

MICROBIOLOGICAL AND FUNCTIONAL QUALITY OF READY-TO-EAT ARUGULA AS TREATED BY COMBINATIONS OF UV-C AND NONCONVENTIONAL MODIFIED ATMOSPHERES

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ABSTRACT

The industry of ready-to-eat vegetables is interested in developing environmentally friendly sanitization techniques such as the ultraviolet light (UV-C). The effect of UV-C (5, 10, 15, 20 and 25 kJ/m⁻²) and nonconventional modified atmospheres (MA) using helium, argon, nitrous oxide and high oxygen were evaluated on arugula leaves inoculated with *Escherichia coli*. According to preliminary tests, 10 kJ/m⁻² UV-C was the most effective in reducing *E. coli*. The combinations of UV-C (10 or 15 kJ/m⁻²) with the He or Ar-enriched atmospheres were selected. The UV-C factor significantly affected enterobacteria counts and the total antioxidant activity showed an increase on the second day, mostly for the 15 kJ/m⁻² dose combined either with He or Ar (1.28 and 1.31 mg trolox equivalent/g (fw), respectively). UV-C was the determining factor on the effectivity of the combined treatments, delaying the microbial growth and preserving the functional quality of ready-to-eat arugula.

PRACTICAL APPLICATIONS

There is increasing interest in developing environmentally friendly sanitization techniques as an alternative to chlorine-based washing systems. This study gives an insight with regard to the effect of UV-C light combined with nonconventional modified atmospheres on the microbiological, functional and sensory quality of ready-to-eat arugula. The results demonstrated that UV-C was the determining factor on the effectivity of the combined treatments delaying the microbial growth, preserving the functional and sensory quality of arugula. Therefore, data from this study may be useful for vegetable producers and manufacturers to consider the implementation of a low cost sanitization technology such as UV-C light for extending the shelf life and maintaining the overall quality of ready-to-eat leafy salads.

INTRODUCTION

Increasing popularity of nontraditional leafy vegetables has created a new opportunity for innovation in the field of the minimally processed (MP) produces. The leaves and young stems of arugula (*Eruca vesicaria* Mill.) are specially appreciated due to their unique and slightly spicy flavor (Tomás-Callejas *et al.* 2011a). Furthermore, they are a good source of biologically active sulfur and nitrogenated compounds, which are associated with several health benefits (Manchali *et al.* 2012).

The use of active modified atmosphere (MA) with such noble gases as argon (Ar), helium (He), xenon (Xe), or other nonconventional atmospheres, such as nitrous oxide (N₂O) and superatmospheric oxygen, has gained interest in recent years (Artés-Hernandez *et al.* 2009; Rocculi *et al.* 2009; Char *et al.* 2012). The noble gases are characterized by their lack of or low reactivity; however, some of them have diffusivity characteristics that might modify the diffusion of O₂, CO₂ and C₂H₄ in some commodities, thus affecting the vegetable physiology. In this sense, Ar and He are monoatomic and smaller than

diatomic N₂ and may modify the diffusion of respiratory gases (Jamie and Saltveit 2002).

Nitrous oxide (50–100%) reduced the respiration of onion bulbs by 50% after 5 days at 18C compared to air storage (Benkeblia and Varoquaux 2003). MA with N₂O (90%) maintained the best quality of kiwifruit slices, delaying firmness loss and browning; whereas, an acceptable quality maintenance until the eighth day was found in Ar (90%) atmosphere, compared with a rapid quality loss for samples in air and in N₂ (90%) (Rocculi *et al.* 2005).

A compressed mix of Ar and Xe at 2:9 (v:v) and 1.1 MPa for 24 h reduced the respiration rate of green asparagus spears delaying senescence for 18 days of storage at 4C and 90% RH. The same authors also informed a shelf life of 12 and 3 days for the MA treatment (compressed mix of 5% O₂ and 5% CO₂) and the control in air, respectively (Zhang *et al.* 2008).

The application of UV-C light has been studied for reducing the microbial load in vegetables such as watercress applying doses of 6 and 18 kJ/m⁻²; lettuce using from 0 to 8 kJ/m⁻²; spinach leaves with doses from 0 to 24 kJ/m⁻²; cabbage leaves with doses in the range of 0–12 kJ/m⁻² (Allende *et al.* 2006; Escalona *et al.* 2010; Ruiz *et al.* 2010; Hinojosa *et al.* 2013). These studies reported that UV-C light caused a slight increase in the respiratory rate due to the stress generated by the treatment, but also delayed bacterial growth, increased phenolic compounds and antioxidant activity, and reduced tissue damage compared to the chemical treatments, maintaining the sensory quality.

UV-C light should be used in combination with other preservation techniques since the reduction of microbial growth caused by the cumulative damage of the DNA reduces the total number of bacterial cells, but does not result in complete sterilization (Char *et al.* 2010). Studies of the combined use of UV-C light with modified atmosphere packaging on lettuce leaves performed well in reducing microbial load and prolonged its life, without affecting their sensory attributes (Allende and Artés 2003).

