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Combined Effect of UV-C and Modified Atmosphere Packaging for Keeping Antioxidant Compounds and Extend to Shelf-Life of Fresh-Cut Rocket Leaves

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***Abstract*— The objective of this study was to investigate the effect of UV-C treatments (7.5, 15 and 30 kJ/m2) combined with modified atmosphere packaging (MAP) on phenolic compounds, antioxidant capacity and shelf-life of fresh-cut rocket leaves during 12 days of storage at 5 °C. Samples no UV-C irradiation under passive MAP were used as a control. According to the sensory quality attributes, all combination treatments resulted in a shelf-life for up to 12 days, with the exception of higher doses of 30 kJ UV-C/m2 which resulted in a shorter shelf-life. The results showed that combined treatments had no adverse effects on ascorbic acid content, phenolic compound and antioxidant capacity of fresh-cut rocket leaves. The application of 15 kJ/m2UV-C combined with MAPdelayed the degradation of total chlorophyll content throughout shelf-life. UV-C decreased microbial counts after illumination. Until 8 days at 5 ºC, mesophilic, psycrophilic, enterobacteria and yeast and moulds populations were significantly lower in treated samples with UV-C with MAP.As a main conclusion, UV-C light treatment combined with MAP was demonstrated to be a high potential novel technology for surface decontamination and keeping the overall quality and bioactive compounds of fresh-cut rocket leaves.**

***Index Terms*— UV-C,rocket,total phenolics,antioxidant capacity..**

I. INTRODUCTION

In recent years the market sales of ready-to-use vegetables and fresh produce have grown rapidly due to the health benefits associated with the consumption of these foods [1]. The rocket (Eruca sativa Mill) is one of the popular vegetables consumed in Mediterranean countries and it is consumed in raw salads either alone or in a mixture with other vegetables and it is usually marketed as leaf bunches [2, 3]. It is a member of the Brassica plant family, well known for its pleasant bitter flavor and contains a wide range of phytonutrients, such as provitamin A, vitamin C, flavonoids and glucosinolates, as well as potassium, sulfur and fiber [4-6]. The processing of fresh-cut fruits and vegetables promote faster deterioration in comparison with their intact counterparts [6]. Besides, the major postharvest problem of this vegetable is its rapid senescence, that results in a loss of green color or yellowing, as a consequence of chlorophyll degradation [7, 8]. In addition, Brassica vegetables are also a good source of other natural antioxidants such as vitamin C and phenolic compounds, which are also recognized for their beneficial health properties as anti-inflammatory, antihistaminic and antitumoral [9, 10]. It is well known that the antioxidant content of the vegetables may greatly vary depending not only on the cultivar and farming methods, but mostly on the post-harvest handling practices [10, 11].Therefore, developing effective methods for prolonging the fresh status as well as preserving or even increasing the content and activity of the antioxidant compounds of the fresh produce through the post-harvest handling and processing, could be of importance for the improvement in the commerciality of fresh produce, as well as for the improvement of the positive effects of the consumption of fruits and vegetables on human health [10].

The modified atmosphere packaging (MAP) is a technique used for prolonging the shelf life of fresh-cut vegetables, provided that levels of O_2 are high enough to prevent anaerobic conditions. Besides, MAP decreases microbial development and reduces cross-contamination, and in this way improves food safety [8, 12]. Artés-Hernández et al. [12] reported that the efficacy of MAP requires an atmosphere of 3-5 kPa O_2 and 8-10 kPa CO_2 and that a temperature below 5 ºC is quickly reached. However, this effect can be modified if the characteristics of film permeability induce non-beneficial results [13].

On the other hand, ultraviolet (UV) radiation has been used to extend the shelf life of several fresh fruits and vegetables [14]. The UV wavelength band ranges from 100 to 400 nm and the UV radiation is divided into three sub-bands: UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm) wavelength ranges [15, 16]. The shortwave ultraviolet light range of (UV-C) (200–280 nm) is known as a germicidal range and has been found to be efficient for control of postharvest ripening and diseases in tomatoes, strawberries, baby spinach, broccoli, peppers, and blueberries among others [1]. Besides the exposure to UV-C light has been shown to cause plant tissues stress, which induces defense mechanisms in plants that include the accumulation of antimicrobial compounds, decrease in cell wall degrading enzymes, increase in activity of defense enzymes and increase in antioxidant activity ([10, 17].These compounds are highly desirable as they can contribute to prolong the life and maintain the quality of vegetable and fruits by delaying

