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# UV-C and ozone treatment influences on the antioxidant capacity and antioxidant system of minimally processed rocket (*Eruca sativa Mill.*)

Diego R. Gutiérrez[a], Alicia R. Chaves[b], Silvia del C. Rodríguez[a,c,*]

[a] Centro de Investigaciones y Transferencia de Santiago del Estero (CITSE-CONICET), Facultad de Agronomía y Agroindustria (FAyA), Universidad Nacional de Santiago del Estero (UNSE), RN 9 Km 1125, El Zanjón, (CP 4206), Santiago del Estero, Argentina
[b] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Universidad Nacional de la Plata (UNLP-CONICET). 47 y 116, Facultad de Ciencias Exactas, UNLP, 47 y 115, 1900, La Plata, Argentina
[c] Instituto de Ciencia y Tecnología de Alimentos (ICyTA- FAyA-UNSE), Av. Belgrano (S), 1912, Santiago del Estero, Argentina

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ABSTRACT

In this work, treatments with UV-C light (20 kJ $m^{-2}$) and gaseous ozone (2 mg $L^{-1}$ $O_3$) were applied to minimally processed rocket (*Eruca vesicaria subsp. Sativa*) leaves to investigate their effects on the antioxidant capacity and antioxidant system throughout 8 ds at 5 °C. As control, water washing was used. On 8 d, the ascorbic acid content was reduced about 50% both control and treated samples. Treatments with 20 kJ UV-C $m^{-2}$ or 2 mg $L^{-1}$ $O_3$, did not affect the phenolic content neither the total antioxidant capacity, remaining almost constants during storage. The phenylalanine ammonia lyase (PAL) activity had no significant variations during storage, in correlation with the content of phenolics. As to the enzymes involved in the antioxidant system, an increase in its superoxide dismutase (SOD) activity with respect to uncut rocket leaves was detected after the process. In turn, enzymes that remove $H_2O_2$ like ascorbate peroxidase (APX) and catalase (CAT) showed no significant changes. During storage at 5 °C, the SOD activity remained unchanged while APX and CAT showed a gradual increase in both treated and untreated samples. In conclusion, the UV-C and ozone treatments applied inhibited the growth of spoilage by bacteria as well as by yeasts, extended shelf-life, without exerting significant additional stress with respect to the cutting stage of the leaves, reason by which they did not trigger a greater activation of the antioxidant system.

## 1. Introduction

The consumption of fruits and vegetables has increased in recent times due to its content in phytochemicals and antioxidant compounds that are beneficial for human health (Lemoine et al., 2010). Koukounaras et al. (2009) reported that in the Mediterranean countries, the rocket (*Eruca sativa* Mill.) is a vegetable very popular with a high interest by consumers. The rocket is a vegetable that is distinguished by its pleasant bitter taste and also by its content of phytonutrients that stimulate health such as provitamin A, vitamin C, flavonoids, sulfur, potassium and fiber (Nunes et al., 2013; Gutiérrez et al., 2016).

Lemoine et al. (2008) reported that both harvesting and processing of vegetables cause severe stress leading to the symptoms of senescence in them. The postharvest chemical treatments used to preserve vegetables and fruits are being less accepted by consumers because of their possible contaminating effects (Shen et al., 2013). Thus in the last years, new physical technologies are of interest in to extend the postharvest life of fruits and vegetables. Among new physical technologies, treatments with low dose UV-C can be effective due to that delaying ripening, producing no pollution and extending the shelf life of various fruits and vegetables (Lemoine et al., 2007). Artés-Hernández et al. (2010) reported that radiation UV-C has germicidal effect and is due to the fact that it damages the nucleic acids of the microorganisms affecting their multiplication. There have been studies in different vegetables on how UV-C radiation influences changes in sensory quality, bioactive compounds and microbial development in vegetables in fresh-cut melon (Manzocco et al., 2011), broccoli (Martínez-Hernández et al., 2011), pineapple (Pan and Zu, 2012) and rocket (Gutiérrez et al., 2015). Different studies have shown that the application of UV-C light improved total phenolics contents and antioxidant capacity of several fruits and vegetables along storage (Erkan et al., 2008; Perkins-Veazie et al., 2008). This agrees with Allende et al. (2007) who reported that UV-C radiation reduced the deterioration of tomatoes and strawberries and produced an increase in antioxidants (Erkan et al., 2008). Besides, Wang et al. (2009) reported an increase of phenolic compounds in UV-C treated blueberries.

* Corresponding author at: Instituto de Ciencia y Tecnología de Alimentos (ICyTA- FAyA-UNSE), Av. Belgrano (S), 1912, Santiago del Estero, Argentina.
*E-mail address:* silviadepece@hotmail.com (S.d.C. Rodríguez).