However, there is still insufficient relevant information about the use of nonconventional gases in combination with other disinfection techniques such as UV-C light to maintain the overall quality of MP produces. Therefore, the objective of this work was to study the combined effect of UV-C light and MA with nonconventional gases (He, Ar and N₂O) and high oxygen on the microbiological and functional quality of MP arugula stored under refrigeration.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Arugula (*Eruca vesicaria* Mill.) was purchased from a commercial grower located in the Calera de Tango area (Hidro-

huerta Tango, Ltd., Santiago, Chile). The cultures were grown in soil by staggered planting. The leaves were hand harvested in February–March (summer) at 30 days from sowing; the commercial maturity state was defined as a minimal leaf length of 10 cm. The leaves were immediately transported in a portable box to the laboratory, where they were stored at 5C in darkness and 95% RH. The following day, the leaves were processed in a clean room at 8C, as described below.

Sample Preparation

Arugula leaves were pre-conditioned and selected by removing the physically damaged, dehydrated or colorless leaves. Then, the leaves were washed with tap water at 5C for 1 min, immersed in a solution of 100 mg/L NaOCl (Clorox, Santiago, Chile) adjusted to pH 6.5 with citric acid (Merck, Darmstadt, Germany) and rinsed in water at 5C. For each step the immersion was for 1 min under constant agitation using 10 L of solution per 1 kg of arugula. The leaves were drained on a stainless-steel mesh for 3 min and the remaining water was removed using a hand centrifuge.

Inoculum Preparation and Inoculation Procedure

The inoculum of *Escherichia coli* ATCC 35218 was prepared by transferring a loopful of a stock culture maintained in tryptone soy agar (TSA) slants to a 50 mL Erlenmeyer flask of tryptone soy broth (TSB). The culture was incubated with agitation at 37C for 24 h. The final inoculum solution was prepared by dilution (1:1,000) in 0.1% peptone water obtaining a final inoculum count of 10⁷ CFU/mL. The leaves were inoculated by immersion (1 min) and drained (15 min) in a safety cabinet with forced air circulation. All microbiological media were purchased from Merck (Darmstadt, Germany).

Treatments

UV-C Light Preliminary Test. Preliminary UV-C treatments were conducted to determine the most appropriate doses of UV-C light. The effect of UV-C light (0, 5, 10, 15, 20 and 25 kJ/m⁻²) was evaluated on arugula leaves inoculated with *E. coli*, as a model microorganism. UV-C doses were applied using an experimental chamber (Model G-636, KBM Co., Santiago, Chile), which consisted of a metallic box (1.28 m × 0.56 m × 0.60 m) with six unfiltered UV-C germicidal lamps (TUV 36W/G36 T8, Philips, Santiago, Chile) with an emission peak at 253.7 nm. The lamps were placed three of them at the top and the others at the bottom of the chamber. A central grid located at 0.21 m from the lamps would support the samples. The UV-C doses (5, 10,

15, 20 and 25 kJ/m⁻²) were applied by varying the exposure time (from 2 to 10 min, respectively). The intensity of the lamps at the central grid was ≥ 26 W/m⁻² as measured with a radiometer (Blak-Ray J-225, UVP, Upland, CA). After UV-C treatment, the leaves (20 g) were packed in perforated bags (with seven holes in each side made with a 0.8 mm diameter needle) to get air atmosphere and stored at 5 ± 0.5C and 95% RH for 10 days. The *E. coli* counts were performed on days 0, 2, 6 and 10. A total of 16 bags of each treatment (four for each measuring day) were prepared.

Nonconventional Modified Atmospheres Preliminary Test.

Preliminary, different nonconventional modified atmospheres were assessed to determine the most appropriate packaging atmosphere. The effect of argon (53% Ar + 41% O₂ + 6% N₂), helium (53% He + 42% O₂ + 5% N₂), nitrous oxide (53% N₂O + 42% O₂ + 5% N₂) and high oxygen (80% O₂ + 20% N₂) enriched atmospheres was evaluated on arugula leaves inoculated with *E. coli*. The untreated control consisted in perforated bags (as described above) to get air atmosphere with high humidity. After inoculation, the leaves (20 g) were packed in polypropylene bags (0.10 m × 0.20 m) provided by Plaspack (Santiago, Chile). The film had a permeability of 25 mL O₂/m⁻²/day and 71 mL CO₂/m⁻²/day at 20C (data provided by the supplier). The gases (Ar, He and O₂) were injected into the bags using a gas mixer panel just before completing the heat sealing. Finally, the bags were stored at 5 ± 0.5C and 95% RH for a maximum of 10 days. Measurements of the gas composition of the atmospheres and the *E. coli* counts were performed on days 0, 2, 6 and 10. A total of 16 bags of each treatment (four for each measuring day) were prepared.

