APPLICATION OF UV-C RADIATION IN THE CONSERVATION OF MINIMALLY PROCESSED ROCKET (*ERUCA SATIVA* MILL.)

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ABSTRACT

The effects of UV-C pretreatments (10, 20 and 30 kJ/m^2), compared with modified atmosphere packaging (MAP), on sensory change, microbial and bioactive profile of minimally processed rocket leaves throughout 12 days at 5C were investigated. Nonirradiated samples were used as controls. All UV-C treatments reduced the natural microflora growth. In relation to sensory quality, all treatments resulted in a shelf life of 8 days. Total antioxidant activity and total phenol content decreased after 8 days for all treatments. Generally, UV-C slightly reduced the initial total chlorophyll content; however, during storage, its degradation was similar to that of the control. It is concluded that UV-C pretreatment could be useful to the industry to reduce the natural microflora growth, keeping the overall quality without affecting the bioactive compound profile of fresh rocket leaves during 8 days of storage at 5C.

PRACTICAL APPLICATIONS

The information provided in this study shows that the use of UV-C radiation could be a useful tool for the food industry for maintaining the overall quality and safety of fresh rocket leaves, while minimizing water consumption and wastewater discharge rates.

INTRODUCTION

The fresh-cut fruit and vegetables offer great advantages for consumers owing to their convenience and ready-to-use properties, although they provide an ideal medium for microbial development (Artés *et al.* 2009).

Rocket is well known for its pleasant bitter flavor, and like other cruciferous vegetables, rocket contains a range of health-promoting phytonutrients including vitamin C, fiber, flavonoids and glucosinolates (Barillardi *et al.* 2005; Martínez-Sánchez *et al.* 2006a). Color is the most important quality characteristic of rocket leaves, as leaves with intense uniform green color are highly attractive. Any alteration of the normal green color could be a limiting factor for their marketability. The major postharvest problem of this vegetable is its rapid senescence, expressed primarily as yellowing, which is the result of chlorophyll degradation, although wilting is also a serious problem (Koukounaras *et al.* 2007).

The modified atmosphere packaging (MAP) is a technique used for prolonging the shelf life of fresh-cut vegetables. However, their beneficial effect can be compromised if the film permeability characteristics are improper, leading to undesirable atmospheres inside the package, adversely affecting product quality (Martínez-Sánchez *et al.* 2006a,b).

The fresh-cut industry commonly uses sodium hypochlorite (NaOCl) and acids as sanitizer agents; however, by-products of this procedure such as trihalomethanes and chloramines are potentially harmful to humans. Therefore, alternative disinfectant agents must be studied (Escalona *et al.* 2010; Nogales-Delgado *et al.* 2012).

Physical methods include ultrasound, high pressure, high-intensity electric field pulses, UV radiation, radio frequency and ionizing radiation, and these methods have been shown to be capable of killing or inhibiting bacterial growth. The use of nonionizing, germicidal and artificial ultraviolet (UV) light at a wavelength of 190-280 nm (UV-C) could be effective for surface decontamination of fresh-cut vegetables and fruits (Artés-Hernández *et al.* 2009; Tomás-Callejas *et al.* 2012).

Treatment with UV-C offers several advantages to food processors as it leaves no residues, does not have legal restrictions, is easy to use and lethal to most types of microorganisms (Bintsis *et al.* 2000), and does not require extensive safety equipment to be implemented (Yaun *et al.* 2004). Lado and Yousef (2002) reported that UV-C radiation from 0.5 to 20.0 kJ/m^2 inhibited microbial growth by inducing the formation of pyrimidine dimers, which alter the DNA helix and block microbial cell replication. Therefore, cells that are unable to repair radiation-damaged DNA die and sublethally injured cells are often subject to mutations. The effective UV-C dosage depends on crop types and doses too high may cause deleterious effects on fruit quality (Perkins-Veazie *et al.* 2008).

Another advantage underlying the effectiveness of UV-C radiation seems to be its independence of temperature in the 5-37C range, although it depends on the incident irradiation (Bintsis *et al.* 2000; Gardner and Shama 2000; Lado and Yousef 2002). In recent years, many studies have been conducted to ascertain the effect of UV-C light on different plants, with the aim of reducing the microbial load on vegetable surfaces (Allende and Artés 2003a), controlling diseases (Pan *et al.* 2004), slowing down processes related to maturation (Barka *et al.* 2000) and also to study how the stress of this radiation affects the development of chilling injury in some sensitive products (Vicente *et al.* 2005). Other studies have reported that UV-C inhibited microbial growth, retarding decay and senescence of zucchini squash (Erkan *et al.* 2001), tomatoes (Maharaj *et al.* 1999; Charles *et al.* 2008), strawberries (Baka *et al.* 1999; Marquenie *et al.* 2002), carrots (Mercier *et al.* 1993a,b, 2000), sweet potatoes (Stevens *et al.* 1999) and fresh-cut melon (Manzoco *et al.* 2011).

The purpose of this study was to evaluate the effects of UV-C pretreatment compared with a conventional passive MAP on sensory change, microbial and bioactive profile of fresh rocket leaves during refrigerated storage at 5C.