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Another sanitizing agent approved by the US-FDA (2001) that is being tested in food industry today is ozone. Ozone breaks down quickly into oxygen and leaves no toxic residue which makes it appealing and useful to food industry (Karaca and Velioglu, 2014). Treatments that use ozone have proven to be effective in extending the shelf life of different fruits such as grapes, pears, oranges and apples (Ali et al., 2014). Furthermore, Jay et al. (2005) reported that 0.15–5.0 mg $L^{-1}$ ozone treatments seemed to inhibit both spoilage bacteria and yeasts development. On the other hand, ozone can cause oxidative stress in plant tissues and in this way induce diverse physiological responses such as synthesis phenolic compounds, antioxidants and other secondary metabolites (Forney, 2003).

Martínez-Hernández et al. (2013) reported that these types of stress can induce the accumulation of reactive oxygen species (ROS) in plant cells and to prevent these damages, both enzymatic and non-enzymatic antioxidant systems are activated. Boonkorn et al. (2012) found that the most important enzymes are catalases (CAT; EC 1.11.1.6), superoxide dismutases (SOD; EC 1.15.1.1) and ascorbate peroxidases (APX; EC 1.11.1.11). The function of these enzymes is that they are ROS scavengers, which cause oxidative damage that are induced by various biotic or abiotic stress such as low or high temperatures, UV radiation, exposure to ozone, pathogenic attack and mechanical damage (Lemoine et al., 2010; Boonkorn et al., 2012). The enzymes act as follows: the superoxide radical ($O_2-$) is dismutated to $H_2O_2$ by the enzyme SOD, while that the enzymes CAT and APX metabolize $H_2O_2$ to $H_2O$ (Martínez-Hernández et al., 2013).

The objective of this study was to analyze the effects of UV-C and ozone on several chemical components and on enzymes related to the antioxidant systems of minimally processed rocket stored at 5 °C.

## 2. Materials and methods

### 2.1. Plant material and chemicals

The rocket (*Eruca Sativa* Mill.) was harvested from the field of a farmer in the province of Santiago del Estero, Argentina. Its stemless leaves were transported to the laboratory for their processing.

### 2.2. Sample preparation, treatments and storage conditions

The rocket leaves were sorted at 8 °C in a disinfected area in order for the removal of damaged or dehydrated leaves. After selection, the leaves were washed with running water 5 °C for 1 min and drained on a stainless-steel mesh. They were then cut into 20 mm strips with a sharp stainless-steel knife and were washed for 2 min and drained again. The remaining water was removed using a manual centrifuge and the leaves submitted to sanitizing treatments using UV-C and $O_3$ afterwards.

The treatments applied were as follows: 0 (control); 2 mg $L^{-1}$ for 10 min $O_3$; 20 kJ $m^{-2}$ (303 s) of UV-C dose (the exposure time was calculated according to the radiation intensity). Both the UV-C dose and the $O_3$ concentration were selected according to the results of preliminary experiments (Gutiérrez et al., 2016). In each treatment, 60 g of leaf strips were placed on 600 mL polypropylene (PP) trays wrapped with a 35 μm bi-oriented PP film thermally sealed on the topside to generate a passive MAP.

The transmission rate was $2.58 \times 10^{-6}$ mol $O_2$ $m^{-2} s^{-1}$ and $9.30 \times 10^{-6}$ mol $CO_2$ $m^{-2} s^{-1}$ at 20 °C and 90% RH. The trays were stored in a dark cold room (5 °C). Five trays per treatment and storage time were performed. The various parameters were measured after 1, 4, 5 and 8 d of refrigerated storage.

### 2.3. UV-C and ozone treatments

The UV-C apparatus was a reflective stainless-steel chamber equipped with 6 unfiltered germicidal lamps (254.7 nm, TUV 36W/G36, Philips, Amsterdam, The Netherlands), on its topside and 6 at its bottom. This equipment is fully described in a work by Gutiérrez et al. (2016). A constant light source of 254 nm was used (intensity of radiation 0,066 kW) and applied upon the samples for different exposure times according to the test. The UV-C radiation doses was measured with a digital radiometer (Cole-Parmer Instrument Company, Vernon Hill, IL).

A 1 g $h^{-1}$ ozone generator (Bio3 Ozone Generator, TDZ-1 model, Uruguay) was used for the tests. The ozone concentration within the chamber was recorded via an ozone analyzer (Gas Alert Extreme $O_3$ − BW Technology, Honeywell, Canada). The $O_3$ flow and treatments employed were described by Gutiérrez et al. (2016).