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senescence and fruit ripening, andfor enhancing the induction of natural defenses against fungi and bacteria [1, 10]. It has been reported that exposure to UV radiation resulted in the enhancement of total phenols and polyamine compounds in mangoes[18], anthocyanin, phenolic content, and antioxidant capacity in strawberries [19], flavonoids in blueberries [20] and ascorbic acid, phenolic compounds and antioxidant activity in tomatoes [1, 11, 21]. However, in some cases, high doses of UV-C may produce damage. Pan et al. [22] found that doses from 1 to 4.1 kJ UV-C/m^2 caused effects such as calyx browning, soft spots, and loss of anthocyanin and phenolic content in strawberries.

Therefore, the aim of this study was to investigate the effect of three pre-packaging UV-C treatments under passive MAP on the postharvest quality, total phenolic compoundand antioxidant activity of fresh-cut rocket leaves during shelf life.

II. MATERIALS AND METHODS

A.Plant material and chemicals

Rocket (*Eruca vesicaria subsp. Sativa*Mill) leaves were provided by local producer in Santiago del Estero, Argentina. Immediately after harvest the leaves were transported to the laboratory, where they were stored in a cold room at 5 °C in darkness.Next day, the leaves were minimally processed in a disinfected room at 16 °C.

B. Sample preparation, treatments and storage conditions

Leaves with defects such as physically damaged, dehydrated or yellowing were discarded.Afterwards, the selected leaves were washed with tap water (5 °C) for 1 min and drained on a stainless-steel mesh. The leaves were cut in strips of about 20 mm in size and then were washed for 2 min at 5 °C. All cuts were manually made with sharp and disinfected knives (150 mg/L NaClO). The remaining water of the cut leaves was removed using a manual centrifuge and then the following treatments were applied:Controland UV-C treatment of samples(7.5, 15 and 30 kJ/m^2).The UV-C equipment was earlier described [6].To generate passive modified atmosphere packaging (MAP), samples of about 60 g of cut leaves per treatment were randomly placed in 600 mL polypropylene (PP) trays and thermally sealed on the top with a bi-oriented PP film of 35 µm in thickness.

The O_2 and CO_2 transmission rates at 20 °C and 90 % RH were 5,000 mL O_2/m^2.d.atm and 18,000 mL CO_2/m^2.d.atm and the water vapor transmission rate was 110 g/m^2.d.atm (data provided by INTI, Argentina).Three replicates, each one comprising a PP traywith processing treatment and storage time of (1, 4, 8 and 12 days) were prepared and kept in a cold room at 5 °C.

C. Sensory quality

Overall visual quality and decay was evaluated using a nine-point scale (9 = excellent, 7 = good, 5 = acceptable (limit of acceptability), 3 = poor and 1 = extremely poor). Color and odor were evaluated using a five-point scale, where 5 = full characteristic of the product, 3 = acceptable (limit of acceptability) and 1 = no characteristic, based on that used by Gutierrez et al. [6].

These sensory attributes were evaluated in a sensory room at 20 °C, equipped with individual cabinets on days 1, 4, 8 and 12 by a trained panel (8 members ranging between 25 and 65 years) over a representative sample coming from each treatment, immediately afteropening the packages.

D. Color

A tristimulus colorimeter (Minolta CR-300, Osaka, Japan) was used to measure the color variation of the rocket samples at different times of storage. The colorimeter was calibrated with color standards of the CIE LAB system. Data were collected and recorded during each test with L*a*b* color space values meaning: L* (brightness), a* (red to green color) and b* (yellow to blue color). The combination parameters such as the hue (h°) angle [$(h° = 180 + \tan^{-1}(b^*/a^*)$], expressing the characteristic/dominant color, and the Chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$], quantifying the color intensity, were also determined.Fifteen measurements were performed for each treatment on days 1, 4, 8 and 12 of storage period.

E. Ascorbic acid content

Ascorbic acid was measured by titrating with 2,6-dichlorophenolindophenol according to the method of A.O.A.C.[23].Samples wereprepared with 10 g of rocket leaves on each tray, homogenized in 20 ml of 3.0% metaphosphoric acid (HPO_3) solution andthe filtrate transferred into a 100 ml volumetric flask. The volumewas made up to 100 ml using 3.0% HPO_3. An aliquot of the sample(10 ml) was taken and titrated against 2,6 dichlorophenolindophenoldye until a pink color persisted for 15 s. The ascorbicacid content was expressed as mg/100 g of fresh weight (FW). All measurements were made in triplicate.