MATERIALS AND METHODS

Plant Material and Preparation

Rocket (*Eruca vesicaria* ssp. *sativa*) was obtained from commercial producers located in the Lonquén area in Santiago de Chile, Chile. The leaves were commercially field-grown and hand-harvested with scissors at a marketable development stage, with a minimum length of 10 cm. After the leaves were harvested, they were immediately transported to the laboratory in a portable ice box at 5C, where they were forced-air cooled at 5C and held at 5C until the next day. Rocket leaves were minimally processed in a cold room at 8C and those with defects (physical damage, desiccation or loss of their characteristic green color) were discarded. The selected leaves were washed with tap water for 2 min at 5C and then spin dried for 30 s in a handheld salad spinner to remove excess water.

UV-C Treatment

The equipment used to treat the leaves with UV-C light consisted of a $1.3 \times 0.5 \times 0.7$ m reflective stainless steel chamber, equipped with 12 unfiltered germicidal lamps (254.7 nm, TUV 36W/G36 T8, Philips, Amsterdam, The Netherlands): 6 on the top and 6 at the bottom of the chamber. A stainless steel mesh that would support the samples without significantly blocking the incidence of UV-C radiation was horizontally suspended in the middle of the space between the lamps at a distance of 0.21 m from each other. In order to determine the UV-C radiation intensity of the lamps, a digital radiometer (Cole-Parmer Instrument Company, Vernon Hill, IL) was used. The applied UV-C intensity was calculated as a mean of 15 UV-C readings on each side of the net with nonsignificant differences within both sides, which also shows any possible interference between UV-C and stainless steel mesh. The UV-C light intensity was kept constant, and the applied doses varied by altering the exposure time at the fixed distance. In one side of the steel chamber, a fan was placed to avoid a temperature increase from the lamps. Rocket leaves were carefully arranged in a single layer on the grid for the treatment with doses of 10, 20 and 30 kJ/m^2. UV-C exposure times ranged between 160 and 480 s. Untreated samples were used as control. After the UV-C treatment, rocket leaves were cooled at 5C for about 1 h before packaging.

Rocket Packaging

Samples of approximately 60 g of leaves were manually packed in Sealed Air PD-960 bags (package dimensions: 0.18×0.23 m) (CRYOVAC, Santiago, Chile), with permeability of 6,260 mL O_2/m^2/d/atm and 20,032 mL CO_2/m^2/d/atm at 23C and 0% relative humidity. Bags were heat-sealed and placed at 5C during the storage period.

Gas composition, sensory evaluation, color, chlorophyll (a, b and total), carotenoid content, microbial growth (mesophilic and psychrotrophic bacteria, enterobacteria,

molds and yeasts), phenols and antioxidant capacity were evaluated after processing at 1, 4, 8 and 12 days of storage. The entire experiment was repeated three times, and since the same trends were found, the results from only one experiment are shown.

Changes in Gas Composition within Packages

The atmosphere O_2 and CO_2 concentrations of individual packages were measured during storage using a gas analyzer (Checkpoint, PBI Dansensor, Ringsted, Denmark). Samples of 15 mL of headspace gas were taken through a septum (a patch of silicone sealant applied to the film) using the sensor's needle. Three packages from each treatment were measured each sampling day.

Color

Superficial color was determined by measuring the color parameters L^*, a^* and b^* in the CIE LAB space with a tristimulus colorimeter (Minolta CR 300, Ramsey, NJ), with a viewing aperture of 8 mm in diameter, D65 illuminant and 0° observer angle, previously calibrated using the manufacturer's standard white plate. The color parameters were expressed as lightness (L^*), chroma (C^*) and hue angle ($h°$). The hue angle $[(h° = 180 + \tan^{-1}(b^*/a^*)]$ and chroma values $[C^* = (a^{*2} + b^{*2})^{1/2}]$ were calculated from a^* and b^* values (Koukounaras *et al*. 2007). The measurements were performed on four randomly selected leaves per bag (two measurements per leaf), supporting the leaf on a black surface to prevent color interference, on days 1, 4, 8 and 12 of storage.

Sensory Quality

Sensory evaluation was performed on days 1, 5, 8 and 13 of storage by a nine-member expert panel. The expert panel was trained for their ability to discriminate between small variations in the sensory characteristics. Overall appearance, color, flavor and freshness were evaluated and scored on a 9-1 scale, where 9 = excellent, 7 = very good, 5 = good (limit of consumer acceptability), 3 = fair (limit of usability) and 1 = poor, inedible (Martínez-Sánchez *et al*. 2006a). The samples were coded with random three-digit numbers to mask the treatment identity to minimize subjectivity and ensure test accuracy. All evaluations were performed in a sensory room, equipped with individual cabinets with white and red lights. Flavor and freshness were evaluated under red light, and overall appearance and color were evaluated under white light.

Microbiological Analysis

To determine microbial growth throughout the shelf life, three random samples from each treatment were analyzed on days 1, 4, 8 and 12 of storage.

Samples of 10 g were mixed with 90 mL of 0.1% sterile isotonic peptone water and homogenized in a sterile bag in a stomacher (IUL Instruments, Barcelona, Spain) for 1 min. For the enumeration of each microbial group (mesophilic and psychrotrophic bacteria, enterobacteria, molds and yeasts), serial dilutions were prepared in 0.1% isotonic peptone water as needed, for plating. Culture media and incubation conditions were as follows: (1) aerobic plate count incubated at 37C for 48 h for mesophilic bacteria and at 5C for 7 days for psychrophilic bacteria; (2) eosin methylene blue agar incubated at 37C for 48 h for enterobacteria; (3) potato dextrose with addition of 2 mL/L acid lactic incubated at 27C for 7 days for molds and yeasts. All culture media were purchased from Merck (Darmstadt, Germany). All microbial counts were reported as log colony forming units per gram of product (log cfu/g).