### 2.4. Total phenolic compounds

The total phenolic compounds concentration was determined following Singleton et al. (1999). The phenolic compounds of the samples were extracted as described by Gutiérrez et al. (2015); For each day of measurement, samples (10 g) of each replicate treatment were frozen at −80 °C (Ultrafrezzer Righi, Argentina) and stored until chemical determinations were performed. The 4 g frozen rocket samples were homogenized using 20 mL of methanol and centrifugated for 15 min at 6000 × g at 4 °C. The supernatant of each sample was used as an extract. A 0.5 mL aliquot of each extract mixed with 8 mL of distilled water altogether were mixed in turn with 0.5 mL of the Folin-Ciocalteu reagent (that is, 1:1 v/v, diluted with distilled water). Three minutes later, 1 mL of a 5% $Na_2CO_3$ solution was added to the solution while shaking the solution vigorously. The resultant mixture was then incubated in darkness at 25 °C for 1 h and its absorbance measured at 765 nm using a UV–vis spectrophotometer (JASCO V-630). The total phenolic content was expressed as chlorogenic acid equivalents (CAEq) in g $kg^{-1}$ (expressed on a fresh weight basis). All the measures were made in triplicates.

### 2.5. Antioxidant capacity

The total antioxidant activity of rocket was determined as described by Brand Williams et al. (1995) out of each extract obtained as described above. Thus, a 150 μL aliquot of each extract was added with 2850 μL of a 0.1 mM DPPH solution (prepared with ethanol) and the mixture kept in dark for one hour at room temperature. The absorbance at 515 nm was measured at different times with a spectrophotometer (JASCO V-630, UV-vis). The calibrating curve was depicted using Trolox as standard and the results are expressed as Trolox equivalents (Trolox Eq) in g $kg^{-1}$ (expressed on a fresh weight basis). All the measurements were made in triplicate.

### 2.6. Ascorbic and dehydroascorbic acid content

The samples of rocket leaves (2 g) were added to 20 mL of 6% (w/v) trichloroacetic acid (TCA). The mixture to be extracted was kept in darkness for an hour. The homogenate was centrifuged at 12,000 × *g* at 4 °C for 20 min. The supernatant was used for measurements. Both the ascorbic acid (AA) and dehydroascorbic (DHA) acids contents were determined using the spectrophotometer described above by following Kampfenkel et al. (1995). Thus, a standard AA solution was employed for identifying and quantifying these contents in reference to a standard curve while DHA content resulted out of the difference between the total Vitamin C content and AA. The results were expressed as AA in g $kg^{-1}$ (expressed on a fresh weight basis). All the measures were taken in triplicate.

### 2.7. Superoxide dismutase activity

A solution made from 2 g rocket leaves samples in 2 mL of buffer solution (namely 1 mM phenylmethylsulfonyl fluoride (PMSF), 100 mM sodium phosphate pH 7.8, 0.1 mM EDTA, 10 g $L^{-1}$

polyvinylpolypyrrolidone (PVPP)) was homogenized by stirring it for 30 min and centrifuged at 16,000 × *g* for 20 min. The supernatant was immediately analyzed for SOD, APX, and CAT activities. All the extracting process was carried out at 4 °C.

The SOD activity was determined following Lemoine et al. (2010) with slight modifications. The enzyme mixture assayed contained 50 mM of pH 7.8 sodium phosphate buffer solution, 13 mM of methionine, 75 µM nitro blue tetrazolium (NBT), 2 µM riboflavin, 0.1 mM EDTA and 300 µL of enzyme extract, making altogether a total volume of 3 mL. The mixture was poured into uniformly transparent test tubes, homogenized and exposed for 15 min at room temperature to four 15 W fluorescent lamps located 50 cm above the samples.

The test started or stopped when the lamps were switched on or off respectively and absorbance measured at 560 nm. Non-irradiated assayed mixtures were used as control since they do not develop color. The mixtures without the enzyme extract, in turn, developed maximum color which decreased as increasing quantities of extract were added. An enzyme activity unit (EAU) was defined as the amount of enzyme needed to inhibit NBT photoreduction by 10% under the assay conditions per mg of protein. All the measures were made in triplicate.

### 2.8. Ascorbate peroxidase activity

The APX activity was determined according to the Nakano and Asada's method (1981). The activity at 25 °C of a mixture containing 100 mM of a pH 7 sodium phosphate buffer solution, 0.25 mM of sodium ascorbate, 10 mM $H_2O_2$ and 150 mL of enzyme extract completing altogether a final volume of 3.0 mL was determined. The absorbance reduction at 290 nm was followed and an APX enzyme activity unit (EAU) was defined as the amount of enzyme needed to oxidize 1 µmol per minute of ascorbate under test conditions. The results were expressed as EAU per mg of protein. All the measures were taken in triplicate.

