Modified atmosphere packaging and post-packaging irradiation of *Rumex induratus* leaves: a comparative study of postharvest quality changes

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Abstract The effects of conventional and inert-gas enriched atmospheres, as well as those caused by different γ -irradiation doses, on buckler sorrel (*Rumex induratus*) leaves quality were evaluated and compared after 12 days of storage at 4 °C. The green colour of the abaxial surface of the leaves was relatively stable, as well as the carbohydrates content and the calorific value. The storage time decreased the amounts of fructose, glucose, trehalose, α -tocopherol, and β -tocopherol and increased the levels of total organic acids and δ -tocopherol. The total tocopherols content was higher in air-packaged non-irradiated leaves, antioxidant compounds that may have contributed to the preservation of polyunsaturated fatty acids (PUFA). Some antioxidant properties were also favoured during storage. It was found that the overall postharvest quality of buckler sorrel leaves is better maintained with the argon-enriched atmospheres, while the 6 kGy dose was a suitable option to preserve PUF+A during cold storage. This study highlights the suitability of the applied postharvest treatments and the interest to include buckler sorrel leaves in contemporary diets.

Keywords *Rumex induratus* · Buckler sorrel · Modified atmosphere packaging · Gamma irradiation · Cold storage · Postharvest quality

Introduction

The human longevity associated with the Mediterranean Diet could be partly attributed to the intake of wild plant-based foods (Yannakoulia et al. 2015). The consumption of these plants plays an important role in complementing staple agricultural foods in many regions of the world (García-Herrera et al. 2014). A good example is buckler sorrel (*Rumex induratus* Boiss. & Reut., Fam. Polygonaceae), a native Iberian plant that occurs mainly in dry and stony sites of the thermo-Mediterranean region. Their tender leaves are appreciated and consumed, especially in salads (Carvalho 2010), constituting a promising dietary source of nutrients (Pereira et al. 2011) and biologically active compounds (Ferrerres et al. 2006; Guerra et al. 2008). However, this leafy vegetable is underutilized, due to the altered lifestyle of the modern society and introduction of non-native vegetables, not being found in food composition databases.

The revalorization of traditionally consumed wild species is currently considered as a focus of renewed attention (García-Herrera et al. 2014). Besides being an important input of health-promoting compounds and considered as added-value foods for commercialization, its recovery is an important strategy to improve the diversity of available foods. Additionally, consumers are looking for safe, healthy, more sustainable and convenient foods with different organoleptic properties of those daily consumed (Kühne et al. 2010). Thus, the resurgence of buckler sorrel consumption can meet this demand, and may be achieved if some challenges are accomplished, like quality assurance and innovation.

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To promote buckler sorrel revival and commercialization it is important to improve its shelf-life for an extended period for distribution and storage. This can be achieved using modified atmosphere packaging (MAP), a method which consists of changing the headspace gas composition surrounding the food in the package to prolong the initial fresh state and quality. The consequent reduction in metabolic activity and chemical oxidation prevents compositional changes associated with maturation and senescence, thus retaining the attributes that consumers consider as freshness markers (Pinela and Ferreira 2015). Recently, inert gases such as argon and nitrogen have been tested (Char et al. 2012; Pinela et al. 2016), but the literature describing their application and benefits is still limited. Besides, modified atmospheres can passively evolve within the package as a consequence of product respiration and diffusion of gases through the film (Choi et al. 2014). Postharvest treatments based on ionizing radiation can also be used for preserving fresh fruits and vegetables during shelf-life (Pinela and Ferreira 2015). Low doses of γ -irradiation are ideally applied after packaging to prevent post-contamination and to delay physiological processes (ICGFI 1999). Nevertheless, the commercial application to fresh products is still limited, despite being considered as a safe and effective technology by several international authorities (WHO 1999).

At the present, there are no reports that any preservation technology has previously been applied to buckler sorrel leaves. In this study, the effects induced by different packaging atmospheres (conventional and inert-gas enriched MAP) and γ -irradiation doses (up to 6 kGy) on postharvest quality parameters of buckler sorrel leaves were evaluated after 12 days of storage at 4 °C.

Materials and methods

Standards and reagents

Amber Perspex routine dosimeters, Batch X, were purchased from Harwell Company (Oxfordshire, UK). 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 -, γ -, and δ -isoforms), sugars (D(-)-fructose, D(+)-glucose anhydrous, D(+)-melezitose hydrate, D(+)-sucrose, and, D(+)-trehalose), organic acids (quinic, malic, oxalic, and ascorbic 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 mg/mL, 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).

Sampling and samples preparation

Wild specimens of buckler sorrel or French sorrel (*Rumex induratus* Boiss. & Reut.; syn: *Rumex scutatus* subsp. *induratus* (Boiss. & Reut.) Nyman) were gathered in April 2014 in the Bragança region (North-eastern Portugal), considering local consumers' sites, criteria and preferences (Carvalho 2010). Subsequently, healthy and undamaged leaves were selected, rinsed in tap water and drained to eliminate excess water. A portion was immediately analyzed (non-stored control), and the remaining fresh material was subjected to the postharvest 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.

Samples packaging and irradiation

A low-density polyethylene film (black LDPE resin) with a thickness of 63 μ m and permeability to O₂ and CO₂ at 25 °C of 69 cm³/m²/24 h/atm and 251 cm³/m²/24 h/atm, respectively, was used in the manufacture of packages (VWR, Lisbon, Portugal). Approximately 20 g of buckler sorrel leaves were placed in 11.5 cm $\times$ 20 cm sterilized packages (headspace volume of 0.7 L) and applied four different atmospheres: air-packaging (stored control in passive MAP), vacuum-packaging (no atmosphere), and N₂- and Ar-enriched atmospheres. Briefly, air-packaging consisted of sealing without eliminating the air in the package (20.8 % O₂ and <0.1 % CO₂) and vacuum-packaging was performed by eliminating the air with a vacuum-packaging machine. For non-conventional MAP, the headspace air in the packages was first eliminated and then the target gas (100 % N₂ or Ar) was injected.

For the irradiation treatment, air-packaged samples were divided into four groups: a non-irradiated (0 kGy) control group and three groups irradiated at 1, 2 and 6 kGy of γ -rays (predicted doses). The irradiation was performed one day after packaging in an ⁶⁰Co experimental chamber (Precisa 22, Gravinier Manufacturing Company Ltd., UK) located at C2TN, with four sources and a total activity of 177 TBq (4.78 kCi; February 2014). Amber Perspex routine dosimeters were used to measure the distribution of the absorbed energy and to determine the maximum ($D_{\max}$) and the minimum ($D_{\min}$) dose absorbed by the samples, following the procedure previously described by Fernandes

et al. (2012). Although γ -rays penetrates dense materials and can be used to treat boxed commodities and even those stacked on pallets (Hallman 2016), the samples were rotated upside down half of the time to increase the dose uniformity. The measured average doses were 1.02 ± 0.07 , 2.14 ± 0.08 and 5.99 ± 0.20 kGy for the samples irradiated at the predicted doses of 1, 2 and 6 kGy, respectively. The dose uniformity ratio ($D_{\max}/D_{\min}$) was 1.18. The desired doses were achieved by the time of exposure and by the location of the samples relative to the source. The estimated average dose rate for the irradiation position was obtained with a Fricke dosimeter and was 1.22 kGy/h.