Three replicates were performed on each of three bags for each disinfection treatment. The initial microbial load was determined after treatments.

Total Phenolic Content

The analysis of total phenolics was carried out using the Folin–Ciocalteu method (Singleton *et al*. 1999). About 3 g of ground-frozen (−80C) sample of rocket leaves was homogenized with an aqueous alcoholic solution (MeOH/H_2O, 1:1 by volume) and centrifuged at 12,000 × g for 30 min. A 0.5 mL aliquot of this extract was added into a 10-mL volumetric flask with 8 mL of distilled water and mixed together with 0.5 mL of Folin–Ciocalteu phenol reagent. After 3 min, 1 mL of 5% Na_2CO_3 solution was added to the solution. The absorbance was measured at 750 nm after 10 min at 25C using a UV-vis spectrophotometer (T70 PG Instruments, Ltd., Leicester, U.K.). Gallic acid was used as a standard and the results were expressed in grams of gallic acid equivalent (GAE) per kilogram of fresh weight. All measurements were made in triplicate.

Antioxidant Capacity

The total antioxidant activity was determined based on the evaluation of the free radical-scavenging capacity according to Brand-Williams *et al*. (1995). A solution of 0.7 mM 2,2-diphenyl-1-picrylhydrazil radical (DPPH) in methanol was prepared before the assay and the absorbance was adjusted to 1.10 ± 0.02 immediately before use. Sample extraction was conducted as described in the Total phenolic content

section. A 150 μL aliquot of the previously described extract and 2,850 μL of absorbance-adjusted DPPH solution were added. The absorbance was measured at 515 nm in a UV-vis spectrophotometer (T70 PG Instruments, Ltd.). The calibration curve was performed using Trolox as a standard. The results are expressed in grams of Trolox equivalent per kilogram of fresh weight. All measurements were made in triplicate.

Chlorophyll Content, Carotenoid Content

For tissue preparation, 30 g of leaves of each treatment was frozen at −80C. Frozen samples (0.4 g) were triturated with 15 mL of acetone : water (80:20, v/v) and then centrifuged at 5,000 × g for 15 min. The supernatant was used to determine the total chlorophyll content, chlorophyll a and b, and total carotenoids according to Lichtenthaler (1987). The absorbance (A) at 663.2, 646.8 and 470 nm was measured using a UV-visible spectrophotometer (T70 PG Instruments, Ltd.). The equations developed by Lichtenthaler (1987) were used to determine the individual levels of chlorophyll a ($C_a = 12.25A_{663.2} - 2.79A_{646.8}$), chlorophyll b ($C_b = 21.5A_{646.8} - 5.1A_{663.2}$), total chlorophyll amount ($C_a + C_b$) and total carotenoids [$C_{x+c} = (1000A_{470} - 1.82C_a - 85.02C_b)/198$]. Chlorophyll and carotenoid contents were expressed as mg/100 g fresh weight. All measurements were made in triplicate.

Statistical Analysis

The experiment was based on a 4 × 4 bifactorial (UV-C pretreatment × storage time), which was subjected to analysis of variance (ANOVA) using Infostat (Version 2011. FCA. Universidad Nacional de Córdoba. Córdoba, Argentina) software. Mean values were subjected to the least significant difference test at $P < 0.05$.

RESULTS AND DISCUSSION

Changes in Gas Composition within Packages

The changes in CO_2 and O_2 concentrations inside the bags during refrigerated storage are shown in Fig. 1. The initial gas composition of passive MAP was air (20.9 kPa O_2 and 0.03 kPa CO_2). As expected, a decrease in the headspace O_2 concentration, along with an increase in the headspace CO_2 concentration, was detected during storage. The O_2 and CO_2 changes within rocket bags were natural consequences of respiratory activity and the gas diffusion across the film (Gil *et al.* 1998; Nogales-Delgado *et al.* 2012).

After 8 days, the O_2 level ranged from 9 to 13% and CO_2 level was in the range of 2–3% inside the bags for the different treatments, and the levels remained steady thereafter until the end of the storage period at 5C. After day 4, O_2 level in the control was significantly above those found in leaves treated with UV-C. This difference, of approximately 2%, remained stable until the end of the storage period. In addition, no significant differences were observed in terms of O_2 concentration inside the bags among the UV-C-treated products (Fig. 1A).

With respect to CO_2, UV-C-treated rocket exhibited higher values than those of the control. No significant differences ($P > 0.05$) were found among the samples treated with different doses of UV-C (Fig. 1B). This could be due to UV-C radiation causing stress on the leaves, increasing their respiratory rate and thus leading to higher concentrations of CO_2 and lower concentrations of O_2 inside the bags.