### 2.9. Catalase activity

The CAT activity was determined by measuring the $H_2O_2$ disappearance rate using the method by Maehly and Chance (1959) with slight modifications. The reaction mixture was made out of 50 µL of the enzyme extract added to 50 mM of a pH 7.4 phosphate buffer solution and to 100 µL of 1% $H_2O_2$. The decline in absorbance at 240 nm, equaled to a decrease in $H_2O_2$, was monitored every 5 min per hour. A decrease in one enzyme activity unit (EAU) was defined as the amount of enzyme needed to consume 1 µmol $H_2O_2$ per minute. The results were expressed in EAU per mg of protein. All the measures were made in triplicate.

### 2.10. Phenylalanine ammonia lyase activity

Four-gram samples of frozen rocket leaves were homogenized using 20 mL of 0.1 M $Na_2B_4O_7.10H_2O$, 2 mM of EDTA, 5 mM of 2-mercaptoethanol and 30 g $L^{-1}$ of pH 8.8 PVPP. The solution was stirred for 1 h at 4 °C and then centrifuged for 20 min at 16,000 × *g* at 4 °C. The supernatant was tested for PAL activity. The PAL activity was determined according to the method by Lemoine et al. (2010). The reaction mixture contained 600 mL of 0.01 M phenylalanine, 3400 mL of 30 mM sodium borate buffer solution and 2000 mL of crude enzyme extract. The reaction was incubated at 37 °C for 4 h. The reaction was stopped by adding 400 µL of 5 M HCl immediately after being cooled and centrifuged. The PAL activity was determined spectrophotometrically at 290 nm by following the formation of the transcinnamic acid from L-phenylalanine. One unit of PAL activity was defined as the amount of enzyme bringing about a 0.01 absorbance increase at 290 nm per h, per milligram of protein. All the measures were made in triplicate.

### 2.11. Protein determination

The protein content was determined by the Bradford's method (Bradford, 1976) in the different extracts using bovine serum albumin as the standard protein.

### 2.12. Statistical analysis

The experiments were performed using a randomized design based on a bifactorial design. Their statistical analyses were performed using the Infostat software (version 2011, National University of Cordoba, Argentina). Both the analysis of variance (ANOVA) and the Least Significant Difference (LSD) tests were applied to evaluate the influence of either the treatment and the storage time upon data of phenols, antioxidant capacity, ascorbic and dehydroascorbic acid and antioxidant enzymes activity. A LSD test with a significance level at $P < 0.05$ was used to determine significant differences among means.

## 3. Results and discussion

In a previous work, treatments with UV-C light (20 kJ $m^{-2}$) and gaseous ozone (2 mg $L^{-1}$) are reported to inhibit not only spoilage bacteria but also yeasts growth and to maintain fresh-cut rocket general quality through 8 d storage at 5 °C. Applying these treatments, an extended shelf-life of rocket is recorded in comparison to untreated samples (Gutiérrez et al., 2016). Due to the importance of antioxidant systems in preserving plant tissue integrity together with their potential benefits to human diet, in this work the effect of these treatments on several enzymatic and non-enzymatic mechanisms related to the maintenance of the antioxidant status is analyzed.

### 3.1. Phenolic compounds and antioxidant capacity

Initially, no significant differences between the UV-C and ozone treatments as to the control were observed in the total phenolic content which ranges from 1.33 to 1.36 g $kg^{-1}$ CAEq; this range remained practically constant for all the treatments at 5 °C. No significant differences between the control and treated samples were either found at the end of storage (Fig. 1A). Phenolic compounds play an important role in the protection of plant tissues against biotic and abiotic stresses, as well as ascorbic acid (Lemoine et al., 2010).

At first, the antioxidant capacity was similar in all the treatments; it ranged from 1.36 to 1.42 g $kg^{-1}$ Trolox Eq. As observed for total phenol content, treatments did not show significant variations along storage at 5 °C. No significant differences between the control and the samples treated with UV-C and ozone were found (Fig. 1B). The results obtained in this paper agree with Lemoine et al. (2007) since are not found changes after UV-C treatment in the total phenol content of broccoli or during 14 d storage at 4 °C. However, González-Aguilar et al. (2007) reported that radiation UV-C increased the phenolic compounds of entire and fresh-cut mangoes.

Besides, Tomás-Callejas et al. (2012) reported on Tatsoi baby leaves treated with UV-C radiation (4.54 kJ $m^{-2}$) a significant increase in both total phenolics content and total antioxidant capacity after 4 d at 5 °C. Others authors, e.g. Artés-Hernández et al. (2009) found in spinach treated with doses of 4.54, 7.94 and 11.35 kJ UV-C $m^{-2}$ a 50% decrease in the total antioxidant activity after 13 d at 5 °C.

