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

37 **Abstract**

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

47
48 **Keywords:** antioxidant activity, pre-treatments, quality, rocket, storage
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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.

Colour variation of green leafy vegetables after harvest is a result of high biological variance and heterogeneity of the product. An uneven loss of green colour was found in packaged wild rocket, (Løkke et al., 2012). Anaerobic conditions inside the package lead to the development of off-odours and tissue degradation for respirationnal fermentation (Allende et al., 2004; Kim et al., 2004), and finally, to acidic degradation of chlorophyll leading to severe colour 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 x 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 cm³ m⁻² 24 h⁻¹ atm⁻¹ according to ASTM D3985, and the water transmission rate (WVTR) of 6 g m⁻² 24 h⁻¹ 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 hours from production, three, seven, ten, and fourteen storage days over the recommended seven 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⁻¹ (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⁻¹, 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. Colour 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):

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

150 Browning index represents the purity of brown colour and is calculated using L^* , a^* , b^* according to Mohammadi et al.
151 (2008):

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

153 where:

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

155

156 **Quantification of total phenolic content (TPC)**

157 Total phenolic content (TPC) was determined spectrophotometrically using the Folin–Ciocalteu reagent (Carlo Erba,
158 Milan, Italy), according to the methodology described by Singleton and Rossi (1965) with some modifications. 100 µL of
159 methanol extract was reacted with Folin-Ciocalteu solution and spectrophotometrically analyzed at 760 nm. The results
160 are reported as mg gallic acid kg⁻¹.

161

162 **Determination of antioxidant activity**

163 The antioxidant activity was determined by different methods, the scavenging activity of the stable 2,2-diphenyl-1-
164 picrylhydrazyl (DPPH) radical (Alfa Aesar, Karlsruhe, Germany) and Trolox equivalent antioxidant capacity (TEAC)
165 that was determined using the ABTS⁺ radical (Alfa Aesar, Karlsruhe, Germany).

166 **2,2-Diphenyl-1-picrylhydrazyl (DPPH)**

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

171

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

173 where:

174 %Inhibition was the percentage of DPPH radical inhibition; A_{t0} is the value of absorbance of DPPH solution at initial time
175 while, A_{tend} is the value of the absorbance measured after fifteen minutes.

176

177 **Trolox equivalent antioxidant capacity (TEAC)**

178 ABTS(2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation decolourisation assay was prepared
179 according to the method of Re et al. (1999) and then the Trolox equivalent antioxidant capacity (TEAC) was determined.
180 25 µL of methanol extract was reacted with 2975 µL of ABTS solution for 6 min in the dark. The absorbance was
181 measured at 734 nm. The results were expressed as µM TE g⁻¹.

182

183 **Statistical analysis**

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

189

190 **Results and discussion**

191 Initial average microbial counts ranged from 6.07 to 6.22 log cfu g⁻¹ and this is in agreement with the initial microbial load
192 reported for the fresh-cut washed vegetables (TBC < 8 log cfu g⁻¹). According to French regulations, an aerobic plate count
193 of 5x10⁷ cfu g⁻¹ is the maximum acceptable value at the end of the microbiological shelf-life of numerous fresh-cut
194 vegetables as reported by Fan and Song (2008).

195 Nguyen-the et al. (1994) indicated that mesophilic bacteria counts on different fresh-cut vegetables range from 10³ to 10⁹
196 log cfu g⁻¹, whereas densities on products evaluated soon after processing range from 10³ to 10⁶ log cfu g⁻¹. There were
197 significantly statistical differences among the three samples and during the storage with $p < 0.01$ after three, seven and ten
198 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 to 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 fourteen 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 $8 \log \text{cfu g}^{-1}$; the light influence on this microbial parameter was confirmed by multivariate statistical analysis with $p < 0.01$ (Table 3).