Earlier studies on leaf vegetables such as "Red Oak Leaf" lettuce-treated UV-C with doses of 0.81, 4.07 and 8.14 kJ/m^2 yielded similar results: higher concentrations of CO_2 and lower concentrations of O_2 inside the bags of

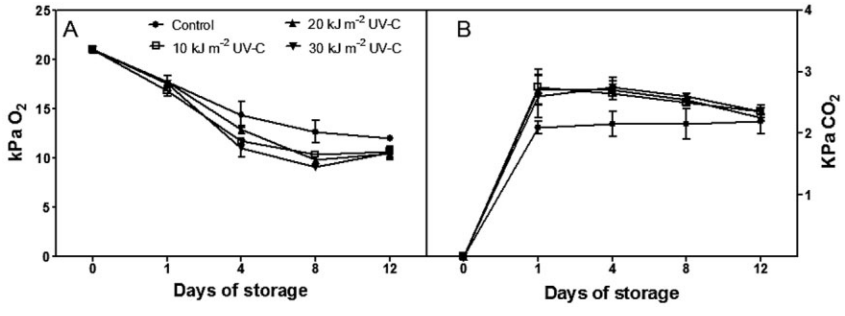

FIG. 1. GAS CONCENTRATION CHANGES WITHIN BAGS OF FRESH-CUT ROCKET LEAVES UNTREATED AND TRATED WITH DIFFERENT DOSES OF UV-C RADIATION AND STORED UP TO 12 DAYS AT 5C: (A) O_2 LEVELS AND (B) CO_2 LEVELS. DATA REPRESENT MEANS OF THREE REPLICATES ($N = 3 \pm SD$)

UV-C-irradiated leaves with respect to the control (Allende and Artés 2003b).

However, these results differ from those reported by Artés-Hernández *et al.* (2009), who observed that spinach leaves treated with different doses of UV-C (4.54, 7.94 and 11.35 kJ/m^2) and untreated samples shared similar patterns of headspace gas change inside the bags during their storage at 5C.

Color

Surface color parameters for rocket leaves treated with 10, 20 and 30 kJ UV-C/m^2 were evaluated during storage. The initial lightness value (L^*) ranged between 43.0 and 45.7 for all samples. The lightness (L^*) increased with time, suggesting incipient yellowing of the leaves at the end of storage for both UV-C-treated and non-treated leaves. No significant differences were found either among the different treatments or with respect to the control (Table 1).

Regarding the chroma parameter (C^*), it increased significantly throughout the shelf life for all treatments and the control relative to the initial values. No significant differences ($P > 0.05$) were found between the irradiated samples and the control during all storage. On the contrary, the hue angle ($h°$) decreased significantly in all samples stored at 5C. No significant differences were found between the control and the treated samples.

These results are in agreement with earlier studies reporting a slight increase of L^* and a decrease in the hue angle after 4 days at 20C in broccoli that was treated with 10 kJ/m^2 UV-C (Costa *et al.* 2006). However, Artés-Hernández *et al.* (2009) noted that spinach leaves irradiated with different UV-C doses (4.54, 7.94, and 11.35 kJ/m^2) showed a significant reduction of about 10–15% in the initial L^* value, as well as a slight reduction of C^*, while hue remained stable. The authors attributed the reduction in the L^* value to possible cellular damage in the leaves caused by the UV-C radiation.

Sensory Analysis

The sensory quality parameters affected most were overall appearance and color (Fig. 2). During the first 8 days of storage at 5C, the overall appearance for the UV-C-treated and untreated samples scored above the acceptable limit of usability for fresh consumption (Fig. 2A). However, all treatments showed an important decrease in overall appearance at 12 days at 5C, being unacceptable for fresh consumption.

When analyzing visual color scores, no significant difference between UV-C-treated and untreated samples were observed until day 8. All samples treated with UV-C showed values above the limit for consumer acceptability during the 12 days of storage and were significantly different from the control at day 12 (Fig. 2B).

According to these sensory quality attributes, a maximum shelf life of 8 days at 5C was established in our conditions for all treatments. Looking at these results, it could be concluded that all tested UV-C doses did not affect the sensory quality of fresh processed rocket.

These results coincide with the reports by other authors, who found no difference between controls and irradiated products such as lettuce (Allende and Artes 2003a,b) and spinach (Artés-Hernández *et al.* 2009). However,

TABLE 1. COLOR PARAMETERS [LIGHTNESS (L^*), CHROMA (C^*) AND HUE ANGLE ($H°$)] OF FRESH-CUT ROCKET LEAVES UNTREATED AND TREATED WITH DIFFERENT DOSES OF UV-C RADIATION AND STORED UP TO 12 DAYS AT 5C

Storage time (days)	UV-C (kJ/m^2)			
	Control	10	20	30
L^*				
1	43.9 ± 2.0^{a}B	45.7 ± 2.5^{a}B	44.4 ± 2.1^{a}B	44.9 ± 2.4^{a}C
4	44.8 ± 2.2^{a}B	45.9 ± 3.5^{a}AB	46.3 ± 4.1^{a}AB	45.8 ± 3.4^{a}BC
8	48.8 ± 3.9^{a}A	47.5 ± 3.8^{a}AB	47.9 ± 3.6^{a}A	48.8 ± 3.3^{a}A
12	48.3 ± 3.3^{a}A	47.7 ± 3.8^{a}A	47.0 ± 3.8^{a}A	47.3 ± 3.4^{a}AB
C^*				
1	28.1 ± 2.2^{a}C	28.3 ± 3.5^{a}A	27.5 ± 2.3^{a}B	27.6 ± 2.1^{a}B
4	28.3 ± 2.8^{a}BC	25.3 ± 1.5^{abc}B	25.5 ± 3.8^{ab}C	23.7 ± 2.1^{c}C
8	29.6 ± 2.5^{a}AB	28.8 ± 3.1^{a}A	29.4 ± 3.2^{a}A	29.7 ± 3.5^{a}A
12	30.9 ± 2.7^{a}A	28.3 ± 3.2^{ab}A	28.3 ± 3.4^{ab}AB	29.2 ± 4.1^{ab}AB
Hue				
1	127.2 ± 1.4^{a}A	126.3 ± 2.3^{a}AB	127.5 ± 1.6^{a}A	126.7 ± 1.8^{a}AB
4	126.4 ± 2.1^{a}AB	127.4 ± 2.6^{a}A	126.3 ± 2.7^{a}AB	127.3 ± 2.4^{a}A
8	125.8 ± 1.9^{ab}B	126.7 ± 2.4^{ab}AB	126.3 ± 1.5^{ab}AB	125.5 ± 2.0^{b}C
12	125.5 ± 2.1^{b}B	125.9 ± 2.7^{ab}B	125.8 ± 1.9^{ab}B	125.8 ± 1.5^{ab}BC