On the other hand, the result in ozone treatments coincides with those reported by Tzortzakis et al. (2007) who found that ozone treatments with concentrations up to 1 µmol $mol^{-1}$ did not influence on phenolics compounds and antioxidant activities. On the other hand, Karaca and Velioglu (2014) reported that treatments with gaseous ozone of 0.95 µg $L^{-1}$ for 20 min in parsley, decreased 12% and 41% in total phenolic content and antioxidant activity, respectively ($P < 0.05$).

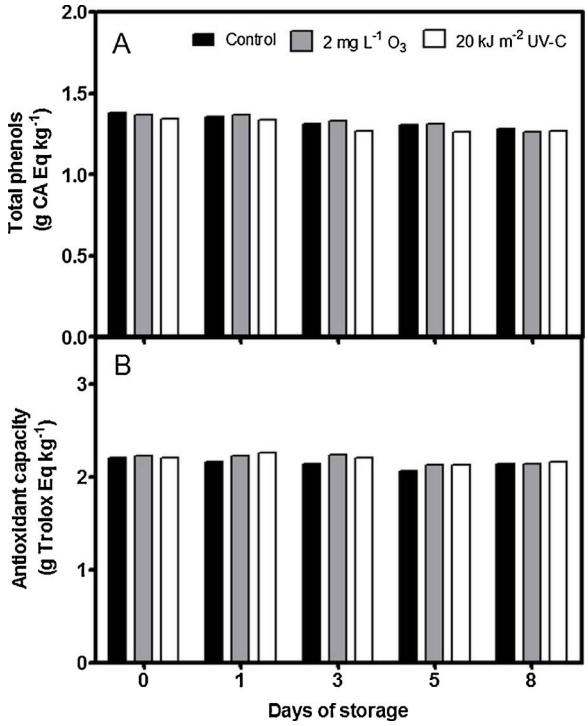


**Fig. 1.** Content of total phenolics (A) and antioxidant capacity (B) of minimally processed rocket treated with UV-C radiation (20 kJ $m^{-2}$) and gaseous $O_3$ (2 mg $L^{-1}$) stored at 5 °C for 8 d. There were no significant differences among treatments at each storage time at $P < 0.05$ according to LSD test.
(A) SD = 0.05, LSD ($P \leq 0.05$) = 0.07.
(B) SD = 0.07, LSD ($P \leq 0.05$) = 0.09.

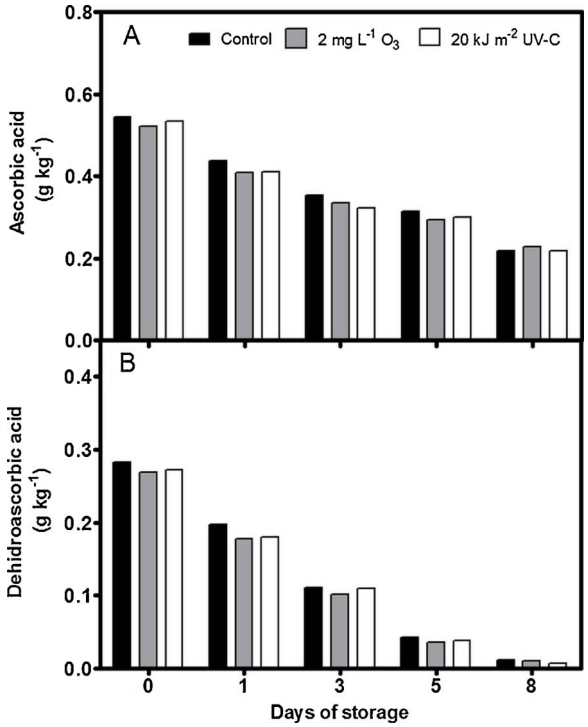


**Fig. 2.** Content of ascorbic acid (A) and dehydroascorbic acid (B) of minimally processed rocket treated with UV-C radiation (20 kJ $m^{-2}$) and gaseous $O_3$ (2 mg $L^{-1}$) stored at 5 °C for 8 d. There were no significant differences among treatments at each storage time at $P < 0.05$ according to LSD test.
(A) SD = 0.02, LSD ($P \leq 0.05$) = 0.03.
(B) SD = 0.01, LSD ($P \leq 0.05$) = 0.01.

### 3.2. Ascorbic acid content

Among foods rich in vitamin C are fresh fruits and vegetables whose main biologically active form is the L-ascorbic acid (AA) though its oxidation product, i.e. L-dehydroascorbic acid (DHA) is active as well (Lemoine et al., 2010). Immediately after being processed, all the treatments showed a significant ($P < 0.05$) decrease of approximately 15% in the AA content of rocket respect to that of whole leave samples ($0.61 \pm 0.028$ g $kg^{-1}$ AA). In turn, no differences between treatments were found. Different authors reported that this decrease may be due to minimal processing that cause's oxidative degradation (Shen et al., 2013). In sum, the AA content decreased up to 50% of its initial after 8 d of refrigerated storage in both control and treated samples. However, no significant differences ($P > 0.05$) between treatments were found (Fig. 2A).