A total of 70 packages were prepared (10 for each treatment) and stored at 4 °C for 12 days.

Headspace gas composition analysis

The O₂, CO₂ and N₂ concentrations inside the packages were monitored using a portable gas analyzer (model Oxybaby 6.0, WITT, Denmark) previously calibrated by sampling atmospheric air. The Ar concentration was calculated according to the Eq. (1).

$$[\text{Ar}] = 100 - ([\text{O}_2] + [\text{CO}_2] + [\text{N}_2]) \quad (1)$$

Measurements were performed after packaging and at the end of the storage period and the values were expressed as a percentage.

Quality analysis

Colour parameters

The CIE $L^*a^*b^*$ colour values were measured on both sides (adaxial and abaxial surfaces) of nine randomly selected leaves with a colorimeter (model CR-400; Konica Minolta Sensing Inc., Japan) previously calibrated using a standard white plate (Pinela et al. 2015). 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.

Proximate composition

Samples were analyzed using the AOAC procedures (AOAC 2005). Briefly, the crude protein content ($N \times 6.25$) 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

the difference according to the Eq. (2). The results were expressed as g per 100 g of fresh weight (fw).

$$m_{\text{carbohydrates}} = 100 - (m_{\text{moisture}} + m_{\text{proteins}} + m_{\text{fats}} + m_{\text{ashes}}) \quad (2)$$

The calorific value was calculated according to the Eq. (3) and expressed as kcal per 100 g of fresh weight (fw).

$$\text{Energy} = 4 \times (m_{\text{proteins}} + m_{\text{carbohydrates}}) + 9 \times (m_{\text{fats}}) \quad (3)$$

Hydrophilic compounds

Free sugars were determined by high performance liquid chromatography (HPLC) coupled to a refraction index detector as described by Pereira et al. (2011). The identification was made by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method, using melezitose as internal standard (IS). The results were expressed in mg per 100 g of fresh weight (fw).

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 °C 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, i.e., quinic, malic, oxalic, and ascorbic acids. The results were expressed in mg per 100 g of fresh weight (fw).

Lipophilic compounds

Fatty acids were analyzed by gas chromatography with flame ionization detection (GC-FID)/capillary column according to 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.

Tocopherols were determined by HPLC coupled to a fluorescence detector (FP-2020; Jasco) following the procedures 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, using tocol as IS. The results were expressed in mg per 100 g of fresh weight (fw).

Bioactive properties

Four *in vitro* assays were performed to evaluate the extracts antioxidant activity (Pinela et al. 2015), which were prepared according to Pereira et al. (2011) using a mixture of methanol:water (80:20, v/v) as extraction solvent. Briefly, the DPPH[•] 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[•] was determined by measuring the absorbance at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH[•] discoloration using the Eq. (4).

$$\text{RSA (\%)} = [(A_{\text{DPPH}} - A_{\text{S}})/A_{\text{DPPH}}] \times 100 \quad (4)$$

where A_{DPPH} is the absorbance of the DPPH[•] 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 (CBI) 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 Eq. (5).

$$\text{CBI (\%)} = (A_{\beta\text{T}2}/A_{\beta\text{T}0}) \times 100 \quad (5)$$

where $A_{\beta\text{T}2}$ is the absorbance of the emulsion after 2 h of incubation at 50 °C and $A_{\beta\text{T}0}$ is the initial absorbance. The thiobarbituric acid reactive substances (TBARS) formation inhibition capacity was evaluated in porcine brain homogenates. The colour intensity of the malondialdehyde-thiobarbituric acid complex formed during heating at 80 °C was measured at 532 nm, and the inhibition ratio calculated using the Eq. (6).

$$\text{Inhibition ratio (\%)} = [(A - B)/A] \times 100 \quad (6)$$

where A and B correspond to the absorbance of the control and the sample solution, respectively. Results were expressed in EC_{50} values (mg/mL), i.e., sample concentration providing 50 % of antioxidant activity or 0.5 of absorbance in the reducing power assay. Trolox was used as a positive control.

Total phenolics (Wolfe et al. 2003) and flavonoids (Jia et al. 1999) were quantified in the hydromethanolic extracts concentrated at 0.625 or 1.25 mg/mL by reading the absorbance at 765 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 mg of gallic acid equivalents (GAE) or catechin equivalents (CE) per g of extract.

Statistical analysis

For each postharvest treatment, three independent samples were analyzed. Data were expressed as mean $\pm$ 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 of 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.

Principal components analysis (PCA) was applied as pattern recognition unsupervised classification method. The number of dimensions to keep for data analysis was assessed by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha parameter (that must be positive) and also by the total percentage of variance (that should be as higher as possible) explained by the number of components selected. The number of plotted dimensions (two) was chosen in order to allow meaningful interpretations.

Results and discussion

Headspace gas composition

The initial levels of N_2 and Ar inside the packages reached values of above 95 % and were lower at the end of the storage period. In both non-conventional MAP, the levels of CO_2 and O_2 were lower than 10 and 15 %, respectively. The N_2 concentration inside the N_2 -enriched MAP decreased approximately 25 %, while this gas evolved within the Ar-enriched MAP (~ 35 %). The final headspace gas composition of air-packaged buckler sorrel leaves (non-irradiated and irradiated) revealed comparable values of N_2 (>75 %), while the percentages of CO_2 and O_2 were ~ 5 and 20 %, respectively. The observed changes can be attributed to the plant respiration process and diffusion of gases through the film.

Effect of the packaging atmosphere

Colour parameters

The colour is a very important quality parameter that plays a key role in establishing consumer acceptability of the product, being more important than flavour and texture in the initial food-selection process. Consequently, colour loss is one of the major external postharvest problems. Based on the one-way ANOVA p values (Table 1), it was possible to conclude that the assayed packaging atmospheres induced significant changes in all colour parameters, except for greenness (a^*) registered in the abaxial surface of the leaves. Vacuum and non-conventional MAP increased the leaves lightness (L^*) in both surfaces, compared to the non-stored control and to air-packaged leaves. The lower a^* values were registered in the adaxial surface of the leaves stored under N_2 -enriched atmospheres, but without statistical difference from those stored in air and Ar-enriched atmospheres. Furthermore, N_2 -enriched MAP and air-packaging were the less effective treatments in preventing the leaves yellowing (higher b^* values) in the adaxial and abaxial surfaces, respectively. Additionally, the adaxial surface was more propitious for colour changes than the abaxial one, which could be related to the mesophyll structure and presence of cuticle.

Proximate composition

The proximate composition of the buckler sorrel leaves stored under different packaging atmospheres is presented in Table 2. The proximate composition of this species collected in the same geographical region was previously described by Pereira et al. (2011). The authors reported lower protein content (1.31 g/100 g fw) and higher amounts of carbohydrates (6.93 g/100 g fw). Despite this, the moisture (~ 90.29 g/100 g fw), ash (~ 1.07 g/100 g fw) and fat (~ 0.39 g/100 g fw) contents and the energetic contribution (~ 36.47 kcal/100 g fw) were similar to those described in this study. According to the present results, it can be concluded that some of the assayed packaging atmospheres induced significant changes on the protein, fat and ash contents ($p < 0.05$), while moisture, carbohydrates and the energetic contribution were not affected. Air-packaged buckler sorrel leaves presented slightly higher protein and ash levels, contrarily to the observed regarding fat content. In fact, when the plant metabolism is not slowed down by the applied postharvest treatment, the organic reserves of fat may be consumed faster and new compounds will be synthesized and accumulated in the leaves tissues.