Data represent means ($n = 24$ ± SD). Means in the same row with different superscript letters or in the same column for the same type of sample with different uppercase letters were significantly different according to the least significant difference test at $P < 0.05$.

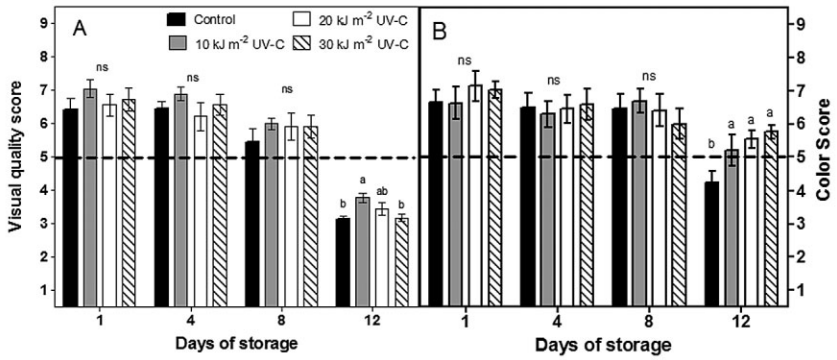

FIG. 2. SCORE MEANS FOT VISUAL QUALITY (A) AND COLOR (B) OF FRESH-CUT ROCKET LEAVES UNTREATED AND TREATED WITH DIFFERENT DOSES OF UV-C RADIATION AND STORED UP TO 12 DAYS AT 5C. THE DASHED HORIZONTAL LINE INDICATES THE LIMIT OF ACCEPTANCE FROM THE CONSUMER'S POINT OF VIEW. BARS ARE THE MEAN OF 3 REPLICATES PLUS STANDARD DEVIATION. DIFFERENT LETTERS AT EACH STORAGE TIME REPRESENT SIGNIFICANT DIFFERENCES ($P < 0.05$) ACCORDING TO THE LSD (LEAST SIGNIFICANT DIFFERENCE) TEST. NS, NOT SIGNIFICANT

Martínez-Hernández *et al.* (2011) observed that the shelf life of broccoli treated with UV-C (1.5–15 kJ/m^2) and stored at 5C reached 19 days, with the exception of 15 kJ UV-C/m^2 treatment, which shortened the broccoli's shelf life. The authors argue that such shortening of the shelf life may be attributed to high UV-C radiation damaging the plant tissue.

Microbiological Analysis

The initial microbial load for mesophilic counts was 5.2 log cfu/g. As expected, microbial populations on the rocket leaves increased during storage. The decontamination effect of UV-C reduced the initial microbial load by 0.6 log cfu/g compared with the control. An antimicrobial effect of the UV-C light was observed during the first 8 days compared with the control. However, at 12 days, no significant differences among treatments nor with the control were found (Fig. 3A). The microbial limit of 7 log cfu/g of aerobic counts established by the Spanish legislation (BOE 2000) was exceeded on day 6 for control, and by day 8 for leaves treated with 10, 20 and 30 kJ/m^2.

Similar results were found for psychrophilic bacterial growth. Initially, UV-C reduced the psychrophilic counts by 0.6–0.9 log cfu/g compared with the control (Fig. 3B). The UV-C treatments inhibited psychrophilic and mesophilic bacterial growth during the first 8 days at 5C. However, at 12 days, no significant differences among treatments were found. These results coincide with those of Allende and Artés (2003b) who reported a small reduction (0.5 log cfu/g) in the growth of psychrophilic bacteria in "Lollo Rosso" lettuce treated with 8.14 kJ UV-C/m^2 and stored in a passive modified atmosphere at 5C.