These results with UV-C radiation are consistent with that reported by Shen et al. (2013). They observed that UV-C treatments with doses of 3.0, 0.75 and 1.5 kJ $m^{-2}$ in minimally processed Satsuma mandarin did not influence on their ascorbic acid content through the storage at 4 °C. Other authors also reported similar results in fruits treated with UV-C radiation such as blueberries (Perkins-Veazie et al., 2008) and fresh-cut watermelon (Artés-Hernández et al., 2010).

In contrast to the ozone results obtained in the present study, Karaca and Velioglu (2014) showed that treatments with gaseous ozone of 0.95 μg $L^{-1}$ for 20 min, reduced the ascorbic acid content by about 40% in parsley. Other authors also reported on the reduction of the ascorbic acid content in different vegetables such as strawberries (Allende et al., 2007) and rice leaves (Imai and Kobori, 2008).

### 3.3. Dehydroascorbic acid content

Changes in the levels of dehydroascorbic acid during storage showed a pattern similar to that of AA content (Fig. 2B). The DHA acid content showed, in general, a decreasing trend throughout shelf-life at 5 °C for all the treatments. No significant differences in DHA acid content between the control and samples treated either with UV-C or ozone, both immediately after being processed and during storage. Lemoine et al. (2010) reported that in general the concentration of DHA is lower than that of L-ascorbic acid and during storage the concentration of DHA can be increased due to its oxidation.

In this work, the DHA content represented up to 32% of the total AA content although it was not constant in time. The decreased AA levels observed could mainly be due to the oxidative degradation caused by the minimal processing even though the treatments with UV-C and ozone did not have additional effects on their degradation.

### 3.4. SOD

The different types of plant-induced stress could lead to an increase in reactive oxygen species (ROS) exacerbating the damage or by the activation of defense mechanisms (Neill et al., 2002; Ong et al., 2014). Besides, it was considered opportune also in this work to measure the activity of the enzymes involved in the antioxidant system in whole leaves of rocket. Thus, the enzymatic activities of the control and the samples immediately to be treated with ozone or UV-C were evaluated and compared with the whole leaves.

Immediately after processing, all the samples showed significant increase in their SOD enzyme activity as to the values of whole leaves samples ($1.1 \pm 0.1$ UAE $mg^{-1}$ protein) while no significant differences were observed between the samples treated either with UV-C or $O_3$ and the control (Fig. 3).

This increase in the activity of the SOD enzyme after the treatments could inhibit the accumulation of superoxide free radicals, since it is a primary scavenger of the same (Lemoine et al., 2010). Besides, these radicals can be dismuted to $H_2O_2$ and these in turn are converted to non-toxic molecules by other enzymes like CAT or APX (Sala and

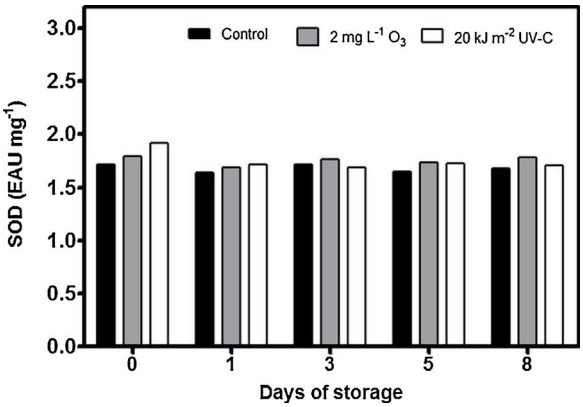


**Fig. 3.** Superoxide dismutase (SOD) activities of minimally processed rocket treated with UV-C radiation (20 kJ $m^{-2}$) and gaseous $O_3$ (2 mg $L^{-1}$) stored at 5 °C for 8 d. There were no significant differences among treatments at each storage time at $P < 0.05$ according to LSD test.
SD = 0.12, LSD ($P \leq 0.05$) = 0.14.

Lafuente, 2004; Boonkorn et al., 2012).

In this work, the activity of the SOD enzyme remained practically constant ($P > 0.05$) during storage at 5 °C and no significant differences between the treatments were found. However, Starzyńska et al. (2003) reported an increase in the activity of the SOD enzyme in broccoli after 7 and 10 d of storage at 5 °C. In studies carried out by Toivonen and Sweeney (1998), reported in broccoli a correlation between the yellowing and the antioxidant enzymes, presenting a greater activity of the SOD enzyme in samples that remained greener through the storage.

On the other hand, Boonkorn et al. (2012) found that treatments with ozone (200 µg $L^{-1}$ for 0, 2, 4 or 6 h) in tangerine fruits induced an increase in the activity of the SOD enzyme and was significantly higher than the control fruit stored at 25 °C.

