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# EFFECT OF ULTRAVIOLET-C RADIATION COMBINED WITH UNCONVENTIONAL ATMOSPHERE PACKAGING ON THE QUALITY OF FRESH-CUT ARUGULA (*ERUCA SATIVA* MILL.)

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## ABSTRACT

The combined effects of ultraviolet (UV) light C (0.34 to 20.13 kJ/l$^2$) and superatmospheric $O_2$ (>85%) packaging on the respiration rate, atmospheric composition, microbiological growth and nutritional quality of fresh-cut arugula stored for 10 days at 5C were studied. All treatments performed under increased $O_2$ levels exhibited a reduction in the respiration rate throughout the cold storage. High microbial load of the raw material were found. UV-C radiation enabled an initial reduction of about 1 and 1.5 log units on mesophilic and psychrotrophic counts, respectively. However, this treatment was not effective for mold control. In addition, a noticeable increase occurred in the total antioxidant capacity and polyphenol content without affecting the visual appearance of the leaves. UV-C radiation, as a sanitizing method, in combination with superatmospheric $O_2$ conditions maintains the overall quality of fresh-cut arugula and is a feasible alternative to industrial-scale enforcement.

## PRACTICAL APPLICATIONS

Our finding indicates that ultraviolet (UV)-C doses between 15.14 and 20.13 kJ/m$^2$ combined with packaging in high $O_2$ atmosphere (>85%) are an environmentally friendly disinfection method, effective in preserve fresh-cut arugula during 10 days at 5C. Additionally, the treatments determined an increased compounds considered interesting for consumer health. In fact, UV-C radiation can be applied by a cheap and simple chamber with a relative low maintenance cost being a feasible alternative to small industry.

## INTRODUCTION

Arugula (*Eruca vesicaria* Mill.) is an annual plant that belongs to the Brassicaceae family; this plant is usually consumed as the young leaf and has a strong and spicy characteristic flavor. Arugula is largely appreciated for its numerous beneficial compounds for human health, such as vitamins A and C, folic acid, carotenoids, flavonoids and glucosinolates (Martínez-Sánchez *et al.* 2006a,b; Manchali *et al.* 2012). Its short shelf life of approximately 8–12 days when stored under optimum conditions (0C and 100% Relative Humidity (RH)) makes it necessary to use a combination of preservation methods (sanitization, temperature, relative humidity, atmospheric composition) to ensure product quality, especially during transport when the temperature rises and the relative humidity is variable (Nielsen *et al.* 2008; Koukounaras *et al.* 2009). To address these critical challenges, several alternative techniques, including ultraviolet light C (UV-C) and superatmospheric $O_2$ packaging, appear to be useful to preserve the quality of horticultural products (Jacxsens *et al.* 2001; Allende *et al.* 2006; Artés-Hernández *et al.* 2009; Odriozola-Serrano *et al.* 2010;

Tomás-Callejas *et al.* 2012). Additionally, the application of combined preservation treatments, according to the hurdle technology (Leistner 2000), could have a synergistic effect leading to remarkable microbial reduction. UV-C comprises the radiation located in the range from 200 to 280 nm, belonging to the nonionizing region of the electromagnetic spectrum, and it acts directly or indirectly as an antimicrobial agent, causing bacterial DNA damage or inducing resistance mechanisms against pathogens in various fruits and vegetables (Ben-Yehoshua and Mercier 2005).

UV-C has been successfully used for microbial growth reduction as well as to delay senescence and ripening in various whole and fresh-cut vegetable products, such as lettuce, baby spinach, melon, grape berries and mushrooms (Allende *et al.* 2006; Artés-Hernández *et al.* 2010; Escalona *et al.* 2010; Jiang *et al.* 2010; Fava *et al.* 2011; Manzocco *et al.* 2011; Hinojosa *et al.* 2013). UV-C treatments are generally applied by high-pressure lamps of mercury, which emit radiation at a wavelength of 254 nm, coinciding with the maximum germicidal effectiveness (Artés-Hernández *et al.* 2009) and easily coupled to the process line.

In addition, elevated $O_2$ atmospheres have been used as an alternative to traditional low $O_2$ and high $CO_2$ atmospheres to maintain the quality and safety of fresh-cut products (Day 1996) and have been suggested as an effective method to inhibit the growth of microorganisms and prevent undesirable anoxic fermentation in various products, including leafy vegetables (Amanitidou *et al.* 1999; Kader and Ben-Yehoshua 2000; Jacxsens *et al.* 2001; Odriozola-Serrano *et al.* 2010; Oms-Oliu *et al.* 2008a; Tomás-Callejas *et al.* 2012).

The primary objective of the present study was to evaluate the synergic effect of UV-C irradiation, as an alternative sanitizer method, combined with subsequent high $O_2$ atmosphere packaging on the quality characteristics of fresh-cut arugula stored at 5C for 10 days.