F. Extraction and determination of phenolics

Polyphenol extraction was conducted according to the procedure described by Gutiérrez et al.[8].The analysis of total phenolics was determined using the Folin-Ciocalteu method [24].The absorbance was measured at 760 nm after 1 h at 25°C using a UV-vis spectrophotometer(JASCO, model V-630, Japan). A standard curve was prepared by using a standard solution of chlorogenic acid. Results were expressed as mg chlorogenic acid equivalents (CAE)/g FW. All measurements were made in triplicate.

G. Total antioxidant capacity

Total antioxidant capacity was tested based on the evaluation of the free radical scavenging capacity according to the DPPH assay [25].An aliquot of 150 µL of the extract obtained from the preparation of phenolic compounds was added to the 2,850 µL of 0.1 mM DPPH solution (prepared with ethanol) and was stored in the dark for 1 h at ambient temperature. The absorbance at 515 nm was measured at different times with a spectrophotometer (JASCO V-630, UV-vis). The calibration curve was performed using Trolox as a standard, and the results were expressed in mg of Trolox equivalent (TE)/g FW. All measurements were made in triplicate.

H. Chlorophyll and Carotenoid Content

The sample preparation for chlorophyll and carotenoides determination was conducted according to Gutiérrez et al. [6]. A 0.4 g of frozen rocket was triturated with 15 mL of a mixture of acetone/water (80:20), and then centrifuged at 6,000 x g for 20 min. After centrifugation, the supernatant was used to determine the total chlorophyll content, chlorophyll a and b and total carotenoids and the absorbance (A) at 663.2, 646.8 and 470 nm was measured using a UV-visible spectrophotometer (JASCO, model V-630, Japan). The equations developed by Lichtenthaler [26]were used to determine the individual levels of chlorophyll a ($Ca = 12.25\ A_{663.2} - 2.79\ A_{646.8}$), chlorophyll b ($C = 521.5\ A_{646.8} - 5.1\ A_{663.2}$), where the total chlorophyll amount was calculated as (Ca + Cb) and total carotenoids [$C_{x+c} = (1000\ A_{470} - 1.82\ Ca - 85.02\ Cb)/198$]. Chlorophyll and carotenoids contents were expressed as mg /100 gFW. All measurements were made in triplicate.

I.Microbiological analysis

To determine each microbial group (mesophilic, psychrotrophic and enterobacteria, molds and yeasts), 10 g of the sample was placed in a stomacher bag under sterile conditions.After adding 90 mL of sterile buffered peptone, the mixture was homogenized in a masticator (Bioamerican Science, Argentina) for 2 min and aliquot diluted were prepared in 0.1% isotonic peptone water as needed. To determine aerobic mesophilic count, 100 µL of the diluted sample was spread on plate count agar (PCA) and incubated at 37 ºC for 2 days and at 5 ºC for 7 days for aerobic psychrotrophic counts. In order to determine enterobacteria counts, 100 µL of the diluted sample was spread on eosin methylene blue agar (EMB) and incubated at 37 ºC for 2 days; and for determine yeast and mould counts, 100 µL of the diluted sample was spread on potato dextrose (PD) with addition of 2 mL/L of lactic acid incubated at 27 ºC for 7 days.The analysis was repeated three times for each replication and results expressed on fresh weight basis as log CFU/g.

I. Statistical analysis

The experiment was a 4 × 4 factorial design (UV-C treatments × storage time) which was subjected to analysis of variance (ANOVA) using Infostat Versión 2011 software (National University of Cordoba, Argentina). Mean values were subjected to the Least Significant Difference (LSD) test at $p< 0.05$.

III. RESULTS AND DISCUSSION

A. Sensory Analysis

After 6 days at 5 °C, fresh-cut rocket leaves presented a slight decrease in overall appearance, color and odor without significant differences between treatments, but not exceeding the acceptable limit of usability for fresh consumption (Fig.1). However, at 5 °C all treatments didnot reach the limit of marketability after 12 days, with the exception of the 30 kJ/m^{2}UV-C combined treatment, which registered a shorter shelf-life mainly due to the moderate unpleasant odors after 8 days. This decrease of shelf life could be due to high UV-C doses provoking cell damage that could have helped microbial growth inducing softening[27].This result of UV-C coincides with reports by Artés-Hernández et al. [28] who established a maximum shelf life of 11 days at 5 °C in fresh-cut 'Fashion' watermelon treated with low UV-C doses of 1.6 and 2.8 kJ/m^2, while the treated with higher UV-C doses of 4.8 and 7.2 kJ/m^2, were considered acceptable for fresh consumption only up to 8 days at 5 °C.