Table 1 Colour parameters (indicated for adaxial and abaxial surface of leaves) for buckler sorrel leaves exposed to different postharvest treatments

Harvest (day 0)	12 days of storage at 4 °C								p values	
	Non-conventional MAP				Conventional packaging				1-way ANOVA ^a	
	N_2	Ar	Vacuum	Air	0 kGy	1 kGy	2 kGy	6 kGy	PA	ID
Adaxial surface										
L^*	39 ± 1^{bD}	46 ± 3^a	45 ± 4^a	45 ± 2^a	41 ± 2^{bCD}	43 ± 2^{BC}	46 ± 4^A	46 ± 2^{AB}	<0.001	<0.001
a^*	-14 ± 1^{aA}	-17 ± 2^b	-15 ± 2^{ab}	-14 ± 2^a	-16 ± 1^{abAB}	-16 ± 1^{BC}	-16 ± 1^{BC}	-17 ± 1^C	0.002	<0.001
b^*	19 ± 1^{bCD}	25 ± 5^a	20 ± 4^{bc}	17 ± 2^c	23 ± 3^{abC}	24 ± 2^{BC}	26 ± 2^{AB}	27 ± 4^A	<0.001	<0.001
Abaxial surface										
L^*	50 ± 2^{bC}	55 ± 1^a	55 ± 4^a	56 ± 2^a	50 ± 2^{bC}	51 ± 1^{BC}	53 ± 2^{AB}	54 ± 2^A	<0.001	<0.001
a^*	-14 ± 1	-14 ± 1	-13 ± 1	-14 ± 2	-14 ± 1	-15 ± 1	-14 ± 1	-15 ± 1	0.114	0.058
b^*	20 ± 2^{bB}	20 ± 1^b	20 ± 3^{ab}	22 ± 4^{ab}	23 ± 1^{aA}	25 ± 3^A	23 ± 3^{AB}	25 ± 3^A	0.015	<0.001

The results are presented as the mean $\pm$ SD

L^* (lightness $\leftrightarrow$ darkness); a^* (redness $\leftrightarrow$ greenness); b^* (yellowness $\leftrightarrow$ blueness)

^a $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one packaging atmosphere (PA) or irradiation dose (ID) differs from the others (in this case multiple comparison tests were performed). For each preservation treatment, means within a row with different letters (lower case for PA and capital letters for ID) differ significantly ($p < 0.05$)

Table 2 Proximate composition, calorific value, and individual sugars and organic acids content for buckler sorrel leaves exposed to different postharvest treatments

	12 days of storage at 4 °C												<i>p</i> values					
	Non-stored control																	
	Non-conventional MAP						Conventional packaging						1-way ANOVA ^a					
	N ₂		Ar		Vacuum		Air		0 kGy		1 kGy				2 kGy		6 kGy	
Harvest (day 0)																		
Moisture content (g/100 g)	90 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	90 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1	0.135	0.103
Ash content (g/100 g)	0.91 ± 0.02 ^{bb}	0.96 ± 0.05 ^{ab}	0.91 ± 0.05 ^b	0.94 ± 0.05 ^{ab}	0.94 ± 0.05 ^{ab}	0.94 ± 0.05 ^{ab}	1.00 ± 0.05 ^{ab}	0.99 ± 0.05 ^{ab}	0.99 ± 0.05 ^{ab}	0.99 ± 0.05 ^{ab}	0.99 ± 0.05 ^{ab}	0.99 ± 0.05 ^{ab}	0.98 ± 0.05 ^{ab}	0.98 ± 0.05 ^{ab}	0.98 ± 0.05 ^{ab}	0.98 ± 0.05 ^{ab}	0.026	0.015
Proteins content (g/100 g)	2.9 ± 0.1 ^{bBC}	2.9 ± 0.1 ^b	2.9 ± 0.1 ^b	2.9 ± 0.1 ^b	2.9 ± 0.1 ^b	2.9 ± 0.1 ^b	3.3 ± 0.1 ^{ab}	3.0 ± 0.1 ^b	3.0 ± 0.1 ^b	3.0 ± 0.1 ^b	3.0 ± 0.1 ^b	3.0 ± 0.1 ^b	3.0 ± 0.1 ^{BC}	2.9 ± 0.1 ^C	2.9 ± 0.1 ^C	2.9 ± 0.1 ^C	<0.001	<0.001
Fat content (g/100 g)	0.39 ± 0.02 ^{aA}	0.38 ± 0.03 ^{ab}	0.39 ± 0.03 ^a	0.36 ± 0.02 ^b	0.36 ± 0.02 ^b	0.36 ± 0.02 ^b	0.35 ± 0.02 ^{bb}	0.29 ± 0.04 ^C	0.29 ± 0.04 ^C	0.29 ± 0.04 ^C	0.29 ± 0.04 ^C	0.31 ± 0.04 ^{BC}	0.30 ± 0.03 ^C	0.30 ± 0.03 ^C	0.30 ± 0.03 ^C	0.30 ± 0.03 ^C	0.001	<0.001
Carbohydrates content (g/100 g)	5.5 ± 0.5	5.0 ± 0.5	5.1 ± 0.5	5.1 ± 0.5	5.1 ± 0.5	5.1 ± 0.5	5.3 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	4.9 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	0.537	0.403
Calorific value (kcal/100 g fw)	36 ± 3	34 ± 2	34 ± 2	34 ± 2	34 ± 2	34 ± 2	36 ± 2	33 ± 3	33 ± 3	33 ± 3	33 ± 3	33 ± 3	33 ± 3	33 ± 3	33 ± 3	33 ± 3	0.170	0.054
Fructose (mg/100 g)	399 ± 19 ^{aA}	269 ± 9 ^b	234 ± 12 ^c	240 ± 9 ^c	240 ± 9 ^c	240 ± 9 ^c	165 ± 7 ^{dc}	176 ± 8 ^C	176 ± 8 ^C	176 ± 8 ^C	176 ± 8 ^C	191 ± 7 ^B	135 ± 9 ^D	135 ± 9 ^D	135 ± 9 ^D	135 ± 9 ^D	<0.001	<0.001
Glucose (mg/100 g)	385 ± 17 ^{aA}	184 ± 6 ^b	157 ± 5 ^c	173 ± 5 ^b	173 ± 5 ^b	173 ± 5 ^b	127 ± 7 ^{db}	113 ± 6 ^C	113 ± 6 ^C	113 ± 6 ^C	113 ± 6 ^C	124 ± 7 ^{BC}	86 ± 4 ^D	86 ± 4 ^D	86 ± 4 ^D	86 ± 4 ^D	<0.001	<0.001
Sucrose (mg/100 g)	66 ± 4 ^{cA}	26 ± 2 ^d	72 ± 4 ^b	94 ± 4 ^a	94 ± 4 ^a	94 ± 4 ^a	24 ± 2 ^{dc}	24 ± 4 ^C	24 ± 4 ^C	24 ± 4 ^C	24 ± 4 ^C	27 ± 3 ^C	53 ± 4 ^B	53 ± 4 ^B	53 ± 4 ^B	53 ± 4 ^B	<0.001	<0.001
Trehalose (mg/100 g)	61 ± 3 ^{aA}	37 ± 3 ^b	34 ± 4 ^b	29 ± 3 ^c	29 ± 3 ^c	29 ± 3 ^c	17 ± 2 ^{db}	15 ± 2 ^B	15 ± 2 ^B	15 ± 2 ^B	15 ± 2 ^B	6 ± 1 ^C	17 ± 2 ^B	17 ± 2 ^B	17 ± 2 ^B	17 ± 2 ^B	<0.001	<0.001
Total sugars (mg/100 g)	912 ± 33 ^{aA}	516 ± 17 ^{bc}	498 ± 21 ^c	536 ± 11 ^b	536 ± 11 ^b	536 ± 11 ^b	332 ± 15 ^{db}	328 ± 12 ^B	328 ± 12 ^B	328 ± 12 ^B	328 ± 12 ^B	348 ± 15 ^B	291 ± 14 ^C	291 ± 14 ^C	291 ± 14 ^C	291 ± 14 ^C	<0.001	<0.001
Oxalic acid (mg/100 g)	0.70 ± 0.04 ^{abB}	0.64 ± 0.05 ^{bc}	0.69 ± 0.05 ^{ab}	0.58 ± 0.05 ^c	0.58 ± 0.05 ^c	0.58 ± 0.05 ^c	0.76 ± 0.05 ^{ab}	0.80 ± 0.05 ^A	0.80 ± 0.05 ^A	0.80 ± 0.05 ^A	0.80 ± 0.05 ^A	0.68 ± 0.05 ^B	0.51 ± 0.05 ^C	0.51 ± 0.05 ^C	0.51 ± 0.05 ^C	0.51 ± 0.05 ^C	<0.001	<0.001
Quinic acid (mg/100 g)	2.0 ± 0.2 ^{dc}	3.2 ± 0.2 ^b	3.5 ± 0.1 ^a	3.0 ± 0.1 ^c	3.0 ± 0.1 ^c	3.0 ± 0.1 ^c	2.9 ± 0.1 ^{cb}	3.0 ± 0.1 ^{AB}	3.0 ± 0.1 ^{AB}	3.0 ± 0.1 ^{AB}	3.0 ± 0.1 ^{AB}	3.1 ± 0.1 ^A	2.8 ± 0.2 ^B	2.8 ± 0.2 ^B	2.8 ± 0.2 ^B	2.8 ± 0.2 ^B	<0.001	<0.001
Malic acid (mg/100 g)	0.31 ± 0.03 ^{cA}	0.43 ± 0.05 ^b	0.42 ± 0.05 ^b	0.56 ± 0.05 ^a	0.56 ± 0.05 ^a	0.56 ± 0.05 ^a	0.26 ± 0.04 ^{cb}	0.35 ± 0.04 ^A	0.35 ± 0.04 ^A	0.35 ± 0.04 ^A	0.35 ± 0.04 ^A	0.14 ± 0.02 ^C	0.14 ± 0.02 ^C	0.14 ± 0.02 ^C	0.14 ± 0.02 ^C	0.14 ± 0.02 ^C	<0.001	<0.001
Ascorbic acid (mg/100 g)	0.020 ± 0.002 ^{aA}	0.005 ± 0.001 ^d	0.008 ± 0.001 ^c	0.002 ± 0.001 ^e	0.002 ± 0.001 ^e	0.002 ± 0.001 ^e	0.011 ± 0.001 ^{bb}	0.009 ± 0.002 ^C	0.009 ± 0.002 ^C	0.009 ± 0.002 ^C	0.009 ± 0.002 ^C	0.008 ± 0.002 ^C	0.007 ± 0.002 ^C	0.007 ± 0.002 ^C	0.007 ± 0.002 ^C	0.007 ± 0.002 ^C	<0.001	<0.001
Total organic acids (mg/100 g)	3.0 ± 0.1 ^{dd}	4.3 ± 0.3 ^b	4.6 ± 0.2 ^a	4.1 ± 0.2 ^{bc}	4.1 ± 0.2 ^{bc}	4.1 ± 0.2 ^{bc}	3.9 ± 0.2 ^{cb}	4.2 ± 0.2 ^A	4.2 ± 0.2 ^A	4.2 ± 0.2 ^A	4.2 ± 0.2 ^A	3.9 ± 0.2 ^B	3.5 ± 0.2 ^C	3.5 ± 0.2 ^C	3.5 ± 0.2 ^C	3.5 ± 0.2 ^C	<0.001	<0.001

