Physico-chemical and microbiological quality of ready-to-eat rocket (*Eruca vesicaria* (L.) Cav.) treated with organic acids during storage in dark and light conditions

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Abstract The effect of alternative dipping solutions to chlorinated water was studied on qualitative parameters of ready-to-eat rocket: sanitised tap water, 1% of citric acid solution and a mixture of citric and ascorbic acids solution. After packaging in normal atmosphere, a monitoring of total bacterial count and physico-chemical parameters was carried out to 14 storage days in dark and light storage conditions. The dark exposure of the rocket leaves contributed to preserve a lower microbiological growth and the green color: light presence highly influenced total bacterial count and Hue angle ($p < 0.01$). Treatments with acids can be recommended to preserve antioxidant compounds and color leaves: this factor also influenced other studied parameters as acidity, total bacterial count, and antioxidant activity ($p < 0.01$). The studied alternative dipping solutions and the storage in darkness involved a better quality of rocket up to 14 days of shelf life respect the commercial shelf life of 7 days.

Keywords Antioxidant activity · Pre-treatments · Quality · Rocket · Storage

Introduction

In recent years the market of minimally processed vegetables such as romaine lettuce, iceberg lettuce, red leaf lettuce, oak leaf lettuce and escarole, endive, radicchio, rocket, spinach has grown rapidly due to its convenience degree. Together with the reduced time for preparation, lower transportation and less storage cost make it possible to eat vegetables favourable, not only for the home consumption but also for the gastronomy.

Minimal processing of the vegetables includes the steps of harvesting, cold storage, trimming, shredding, washing/rinsing, draining, packaging, cold storage and finally distribution (Baur, 2005). The main decontamination step of these vegetables is washing. The use of chlorine as sanitizer in minimally processed vegetables is among the most common techniques applied in food industry (Gil et al., 2009). However, health- and environment-related concerns regarding the carcinogenic by-products of chlorine have promoted the search for alternative methods to decontaminate fresh-cut products (Ongeng et al., 2006; Rico et al., 2007). Zhang and Farber (1996) reported the effect of organic acids (ascorbic acid, citric acid and lactic acid) for the decontamination of vegetables.

Ready-to-eat vegetables are usually pre-packed for convenience and retaining freshness: in particular, they are highly perishable with a storage life of about 7–10 days at refrigeration temperatures (Krasaekoopt and Bhandari, 2011). Tsironi et al. (2017) highlighted that the principal qualitative markers for the shelf-life of ready-to-eat vegetables are microbiological and physico-chemical parameters.

Color variation of green leafy vegetables after harvest is a result of high biological variance and heterogeneity of the product. An uneven loss of green color was found in

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packaged wild rocket, (Løkke et al., 2012). Anaerobic conditions inside the package lead to the development of off-odours and tissue degradation for respirational fermentation (Kim et al., 2004), and finally, to acidic degradation of chlorophyll leading to severe color changes (Toivonen and Brummell, 2008). In fact, a correct packaging can prevent these damages because of the packaging alters the atmosphere surrounding the vegetable, as the living cells of the wild rocket leaves respire, which means that they use O₂ and produce CO₂ resulting in modified atmospheres (MA) inside the package. The aims of the experiments were to evaluate the effects of storage conditions on the shelf life and antioxidant characteristics of a vegetable species, such as the salad rocket.

Materials and methods

Experiments were carried out on a commercial farm (COF spa, Vibo Valentia) where rocket leaves were minimally processed by different dipping for 5 min. The dipping solutions were the following: R1, control sample dipped in sanitized tap water; R2, sample dipped in 1% (w/v) citric acid (FU -E330, A.C.E.F, Piacenza, Italy), and R3, sample dipped in 0.5% (w/v) citric acid and 0.5% (w/v) ascorbic acid (Ph. Eur. -E300, A.C.E.F, Piacenza, Italy). After these treatments, the rocket was packaged (125 ± 5 g) in Polypropylene antifog bags (25×20 cm of size; 35 µm of thickness) in normal atmosphere. Concerning the diffusional properties of packaging material, the oxygen transmission rate (OTR) was $1600 \text{ cm}^3/(\text{m}^2 \times 24 \text{ h} \times \text{atm})$ according to ASTM D3985, and the water transmission rate (WVTR) of $6 \text{ g}/(\text{m}^2 \times 24 \text{ h})$ according to ASTM F1249. The packages were transported in a commercial cooling truck at 4 ± 1 °C to Mediterranean University of Reggio Calabria and were stored at 4 °C in dark and light conditions (by use of three fluorescence lamps of 8 W, 430 lm for each one) and the experiment started the following day. The microbiological and physico-chemical analyses were performed after 18 h from production, three, seven, ten, and fourteen storage days over the recommended 7 days by producer.