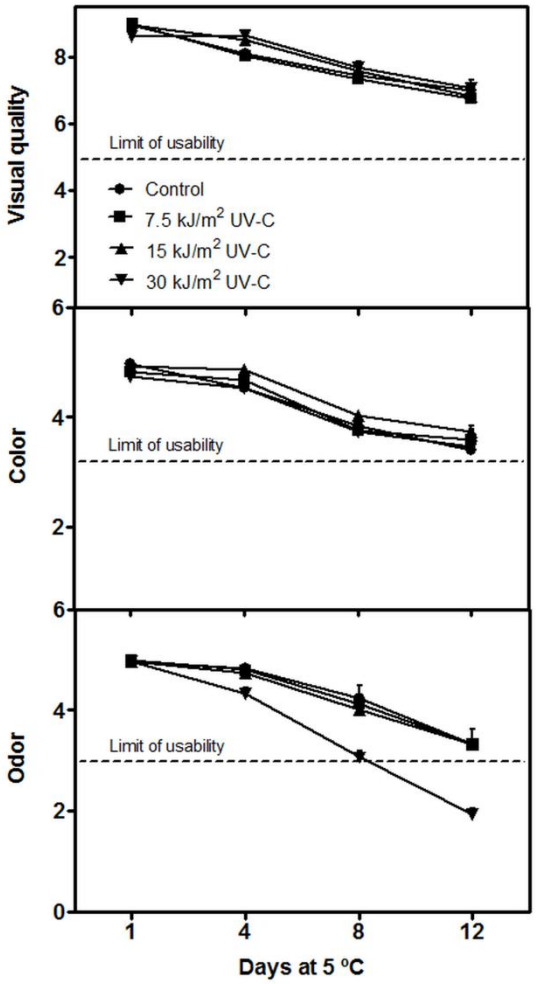

Fig. 1: Sensory evaluation of visual quality (A), color (B) and odor (C) of fresh-cut rocket leaves untreated (Control) and treated with several UV-C doses and stored under passive MAPup to 12 days at 5 °C. Error bar shows standard deviation (SD).

B. Color

Table 1 show the effect combined of UV-C radiation with MAP on the surface color parameters on fresh-cut rocket leaves during storage. It can be observed that the initial lightness value (L*) was between 66.7 and 68.2, preserving the initial value for all treatments at the end of storage. Surface color saturation (chroma) of fresh-cut rocket leaves did not significantly change during storage at 5 °C as well as the hue angle. No significant differences showed in L*, Chroma and Hue values among treatments throughoutcold storage, which indicated that UV-C radiation did not have any negative effect on the color changes of rocket leaves.

Similar results have been reported, where treatment with 4.54 kJ UV-C/m^2 on Tatsoi baby leaves kept L*, C* and Hue angle values during shelf life [29] and lettuce [30].
In other work reported by Fonseca and Rushing [31], slight changes in color were found in fresh-cut watermelon treated with 1.4-13.7 kJ UV-C/m^2 after 5 days at 3 °C. In the same way, slight changes in color found after 18days at 10 °C on red peppers treated with 7 kJ UV-C/m^2 and untreated[32].
However Alexandre et al. [33] showed that 12.36W/m^2 UV-C for 2 min induced a negative impact on red bell pepper color changes. Color changes of fresh-cut leafy vegetables during storage can be induced by storage under light or dark conditions [25, 34].

Table 1: Color parameters L*, Chroma and hue changes for fresh-cut rocket leaves untreated (Control) and treated with several UV-C doses and stored under passive MAP up to 12 days at 5 °C.