The results are presented as the mean ± SD

^a *p* < 0.05 indicates that the mean value of the evaluated parameter of at least one packaging atmosphere (PA) or irradiation dose (ID) differs from the others (in this case multiple comparison tests were performed). For each preservation treatment, means within a row with different letters (lower case for PA and capital letters for ID) differ significantly (*p* < 0.05)

Hydrophilic compounds

From the one-way ANOVA *p* values, it can be concluded that the assayed packaging atmospheres induced significant changes in the free sugars content (Table 2). Fructose, glucose, sucrose and trehalose were identified in the buckler sorrel leaves, being fructose the most abundant. The non-stored control revealed the highest contents in free sugars, except for sucrose, which values decreased under air and N₂-enriched atmospheres and were higher under vacuum and Ar-enriched atmospheres. Among treatments, air-packaged buckler sorrel leaves revealed the lowest sugars content, presenting a total sugars decrease of 63.6 %. Vacuum-packaged and N₂-enriched MAP samples presented the highest amounts of glucose and total sugars, while sucrose was particularly high in vacuum-packaged and Ar-enriched MAP samples, and fructose in N₂-enriched MAP.

All fresh vegetables remain biologically living after harvest. Therefore, the decrease of fructose and glucose levels in stored buckler sorrel leaves can be related to their use by the plant to produce the energy required for metabolism, since reducing sugars are the main substrates in the respiration process (Dey and Harborne 1997). Thus, air-packaging, where the total sugars content was lower, can be associated to some incapacity in slowing down those physiological processes. Likewise sugars are considered to be postharvest quality markers.

Besides these sugars, Pereira et al. (2011) also reported the presence of raffinose (~19 mg/100 g fw) in buckler sorrel leaves, as well as a total sugars content (~483 mg/100 g fw) ~47 % lower than that found in our non-stored control leaves. Despite the higher levels of sucrose (~121 mg/100 g fw), lower amounts of fructose (166 mg/100 g fw), glucose (122 mg/100 g fw) and trehalose (53 mg/100 g fw) were reported. Differences in the specimens phenological stage at harvest time, as well as disparity on soil and annual weather conditions of the gathering sites, may affect the chemical composition (Nikolopoulou et al. 2007), justifying the observed variations.

The organic acids content was also affected by the different packaging atmospheres (Table 2). Oxalic, quinic, malic and ascorbic acids were detected, and quinic acid was the most abundant. The refrigerated storage decreased the ascorbic acid content, while quinic acid and the total organic acids contents were higher in packaged buckler sorrel leaves. Air-packaging preserved better the ascorbic acid but increased the oxalic acid content, while vacuum-packaging showed opposite results. Ar- and N₂-enriched MAP also revealed higher amounts of malic and total organic acids compared to the non-stored control leaves. The oxalic acid was previously reported by Ferreres et al.