Microbiological analysis

For the microbiological enumeration, a representative sample (10 g) was diluted with a sterile Ringer's solution and was homogenized with a Stomacher (BagMixer[®] interscience, Saint Nom, France). Decimal serially dilutions were prepared and plated on Petri plates and Total bacterial count (TBC) was enumerated on PCA-Plant Count Agar- growth land (Oxoid, Milan, Italy) at 26 °C for 48 h and expressed as Log₁₀ CFU/g (Fan and Song, 2008).

Physico-chemical analysis

Headspace gas composition was determined using a CheckPoint handheld Gas Analyser (PBI Dansensor, Milan, Italy) and was expressed as oxygen and carbon dioxide percentages. Titratable acidity, expressed as % of citric acid/g, and pH (with a pH meter Crison GLP, Barcellona, Spain) were measured according to the AOAC method (2000). Dry matter (% d.m.) was evaluated by loss weight in an oven at 70 °C to constant weight and water activity (*a_w*) was measured by means of Aqualab LITE (Decagon, Inc., Washington, USA) instrument. Color was measured using of a tristimulus colorimeter (model CM-700d, Konica Minolta, Osaka, Japan), based on the CIELab scale and by determination of L* (lightness, black/white from 0 to 100), a* (green/red from - 60 to 60) and b* (blue/yellow from - 60 to 60). Measures were individually performed on ten leaves for each sample (R1, R2, R3) in duplicate. Hue angle (H°) was calculated from a* and b* values according to the formula reported by Wrolstad and Smith (2010):

$$\text{Hue angle (}^\circ\text{)} = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

Browning index represents the purity of brown color and is calculated using L*, a*, b* according to Mohammadi et al. (2008):

$$\text{Browning index (BI)} = \left[\frac{100(x - 0.31)}{0.17} \right] \quad (2)$$

where

$$x = \left[\frac{(a^* + 1.75L^*)}{5.6645L^* + a^* - 3.012b^*} \right] \quad (3)$$

Quantification of total phenolic content (TPC)

Total phenolic content (TPC) was determined spectrophotometrically in a UV-VIS spectrophotometer (Agilent, Santa Clara, California, USA) using the Folin-Ciocalteu reagent (Carlo Erba, Milan, Italy), according to the methodology described by Singleton and Rossi (1965) with some modifications. 100 µL of methanol extract was reacted with Folin-Ciocalteu solution and spectrophotometrically analyzed at 760 nm. The results are reported as mg gallic acid/kg.

Determination of antioxidant activity

The antioxidant activity was determined in a UV-VIS spectrophotometer (Agilent) by different methods, the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Alfa Aesar, Karlsruhe, Germany)

and Trolox equivalent antioxidant capacity (TEAC) that was determined using the ABTS⁺ radical (Alfa Aesar, Karlsruhe, Germany).

2,2-Diphenyl-1-picrylhydrazyl (DPPH)

The DPPH assay was conducted according to the method reported by Brand-Williams et al. (1995). 50 μ L of aliquot of methanol extract was reacted into a cuvette with 2950 μ L of DPPH solution for 15 min in the dark. The absorbance was measured at 515 nm. The antioxidant capacities of extracts were expressed as percentage of inhibition according to the following formula:

$$\%Inhibition = \left(\frac{A_{t0} - A_{tend}}{A_{t0}} \right) \times 100 \quad (4)$$

where % Inhibition was the percentage of DPPH radical inhibition; A_{t0} is the value of absorbance of DPPH solution at initial time while, A_{tend} is the value of the absorbance measured after 15 min.

Trolox equivalent antioxidant capacity (TEAC)

ABTS(2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation decolourisation assay was prepared according to the method of Re et al. (1999) and then the Trolox equivalent antioxidant capacity (TEAC) was determined. 25 μ L of methanol extract was reacted with 2975 μ L of ABTS solution for 6 min in the dark. The absorbance was measured at 734 nm. The results were expressed as μ M TE/g.