Color parameter and UV-C dose	Storage time at 5 °C			
	Day 1	Day 4	Day 8	Day 12
L*				
Control	68.2 $_1^A$	67.6 $_1^A$	68.8 $_1^A$	70.0 $_1^A$
7.5 kJ/m^2 UV-C	66.9 $_1^B$	66.9 $_1^B$	68.3 $_1^{AB}$	70.1 $_1^A$
15 kJ/m^2 UV-C	67.3 $_1^B$	67.2 $_1^B$	69.2 $_1^A$	69.3 $_1^A$
30 kJ/m^2 UV-C	66.7 $_1^A$	67.8 $_1^A$	68.9 $_1^A$	69.1 $_1^A$
Chroma				
Control	23.5 $_1^A$	22.5 $_1^A$	21.5 $_1^A$	22.7 $_1^A$
7.5 kJ/m^2 UV-C	22.3 $_1^{AB}$	20.7 $_1^B$	21.3 $_1^B$	23.2 $_1^A$
15 kJ/m^2 UV-C	22.9 $_1^A$	21.4 $_1^A$	22.6 $_1^A$	23.0 $_1^A$
30 kJ/m^2 UV-C	22.0 $_1^A$	20.8 $_1^A$	21.8 $_1^A$	21.9 $_1^A$
Hue				
Control	152.5 $_1^A$	154.2 $_1^A$	157.7 $_1^A$	156.5 $_1^A$
7.5 kJ/m^2 UV-C	157.3 $_1^A$	159.1 $_1^A$	159.1 $_1^A$	155.1 $_1^A$
15 kJ/m^2 UV-C	154.7 $_1^A$	157.5 $_1^A$	154.7 $_1^A$	156.7 $_1^A$
30 kJ/m^2 UV-C	156.6 $_1^A$	155.9 $_1^A$	156.8 $_1^A$	154.5 $_1^A$

Different letter among each row denotes significant difference ($p<0.05$). Different numbers within each column denotes significant difference ($p<0.05$).

C. Ascorbic acid

The ascorbic acid content in all samples decreased significantly ($p< 0.05$) during the 12 days of storage at 5 °C (Fig. 2).The application of combined treatments had no significant influence on ascorbic acid content throughout the storage period, and no significant differences between the control and the treated samples with UV-C. Similar results have been found in UV-C treated broccoli [35], blueberries [36], fresh-cut watermelon [28] and Satsuma mandarin [37], where the UV-C treatments showed no adverse effect on ascorbic acid content during refrigerated storage.

However, our result was in contrast to previous findings in tomato [38, 39] and fresh-cut mango[18]in which UV-C treatment decreased ascorbic acid levels. This negative effect of UV-C on ascorbic acid content was probably due to heat-sensitivity of vitamin C and oxidation of vitamin C, which are stimulated in the presence of light, oxygen and enzymes, e.g., ascorbate oxidase and peroxidase [40].

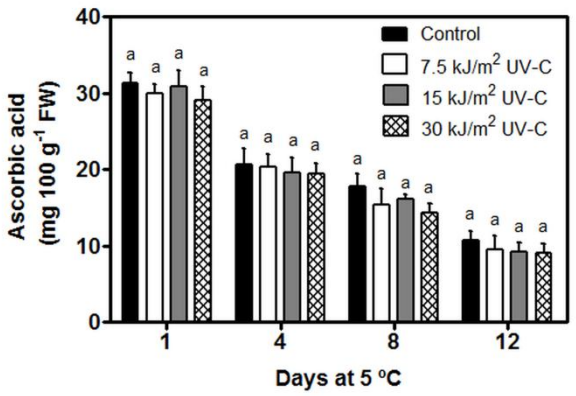

Fig. 2: Ascorbic content of fresh-cut rocket leaves untreated (Control) and treated with several UV-C doses and stored under passive MAPup to 12 days at 5 °C. Different letters at each storage time represent significant differences at $p < 0.05$ according to LSD test.

D. Total polyphenol content

Initial total phenolic content for all treatments was similarlyranged between 2.0 and 2.2 mg CAE/g FW (Table 2).The UV-C treatments had no significant influence on total phenoliccontent throughout shelf-life at 5 °C. It wasmaintained practically constant without significant differences among the treated samples with UV-C and control sample.

These results agree with what was reported by Tomás-Callejas et al. [41], who found that the phenolic content was kept throughout the shelf life after minimal processing on freshcut mizuna baby leaves, a different cultivar of B. rapa. In Brassica vegetables, the different polyphenol contents may be influenced by several factors, including genetic and environmental influences, growing period and maturity stage atharvest time[29, 42].However many studies have shown that UV-C treatment is effective in increasing total phenolic content of fruit and vegetables [19, 29, 36, 43]. Shen et al.[37] reported that UV-C doses of 1.5 and 3.0 kJ/m^2 increased the total phenolic content in Satsuma mandarin during storage, while the lower dose (0.75 kJ/m^2) has not had such an effect. Erkan et al. [19] also reported an increase of phenolic contentin strawberry fruittreated with 0.43-4.3 kJ/m^2.