(2006) in aqueous extracts of buckler sorrel leaves collected in the same region. After that, Guerra et al. (2008) revealed the additional presence of ascorbic, citric, malic and shikimic acids, and demonstrated that the growing conditions and phenological stage affect the total amount of organic acids.

Lipophilic compounds

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 omega-6/omega-3 (n-6/n-3) are shown in Table 3. The assayed packaging atmospheres induced significant changes in the 22 detected fatty acids and given ratios (*p* < 0.05). In non-stored control buckler sorrel leaves, α -linolenic (C18:3n3), palmitic (C16:0) and linoleic (C18:2n6) acids were the 3 most abundant, in agreement to Pereira et al. (2011). C16:0 gave the lower values in the non-stored control leaves and increasing in the packaged ones especially under Ar-enriched MAP. In turn, the amounts of C18:2n6 and C18:3n3 were lower after the 12 days of refrigerated storage, being the air-packaging treatment the one which preserved better these fatty acids. In general, Ar-enriched MAP and vacuum-packaging induced negative effects, namely increasing the amounts of SFA (mainly due to the contribution of arachidic acid (C20:0)) and the n-6/n-3 ratios, and giving the low PUFA/SFA ratios. Despite MUFA also increased in packaged samples (except in the air-packaged ones), PUFA decreased under these conditions. Air-packaging was the most appropriate treatment to retain high values of PUFA and PUFA/SFA ratios, and low n-6/n-3 ratios as recommended for good nutritional quality.

The fatty acids content can be used as indicator that freshly harvested plants may have entered into stress or senescence (Yi et al. 2009). The onset of this process is connected with the loss of unsaturated fatty acids and accumulation of peroxidation products. As targets of free radicals and therefore substrates for peroxidation, elevated levels of PUFA are unfavourable and its uncoupling may be activated in mitochondria leading to an overall decrease in the double bond index (Yi et al. 2009). In addition, the elimination of reactive oxygen species is strongly linked to the activity of the enzymes that catalyze the peroxidation of unsaturated fatty acids from the cellular wall (Baysal and Demirdöven 2007). In our work, the increase in SFA and the decrease of PUFA under vacuum and Ar-enriched atmospheres can therefore be linked to unfavourable preservation conditions. The air-packaging treatment showed the most desired ratios and the higher values of PUFA, despite the initial headspace gas composition surrounding the buckler sorrel leaves in the package contained

Table 3 Fatty acids and tocopherols content for buckler sorrel leaves exposed to different postharvest treatments