## MATERIALS AND METHODS

### Plant Material

The arugula (*Eruca sativa* Mill cv. Coltivata; South World Seeds) leaves used in this study were produced by the company Hydro Huerta located in Calera de Tango (Provincia del Maipo, Región Metropolitana, Chile). The cultivation was performed directly on the soil inside a greenhouse during the winter period (June to August). During the growing period, minimum temperatures ranged from −0.9 to 5C while maximum temperatures ranged from 12 to 20C. The harvest was performed manually, when the leaves had reached a mean length of 10 cm (45 days from planting to harvest).

### Sample Preparation and Establishment of the Treatment Process

Once harvested, the arugula leaves were transported to the facilities of the Centro de Estudios Postcosecha of the Facultad de Ciencias Agronómicas, Universidad de Chile, stored in plastic trays lined with perforated polythene and kept in a cold chamber at 5C for 24 h until treatment.

After selection (eliminating leaves with physical of pathological damages), the leaves were rinsed with tap water at 5C for 3 min and then slipped into a stainless steel mesh and centrifuged using manual centrifuge (Ilko, Santiago, Chile). After this initial washing and drying, the NaClO washing (control) and UV-C treatments were applied. UV-C irradiation was applied in a closed chamber ($60 \times 60 \times 125$ cm) provided with six germicidal tubes of 36 W (TUV 36W/G36 T8, Philips, Eindhoven, Netherland), three of were located at the top with a separation distance of 0.12 m. The other three were located in the bottom (layout) and there was a central stainless steel mesh, equidistant to the emission sources of radiation (0.25 m), which enabled the arugula leaves to be placed without overlapping to ensure the homogeneity of the incident radiation.

Radiation doses were calculated using the following equation: $D$ = intensity × exposure time (s)

Intensity was measured using a radiometer VLX 254 (Vilber Lourmat, Torcy, France). Measurements were performed in nine points and the value used to calculate the intensity was the average value as the variation was less than 1%. The following doses: 0.34, 5.16, 10.15, 15.14 and 20.13 kJ/l$^2$, were obtained by varying the exposure time (100, 120, 240, 360 and 480 s).

The values used were selected according to the reports from different authors (Allende and Artés 2003; Escalona *et al.* 2010; Hinojosa *et al.* 2013).

The control sample was washed with stirring in a NaClO solution (100 mg/L) for 3 min at 5C and a pH of 6.5 (adjusted with 2 N citric acid) and then it was rinsed in tap water at 5C for 1 min. Excess water was removed in the same way as was performed in the washing prior to the treatment application.

Approximately, 50 g of leaves were packaged in nylon bags ($0.15 \times 0.25$ m) with an $O_2$ and $CO_2$ permeability of 25 mL/m$^2$/day and 71 mL/m/day at 23C, respectively, according to the data provided by the supplier (San Jorge Packaging, Santiago, Chile). Then, the bags were placed in a packaging machine (Plaspak, mod. KVP420T, Buin, Chile) that injected $O_2$ to a concentration of 88 to 90% and subsequently heat-sealed them. The $CO_2$ and $N_2$ concentration showed average values of 0 and 11.5%, respectively.

Three replicates of each treatment were analyzed after 1, 4, 7 and 10 days of storage at 5C.

## Respiration Rate

In order to determine the respiration rate evolution, samples of 200 g of arugula leaves of each UV-C and control treatment were placed in a plastic container (4 L).

The containers, fitted with a silicone septum on the lid, were hermetically sealed. After that, pure $O_2$ was injected until a concentration of 88–90%. The $CO_2$ and $N_2$ concentration showed average values of 0 and 11.5%, respectively. The storage was performed at 5C. Through the septum, gas samples of 10 mL were extracted and injected into a gas chromatograph (Hewlett Packard 5890 series II, Palo Alto CA) equipped with a thermal conductivity detector and a packed column (Q PoraPack 810–1000, Milford, MA) with an injector at 50C and a detector at 200C. The evaluation was performed in triplicate on days 1, 4, 7 and 10 and the results were expressed as mg $CO_2$/kg/h, calculated by the difference between two consecutive assessments. Additionally, the evolution of the $O_2$ and $CO_2$ concentration inside the plastic container were periodically monitored using a portable gas analyzer (Checkpoint, PBI Dansensor, Ringsted, Denmark).

## Atmosphere Composition

The gas concentration changes inside the bags were monitored using the same portable gas analyzer described previously. Gas samples were obtained through a silicone septum affixed outside of the bags. The evaluations were performed on days 1, 4, 7 and 10 and the results were expressed as $O_2$ and $CO_2$ percentages.