Regarding enterobacteria (Fig. 3C), the initial counts were significantly inhibited with the UV-C treatments; however, the reduction was slightly less than those of mesophilic and psychrophilic bacteria, ranging from 0.3 to 0.4 log cfu/g. The enterobacteria population increased from

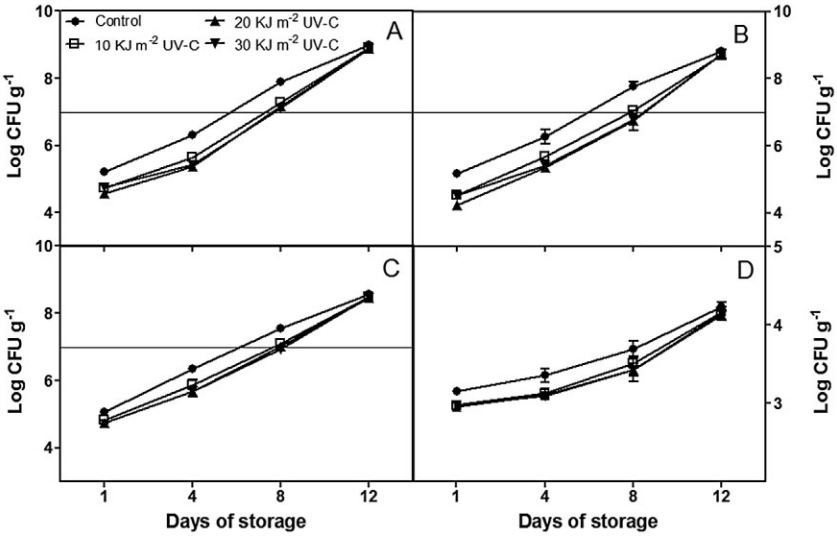

FIG. 3. MESOPHILIC (A), PSYCHROPHILIC (B), ENTEROBACTERIA (C) AND MOLD AND YEAST (D) COUNT (LOG CFU G^{-1}) OF FRESH-CUT ROCKET LEAVES UNTREATED AND TREATED WITH DIFFERENT DOSES OF UV-C RADIATION AND STORED UP TO 12 DAYS AT 5C. DATA REPRESENT MEANS OF THREE REPLICATES ($N = 3 \pm$ SD)

5.1 to 8.5 log cfu/g in control leaves during storage at 5C. The samples treated with UV-C light had enterobacterial counts significantly lower than those of the control until day 12 when no significant difference between treated and control leaves was observed.

Therefore, UV-C can reduce the initial counts, which could be considered as a good alternative to chlorine during the processing line before packaging (Escalona *et al.* 2010). Many research studies report the antimicrobial effect of disinfectant agents immediately after their application. However, the maintenance of the microbiological reduction during storage is even more important. An important point is that the effectiveness of UV-C depends on the incident irradiation, determined by the structure and topography of the surface of the product (Gardner and Shama 2000). It is also known that the sensitivity of bacteria to UV-C varies with species and also among different strains of the same species (Block 1977). Several bacteria and yeast have a potent repair mechanism of photoreactivation. The exposure of cells to visible light after UV-C treatment induces enzymatic photorepair and expression of excision-repair genes that may restore DNA integrity (Sommer *et al.* 2000; Lado and Yousef 2002). Therefore, by this repair mechanism, they recover viability following UV radiation (Mercier *et al.* 2000).

For mold and yeast counts, similar results were found (Fig. 3D). Compared with control, mold and yeast growth was significantly inhibited after UV-C treatments; however, by day 12 at 5C, no significant differences in mold and yeast growth between control and UV-C treated rocket were found. Our results agree with Erkan *et al.* (2001), who showed that yeast growth in zucchini squash slices was inhibited when treated with 4.93 and 9.86 kJ/m^2 UV-C radiation doses. On the contrary, Martínez-Hernández *et al.* (2013) and Lemoine *et al.* (2007) did not find any fungistatic effects on broccoli after radiating it with doses of 6.0 and 8.0 kJ UV-C/m^2, respectively.

An inadequate control of the UV-C light management during treatment of leafy greens might damage the superficial tissues of the leaves and cause nutrient release and promote microbiological growth during storage (Tomás-Callejas *et al.* 2012). It has been suggested that UV-C light could alter the cell permeability, causing leakage of electrolytes, amino acids and carbohydrates, which might stimulate microbial growth (Nigro *et al.* 1998). In addition, it might have an impact on the sensory quality. The crucial point is whether a safe dose can be found which would greatly impair microbial growth without damaging the product (Ben-Yehoshua and Mercier 2005).

We conclude that the studied UV-C treatments prolonged the shelf life of the leaves by 2 days based on the reduction in microbial growth and maintenance of the sensorial quality during 8 days at 5C.

Total Phenolic Content

Phenolic compounds are important secondary metabolites with antioxidant activities (Bravo *et al.* 2012) and are important antioxidants in human nutrition (Park and Kim 2015). The initial total phenolic content was 2.06 g GA eq/kg fresh weight (FW) in control leaves. For total phenolic content, samples treated with 10 kJ UV-C/m^2 followed the same behavior as that of the control throughout storage at 5C. A slight decrease in both control and UV-C treatments occurred by day 4, although no further decrease was observed through day 12 (Fig. 4A).

The higher intensity UV-C treatments caused an initial stress in the leaves inducing a significant increase in total phenolic content of approximately 8% for 20 and 30 kJ UV-C/m^2, compared with the initial content of the control at days 1 and 4. Then, the total phenolic content slightly decreased, reaching values similar to the remaining treatments at 8 days, and yet on days 8 and 12, no differences either among treatments or compared with the control were found.

Our results agree with Martínez-Hernández *et al.* (2011), who reported an increase of up to 14% in total phenolic content on the processing day in broccoli florets after irradiating them with doses of 1.5, 4.5, 9.0 and 15.0 kJ UV-C/m^2.