### 3.5. APX

The function of this enzyme APX is that it prevents the accumulation of $H_2O_2$ produced by both the SOD enzyme and the dismutation of superoxide radicals, and reduces it to $H_2O$ using the capacity of reduction of AA (Cocetta et al., 2014). Whole leaves samples showed an activity of 339 ± 27 UAE $mg^{-1}$ protein and no significant differences ($P > 0.05$) with respect to the control and the samples treated with ozone or UV-C immediately after being processed were found (Fig. 4A). During storage at 5 °C, the APX activity increase on 1 d and remained practically stabile up to 5 d. Subsequently, a significant increase ($P < 0.05$) in the activity of APX was detected at the end of storage. This increase was of about 22, 18 and 17% in the control, $O_3$ and UV-C treated samples respectively. Conversely, no significant differences between the control and the treated samples were found. This trend is common since the activity of APX as much as that of the enzymes involved in oxidative stress tends to increase as the advance of senescence.

This increase in APX enzyme activity in response to different types of stress has also been found in other vegetables. For example, Martínez-Hernández et al. (2013) reported in broccoli that UV-C treatment with doses of 6 kJ $m^{-2}$ caused an increase in the activity of the APX enzyme during the first 9 d of storage at 5 °C, which then decreased towards the end. Besides, Boonkorn et al. (2012) reported that tangerine fruit exposed to ozone at a concentration of 200 µg $L^{-1}$ showed an increase in the activity of the APX enzyme with respect to the control.

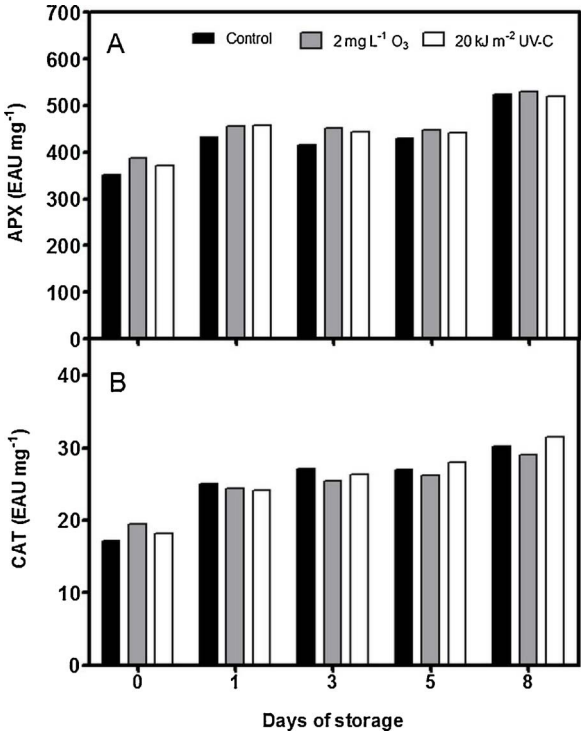


**Fig. 4.** Ascorbate peroxidase (APX) (A) and catalase (CAT) (B) activities of minimally processed rocket treated with UV-C radiation (20 kJ $m^{-2}$) and gaseous $O_3$ (2 mg $L^{-1}$) stored at 5 °C for 8 d. There were no significant differences among treatments at each storage time at $P < 0.05$ according to LSD test.
(A) SD = 22.34, LSD ($P \leq 0.05$) = 27.50.
(B) SD = 1.76, LSD ($P \leq 0.05$) = 2.17.

### 3.6. CAT

The function of the CAT enzyme is to eliminate $H_2O_2$ and thus avoid its toxicity (Lemoine et al., 2010). Immediately after being processed, the whole leaves samples showed an activity of 16.3 ± 1.7 UAE $mg^{-1}$ protein and no significant differences with respect to the control and the samples treated with ozone or UV-C were found (Fig. 4B).

During storage at 5 °C, the activity of CAT increased on d 1 and remained practically stabile for 5 d. Subsequently, a significant increment ($P < 0.05$) in the activity of the enzyme was detected toward the end of storage. Such an increment was of about 20, 19 and 30% in control, $O_3$ and UV-C treated samples respectively. However, no significant differences between the control and the treated samples were recorded after their processing and during storage. This increased activity of the CAT enzyme could be due to the advance of product senescence that would thus generate ROS which in turn actives the antioxidant enzymes (APX and CAT).

The results obtained agree with those of Starzyńska et al. (2003) who reported that an increase in CAT activity in broccoli after 10 d of storage at 5 °C. Besides, Martínez-Hernández et al. (2013) also found that treatment with UV-C radiation (6 kJ $m^{-2}$) induced an increase of the enzyme CAT in broccoli after 9 d at 5 °C. However, studies by Sala and Lafuente (2004) found in "Navelina" oranges stored for 21 d at 22 °C, a decrease in the activity of the CAT enzyme in response to oxidative stress.