Colour 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 no 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 colour 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 (Kortei et al., 2015). Hue angle (H) and browning index (BI) were in values of ranges from $109.9^\circ - 111.7^\circ$ and $36.97 - 46.48$ in dark and from $109.9^\circ - 116.1^\circ$ 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. (2006) 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 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 $780.86 \pm 5.68 - 1209 \pm 75.4$ mg gallic acid kg^{-1} fresh weight in dark and of $774.8 \pm 27 - 1209 \pm 75.4$ mg gallic acid kg^{-1} fresh weight in light. Tiveron et al. (2012) reported a TPC of 110 mg gallic acid 100 g^{-1} for rocket, Mazzucotelli et al. (2017) about 104 mg gallic acid 100 g^{-1} 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 g^{-1} 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 ($399.61 \pm 136 \mu\text{M TE g}^{-1}$ and 16.50 ± 0.93 % inhibition) and in light conditions ($399 \pm 101 \mu\text{M TE g}^{-1}$ and 18.16 ± 1.7 % inhibition); this can be correlated with the highest amount of TPC confirmed by Pearson's correlation coefficient ($r = 1.00$ $p < 0.05$). Mazzucotelli et al. (2017) 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 fourteen days after harvest when stored at 4°C (Martínez-Sánchez et al., 2006a; 2006b) and twelve 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., 2018). In this study, rocket leaves washing in different dipping solutions and packaged in Polypropylene in normal atmosphere had a shelf life of fourteen days in dark and ten days in light at 4°C . Treatments didn't influence TBC during the storage but the presence or absence of O_2 inside the package: in fact the R3 sample treated with a mix solution of acids and stored for fourteen days in dark, had the highest O_2 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, ($\text{TBC} < 8 \log \text{cfu g}^{-1}$). Also colour changes were influenced by O_2 % for the correlated decrement of lightness and the better green colour (a^* parameter) of rocket leaves

258 in R3 sample. R2 showed the highest antioxidant activity expressed by ABTS and DPPH methods and TPC. Finally, the
259 samples treated with the acids (R2 and R3) preserved a good quality during the fourteen storage days at 4°C in dark
260 condition, in particular for the better lightness and leaves green colour (on R3 sample treated with a mix of citric and
261 ascorbic acids) and the highest antioxidant activity and total phenolic compounds (on R2 sample treated with citric acid).
262

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267 Compliance with ethical standards