Harvest (day 0)		12 days of storage at 4 °C										
Non-stored control		Non-conventional MAP				Conventional packaging						
N ₂		Ar		Vacuum		Air		0 kGy		1 kGy	2 kGy	6 kGy
C6:0	0.056 ± 0.004 ^{bcD}	0.32 ± 0.05 ^a	0.032 ± 0.005 ^c	0.073 ± 0.005 ^b	0.083 ± 0.005 ^{bc}	0.11 ± 0.01 ^B	0.13 ± 0.01 ^A	0.08 ± 0.01 ^C				
C8:0	0.10 ± 0.01 ^{cBC}	0.48 ± 0.05 ^a	0.22 ± 0.04 ^b	0.06 ± 0.01 ^d	0.11 ± 0.02 ^{cB}	0.18 ± 0.02 ^A	0.18 ± 0.01 ^A	0.08 ± 0.01 ^C				
C10:0	0.31 ± 0.04 ^{dD}	1.9 ± 0.1 ^b	2.7 ± 0.1 ^a	0.36 ± 0.04 ^d	0.46 ± 0.04 ^{cC}	0.78 ± 0.05 ^A	0.61 ± 0.04 ^B	0.33 ± 0.01 ^D				
C12:0	1.6 ± 0.1 ^{dC}	3.0 ± 0.1 ^c	4.6 ± 0.1 ^a	3.6 ± 0.1 ^b	1.4 ± 0.2 ^{eD}	2.6 ± 0.2 ^A	2.0 ± 0.1 ^B	1.5 ± 0.1 ^{CD}				
C13:0	0.034 ± 0.004 ^{dBC}	0.076 ± 0.004 ^b	0.146 ± 0.005 ^a	0.065 ± 0.005 ^c	0.033 ± 0.003 ^{cC}	0.071 ± 0.005 ^A	0.041 ± 0.005 ^B	0.023 ± 0.004 ^D				
C14:0	2.3 ± 0.1 ^{dC}	5.2 ± 0.2 ^c	12.0 ± 0.4 ^a	6.9 ± 0.2 ^b	1.9 ± 0.1 ^{eD}	3.4 ± 0.2 ^A	3.1 ± 0.2 ^B	1.5 ± 0.1 ^E				
C15:0	0.29 ± 0.01 ^{cBC}	0.45 ± 0.04 ^b	0.96 ± 0.05 ^a	0.41 ± 0.04 ^b	0.25 ± 0.04 ^{cBC}	0.43 ± 0.05 ^A	0.31 ± 0.05 ^B	0.23 ± 0.04 ^C				
C15:1	0.037 ± 0.003 ^{cB}	0.057 ± 0.003 ^c	0.18 ± 0.03 ^b	0.26 ± 0.03 ^a	0.030 ± 0.003 ^{cC}	0.058 ± 0.005 ^a	0.042 ± 0.003 ^B	0.013 ± 0.002 ^D				
C16:0	19.5 ± 0.4 ^{dC}	21.4 ± 0.4 ^c	30.4 ± 0.4 ^a	26.5 ± 0.4 ^b	21.8 ± 0.4 ^{cB}	24.8 ± 0.5 ^A	16.8 ± 0.4 ^D	13.7 ± 0.4 ^E				
C17:0	0.31 ± 0.02 ^{dC}	0.48 ± 0.04 ^b	0.86 ± 0.05 ^a	0.53 ± 0.05 ^b	0.38 ± 0.04 ^{cB}	0.51 ± 0.05 ^A	0.39 ± 0.04 ^B	0.28 ± 0.04 ^C				
C18:0	5.2 ± 0.2 ^{dC}	9.6 ± 0.2 ^c	15.0 ± 0.2 ^a	14.2 ± 0.5 ^b	4.1 ± 0.2 ^{eD}	7.3 ± 0.4 ^A	5.9 ± 0.4 ^B	3.9 ± 0.2 ^D				
C18:1n9	3.1 ± 0.1 ^{dA}	6.2 ± 0.2 ^b	12.2 ± 0.3 ^a	3.5 ± 0.3 ^c	2.9 ± 0.2 ^{dA}	3.2 ± 0.2 ^A	2.6 ± 0.3 ^B	2.0 ± 0.2 ^C				
C18:2n6	14.7 ± 0.4 ^{aA}	9.1 ± 0.3 ^c	0.9 ± 0.1 ^e	4.2 ± 0.2 ^d	10.5 ± 0.4 ^{bC}	9.0 ± 0.4 ^D	14.2 ± 0.2 ^B	13.9 ± 0.4 ^B				
C18:3n3	38.7 ± 0.5 ^{aA}	23.9 ± 0.5 ^c	1.9 ± 0.1 ^e	6.3 ± 0.2 ^d	33.7 ± 0.5 ^{bC}	16.3 ± 0.5 ^D	35.1 ± 0.5 ^B	34.9 ± 0.5 ^B				
C20:0	4.7 ± 0.2 ^{dD}	8.4 ± 0.2 ^b	8.3 ± 0.2 ^b	17.3 ± 0.2 ^a	5.2 ± 0.1 ^{cC}	10.3 ± 0.3 ^A	7.7 ± 0.1 ^B	7.5 ± 0.2 ^B				
C20:1	0.23 ± 0.02 ^{cA}	0.03 ± 0.01 ^d	0.42 ± 0.05 ^b	0.63 ± 0.05 ^a	0.08 ± 0.01 ^{dC}	0.12 ± 0.01 ^B	0.02 ± 0.01 ^D	0.02 ± 0.01 ^D				
C20:2	0.84 ± 0.05 ^{bBC}	0.63 ± 0.05 ^c	0.23 ± 0.01 ^d	0.85 ± 0.05 ^b	1.1 ± 0.1 ^{aA}	1.0 ± 0.2 ^{AB}	0.5 ± 0.1 ^D	0.8 ± 0.1 ^C				
C20:3n3	0.9 ± 0.1 ^{bB}	0.53 ± 0.04 ^c	0.19 ± 0.02 ^e	0.37 ± 0.05 ^d	1.2 ± 0.1 ^{aA}	0.38 ± 0.05 ^D	0.59 ± 0.05 ^C	1.2 ± 0.3 ^A				
C20:5n3	0.41 ± 0.05 ^{bC}	0.61 ± 0.05 ^a	0.13 ± 0.05 ^c	0.48 ± 0.05 ^b	0.43 ± 0.05 ^{bC}	0.29 ± 0.03 ^C	1.6 ± 0.1 ^B	5.7 ± 0.3 ^A				
C22:0	3.4 ± 0.2 ^{eE}	4.8 ± 0.2 ^d	5.1 ± 0.2 ^c	8.5 ± 0.1 ^a	8.1 ± 0.4 ^{bB}	10.2 ± 0.4 ^A	5.4 ± 0.2 ^D	6.9 ± 0.3 ^C				
C22:2	0.41 ± 0.05 ^{cC}	0.018 ± 0.002 ^d	0.55 ± 0.03 ^b	0.020 ± 0.003 ^d	1.6 ± 0.2 ^{aA}	0.75 ± 0.04 ^B	0.39 ± 0.04 ^C	0.045 ± 0.004 ^D				
C24:0	2.7 ± 0.1 ^{dD}	3.0 ± 0.1 ^c	2.9 ± 0.2 ^{cd}	5.0 ± 0.2 ^a	4.6 ± 0.2 ^{bC}	8.2 ± 0.5 ^A	2.3 ± 0.2 ^D	5.3 ± 0.3 ^B				
Total SFA (relative %)	40.6 ± 0.4 ^{dE}	58.9 ± 0.5 ^b	83.3 ± 0.2 ^a	83.4 ± 0.4 ^a	48.4 ± 0.5 ^{cB}	70.0 ± 0.5 ^A	45.0 ± 0.5 ^C	41.4 ± 0.5 ^D				
Total MUFA (relative %)	3.4 ± 0.2 ^{dA}	6.3 ± 0.2 ^b	12.8 ± 0.2 ^a	4.4 ± 0.2 ^c	3.0 ± 0.2 ^{cB}	3.4 ± 0.1 ^A	2.7 ± 0.3 ^C	2.0 ± 0.2 ^D				
Total PUFA (relative %)	56.0 ± 0.4 ^{aA}	34.8 ± 0.5 ^c	3.9 ± 0.2 ^e	12.2 ± 0.3 ^d	48.5 ± 0.4 ^{bC}	27.7 ± 0.5 ^D	52.3 ± 0.5 ^B	56.6 ± 0.5 ^A				
PUFA/SFA	1.38 ± 0.02 ^{aA}	0.59 ± 0.02 ^c	0.05 ± 0.01 ^e	0.15 ± 0.01 ^d	1.00 ± 0.02 ^{bC}	0.40 ± 0.01 ^D	1.16 ± 0.03 ^B	1.37 ± 0.05 ^A				
n-6/n-3	0.40 ± 0.02 ^{cB}	0.39 ± 0.02 ^{cd}	0.78 ± 0.02 ^a	0.71 ± 0.02 ^b	0.37 ± 0.02 ^{dC}	0.63 ± 0.03 ^A	0.41 ± 0.01 ^B	0.35 ± 0.02 ^C				
α-tocopherol (mg/100 g)	3.8 ± 0.2 ^{aA}	2.7 ± 0.1 ^b	2.9 ± 0.2 ^b	2.8 ± 0.1 ^b	2.8 ± 0.1 ^{bb}	1.3 ± 0.1 ^C	1.1 ± 0.1 ^D	0.9 ± 0.1 ^E				
β-tocopherol (mg/100 g)	0.10 ± 0.02 ^{aA}	0.08 ± 0.01 ^b	0.09 ± 0.01 ^b	0.08 ± 0.01 ^b	0.06 ± 0.01 ^{cB}	0.03 ± 0.01 ^D	0.04 ± 0.01 ^C	0.03 ± 0.01 ^D				
γ-tocopherol (mg/100 g)	1.6 ± 0.1 ^{dC}	2.2 ± 0.1 ^b	2.2 ± 0.1 ^b	1.8 ± 0.1 ^c	3.2 ± 0.2 ^{aA}	1.7 ± 0.1 ^B	1.5 ± 0.1 ^C	1.2 ± 0.1 ^D				
δ-tocopherol (mg/100 g)	0.33 ± 0.04 ^{eE}	1.3 ± 0.1 ^c	1.7 ± 0.1 ^a	0.96 ± 0.05 ^d	1.6 ± 0.1 ^{bb}	1.5 ± 0.1 ^C	1.8 ± 0.1 ^A	1.1 ± 0.1 ^D				

Table 3 continued

Total tocopherols (mg/100 g)	Harvest (day 0)		12 days of storage at 4 °C		Conventional packaging			
	Non-stored control		Non-conventional MAP		Vacuum		Air	
	N ₂		Ar		0 kGy		1 kGy	
	5.8 ± 0.3 ^{dB}		6.3 ± 0.3 ^c		7.0 ± 0.1 ^b		5.7 ± 0.1 ^d	
					7.7 ± 0.3 ^{AB}		4.5 ± 0.2 ^C	
							4.5 ± 0.2 ^C	
							3.2 ± 0.1 ^P	

The results are presented as the mean ± SD. The one-way ANOVA *p* values were <0.001 in all cases, indicating that at least one packaging atmosphere (PA) or irradiation dose (ID) differs from the others (in this case multiple comparison tests were performed). For each preservation treatment, means within a row with different letters (lower case for PA and capital letters for ID) differ significantly (*p* < 0.05)

Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Tridecanoic acid (C13:0); Myristic acid (C14:0); Pentadecanoic acid (C15:0); *cis*-10-Pentadecenoic acid (C15:1); Palmitic acid (C16:0); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n7); 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); *cis*-11,14,17-Eicosatrienoic acid (C20:3n3); *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); SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids

oxygen, which could have been caused by the protective effect of tocopherols.