Statistical analysis

The analysis of all samples were performed in duplicate. The results were reported as mean $\pm$ standard deviation (mean $\pm$ SD). Statistical significance between the mean values of each sample was evaluated by analysis of variance (Multivariate and Anova analysis) in the SPSS software (version 15) using multiple comparisons and the Tukey method. The statistical differences among means were considered significant at $p < 0.05$. In addition, Pearson's correlation coefficients (r) to determine the relation between two variables were analyzed.

Results and discussion

Initial average microbial counts ranged from 6.07 to 6.22 log cfu/g and this is in agreement with the initial microbial load reported for the fresh-cut washed vegetables (TBC < 8 log cfu/g). According to French regulations, an aerobic plate count of 5×10^7 cfu/g is the maximum

acceptable value at the end of the microbiological shelf-life of numerous fresh-cut vegetables as reported by Fan and Song (2008).

Nguyen-the and Carlin (1994) indicated that mesophilic bacteria counts on different fresh-cut vegetables range from 10^3 to 10^9 log cfu/g, whereas densities on products evaluated soon after processing range from 10^3 to 10^6 log cfu/g. There were significant statistical differences among the three samples and during the storage with $p < 0.01$ after three, seven and 10 days (data not shown). Sapers (2001) in his work concluded that effective washing and sanitising treatments play important roles in reducing the microbial population on fresh fruits and vegetables used either for the fresh market or fresh-cut products. At the end of the storage, the lowest TBC values were found in R1 sample (dipped in tap water) showing that dipping solutions did not contribute an improvement of the microbial quality. Allende et al. (2008) reported that the initial microbial reductions of the fresh-cut products after washing with different sanitizing agents provided little information about the microbial or sensory quality of the product at the time of consumption. Despite, this may be due to lowest a_w values at final storage day in R1 sample (0.977 ± 0.00), the absence of significant differences among the three dipping solutions for a_w parameter, did not allow to correlate this aspect with the TBC amount. TBC trend was reported in Fig. 1: it showed both an acidic decrease and microbial increase during the fourteen storage days as confirmed by Pearson's correlation coefficient after 14 days ($r = -0.887$ $p < 0.05$ in darkness). Furthermore, the highest TBC was found in R2 and R3 pre-treated samples in light conditions exceeding the legal limit of 8 log cfu/g; the light influence on this microbial parameter was confirmed by multivariate statistical analysis with $p < 0.01$ (Table 3).

Color change was described in other salads such as broccoli (Shi et al., 2016), spinach (Dermesonluoglu et al., 2015) and lettuce (Peng et al., 2015) by an increase in lightness (L^* parameter) that probably might be caused by chlorophyll degradation to colourless compounds. In this study there was not significant increase of L^* parameter during storage time as confirmed statistically by multivariate analysis ($p < 0.05$); only in R3 sample stored in dark condition lightness decreased during the time ($p < 0.05$): the highest presence of oxygen inside the package at the end of storage probably preserved a greener color intensity than the other samples as suggested by L^* decrease and by red/green a^* parameter of -9.48 ± 0.79 in dark, like in a study of green lollo (Tsironi et al., 2017). The Hue angle (H°) describes the relative amounts of redness and yellowness where 90° is defined for yellow, 180° for green (Kortey et al., 2015). Hue angle (H) and browning index (BI) were in values of ranges from 109.9° –

Fig. 1 Total bacteria count and titratable acidity of rocket pre-treated leaves stored for 14 days in darkness (A) and for 10 days in lightness (B)

111.7° and 36.97–46.48 in dark and from 109.9°–116.1° and 35.95–45.46 in light respectively. There was a general decrease in Hue values at the end of storage period, but not in R3 sample (dark condition) in which there was a minor lightness as previously described and a major BI. Also, in this last R3 sample, Hue angle values showed significant differences during storage ($p < 0.05$). The light exposure of the samples had influenced only Hue and L^* values with a significance of $p < 0.01$ and $p < 0.05$ by multivariate statistical analysis (Table 3).