268 Conflicts of interest

269 The authors declare that they have no conflict of interest

271 References

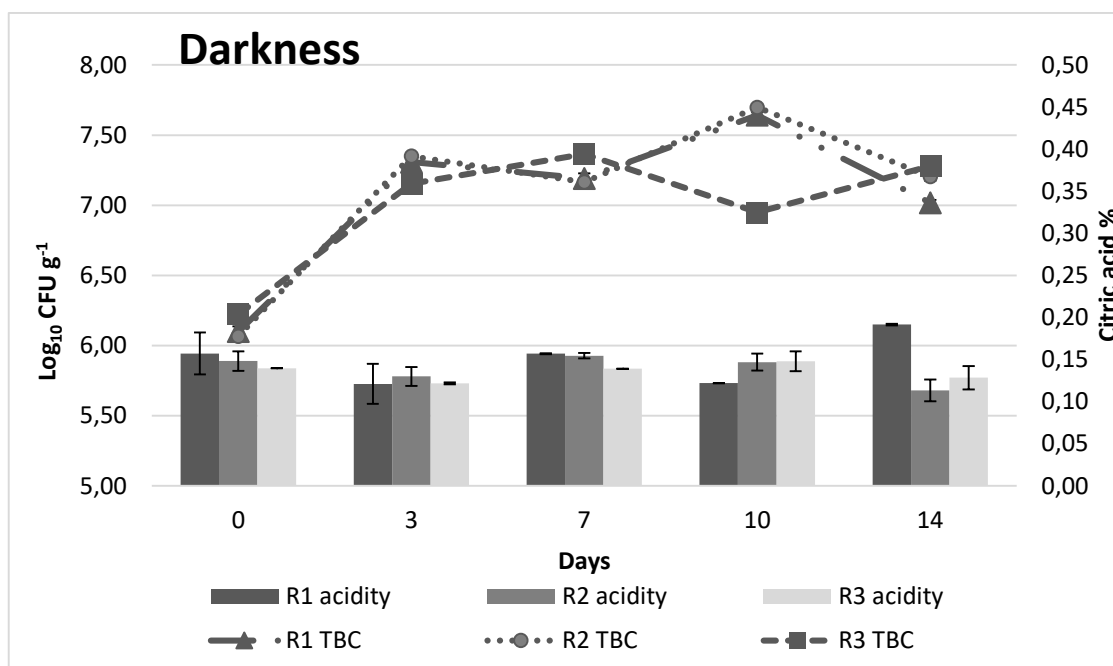
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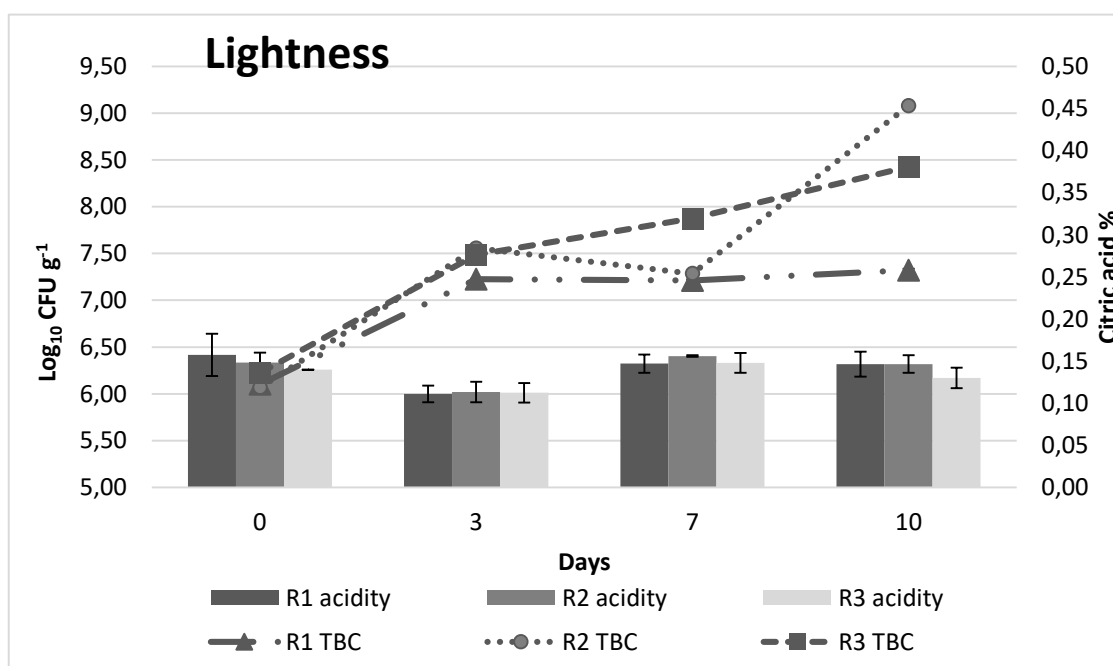
339 **Figure Captions**

341 **Fig.1** Total bacteria count and titratable acidity of rocket pre-treated leaves stored for fourteen days in darkness (a) and for
 342 ten days in lightness (b)
 343