## Microbiological Growth

The microbial growth was determined by a standard determination method. Determinations were performed on NaClO washed leaves, UV-C treated leaves and in the raw material corresponding to the leaves without any treatment as they came to the laboratory. Samples of 10 g of leaves of three random samples at each evaluation time were homogenized in 90 mL of sterile buffered peptone water for 1 min in a sterile stomacher bag using a masticator (Easy Mix, AES Chemunex, Marcy-l'Étoile, France). Serial dilution in sterile buffered peptone water was performed as necessary.

The total quantities of aerobic mesophiles and psychrotrophs were assessed on plate count agar after 48 h or 7 days of incubation at 35 and 7C, respectively. Enterobacteriaceae enumeration was performed on violet red bile dextrose agar incubated for 48 h at 37C, whereas the molds were assessed on potato dextrose agar acidified with 1% tartaric acid and were counted after 7 days of incubation at 25C. All culture media were purchased from Merck (Darmstadt, Germany).

## Color Measurement and Sensory Analysis

Color determination was performed for 10 arugula leaves per bag using a compact tristimulus colorimeter (Konica, Minolta CR 300, Osaka, Japan) with a $D_{65}$ illuminant source and observer angle of 0° and calibrated with a white standard ($Y = 92.6$, $x = 0.3161$, $y = 0.3325$). The values were expressed in the CIE (CIE Lab) system parameters as lightness, chroma and hue angle. Color assessments were performed on days 1, 4, 7 and 10.

In addition to instrumental color measurements, a sensory evaluation of this parameter and the overall product quality were performed by a trained panel of 12 people. The results were collected using an unstructured pattern, a scale of 0 to 15 cm (Ng'ong'ola-Manani *et al.* 2014).

## Total Antioxidant Capacity and Total Phenol Content Determinations

The antioxidant capability was evaluated by the ferric-reducing antioxidant power assay (FRAP), following the method proposed by Benzie and Strain (1996) with some modifications. For the extract preparation, 1 g of each replicate was crushed with liquid nitrogen into a fine powder. Afterwards, 4.5 mL of an ethanol : water solution (1:1 v/v) was added. The mixture was homogenized in an Ultraturrax during 3 min and was then centrifuged at 10 000× $g_N$ for 30 min at 4C. FRAP reagent was prepared from 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.{}_3H_2O$ + 16 mL $C_2H_4O_2$ per liter, pH 3.6), 2, 4, 6-tripyridyl-s-triazine solution (10 mM in 40 mM HCl) and ferric chloride ($FeCl_3.{}_6H_2O$) solution (20 mM in distilled water) in the proportion of 10:1:1 (v/v), respectively. Extracted samples (40 µL) were obtained in discardable cuvettes and the FRAP reagent (900 µL) was added before measuring the absorbance using a spectrophotometer UV-Vis (T 70, PG Instruments Ltd., Leicester, UK) at $\lambda = 593$ nm. The calibration curve was obtained using Trolox as a standard and the results were expressed as Trolox equivalents (T equiv.) in mg/g fresh weight (fw).

To the total phenol contents, the same extract to total antioxidant capability determination was used. Total phenolic compounds was determined using 0.5 mL of the extract and 1 mL of the Folin–Ciocalteu reagent : water solution (1:1 v/v) with vortexing for 15–20 s. After 3 min, 1 mL of saturated sodium carbonate (75 g/L) and 1 mL of distilled water were added. The reaction mixture was incubated in the dark for 1 h and its absorption was measured at $\lambda = 765$ nm. The results were expressed as *p*-coumaric acid equivalents (GA equiv.) in mg/g (fw) (Singleton and Rossi 1965).

### Statistical Analysis

The experiment followed a completely randomized design ($n = 3$). The data were processed by analysis of variance and reported as the mean ± standard error of three replicates. The Infostat, version 2012 (Universidad Nacional de Córdoba, Córdoba, Argentina) software package was used for the analysis. Significant differences among the treatments were analyzed by Tukey's test ($P \leq 0.05$).

## RESULTS AND DISCUSSION

### Respiration Rate

In Fig. 1, the respiration rate evolution was shown. In all of them, including the control, the highest values were observed at the beginning of the experiment where the values were between 51.34 and 62.61 mg $CO_2$/kg/h. This behavior was observed in various fresh-cut products, such as spinach (Escalona *et al.* 2010) and broccoli (Martínez-Hernández *et al.* 2013), and was attributed to the tissue stress probably caused by the processing.

At this time, the highest UV-C radiation doses applied (15.14 and 20.13 kJ/mol) presented significantly low $CO_2$ production, with values of 52.19 and 51.34 mg $CO_2$/kg/h, respectively. From this moment, respiration began to fall steadily until the end of storage period. After 7 days, the higher UV-C doses determined the lowest respiration activity, even though no differences among treatments were registered at the end of the storage where the respiration rate reached 18 to 23% of the initial value.