Atmosphere composition (O_2 and CO_2 %) is shown in Fig. 2. Packaging alters the atmosphere surrounding the plant product, as the living cells of the wild rocket leaves respire, which means that they use O_2 and produce CO_2

resulting in modified atmospheres inside the package. Film materials used for packaging of fresh fruit and vegetables permit O_2 influx and CO_2 efflux (Løkke et al., 2013). CO_2 % increased obviously for tissues respiration as expected, especially in R3 sample. In this last sample the highest O_2 percentage was found: this probably affected the higher TBC in this sample compared to the others as showed by Pearson's correlation coefficient ($r = 0.889$ $p < 0.05$ in darkness) and as just suggested by Martínez-Sánchez et al. (2006a) in a study on rocket leaves underlining that mesophilic counts were maintained throughout the storage under low O_2 .

The different storage conditions (dark and light) did not influence the gas composition, but only the storage time

Fig. 2 Headspace gas composition of rocket pre-treated leaves stored for 14 days in darkness (A) and for 10 days in lightness (B)

both in dark and light conditions by multivariate analysis with a significance $p < 0.01$ (Table 3) and confirmed for each sample ($p < 0.01$) by one-way anova as showed in Tables 1 and 2.

TPC of treated rocket samples is shown in Tables 1 and 2 in dark and light conditions respectively: the values were in the range of 780.86 ± 5.68 – 1209 ± 75.4 mg gallic acid/kg fresh weight in dark and of 774.8 ± 27 – 1209 ± 75.4 mg gallic acid/kg fresh weight in light. Tiveron et al. (2012) reported a TPC of 110 mg gallic acid/100 g for rocket, Mazzucotelli et al. (2018) about of 104 mg gallic acid/100 g similar to the values obtained in this study; while Char et al. (2012) reported a six fold higher polyphenols content. It should be mentioned that beet greens are not considered a vegetable of traditional consumption, so studies about its phenolic content or

antioxidant capacity are relatively scarce. The vegetable with the highest TPC was R2 sample (994.1 ± 27 mg gallic acid/kg fresh weight) in light though light exposure did not influence these components by multivariate analysis. Time and treatments affected TPC with a significance of $p < 0.01$ by multivariate analysis (Table 3) contrarily to Beltrán et al. (2005) who did not find any differences in the total polyphenol content among different washing solutions. The antioxidant capacity of the vegetable was measured by DPPH and ABTS methods. Between the samples treated with acids, the R2 sample, treated with citric acid, showed the highest antioxidant capacity obtained with ABTS and DPPH assays both in dark (399.61 ± 136 μ M TE/g and $16.50 \pm 0.93\%$ inhibition) and in light conditions (399 ± 101 μ M TE/g and $18.16 \pm 1.7\%$ inhibition); this can be correlated with the

Table 1 Physico-chemical parameters of pre-treated rocket leaves stored in dark condition