On the other hand, Boonkorn et al. (2012) also reported an increase in the activity of the CAT enzyme in mandarin exposed to an ozone concentration of 200 µg $L^{-1}$ after 2 d of storage at 25 °C. These authors affirmed that it is important for vegetables to maintain a high activity of this enzyme since it is their defense mechanism against the oxidative stress that ozone can cause.

The different observed activities of CAT and APX enzymes may be due to the fact that these enzymes have different affinities with respect

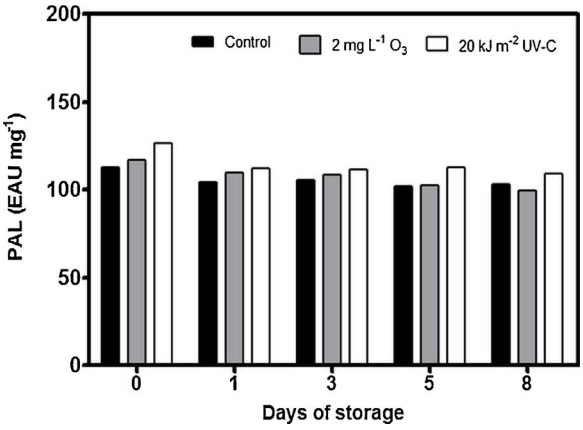


**Fig. 5.** Phenylalanine ammonia lyase (PAL) activities of minimally processed rocket treated with UV-C radiation (20 kJ $m^{-2}$) and gaseous $O_3$ (2 mg $L^{-1}$) stored at 5 °C for 8 d. There were no significant differences among treatments at each storage time at $P < 0.05$ according to LSD test.
SD = 8.01, LSD ($P \leq 0.05$) = 9.86.

to $H_2O_2$ (substrate), since the APX has affinity in the micromolar range and the CAT in millimolar ranges. These differences in affinity might be due to the functions each one performs, APX modulates ROSs as signaling molecules and CAT eliminates ROS when toxic levels are reached (Jacobo-Velázquez et al., 2011; Martínez-Hernández et al., 2013).

### 3.7. Phenylalanine ammonia lyase activity

This enzyme phenylalanine ammonia lyase (PAL) is important in the metabolism of phenylpropanoid, since it catalyzes the formation of transcinnamic acid through the L-deamination of phenylalanine producing the accumulation of phenylpropanoides as phenolic acids and flavonoids. Activation of the PAL enzyme can be induced by different types of stresses, both biotic (bacteria, fungi, etc.) and abiotic (low and high temperature, UV-C, etc.) (Lemoine et al., 2010).

In this paper, it was found that immediately after processed, the whole leaves samples showed an activity of 96.1 ± 10.6 UAE $mg^{-1}$ protein and no significant differences ($P > 0.05$) with respect to the control and the samples treated with ozone or UV-C were found (Fig. 5). During storage at 5 °C, the activity of PAL did not show significant variations in correlation with the total phenolic content which remained practically constant along storage. In contrast to these results, other authors reported an increase of the PAL enzyme in fruits treated with UV-C radiation such as mangoes, peaches and apples (González-Aguilar et al., 2007; Lemoine et al., 2010). On the other hand, Ong et al. (2014) found that ozone treatments at different concentrations (1.5, 2.5, 3.5 and 5.0 μL $L^{-1}$) in papaya induced an increase in the PAL enzyme during their storage for 10 d at room temperature. However, control fruit had lower PAL activity through storage.

According to the data obtained in this paper for cut rocket, the treatments tested in it (that is, 20 kJ UV-C $m^{-2}$ and 2 mg $L^{-1}$ $O_3$) would not induce enough ROS production in order to active PAL and the synthesis of phenolic compounds.

## 4. Conclusions

Based on the findings of this paper, it can be concluded that UV-C light and gaseous ozone treatments would not exert a significant additional stress as to the cutting stage of the leaves. This is the reason why they did not trigger greater activation of the antioxidant system. The increase in the activities of the APX and CAT enzymes during storage would be related to the need of rocket to eliminate high levels of ROS which are toxic to it. Consequently, the reduced levels of ROS due to the action of detoxifying enzymes could contribute to tissue integrity in advanced stages of senescence.

Applying the treatments with 20 kJ UV-C $m^{-2}$ or 2 mg $L^{-1}$ $O_3$ as sanitizing agents in the manufacture of minimally processed cut rocket would be feasible as these treatments kept its sensorial and microbiological quality and did not modify its bioactive compounds with antioxidant activity content during 8 d storage at 5 °C.

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