Different effects of UV-C on the respiratory metabolism of fresh-cut products have been reported (Allende and Artés 2003; Artés-Hernández *et al.* 2009; Tomás-Callejas *et al.* 2012). In some cases, it was found that UV-C radiation causes damage to the tissue that was manifested as respiratory stress, as reported on irradiated lettuce with 0.4, 0.81, 2.44, 4.07 and 8.14 kJ/m$^2$ with a proportional response to the dose applied but without differences between 4.07 and 8.14 kJ/m$^2$ (Allende and Artés 2003). The respiration rates of fresh-cut baby spinach leaves were higher in irradiated (2.4, 7.2, 12 and 24 kJ/m$^2$) than in nonirradiated leaves. However, no significant differences were found among the UV-C doses (Escalona *et al.* 2010). These authors also did not find obvious damage on the epidermal surface because no cellular abnormalities were observed by scanning electron microscopy. On the other hand, Cote *et al.* (2013) found a reduction in the respiratory activity of strawberries treated with 4 kJ/m$^2$ at two UV-C intensities (3 and 33 W/m$^2$) but not in tomato with the same treatment. Additionally, on fresh-cut watercress, doses of 6 and 18 kJ/m$^2$ caused an initial reduction in the respiration that was not maintained over the storage period (Hinojosa *et al.* 2013).

Related to the synergic effect of UV-C and high $O_2$ packaging, Martínez-Hernández *et al.* (2013) reported that high $O_2$ levels (90%) exacerbated the UV-C (6 kJ/m$^2$) stress of the plant cells, manifesting as an increased respiration and ethylene emission in fresh-cut broccoli. This behavior was not in agreement with our finding, suggesting the existence of a complex response that most likely involves various parameters, such as genotype, cropping systems, environmental conditions, physiological stage at harvest and storage conditions.

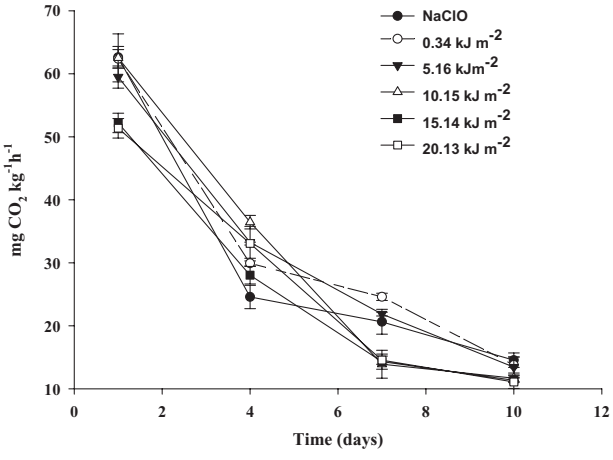


**FIG. 1.** RESPIRATION RATE OF FRESH-CUT ARUGULA STORED AT 5C DURING 10 DAYS (mg $CO_2$/kg/h)
Vertical bars indicate the standard error of the means ($n = 3$).

### Atmospheric Composition Inside the Bags

On day 1 of the experiment, 86 to 90% $O_2$ was attained inside the bags. The $O_2$ levels decreased throughout the storage and stabilized after 7 days, with an average value of 72% and without differences among the treatments until the end (Fig. 2).

On the contrary, the $CO_2$ levels increased during storage and reached an average value of 4.4% on day 1, without any differences among the treatments (Fig. 3). After 10 days, the recorded values were between 20.2 and 24.2%. At this time, the arugula leaves treated with the highest UV-C doses (15.4 and 20.13 kJ/m$^2$) had the lowest $CO_2$ levels (20.2 and 20.8, respectively). As expected, the $O_2$ levels decreased and $CO_2$ increased as a result of the respiratory activity of the products. This behavior was in agreement with the respiration rate finding in this and in similar studies (Tomás-Callejas *et al.* 2011; Martínez-Hernández *et al.* 2013).

According to the results, the bag permeability would be adequate to maintain the required gas concentrations inside, making it possible to maintain a high $O_2$ and moderate $CO_2$ concentration during storage. Many studies

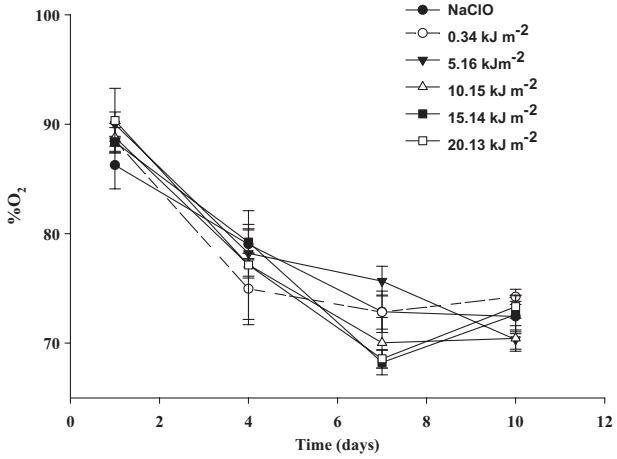


**FIG. 2.** OXYGEN EVOLUTION INSIDE PACKAGING OF FRESH-CUT ARUGULA STORED AT 5C DURING 10 DAYS (%)
Vertical bars indicate the standard error of the means ($n = 3$).