Analyses on samples	Storage days in dark condition					Sig.
	0	3	7	10	14	
Dry matter (%)						
R1	6.96 ± 0.25	7.62 ± 0.18	7.46 ± 0.20	7.70 ± 0.25	7.39 ± 0.28	n.s.
R2	7.01 ± 0.15	7.02 ± 0.12	7.12 ± 0.11	8.20 ± 1.10	7.24 ± 0.08	n.s.
R3	6.79 ± 0.01 ^b	7.41 ± 0.14 ^a	7.21 ± 0.02 ^{ab}	7.25 ± 0.24 ^{ab}	7.35 ± 0.07 ^a	*
pH						
R1	6.22 ± 0.04 ^c	6.61 ± 0.02 ^b	6.56 ± 0.04 ^b	6.54 ± 0.05 ^b	6.88 ± 0.08 ^c	**
R2	6.27 ± 0.00 ^d	6.64 ± 0.01 ^{bc}	6.61 ± 0.01 ^c	6.70 ± 0.02 ^b	6.87 ± 0.03 ^a	**
R3	6.28 ± 0.01 ^c	6.50 ± 0.00 ^d	6.54 ± 0.01 ^c	6.67 ± 0.01 ^b	7.11 ± 0.01 ^a	**
<i>a_w</i>						
R1	0.980 ± 0.00 ^a	0.979 ± 0.00 ^b	0.977 ± 0.00 ^c	0.978 ± 0.00 ^{bc}	0.977 ± 0.00 ^c	**
R2	0.979 ± 0.00	0.977 ± 0.00	0.977 ± 0.00	0.977 ± 0.00	0.979 ± 0.00	n.s.
R3	0.982 ± 0.00	0.977 ± 0.00	0.976 ± 0.00	0.980 ± 0.00	0.980 ± 0.00	n.s.
Index browning						
R1	41.99 ± 4.05	41.99 ± 4.05	36.97 ± 6.49	45.42 ± 7.67	38.43 ± 8.81	n.s.
R2	43.67 ± 6.48	43.67 ± 6.48	46.48 ± 7.20	39.32 ± 5.20	39.93 ± 7.33	n.s.
R3	42.82 ± 5.16	42.82 ± 5.16	41.55 ± 3.52	39.68 ± 4.39	44.13 ± 4.64	n.s.
Hue °						
R1	110.53 ± 1.65	110.53 ± 1.65	111.70 ± 2.91	110.35 ± 1.94	111.43 ± 1.50	n.s.
R2	109.99 ± 1.21	109.99 ± 1.21	109.85 ± 1.97	111.07 ± 1.44	111.52 ± 1.70	n.s
R3	111.32 ± 1.52 ^{bc}	111.32 ± 1.52 ^{bc}	110.95 ± 1.13 ^c	111.39 ± 1.75 ^{ab}	111.72 ± 1.81 ^a	**
<i>L</i> *						
R1	51.97 ± 3.47	50.81 ± 3.27	57.69 ± 6.87	53.44 ± 3.62	52.90 ± 3.07	n.s.
R2	53.34 ± 2.72	53.70 ± 2.92	52.80 ± 2.64	52.92 ± 2.80	53.11 ± 4.41	n.s.
R3	53.26 ± 4.04	51.07 ± 2.77	51.05 ± 3.00	53.77 ± 3.04	51.87 ± 3.15	n.s.
<i>a</i> *						
R1	− 8.08 ± 0.76	− 8.28 ± 0.68	− 8.58 ± 1.18	− 9.09 ± 1.28	− 8.49 ± 1.28	n.s.
R2	− 8.23 ± 0.75	− 8.68 ± 0.90	− 8.80 ± 0.59	− 8.51 ± 0.83	− 8.84 ± 1.07	n.s.
R3	− 8.18 ± 0.91	− 8.89 ± 0.73	− 8.51 ± 0.61	− 8.90 ± 0.85	− 9.48 ± 0.79	n.s.
<i>b</i> *						
R1	21.94 ± 1.80	22.24 ± 1.62	21.65 ± 1.79	24.54 ± 2.13	21.78 ± 3.54	n.s.
R2	22.74 ± 1.99	23.96 ± 2.56	24.61 ± 2.52	22.21 ± 2.20	22.54 ± 2.92	n.s.
R3	22.86 ± 2.90	22.90 ± 1.98	22.31 ± 1.43	22.81 ± 1.55	23.92 ± 1.73	n.s.
TEAC (μM TE/g)						
R1	367.87 ± 127.07	309.67 ± 91.22	296.17 ± 70.28	363.69 ± 137.18	477.51 ± 33.52	n.s.
R2	362.60 ± 17.99	325.25 ± 43.43	318.85 ± 27.68	354.58 ± 33.07	399.61 ± 135.51	n.s.
R3	297.07 ± 59.06	407.08 ± 77.84	290.64 ± 122.82	361.43 ± 91.05	353.08 ± 44.39	n.s.
DPPH assay (% inhibition)						
R1	16.80 ± 1.85 ^{ab}	7.87 ± 1.76 ^c	20.60 ± 0.89 ^a	16.25 ± 0.57 ^{ab}	12.02 ± 0.60 ^{bc}	**
R2	17.54 ± 0.74 ^b	9.59 ± 0.06 ^d	27.94 ± 0.03 ^a	12.85 ± 0.01 ^c	16.51 ± 0.93 ^b	**
R3	15.27 ± 3.49	8.64 ± 2.26	23.28 ± 7.41	16.06 ± 0.97	14.63 ± 1.80	n.s.
TPC (mg gallic acid/kg)						
R1	1155.88 ± 33.72 ^a	1072.05 ± 0.68 ^{ab}	1004.27 ± 34.81 ^b	1102.97 ± 15.58 ^a	853.85 ± 6.21 ^c	**
R2	1208.68 ± 75.43	1011.13 ± 80.24	1166.19 ± 23.71	1074.87 ± 111.47	957.51 ± 9.80	n.s.
R3	1116.66 ± 55.56 ^a	1071.12 ± 0.08 ^b	1162.75 ± 21.55 ^a	997.25 ± 14.12 ^b	780.87 ± 5.68 ^c	**