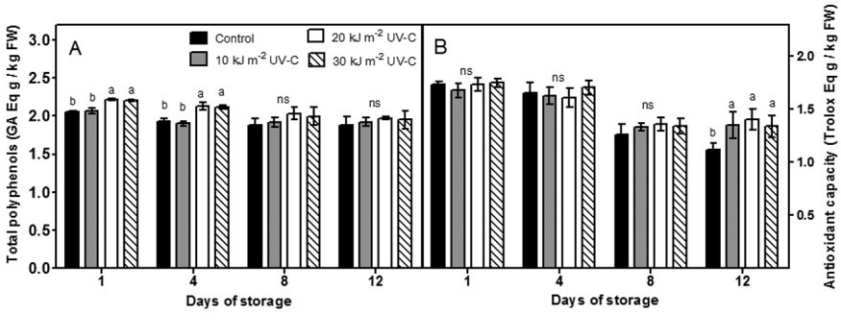

FIG. 4. TOTAL PHENOLIC CONTENT (A) AND TOTAL ANTIOXIDANT CAPACITY (B) OF FRESH-CUT ROCKET LEAVES UNTREATED AND TREATED WITH DIFFERENT DOSES OF UV-C RADIATION AND STORED UP TO 12 DAYS AT 5C. BARS ARE THE MEAN OF 3 REPLICATES PLUS STANDARD DEVIATION. DIFFERENT LETTERS AT EACH STORAGE TIME REPRESENT SIGNIFICANT DIFFERENCES ($P < 0.05$) ACCORDING TO LSD (LEAST SIGNIFICANT DIFFERENCE) TEST. NS, NOT SIGNIFICANT

TABLE 2. CHANGES IN THE CONTENT OF CHLOROPHYLL A, CHLOROPHYLL B AND TOTAL CHLOROPHYLL AND CAROTENOID OF FRESH-CUT ROCKET LEAVES UNTREATED AND TREATED WITH DIFFERENT DOSES OF UV-C RADIATION AND STORED UP TO 12 DAYS AT 5C

	UV-C (kJ/m^2)			
Storage time (days)	Control	10	20	30
Chlorophyll a (mg/100 g FW)				
1	138.1 ± 6.1^{a}A	106.6 ± 3.8^{b}A	109.7 ± 0.4^{b}A	111.5 ± 5.1^{b}A
4	110.9 ± 8.1^{ab}B	101.4 ± 6.2^{b}A	104.8 ± 3.0^{ab}AB	109.5 ± 6.0^{ab}A
8	105.7 ± 6.0^{a}B	98.8 ± 2.2^{a}A	101.0 ± 1.4^{a}B	106.9 ± 4.9^{a}AB
12	92.9 ± 0.8^{ab}C	82.9 ± 2.5^{bc}B	81.8 ± 3.6^{c}C	96.1 ± 5.5^{a}B
Chlorophyll b (mg/100 g FW)				
1	72.3 ± 2.3^{a}A	61.1 ± 7.0^{ab}A	61.3 ± 2.1^{ab}A	60.7 ± 7.1^{ab}A
4	56.1 ± 8.6^{a}B	55.1 ± 10.9^{a}AB	56.5 ± 7.6^{a}A	58.3 ± 5.9^{a}A
8	49.4 ± 6.7^{a}AB	43.5 ± 5.2^{a}BC	43.5 ± 5.7^{a}AB	46.6 ± 2.6^{a}B
12	41.8 ± 1.8^{a}C	39.4 ± 5.4^{a}C	38.4 ± 5.4^{a}B	43.7 ± 3.1^{a}B
Total chlorophyll (mg/100 g FW)				
1	210.5 ± 3.8^{a}A	167.1 ± 3.2^{b}A	170.9 ± 1.6^{b}A	172.2 ± 12.1^{b}A
4	167.1 ± 3.1^{ab}B	156.6 ± 5.1^{b}A	161.2 ± 10.6^{ab}A	167.7 ± 2.4^{ab}A
8	155.1 ± 2.5^{a}C	142.4 ± 6.6^{c}B	144.5 ± 4.3^{bc}B	153.6 ± 2.4^{ab}B
12	134.7 ± 2.7^{a}D	122.4 ± 7.9^{c}C	120.2 ± 7.9^{bc}C	139.8 ± 2.4^{ab}C
Total carotenoids (mg/100 g FW)				
1	38.6 ± 0.4^{a}A	33.0 ± 0.9^{b}A	33.7 ± 1.8^{b}A	34.6 ± 3.4^{b}A
4	26.4 ± 4.5^{a}B	27.6 ± 3.0^{a}AB	30.5 ± 4.8^{a}AB	29.2 ± 3.4^{a}AB
8	29.3 ± 4.2^{a}B	30.5 ± 4.7^{a}AB	30.5 ± 1.5^{a}AB	29.5 ± 1.0^{a}AB
12	23.9 ± 2.7^{a}B	24.3 ± 0.9^{a}B	25.2 ± 1.6^{a}B	26.9 ± 4.7^{a}B

Data represent means (n = 3 ± SD). Means in the same row with different superscript letters or in the same column for the same type of sample with different uppercase letters were significantly different according to the least significant difference test at $P < 0.05$. FW, fresh weight.

All of these results are consistent with the hypothesis of Cisneros-Zevallos (2003) that UV-C radiation may be considered to be a favorable abiotic stress that may be used to enhance phytochemical compounds (Martínez-Hernández *et al.* 2011).