recommended this gas combination to retain the overall quality of fresh-cut vegetables (Day 1996; Kader and Ben-Yehoshua 2000; Artés *et al.* 2009; Escalona *et al.* 2010).

## Microbiological Growth

The psychrotrophic counts are presented in Fig. 4A. The raw material showed a psychrotrophic count of about 5.5 log cfu/g (data not shown). However, the highest UV-C doses possible to reduce the initial load in approximately 1.5 log units. This trend continued until the end of storage, although at this moment, the counts recorded in all of the treatments exceeded 9 log cfu/g.

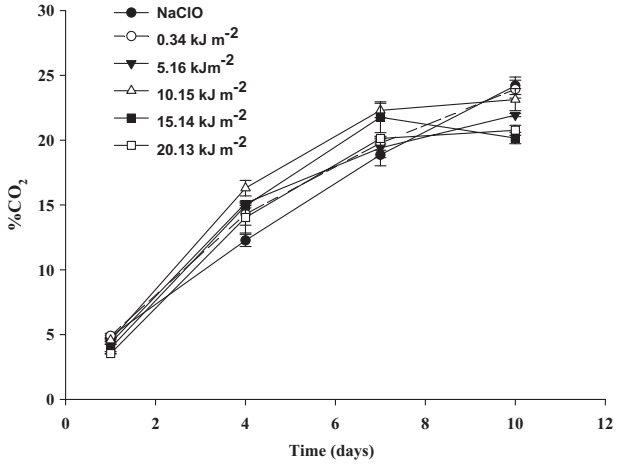


**FIG. 3.** CARBON DIOXIDE EVOLUTION INSIDE PACKAGING OF FRESH-CUT ARUGULA STORED AT 5C DURING 10 DAYS (%)
Vertical bars indicate the standard error of the means ($n = 3$).

Likewise, high initial counts of aerobic mesophiles were also observed in the raw material (data not shown), with values approximate to 5 cfu/g (Fig. 4B). Additionally, in this case, the highest doses of UV-C (15.4 and 20.13 kJ/m$^2$) helped to reduce the initial values by approximately 1 log cfu/g, keeping this lower count compared with NaClO until 10 days of storage. After 4 days of the storage, the lower UV-C doses (0.34, 5.16 and 10.15 kJ/m$^2$) showed higher counts compared with the control treatment. After 7 and 10 days of storage continued to having the higher counts although in this case no differences with the control treatment were found. The results showed high microbial counts with low UV-C doses. Our hypothesis could be that there are wide variations in the bacteria responses to low UV-C doses and subsequent repair strategies, suggesting that UV-C could affect differently the microbial population on the surface vegetal. This could be explained by the fact that bacteria sensitive to UV-C varies with species and also among different strains of the same species (Blatchley and Peel 2001). In addition, it is known that several bacteria and yeast have photoreactivation mechanism that possibility their viability recover after low UV-C radiation exposure (Sommer *et al.* 2000; Lado and Yousef 2002). Another possibility is that UV-C radiation caused slight damage on leaf surface, increasing the nutrient availability for the bacteria growth as mentioned before by Artés-Hernández *et al.* (2009) and Escalona *et al.* (2010).

The initial Enterobacteriaceae counts of the various treatments were approximately 2.5 log cfu/g and the raw material registered values of approximately 3.7 log cfu/g (data not shown). According to the results, the highest UV-C doses reduced the microbial load of the raw material by 0.5 and 0.7 log units. This difference persisted with the other treatments to the end of the storage period, when all of them achieved values higher than 9 log cfu/g (Fig. 4C).

Raw materials showed mold counts of about 2 log cfu/g (data not shown). After UV-C radiation treatment, a reduction was observed and the counts reached values ≤ 1 in all of the doses used (Fig. 4D). The differences among control and UV-C treatment remained more or less unchanged at subsequent analysis points, showing values higher than 5 log cfu/g after 10 days of storage at 5C.

