^{d}	0.46 ± 0.01^{c}	0.47 ± 0.01^{b}	<0.001	<0.001	<0.001
β-carotene blanching inhibition	0.33 ± 0.02^{e}	0.62 ± 0.01^{b}	0.50 ± 0.02^{c}	0.75 ± 0.01^{a}	0.42 ± 0.01^{d}	0.008	0.007	<0.001
TBARS formation inhibition	0.27 ± 0.01^{b}	0.21 ± 0.01^{d}	0.24 ± 0.01^{c}	0.43 ± 0.01^{a}	0.26 ± 0.01^{b}	0.001	<0.001	<0.001
Total phenolics	87 ± 2^{c}	97 ± 2^{b}	99 ± 4^{b}	108 ± 2^{a}	105.8 ± 0.3^{a}	<0.001	0.008	<0.001
Total flavonoids	36 ± 1^{a}	25 ± 1^{c}	25 ± 1^{c}	28 ± 1^{b}	26 ± 1^{c}	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among packaging systems was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one packaging atmosphere differs from the others (in this case multiple comparison tests were performed). For each storage condition, means within a row with different letters differ significantly ($p<0.05$).
GAE - gallic acid equivalents; CE - catechin equivalents.