On the other hand, Artés-Hernández et al. [28] noted in fresh-cut ‘Fashion’ watermelon treated with different UV-C radiation doses (1.60, 2.80, 4.80 and 7.20 kJ/m^2) that the polyphenols content decreased considerably throughout storage without significant differences among treatments. Besides, in other studies noclear UV-C influencewas found in total phenolic content of Collins’and ‘Bluecrop’ blueberries after 7 days at 5 °C plus 2 days at 20 °C [36].

E.Total antioxidant activity

As observed for the total phenolic content, the total antioxidant capacity was maintained stable during storage and was not significantly affected by UV-C treatments (Table 2).This positive correlation among the phenols content and antioxidants capacity has been reported before [44, 27]. A similar pattern was described by Jemni et al. [45], who found that the UV-C radiation stabilized the total antioxidant activity of date fruits (cv. Deglet Nour) during storage at 20

°C. López-Rubira et al. [46]also reported that in pomegranate arils there were no significant differences in antioxidant activity between control and UV-C treated arils after 13 days of MAP storage at 5 °C.Antioxidants are not only phenolic based but alsothe other compounds such as phytic acid, selenium, tocopherol, etc. can contribute to the antioxidant power of plant tissues [40]. However, treatment with UV-C cause stress in plant tissues, which stimulates the biosynthesis of defensive secondary metabolites with antimicrobial and antioxidant activity [10, 17].Sari et al. [17] reported an increase of antioxidant activity in 'Phulae' pineapple fruit treated with UV-C (13.2, 26.4 and 39.6 kJ/m^2) during storage at 10 °C for 28 days. Similar observations have been reported previously in fresh-cut mangoes [18], strawberries [19], blueberries [36], watermelon [28], fresh-cut broccoli [27], Tatsoi baby leaves [29] and tomato [47].

Table 2: Total polyphenols, antioxidant capacity, total chlorophyll content and total carotenoids content changes in fresh-cut rocket leaves untreated (Control) and treated with several UV-C doses and stored under passive MAPup to 12 days at 5 °C.

Parameter and UV-C dose	Storage time at 5 °C			
	Day 1	Day 4	Day 8	Day 12
Total polyphenols (mg CAE g^{-1} F.W)				
Control	2.1 $_1{}^A$	2.1 $_1{}^A$	2.0 $_1{}^A$	2.1 $_1{}^A$
7.5 kJ/m^2 UV-C	2.0 $_1{}^A$	2.1 $_1{}^A$	2.0 $_1{}^A$	2.1 $_1{}^A$
15 kJ/m^2 UV-C	2.1 $_1{}^A$	2.2 $_1{}^A$	2.2 $_1{}^A$	2.1 $_1{}^A$
30 kJ/m^2 UV-C	2.2 $_1{}^A$	2.2 $_1{}^A$	2.1 $_1{}^A$	2.2 $_1{}^A$
Antioxidant capacity (mg TE g^{-1} F.W)				
Control	2.9 $_1{}^A$	2.8 $_1{}^A$	2.8 $_1{}^A$	2.6 $_1{}^A$
7.5 kJ/m^2 UV-C	2.8 $_1{}^A$	2.8 $_1{}^A$	2.7 $_1{}^A$	2.6 $_1{}^A$
15 kJ/m^2 UV-C	2.9 $_1{}^A$	2.8 $_1{}^A$	2.7 $_1{}^A$	2.7 $_1{}^A$
30 kJ/m^2 UV-C	2.9 $_1{}^A$	2.8 $_1{}^A$	2.7 $_1{}^A$	2.7 $_1{}^A$
Total chlorophyll (mg 100 g^{-1} F.W)				
Control	98.4 $_1{}^A$	88.2 $_1{}^B$	90.9 $_1{}^{AB}$	71.2 $_2{}^C$
7.5 kJ/m^2 UV-C	96.5 $_{1,2}{}^A$	90.6 $_1{}^A$	89.3 $_1{}^A$	76.8 $_{1,2}{}^B$
15 kJ/m^2 UV-C	90.8 $_{1,2}{}^A$	88.4 $_1{}^A$	88.5 $_1{}^A$	80.6 $_1{}^B$
30 kJ/m^2 UV-C	87.3 $_2{}^A$	83.7 $_1{}^A$	84.3 $_1{}^{AB}$	73.9 $_{1,2}{}^B$
Total carotenoids (mg 100 g^{-1} F.W)				
Control	24.4 $_1{}^A$	19.2 $_1{}^{BC}$	21.0 $_1{}^{AB}$	15.9 $_1{}^C$
7.5 kJ/m^2 UV-C	24.1 $_1{}^A$	20.6 $_1{}^A$	20.9 $_1{}^A$	16.4 $_1{}^B$
15 kJ/m^2 UV-C	21.9 $_{1,2}{}^A$	21.5 $_1{}^A$	20.7 $_1{}^A$	16.0 $_1{}^B$
30 kJ/m^2 UV-C	21.1 $_2{}^A$	20.4 $_1{}^A$	19.8 $_1{}^A$	14.3 $_1{}^B$