The efficiency of the UV-C irradiation treatment on the various reduced microbial groups has been widely reported in various fresh-cut products and has been confirmed by us in this work. In a study performed by Kim *et al.* (2013), the effect of various UV-C intensities (0.8 to 4.08 kJ/m$^2$) on the microbial load of fresh-cut lettuce inoculated with a cocktail of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *L. monocytogenes* achieved 1.45, 1.35 and 2.12 log reductions at 25C, respectively, and 0.31, 0.57 and 1.16 log reduction at 4C, respectively. Additionally, Birmpa *et al.* (2013) treated fresh-cut lettuce with UV-C (54 kJ/m$^2$),

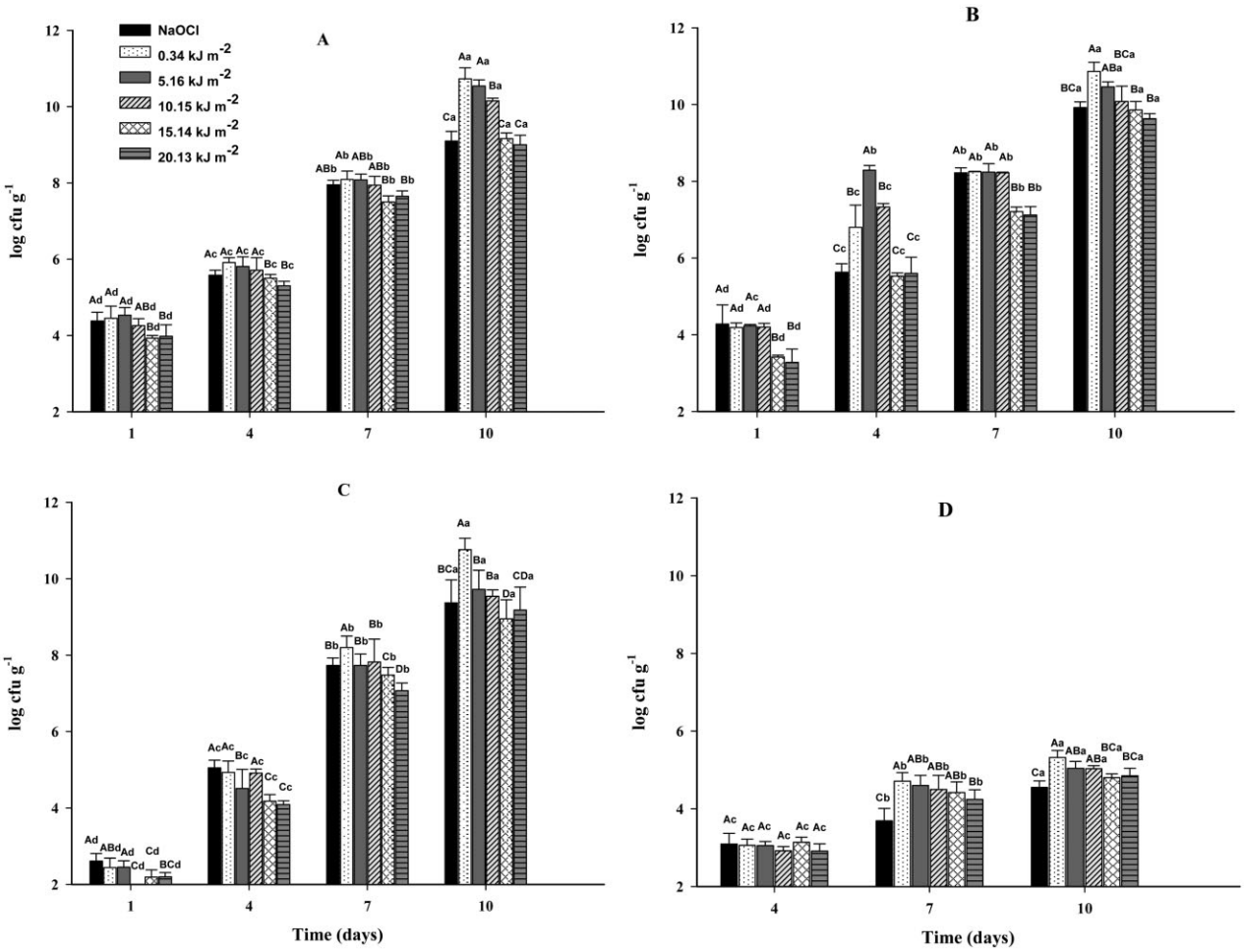


**FIG. 4.** MICROBIAL GROWTH OF FRESH-CUT ARUGULA AT 5C DURING 10 DAYS. (A) PSYCHROTROPHIC, (B) MESOPHILIC, (C) ENTEROBACTERIACEAE AND (D) MOLDS

Vertical bars represent standard error of the means ($n = 3$). Means followed by different letters, uppercase and lowercase for treatment and time, respectively, are statistically different according to Tukey's test at $P \leq 0.05$.

observing significant reductions on *E. coli*, *Listeria innocua*, *Salmonella enteritidis* and *Staphylococcus aureus* populations by 1.75, 1.27, 1.39 and 1.21 log cfu/g, respectively.