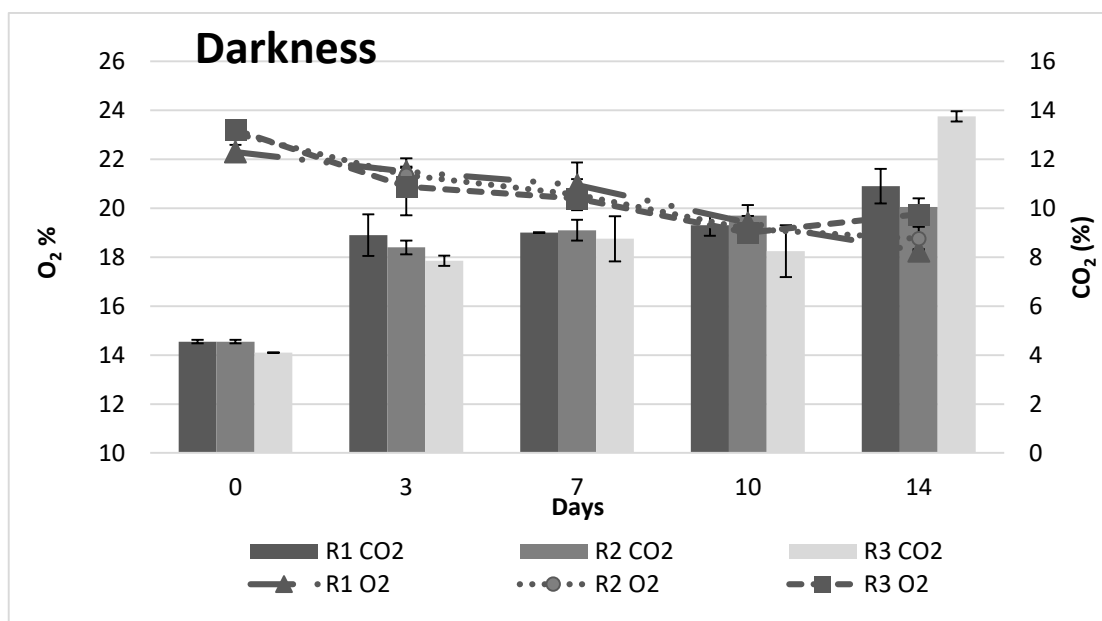
344 **Fig.2** Headspace gas composition of rocket pre-treated leaves stored for fourteen days in darkness (a) and for ten days in
 345 lightness (b)
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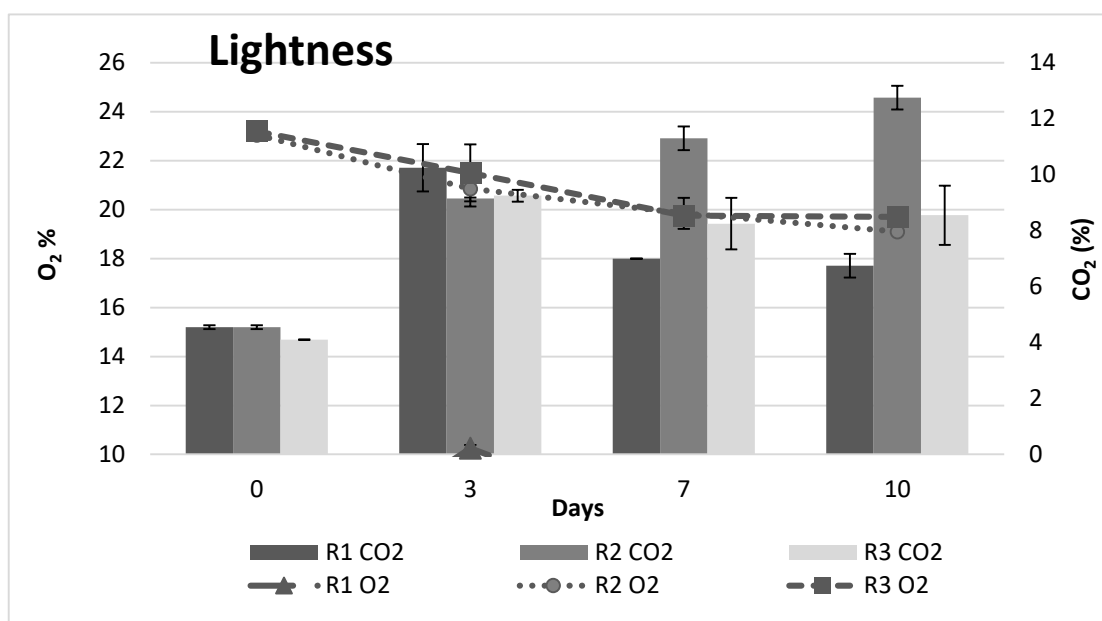
a



b



a



b

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	**
a _w	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	**
L*	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.
a*	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.
b*	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 ⁻¹)	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 (% of 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 ⁻¹)	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

Analysis on	R3	6.49 ± 0.15	6.47 ± 0.24	7.22 ± 0.77	8.40 ± 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 ⁻¹)	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 (% of 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 ⁻¹)	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

Factor	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

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