Different letter among each row denotes significant difference ($p<0.05$). Different numbers within each column denotes significant difference ($p<0.05$).

F.Chlorophyll and Carotenoid Content

The initial chlorophyll amount was in the order of 98.4 mg /100 g FW in the control (Table 2).After radiation, the treatments with UV-C reduced the initial total chlorophyll content (chlorophyll a + chlorophyll b), with decreases of 12% for the higher UV-C dose of 30 kJ UV-C/m^2, while inthe doses of 7.5 and 15 kJ UV-C/m^2 retained the same amount as the control. Our data are in agreement with the reports by Martínez-Hernández et al. [27], who found a reduction of total chlorophyll content in fresh-cut Bimi® broccoli of approximately 23% after treatment with 4.5 kJ UV-C/m^2, and a 31% reduction for treatments with 9.0 and 15.0 kJ/m^2, while with 1.5 kJ UV-C/m^{2}the treated samples retained the same amount as the control. Additionally, these data are in line with the reports by Lemoine et al. [35]who worked with broccoli L. var. Italica.

All treatments showed a decrease of the initial chlorophyll content throughout the shelf-life at 5 °C. The combined treatment had a lower degradation rate than controls. However, at the end of the storage period only the samples treated with 15 kJ UV-C/m^2 had lower chlorophyll reductions when compared to control ($p< 0.05$), thus retaining a higher total chlorophyll content. This result coincides with what was reported by Martínez-Hernández et al. [27], who observed that UV-C pre-treated broccoli samples had lower decreases than controls, retaining the highest total chlorophyll content, in agreement with Tomás-Callejas et al. [29] who also found lower chlorophyll degradation in Tatsoi baby leaves treated with 4.54 kJ UV-C/m^2 throughout the shelf-life at 5 °C.

Regarding carotenoid content immediately after radiation,a decrease of around 3-14% was observed in the treatments with UV-C with respect to the control (38.6 mg 100 g^{-1} FW).During storage a significant decrease ($p< 0.05$) for all treatments was observed. At day 12 the total carotenoids content ranged between 14.3 and 16.4 mg/100 g F.W, with a decrease value of 27-35% regarding the initial value, and no significant differences were observed between the treated samples and the control from day 1 of storage (Table 2).However, Martínez-Hernández et al. [27] found that the total carotenoids content remained constant throughout shelf-life at 5 °C in UV-C pre-treated broccoli, with the exception of the control that showed a decreased of 26% when compared with values on the processing day.

G. Microbiological analysis

Immediately after combined treatment, initial microbial counts were lowered. Counts of mesophilic, psycrophilic, enterobacteria and yeast and moulds in treated samples with 7.5, 15 and 30 kJ/m^2 UV-C were significantly lower than thecontrol (Table 3). Moreover, no significant differences among combined treatments were found. The lower microbial counts found in UV-C treated leaves could be attributed to a direct elimination by DNA denaturation, as reported for melons and watermelon [28, 48].

As expected, during storage microbial populations increased for all treated and untreated samples. UV-C radiation not only decontaminated fresh-cut rocket leaves immediately after processing but also affectedthe growth of microbial population during the following storage. The antimicrobial effect the all treatments were observed until days 8 at 5 ºC. However, by the end of the storage, no significant differences among combined treatments compared to the control treatment were found. In some European countries have been adopted that specific microbiological criteria for minimally processed fruits and vegetables, for example, Spanish legislation established 7 log CFU/g as a maximum limit for total viable count.These data indicate a higher microbial stability of the UV-C treated fresh-cut rocket leaves during storage leading to a higher shelf-life. In particular, the shelf life of treated samples with 7.5, 15 and 30 kJ/m^2 UV-C would be higher than 8 days, whilst that the shelf life for the control samples (determinate by psychrophilic count) was approximately 4 days.These results agree with Gogo et al. [49], who reported that the application of UV-C to vegetable amaranth leaves at 1.7 kJ/m^2 stored at 20 °C significantly reduced the aerobic mesophilic and yeast counts