Moreover, the positive effect of storing fresh-cut products in high $O_2$ atmospheres has also been reported previously. Tomás-Callejas *et al.* (2011) reported that high $O_2$-modified atmosphere packaging (>85% $O_2$) inhibited natural microflora growth on fresh-cut red chard baby leaves for 7 days at 5C. This may be due to an inhibitory effect related to the toxicity of high $O_2$ concentrations to cells (Wszelaki and Mitcham 2000), which may induce DNA and nucleoprotein damage, as well as general protein damage in microorganisms (Moradas-Ferreiras *et al.* 1996). In this sense, the combination of dissimilar preservation techniques determines synergistic effects that are more beneficial than the individual techniques.

Therefore, in our work, the combination of the UV-C treatment and storage in $O_2$-enriched atmosphere packaging will act jointly, making it possible to maintain the initial trend of reduction during the storage period.

In this sense, the combination of UV-C radiation (4.5 kJ/mol$^2$) and enriched $O_2$ MAP (100% initially) achieved the best psychrotrophic growth suppression in fresh-cut tatsoi baby leaves for 6 days at 5C (Tomás-Callejas *et al.* 2012).

According to Martínez-Hernández *et al.* (2013), the UV-C radiation provided initial psychrotrophic and mesophilic reductions of 0.9 and 1.2 log cfu/g, respectively, which added to the high $O_2$ atmosphere storage, making it possible

to maintain low counts up to 19 days of storage. Similarly to our finding, no fungistatic effect was attributed to the UV-C because no effect on the mold load was observed by Lemoine *et al.* (2007) and Martínez-Hernández *et al.* (2013).

## Color Measurement and Sensory Analysis

There was no effect of different UV-C doses on the color parameters of arugula stored under high $O_2$ conditions up to 10 days of storage at 5C. The initial lightness values were approximately 46, whereas the chroma and hue presented average values of approximately 30 and 124, respectively. At the end of the storage, the lightness values were between 43 and 45, whereas the chroma and hue were between 31 and 33 and 122 and 124, respectively (data not shown). The experimental results were validated by the sensory panel that did not find differences in the color and visual appearance of the various treatments (data not shown).

Therefore, the tested UV-C irradiation doses did not affect the color of the fresh-cut arugula and, along with high $O_2$ storage, allows preserving it for 10 days at 5C.

According to the literature, the effect of UV-C radiation on the color of vegetable products was fairly variable. The UV-C radiation (6 and 18 kJ/m$^2$) did not induce large changes in the color parameters of fresh-cut watercress stored for 14 days at 5C (Hinojosa *et al.* 2013). Cote *et al.* (2013) reported color preservation on strawberries irradiated with 4 kJ/m$^2$, but they did not find a significant effect on tomato color parameters, except for L, which was higher in fruit treated with UV-C than in the control.

Related to the possible synergistic effect, Tomás-Callejas *et al.* (2012) reported that the combination of UV-C and an $O_2$-enriched atmosphere resulted in a significant decrease in the lightness parameter on red chard baby leaves after 11 days at 5C, although they argue that differences lower than 3 units are not detectable by the human eye.

## Total Antioxidant Capacity and Total Phenol Content

According to the results shown in Fig. 5, the total antioxidant capacity was not initially affected by the UV-C treatments. However, this parameter increased dramatically over the course of preservation, especially under high UV-C doses (10.15, 15.14 and 20.13 kJ/m$^2$, respectively). After 10 days of storage, the fresh-cut arugula leaves treated with UV-C, at all doses tested, showed between 2 and 2.5-fold higher total antioxidant capacity levels than the untreated leaves (control).

On the other hand, there was no interaction between the treatment and storage time for the total phenol content that statistically differed among the treatments (Fig. 6).

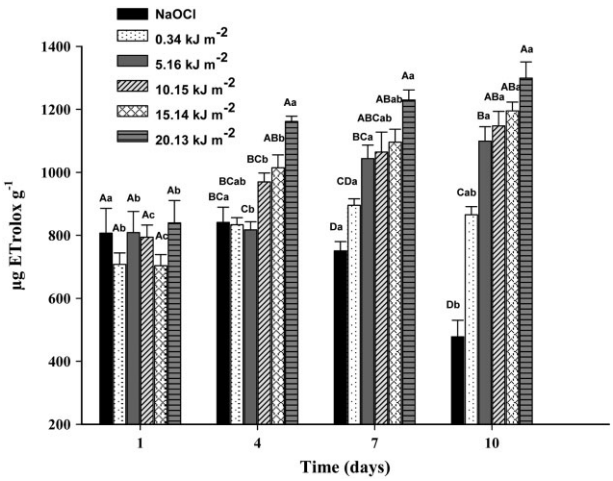


**FIG. 5.** TOTAL ANTIOXIDANT CAPACITY OF FRESH-CUT ARUGULA STORED AT 5C DURING 10 DAYS
Vertical bars represent standard error of the means ($n = 3$). Means followed by different letters, uppercase and lowercase for treatment and time, respectively, are statistically different according to Tukey's test at $P \leq 0.05$.