On the contrary, in spinach treated with doses of 4.54, 7.94 and 11.35 kJ UV-C/m^2, a progressive reduction of the phenolic content was observed during the storage period regardless of the storage temperature (Artés-Hernández *et al.* 2009). However, Costa *et al.* (2006) found that after a 10 kJ UV-C/m^2 radiation, the total phenolic content of broccoli florets increased with respect to the control samples. These levels remained stable for 6 days of storage at 20C. By the end of storage, the total phenol content in the irradiated samples was lower than in the control.

Total Antioxidant Capacity

The initial total antioxidant capacity was of 1.73 g Trolox eq/kg FW in control leaves. All treatments followed the same behavior in total antioxidant capacity until day 8, with a significant decrease of approximately 20% of the initial value (Fig. 4B). However, samples pretreated with UV-C showed significantly higher levels of total antioxidant capacity with respect to the control, by approximately 27% at the end of storage.

Other studies on UV-C-irradiated spinach (Artés-Hernández *et al.* 2009) found different results, i.e., a general tendency for the antioxidant capacity to diminish with the higher doses (4.54, 7.94, 11.35 kJ UV-C/m^2) during their storage at 5C. Additionally, Costa *et al.* (2006) noted that broccoli irradiated with 10 kJ UV-C/m^2 kept its antioxidant capacity for 6 days at 20C, while the untreated samples reduced theirs after 4 days of storage, showing values that were significantly lower than those of the irradiated samples.

However, Martínez-Hernández *et al.* (2011) noted in fresh-cut Bimi broccoli treated with UV-C (1.5, 4.5, 9.0 and 15.0 kJ/m^2), after 19 days at 5C, the higher radiation dose showed the highest total antioxidant content approximately with 1.5-fold higher than the initial content. On the contrary, Tomás-Callejas *et al.* (2012) observed, in fresh-cut Tatsoi baby leaves treated with 4.54 kJ UV-C/m^2, an increase of total phenolic content and total antioxidant activity after 4 days at 5C.

Thus, our results agree with Martínez-Hernández *et al.* (2011) and show how the UV-C treatments did not alter these health-promoting compounds (total phenolic content and total antioxidant capacity) of the fresh rocket leaves for a period of 12 days at 5C.

Chlorophyll and Carotenoid Content

The initial total chlorophyll amount was 210.5 mg/100 g FW in control samples. Chlorophyll a represented around 70% and chlorophyll b around 30% of the total chlorophyll content (Table 2). All UV-C-treated samples reduced, immediately after radiation, the initial total chlorophyll

content (chlorophyll a + chlorophyll b), with decreases ranging from 18 to 21%.

The general trend was a decrease in the chlorophyll content throughout the shelf life for the control and all treatments, as also reported by Tomás-Callejas *et al*. (2012), who worked with fresh-cut Tatsoi baby leaves. However, the UV-C pretreated samples had a lower degradation rate than controls. By the end of the storage period, significant differences were observed between control and samples treated with 10 and 20 kJ/m^2. According to Martínez-Hernández *et al*. (2011), this irreversible breakdown of chlorophyll, due to the effect of the UV-C irradiation, resulted in the appearance of a number of intermediate and final products (Hynninen 1991).

Our data are in agreement with Costa *et al*. (2006) who found a lower chlorophyll degradation rate in 10 kJ UV-C/m^2 pretreated samples of broccoli florets L. var. Italica after 4 days at 20C. Additionally, these data are in line with the reports by Martínez-Hernández *et al*. (2011), who found an initial total chlorophyll content reduction of approximately 23% in broccoli for samples treated with 4.5 kJ/m^2, and a 31% reduction for samples treated with 9.0 and 15.0 kJ/m^2. Additionally, chlorophyll content decreased during cold storage with doses of up to 9.0 kJ/m^2.

Regarding carotenoid content, immediately after irradiation a decrease of around 11-15% was observed in the samples treated with UV-C with respect to the control sample (38.6 mg/100 g FW). After 4 days, the control showed a reduction of about 33% in carotenoid content compared with the initial value; thereafter, no significant differences were observed. However, UV-C pretreated samples showed reductions between 23 and 28% with respect to their initial value. After day 1, no significant differences were observed between the treated samples and the control (Table 2).

These results are in line with those by Martínez-Hernández *et al*. (2011), who reported a reduction of total carotenoid content in broccoli from 6 to 20% with respect to the control values after irradiation with doses of 1.5, 4.5, 9.0 and 15.0 kJ UV-C/m^2. Additionally, the results obtained in this study relate to the variations in the previously analyzed color parameters since a decrease in chlorophyll levels is related to an increase in the L^* and C^* values and a decrease in $h°$, thus resulting in an alteration of the characteristic green leaf color.

CONCLUSIONS

Extending the shelf life of fresh-cut vegetables to meet the highest standard of safety and quality is of paramount importance. For this reason, it is essential to reduce the initial microbial load and maintain the sensory properties during storage.

In this investigation, the effect of UV-C pretreatment compared with conventional passive MAP in the quality of minimally processed rocket leaves was studied. It was concluded that the use of UV-C radiation (10, 20 and 30 kJ/m^2) was effective in retarding the natural microflora growth of fresh rocket leaves. All assayed UV-C treatments did not affect the bioactive compound profile, retaining sensory quality for up to 8 days at 5C. This treatment could be a suitable alternative as a disinfectant agent and a technique for minimizing water consumption in the food industry.

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