at the initial storage day (0 day), while that no significantly different were observed on moulds counts between UV-C treatments and control throughout the storage period. On the other hand, Martínez-Hernández et al. [27]also reported that after UV-C treatment, broccoli initial microbial counts were lowered and this effect was more noticeable for mesophilic and yeast and molds counts. In the same way, Formica-Oliveira et al. [50] reported that moderate UV-C doses initially reduced by approximately 1.5 log units mesophiles and yeasts and molds loads in shredded carrots being such microbial loads below the threshold limit (7 log units), which defines the shelf life of fresh-cut products, after 72 h at 15 ºC.

Table 3: Mesophilic, psychrophilic, enterobacteriaandyeast andmoulds counts (log CFU/g) changes in fresh-cut rocket leaves untreated (Control) and treated with several UV-C doses and stored under passive MAPup to 12 days at 5 °C.

Parameter and UV-C dose	Storage time at 5 °C			
	Day 1	Day 4	Day 8	Day 12
Mesophilic counts (log CFU/g)				
Control	5.34_{1}^{D}	5.71_{1}^{B}	6.14_{1}^{A}	6.57_{1}^{A}
7.5 kJ /m^2 UV-C	4.56_{3}^{D}	4.78_{3}^{C}	5.39_{3}^{B}	6.24_{12}^{A}
15kJ /m^2 UV-C	4.48_{3}^{D}	4.72_{3}^{C}	5.45_{3}^{B}	6.32_{2}^{A}
30 kJ /m^2 UV-C	4.61_{3}^{D}	4.81_{3}^{C}	5.41_{3}^{B}	6.21_{2}^{A}
Psychrophilic count (log CFU/g)				
Control	6.04_{1}^{D}	6.72_{1}^{C}	7.46_{1}^{B}	8.11_{1}^{A}
7.5 kJ /m^2 UV-C	4.79_{3}^{D}	5.81_{3}^{C}	6.74_{3}^{B}	8.14_{1}^{A}
15kJ /m^2 UV-C	4.71_{3}^{D}	5.73_{3}^{C}	6.81_{3}^{B}	8.06_{1}^{A}
30 kJ /m^2 UV-C	4.64_{3}^{D}	5.69_{3}^{C}	6.78_{3}^{B}	8.18_{1}^{A}
Enterobacteriae counts (log CFU/g)				
Control	4.76_{1}^{D}	5.23_{1}^{C}	5.54_{1}^{B}	5.73_{1}^{A}
7.5 kJ /m^2 UV-C	4.03_{3}^{D}	4.47_{3}^{C}	4.91_{3}^{B}	5.62_{1}^{A}
15kJ /m^2 UV-C	3.96_{3}^{D}	4.39_{3}^{C}	4.86_{3}^{B}	5.58_{1}^{A}
30 kJ /m^2 UV-C	4.05_{3}^{D}	4.45_{3}^{C}	4.92_{3}^{B}	5.59_{1}^{A}
Yeasts and moulds counts (log CFU/g)				
Control	4.12_{1}^{D}	4.86_{1}^{C}	5.31_{1}^{B}	5.51_{1}^{A}
7.5 kJ /m^2 UV-C	3.54_{3}^{D}	4.06_{3}^{C}	4.79_{3}^{B}	5.36_{1}^{A}
15kJ /m^2 UV-C	3.43_{3}^{D}	3.94_{3}^{C}	4.72_{3}^{B}	5.38_{1}^{A}
30 kJ /m^2 UV-C	3.46_{3}^{D}	4.02_{3}^{C}	4.78_{3}^{B}	5.41_{1}^{A}

Different letter among each row denotes significant difference ($p<0.05$). Different numbers within each column denotes significant difference ($p<0.05$).

IV. CONCLUSIONS

Our results suggest that the applicationof UV-C radiation combined with modified atmosphere packaging (MAP)could be a satisfactory treatmentfor fresh-cut rocket leaves. All combinationtreatmentstested retarded microbial development until days 8 at 5 ºC, compared to a non-treated controlunder conventional passive MAP.The sensory attributes were preserved up to 12 days at 5 ºConly for the combined of 7.5 and 15 kJ UV-C/m^2.The total polyphenol content and total antioxidant activity were not affected for the combined treatments and were kept during the storage period. Moreover, the application combined of UV-C with MAP delayed the degradation of total chlorophyll content throughout shelf-life.

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