The assayed treatments induced also significant changes in the tocopherols content (Table 3). The non-stored control leaves revealed the highest amounts of α - and β -tocopherols, but the lowest amounts of the γ - and δ -isoforms. While the assayed packaging atmospheres had no effect on the α - and β -tocopherols, γ - and δ -tocopherols were mainly increased under air-packaging and Ar-enriched MAP, respectively. The air-packaged buckler sorrel leaves showed the highest content in total tocopherols, being in agreement with the alterations in the PUFA contents. In fact, these strong lipophilic antioxidants might have preserved the amounts of PUFA.

Considering the literature, stress conditions, such as those induced by unfavourable packaging conditions, may cause an increase in the levels of total tocopherols (Munné-Bosch 2005). In these situations, tocopherols are synthesized to protect the plant tissue from the increased levels of reactive species and to inhibit lipid peroxidation, thus avoiding oxidative damage. Thus, the observed variations in the tocopherols content among the different packaging atmospheres can be related to their ability to provide an adequate protective condition from stressful situations. In this work, the air-packaged buckler sorrel leaves not only showed higher levels of tocopherols, but also low levels of total sugars and organic acids. Seemingly, the passive atmosphere originated inside the package was ineffective in reducing the metabolic activity, and therefore preventing compositional changes associated with senescence.

Bioactive properties

The values for antioxidant activity and total phenolics and flavonoids of the buckler sorrel leaves hydromethanolic extracts are presented in Table 4. The assayed packaging atmospheres induced significant changes (*p* < 0.050) in these parameters. In general, the antioxidant activity was lower in non-stored control buckler sorrel leaves (except for the reducing power), while the ones stored under vacuum exhibited the best antioxidant activity and high amounts of total phenolics and flavonoids. High levels of total phenolics and flavonoids were also detected in air-packaged and Ar-enriched MAP samples, respectively, but decreased under N₂-enriched MAP. Lower phenolic (117 mg GAE/g extract) and higher flavonoid (90 mg CE/g extract) contents and similar reduction power were reported by Pereira et al. (2011) in methanolic extracts. Despite this, these extracts exhibited better performances for the other in vitro assays. Also, the flavonoid synthesis is a plant strategy to withstand stress conditions (Pérez-Gregorio et al. 2011).

Effect of the irradiation dose

The suitability of the γ -irradiation treatment for preserving the postharvest quality parameters of the buckler sorrel leaves during cold storage was investigated and the results are presented below. After harvest, fresh vegetables remain living organisms able to protect its tissues from adverse conditions due to the presence of a wide range of bioactive phytochemicals. Besides, the γ -irradiation treatment can also increase the extractability of certain compounds (Hussain et al. 2016). Therefore, nonlinear dose–response effects of the γ -irradiation treatment on the evaluated quality parameters can be expected after the 12 days of cold storage.

Colour parameters

Based on the one-way ANOVA p values (Table 1), it was possible to conclude that the different γ -irradiation doses also induced significant changes in all colour parameters, except for greenness (a^*) registered in the abaxial surface of the leaves. The applied doses increased the leaves lightness (L^*) in both surfaces. The yellowness (b^*) was also increased with the storage time. In adaxial surfaces, these values were higher with the consequent increase of the irradiation dose, while the greenness (a^*) values were decreased (corresponding to a greener colour) with storage and higher irradiation doses.

Proximate composition

The proximate composition of non-irradiated and irradiated buckler sorrel leaves stored at 4 °C for 12 days is presented in Table 2. The different doses induced significant changes ($p < 0.05$) on the protein, fat and ash contents, while moisture, carbohydrates and the energetic contribution were not affected, as observed for the different packaging atmospheres. The ash content was slightly increased during storage, mainly in non-irradiated leaves. In contrast, the fat content was lower at the end of the storage period and was better preserved in non-irradiated leaves. This reduction in fat levels could have been caused by a reduced activity of the enzymes involved in the *de novo* synthesis of fatty acids induced by the γ -irradiation treatment (Pérez et al. 2007) in combination with the other factors presented in the previous section. Besides, irradiation of high moisture content foods can cause fat oxidation (Cheng et al. 2011).

Hydrophilic compounds

Individual sugars and organic acids profiles are presented in Table 2, showing that γ -irradiation had a significant effect ($p < 0.05$) on these hydrophilic compounds. The

non-stored control buckler sorrel leaves revealed the highest contents of the identified sugars, which were detected in a lesser extent after storage. In general, the 6 kGy dose led to lower amounts of fructose, glucose and total sugars. Nevertheless, the other applied doses did not induced differences in the total amounts of sugars. Lower levels of fructose and glucose were also detected in bananas irradiated at 1, 1.5 and 2 kGy and stored at 16 °C for 21 days (Gloria and Adão 2013). Regarding organic acids, the non-stored control leaves revealed higher values of ascorbic acid and lower values for quinic acid and total organic acids. Among stored samples, the 6 kGy dose induced negative effects on the oxalic, malic, ascorbic and total organic acids contents, and the 1 kGy dose induced the increase in amount these hydrophilic compounds. The decrease of these organic acids may be attributed to the direct effects of γ -rays or to the action of the free radicals possibly generated during radiolysis of water, which promote the conversion (oxidation) of ascorbic acid to dehydroascorbic acid. This may be corroborated by the work done by Hussain et al. (2016) with fenugreek (*Trigonella foenum-graceum* L.) and spinach (*Spinacia oleracea* L.) leaves irradiated at 0.25 and 1.5 kGy and stored at 3 °C for 4 days. The reduction of the ascorbic acid content may also be attributed to its interactive effect with other compounds, which react to protect them against the oxidative damage.

Lipophilic compounds

The results for fatty acids composition, SFA, MUFA, PUFA and the ratios of PUFA/SFA and n-6/n-3 are shown in Table 3. Similar to the assays under different atmospheres, buckler sorrel leaves suffer significant ($p < 0.05$) changes in their fatty acids composition with the applied irradiation doses. Regarding the 3 main fatty acids, C16:0 decreased with the two highest doses and increased with the 1 kGy dose and in non-irradiated leaves, while C18:2n6 and C18:3n3 were better preserved with the two highest doses and negatively affected with the 1 kGy dose, in comparison with the non-stored control. The SFA values were higher in stored samples especially in those irradiated at 1 kGy. The same dose protected MUFA and increased the n-6/n-3 ratio, while decreasing PUFA and the PUFA/SFA ratio. Interestingly, the 6 kGy dose preserved PUFA and the PUFA/SFA and n-6/n-3 ratios during storage. These variations in the fatty acids profile may result from oxidative and radiolytic processes (Fernandes et al. 2016), as well as from variations in the levels of antioxidants (e.g., ascorbic acid and tocopherols). γ -Irradiation can also affect the activity of enzymes involved in the desaturation of fatty acids (Pérez et al. 2007).