Additionally, in this case, the leaves treated with high UV-C doses showed high phenol contents and differences existed among the treatments. The treatments of 15.14 and 20.13 kJ/m$^2$ showed values nearly twice those of the control treatment, were undifferentiated from each other and were followed by smaller-dose treatments.

In a similar way, Hinojosa *et al.* (2013) applied UV-C radiation at 6 and 18 kJ/m$^2$ to fresh-cut watercress, resulting in 70 and 79% increases, respectively, in the antioxidant capability of the raw material trend that was maintained for 14 days at 5C. It was also observed that the highest UV-C

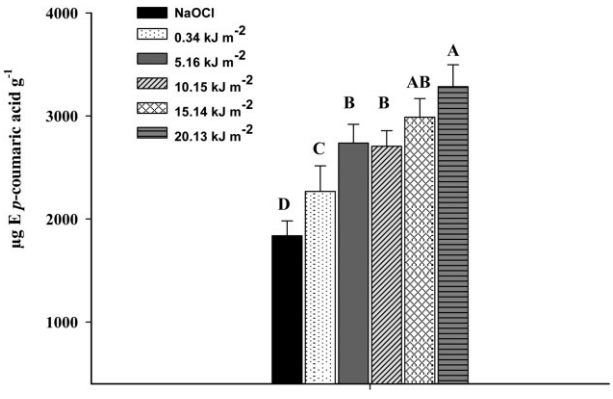


**FIG. 6.** TOTAL POLYPHENOLS CONTENTS OF FRESH-CUT ARUGULA STORED AT 5C DURING 13 DAYS
Vertical bars represent standard error of the means ($n = 3$). Means followed by different letters are statistically different according to Tukey's test at $P \leq 0.05$.

dose allowed preservation of the watercress total phenol content. The observed behavior may be attributed to the damage at the tissue level induced by the UV-C, which leads to oxidative stress, expressed as lipid peroxidation, protein denaturation, carbohydrate oxidation and DNA damage. In response to this stress situation, plants activated defense mechanisms through the induction of enzymes that scavenge reactive oxygen species as well as key enzymes related to the biosynthesis of antioxidant compounds (Strack 1997; Lemoine *et al.* 2010). This effect arises due to increased expression and activity of the enzyme phenylalanine ammonia lyase, a key enzyme in the production of phenylpropanoids, which leads to an increase in phenols, phytoalexins and lignins (Ryalls *et al.* 1996).

Additionally, the use of enriched $O_2$ atmospheres had a positive effect on total antioxidant capacity and the phenol contents of the various products. In this sense, Zheng *et al.* (2007) showed that storage under $O_2$ concentrations higher than 60% increased the total phenolics in strawberries stored for 7 days at 5C. In a similar manner, Tomás-Callejas *et al.* (2012) mentioned that a high-$O_2$ atmosphere increased the total phenol contents of fresh-cut red chard by approximately 80% after 6 days at 5C.

Additionally, in this case, the results differ depending on the product because it also has been found that high $O_2$ concentrations reduce the levels of bioactive compounds. This is true for the results of Oms-Oliu *et al.* (2008a), who reported that high $O_2$ availability in the package headspace could have led to stronger degradation of the main phenolic acids of fresh-cut pears. In addition, a substantial depletion in the antioxidant capacity of fresh-cut melon stored under superatmospheric $O_2$ atmospheres was observed by Oms-Oliu *et al.* (2008b). More recently, Odriozola-Serrano *et al.* (2010) reported that high $O_2$ concentrations (60 and 80%) inside the headspace of packages promoted greater losses of phenolic acids (*p*-coumaric, hydroxybenzoic and ellagic acid) and vitamin C during the storage period on fresh-cut strawberries compared with low $O_2$ levels (2.5, 10 and 21%).

## CONCLUSIONS

High UV-C doses of 15.14 and 20.13 kJ/m$^2$ combined with a high $O_2$ atmosphere present a viable alternative for the quality maintenance of fresh-cut arugula leaves. These UV-C doses enabled the initial reduction of the populations of psychrotrophic, mesophilic and Enterobacteriaceae microbes. However, the high microbial load recorded in the raw material highlights the importance of an integrated management from the culturing step to ensure low microorganism levels at the beginning of the minimal process. This enables UV-C to be a successful disinfection method that is complementary to others and to be a way to increase antioxidant compound levels in vegetal tissues, which constitutes an advantage over other methods. In addition, these doses did not affect the appearance and color parameters of the products.

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