Results are presented as the mean value ± standard deviation, n = 2; Means within a row with different letters are significantly different by Tukey's post hoc test; **Significance at $p < 0.01$; * Significance at $p < 0.05$; n.s. not significant

Table 2 Physico-chemical parameters of pre-treated rocket leaves stored in light condition

Analyses on samples	Storage days in light condition				Sig.
	0	3	7	10	
Dry matter (%)					
R1	6.96 ± 0.25	7.46 ± 0.15	7.31 ± 0.08	7.42 ± 0.09	n.s.
R2	7.01 ± 0.15	6.97 ± 0.24	7.72 ± 0.72	8.00 ± 0.18	n.s.
R3	6.79 ± 0.01	7.11 ± 0.20	7.22 ± 0.07	7.18 ± 0.54	n.s.
pH					
R1	6.22 ± 0.04 ^b	6.62 ± 0.06 ^a	6.51 ± 0.03 ^a	6.67 ± 0.05 ^a	**
R2	6.27 ± 0.00 ^c	6.62 ± 0.04 ^b	6.73 ± 0.03 ^b	6.93 ± 0.04 ^a	**
R3	6.28 ± 0.01 ^c	6.55 ± 0.02 ^b	6.49 ± 0.05 ^b	6.83 ± 0.03 ^a	**
a _w					
R1	0.980 ± 0.000 ^a	0.978 ± 0.001 ^b	0.978 ± 0.000 ^b	0.977 ± 0.000 ^b	**
R2	0.979 ± 0.000 ^a	0.977 ± 0.000 ^b	0.980 ± 0.000 ^a	0.977 ± 0.001 ^b	**
R3	0.982 ± 0.000 ^a	0.977 ± 0.000 ^c	0.980 ± 0.000 ^b	0.977 ± 0.001 ^c	**
Index browning					
R1	41.99 ± 4.05	38.49 ± 4.19	43.30 ± 6.66	45.46 ± 8.14	n.s.
R2	43.67 ± 6.48	43.86 ± 5.58	43.05 ± 5.69	42.26 ± 9.64	n.s.
R3	42.82 ± 5.16	43.02 ± 6.67	45.68 ± 10.3	35.95 ± 7.40	n.s.
Hue °					
R1	110.5 ± 1.65	110.68 ± 1.57	110.03 ± 1.61	110.34 ± 1.03	n.s.
R2	110 ± 1.21	110.00 ± 1.69	109.89 ± 1.92	110.93 ± 2.25	n.s.
R3	111.3 ± 1.52 ^b	110.65 ± 1.52 ^b	110.21 ± 2.13 ^b	116.13 ± 4.44 ^a	**
L*					
R1	51.97 ± 3.47	52.48 ± 2.99	51.62 ± 3.15	52.75 ± 2.46	n.s.
R2	53.34 ± 2.72	53.45 ± 3.58	51.98 ± 3.08	54.08 ± 2.55	n.s.
R3	53.26 ± 4.04 ^a	52.24 ± 2.86 ^a	47.92 ± 4.86 ^{ab}	44.78 ± 7.11 ^b	*
a*					
R1	− 8.08 ± 0.76	− 8.11 ± 0.73	− 8.29 ± 0.74	− 8.96 ± 0.97	n.s.
R2	− 8.23 ± 0.75 ^a	− 8.66 ± 0.72 ^b	− 8.27 ± 1.01 ^{ab}	− 8.98 ± 0.84 ^b	**
R3	− 8.18 ± 0.91	− 8.71 ± 0.88	− 8.03 ± 1.19	− 9.17 ± 1.72	n.s.
b*					
R1	21.94 ± 1.80	21.56 ± 1.57	22.92 ± 2.58	24.24 ± 2.33	n.s.
R2	22.74 ± 1.99	23.95 ± 2.15	22.89 ± 1.39	23.82 ± 3.75	n.s.
R3	22.86 ± 2.90	23.21 ± 2.07	22.13 ± 4.00	19.09 ± 4.13	n.s.
TEAC (μM TE/g)					
R1	367.87 ± 127.07	362.32 ± 64.53	419.34 ± 22.21	388.13 ± 25.03	n.s.
R2	362.60 ± 17.99	379.02 ± 45.92	497.56 ± 135.37	399.05 ± 100.54	n.s.
R3	297.07 ± 59.06 ^{ab}	380.34 ± 48.84 ^a	195.53 ± 8.57 ^b	251.37 ± 22.29 ^{ab}	*
DPPH assay (% inhibition)					
R1	16.80 ± 1.85	13.36 ± 2.25	21.64 ± 3.43	13.36 ± 3.00	n.s.
R2	17.54 ± 0.74 ^b	14.68 ± 1.48 ^b	27.80 ± 3.27 ^a	18.16 ± 1.72 ^b	*
R3	15.27 ± 3.49	13.30 ± 1.64	21.06 ± 3.65	11.52 ± 2.07	n.s.
TPC (mg gallic acid/kg)					
R1	1155.88 ± 33.72	1154.92 ± 61.71	1132.77 ± 49.54	932.28 ± 238.16	n.s.
R2	1208.68 ± 75.43 ^a	1075.69 ± 24.17 ^{ab}	1124.21 ± 3.15 ^{ab}	994.10 ± 26.91 ^c	*
R3	1116.66 ± 55.56 ^a	1093.77 ± 71.29 ^a	991.30 ± 4.59 ^a	774.81 ± 26.95 ^b	**