Concerning tocopherols profile, the non-stored control leaves presented the higher amounts of α - and β -

Table 4 Antioxidant activity and total phenolic and flavonoid content for buckler sorrel leaves exposed to different postharvest treatments

	Harvest (day 0)	12 days of storage at 4 °C						
	Non-stored control	Non-conventional MAP		Conventional packaging				
		N ₂	Ar	Vacuum	Air 0 kGy	1 kGy	2 kGy	6 kGy
DPPH [•] scavenging activity (EC ₅₀ values, mg/mL)	0.67 ± 0.01 ^{aA}	0.60 ± 0.01 ^b	0.60 ± 0.01 ^b	0.53 ± 0.01 ^c	0.59 ± 0.01 ^{bc}	0.53 ± 0.01 ^E	0.65 ± 0.01 ^B	0.57 ± 0.01 ^D
Reducing power (EC ₅₀ values, mg/mL)	0.21 ± 0.02 ^{cB}	0.28 ± 0.03 ^a	0.30 ± 0.02 ^a	0.24 ± 0.02 ^{bc}	0.24 ± 0.02 ^{bb}	0.31 ± 0.03 ^A	0.32 ± 0.04 ^A	0.32 ± 0.03 ^A
β-Carotene bleaching inhibition (EC ₅₀ values, mg/mL)	0.38 ± 0.01 ^{aC}	0.36 ± 0.01 ^b	0.39 ± 0.02 ^a	0.35 ± 0.01 ^b	0.26 ± 0.01 ^{cd}	0.40 ± 0.02 ^B	0.39 ± 0.01 ^{BC}	0.62 ± 0.01 ^A
TBARS formation inhibition (EC ₅₀ values, mg/mL)	0.29 ± 0.01 ^{aA}	0.19 ± 0.01 ^c	0.12 ± 0.01 ^d	0.10 ± 0.01 ^e	0.22 ± 0.02 ^{bc}	0.26 ± 0.01 ^B	0.18 ± 0.01 ^D	0.15 ± 0.01 ^E
Total phenolics (mg GAE/g extract)	144 ± 2 ^{bb}	135 ± 2 ^d	140 ± 4 ^c	146 ± 4 ^{ab}	148 ± 1 ^{aA}	135 ± 2 ^C	124 ± 1 ^E	127 ± 1 ^D
Total flavonoids (mg CE/g extract)	40 ± 1 ^{cA}	37 ± 1 ^d	43 ± 3 ^b	47 ± 2 ^a	36 ± 1 ^{dC}	35 ± 2 ^C	37 ± 1 ^B	36 ± 1 ^C

The results are presented as the mean ± SD. The one-way ANOVA *p* values were <0.001 in all cases, indicating that at least one packaging atmosphere (PA) or irradiation dose (ID) differs from the others (in this case multiple comparison tests were performed). For each preservation treatment, means within a row with different letters (lower case for PA and capital letters for ID) differ significantly (*p* < 0.05)

GAE gallic acid equivalents, CE catechin equivalents

tocopherols, and the lower ones of δ-tocopherol. The α-tocopherol values were reduced with the increase of the irradiation dose, as verified by Di Stefano et al. (2014) in raw unpeeled almond kernels (*Prunus dulcis* (Mill.) D. A. Webb) irradiated up to 10 kGy. Non-irradiated stored leaves reveal the higher total tocopherols content, which might have been stimulated by unfavourable storage condition (since tocopherols are known to be effective lipid-soluble antioxidants involved in the repair of oxidative damage), as explained in the discussion regarding the effects of the different packaging atmospheres. Besides, the irradiation treatment delays physiological processes (ICGFI 1999).

Bioactive properties

The applied doses also induced significant changes (*p* < 0.05) in the antioxidant activity and total phenolics and flavonoids of the hydromethanolic extracts prepared from the buckler sorrel leaves (Table 4). Each of the performed antioxidant activity assays seemed to have been favoured by a specific γ-irradiation dose: the DPPH[•] scavenging capacity was improved with the 1 kGy dose, the β-carotene bleaching inhibition capacity was higher in non-irradiated leaves (which showed also the highest phenolic and tocopherols contents), and the TBARS formation inhibition capacity was higher in those irradiated at 6 kGy. Comparable effects on the DPPH[•] scavenging capacity were observed by Hussain et al. (2016) in fenugreek and spinach leaves irradiated with doses above 0.75 kGy. Buckler sorrel leaves irradiated at 2 and 6 kGy revealed high TBARS formation inhibition capacity, which is in accordance with the higher amounts of PUFA (including C18:2n6 and C18:3n3). Furthermore, changes induced by oxidation and radiolytic processes, and variations in the structure and extractability of phenolic compounds may also affect the antioxidant capacity of the samples (Fernandes et al. 2016; Ito et al. 2016).

Comparative evaluation of the effects on the overall postharvest quality

In the former sections, the effects of packaging the buckler sorrel leaves under different atmospheres, or expose them to different γ-irradiation doses, were studied in several quality parameters. As it could be concluded, all the tested postharvest treatments induced significant changes, hindering the immediate selection of a single process able to maintain the wholesomeness of the fresh buckler sorrel leaves during shelf-life. Nevertheless, it would be useful to find the most suitable treatment when considering the contribution of all assayed quality parameters simultaneously, instead of verifying each parameter one by one.

Fig. 1 Biplot of object scores (postharvest treatments) and component loadings (evaluated quality parameters)

Accordingly, the results were evaluated considering data for all the studied packaging atmospheres and γ -irradiation doses through a categorical principal components analysis (CATPCA).

The plot of object scores (Fig. 1) for the different postharvest treatments indicates that the first two dimensions (first: Cronbach's α , 0.968; eigenvalue, 20.932; second: Cronbach's α , 0.928; eigenvalue, 11.433) account for most of the variance of all quantified variables (44.9 and 29.1 %, respectively). Groups corresponding to each treatment (air, N_2 , Ar, vacuum, 1 kGy, 2 kGy and 6 kGy) were completely individualized, indicating that the assayed postharvest treatments affect the studied quality parameters in a highly specific manner. In fact, all of them had positive and negative effects. Air-packaged buckler sorrel leaves were mainly characterized for their low antioxidant activity and flavonoid content, but kept high amounts of PUFA (mainly due to C18:2n6 and C18:3n3) along the storage

time. Objects corresponding to leaves stored under N_2 -enriched atmospheres were placed close to the origin of coordinates, thereby indicating that they were not characterized by particularly high (or low) levels of none of the assayed parameters. Leaves stored under vacuum, showed low levels of PUFA (especially C18:2n6 and C18:3n3), but high amounts of γ -tocopherol, MUFA (mainly oleic acid (C18:1n9)) and flavonoids. The results for the effects of Ar-enriched atmospheres were similar, with the additional advantages of their high DPPH' scavenging activity and TBARS formation inhibition capacity.

Regarding the effects of γ -irradiation, buckler sorrel leaves treated with the 1 kGy dose presented a low reducing power and β -carotene bleaching inhibition capacity and also low ascorbic acid levels; the major positive points were the high levels of δ -tocopherol. Results for the 2 and 6 kGy doses were somewhat similar, showing low levels of total phenolics, sugars and tocopherols (particularly the α - and β -

isoforms); on the other hand, these leaves presented high L^* values (the leaves showed higher lightness) and low a^* values, i.e., their colour was more green.

Conclusion

Overall, and considering the CATPCA results, it is suggested that buckler sorrel would be more suitably stored under an Ar-enriched atmosphere, since the treatment provided the maintenance of the most relevant postharvest quality attributes; while the 6 kGy dose was a good option to preserve PUFA and the ratios of PUFA/SFA and n-6/n-3 fatty acids. In addition to these findings, the present study highlighted the nutritional and antioxidant properties of buckler sorrel, as well as the interest of its inclusion in contemporary diets. Further studies are of interest to evaluate the effect of preservation treatments on other quality attributes and physiological parameters, as well as different combinations between them and with other technologies.

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