Results are presented as the mean value ± standard deviation, n = 2; Means within a row with different letters are significantly different by Tukey's post hoc test; **Significance at $p < 0.01$; * Significance at $p < 0.05$; n.s. not significant

Table 3 Results of multivariate analysis for some physico-chemical parameters of rocket leaves respect to different Factors and their interactions

Factors	Treatments (T)	Storage time (St)	Storage conditions (dark or light) (Sc)	T × St	T × Sc	St × Sc	T × Sc × St
O ₂ (%)	n.s.	**	n.s.	**	n.s.	*	n.s.
CO ₂ (%)	n.s.	**	n.s.	*	n.s.	n.s.	n.s.
TBC	**	**	**	**	**	**	**
Citric acid (%)	**	**	n.s.	**	n.s.	n.s.	n.s.
TEAC	*		n.s.	n.s.	n.s.	n.s.	n.s.
DPPH assay	**	**	n.s.	n.s.	n.s.	*	n.s.
TPC	**	**	n.s.	n.s.	n.s.	**	n.s.
Hue angle	*	**	**	**	**	**	**
BI	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Lightness	**	n.s.	*	**	**	*	*

**Significance at $p < 0.01$; * Significance at $p < 0.05$; n.s. not significant

highest amount of TPC confirmed by Pearson's correlation coefficient ($r = 1.00$ $p < 0.05$). Mazzucotelli et al. (2018) reported that in rocket vegetable, phenolic compounds were one of the major contributors to antioxidant capacity.

Ready to eat rocket has been reported to have a shelf life of about 14 days after harvest when stored at 4 °C (Martínez-Sánchez et al., 2006b) and 12 days for rocket washed with tap water and stored at 4 °C in unsealed non-MAP commercially available plastic bags in dark (Hall et al., 2013). In this study, rocket leaves washing in different dipping solutions and packaged in Polypropylene in normal atmosphere had a shelf life of 14 days in dark and 10 days in light at 4 °C. Treatments didn't influence TBC during the storage but the presence or absence of O₂ inside the package: in fact the R3 sample treated with a mix solution of acids and stored for 14 days in dark, had the highest O₂ percentage and TBC: all samples, untreated (R1) and treated (R2, R3), stored at 4 °C but in dark condition were within the legal microbiological acceptability limits of for fresh vegetables, (TBC < 8 log cfu/g). Also color changes were influenced by O₂ (%) for the correlated decrement of lightness and the better green color (a* parameter) of rocket leaves in R3 sample. R2 showed the highest antioxidant activity expressed by ABTS and DPPH methods and TPC. Finally, the samples treated with the acids (R2 and R3) preserved a good quality during the 14 storage days at 4 °C in dark condition, in particular for the better lightness and leaves green color (on R3 sample treated with a mix of citric and ascorbic acids) and the highest antioxidant activity and total phenolic compounds (on R2 sample treated with citric acid).

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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