Combined UV-C and Modified Atmosphere Treatments

The UV-C doses and nonconventional modified atmospheres were selected from the results of the preliminary tests. Four combined treatments and one untreated control were evaluated. The untreated control consisted in arugula leaves with no UV-C exposure, packed in perforated bags (with seven holes in each side made with a 0.8 mm diameter needle) to get air atmosphere. The arugula leaves (80 g) were treated with the selected UV-C dose and packed in polypropylene bags (0.20 × 0.28 m). The gases (Ar, He and O₂) were injected into the bags as described before. Finally, the bags were stored at 5 ± 0.5C and 95% RH for a maximum of 10 days. Measurements of the microbial growth counts, the physicochemical parameters, and the sensory evaluation were performed on days 0, 2, 6 and 10. A total of 20 bags of each treatment (five for each measuring day) were prepared.

Microbial Growth

Microbiological determinations were conducted using a sample of 10 g of leaves per replicate. The samples were mixed with 90 mL of 0.1% buffered-peptone water (Merck, Germany) and homogenized in a sterile bag using a stomacher (model Easy Mix, AES Chemunex, Bruz, France) for 40 s. Serial dilutions were prepared, as needed, for plating. Inoculated *E. coli* (for the preliminary tests) was determined using TSA, incubated at 37C for 2 days. The colonies were confirmed using biochemical tests (triple sugar-iron agar (TSI), motility-indol-ornithine (MIO) and lysine-iron agar (LIA)). For the combined treatments, psychrotrophic and total aerobic mesophilic bacteria were determined on plate-count agar (PCA) incubated at 5 or 37C for 7 or 2 days, respectively. Enterobacteriaceae were determined using eosin methylene blue agar (EMB) incubated at 37C for 2 days. Yeasts and molds were determined on acidified potato dextrose agar (PDA) incubated at 25C for 5–7 days. All culture media were purchased from Merck (Darmstadt, Germany).

Modified Atmosphere Gas Composition

The changes of O₂, CO₂ and N₂ concentrations inside the bags were monitored throughout the shelf life using a gas chromatograph (GC) (Hewlett Packard, HP 5890 Series II, Hewlett Packard, Palo Alto, CA) equipped with a thermal conductivity detector (Hewlett Packard, Palo Alto, CA) using injector, oven and detector temperatures of 50, 50 and 200C, respectively. The carrier gas was helium (Indura, Santiago, Chile) at a pressure of 45 psi. Also, a portable gas analyzer (Checkpoint, PBI Dansensor, Ringsted, Denmark) was used. The gas sample was collected using a plastic syringe through a silicone septum placed on the bag. The equipment calibration was performed with a commercial standard (5.0% CO₂ and 10.0% O₂) (Indura, Santiago, Chile) and the values were expressed as percentage of O₂ and CO₂. The Ar and He concentrations in the bags were calculated using Eq. (1)

$$[\text{Ar}] \text{ or } [\text{He}] = 100 - ([\text{O}_2] + [\text{CO}_2] + [\text{N}_2]). \quad (1)$$

Total Polyphenol Content (TP) and Total Antioxidant Activity (TAA)

The determination of total polyphenols was carried out using the Folin–Ciocalteu reagent and measuring the absorption at $\lambda = 660$ nm using the spectrophotometer UV-vis (T 70, PG Instruments, Ltd., Leicester, UK) (Singleton and Rossi 1965). The results were expressed as gallic acid equivalents (GAE) in mg GAE/g (fw).

The total antioxidant activity was determined using the FRAP method (Benzie and Strain 1996) based on the ability

of the substrate to reduce Fe⁺³. The quantification was performed using the spectrophotometer described above at λ = 593 nm. The calibration curve was obtained using trolox (Sigma, St. Louis, MO) as a standard, and the results were expressed as trolox equivalents (TE) in mg TE/g (fw).

Glucose Content

The glucose content was determined following the method proposed by Obando-Ulloa *et al.* (2009) with some modifications. Each measuring day, the arugula samples (40 g of each replicate) were taken and stored at -80C until subsequent determination. The extract was obtained by grinding the leaves in a mortar, filtrating and centrifuging at 3,000 × g_n for 45 min at 4C. Then, the extract was distributed into 2 mL Eppendorf tubes and frozen at -80C until the analysis. The determination day, the samples were thawed and centrifuged (15,000 × g_n for 30 min at 4C), passed through a physical filter (PVDF membrane 0.22 μm; Millex, Barueri, Sao Paulo, Brazil) and activated chemical cartridges (SepPack, Waters, Milford, MS). The sugar content was determined in aliquots of 50 μL of the extract using an ultrahigh performance liquid chromatograph (UPLC-MS) (Waters, Milford, MA) equipped with a triple quadrupole MS detector and an UPLC NH2 column (Waters, Milford, MA). The mobile phase consisted in acetonitrile: deionized water (85:15 v/v).

Color Measurement

The color changes of the arugula leaves were measured using a tristimulus colorimeter (Minolta CR-300, Ramsey, NJ), with an 8 mm diameter of viewing aperture, D65 illuminant and 0° observer angle, previously calibrated with color standards in the CIELab system. The color parameters lightness (L), chroma (C) and hue angle (H_{ab}) were determined. The measurements were performed on the adaxial side of eight leaves per bag, supporting the leaf on a black surface to prevent color interference on days 0, 2, 6 and 10 of cold storage.

Sensory Evaluation

For the sensory evaluation, the descriptive quantitative analysis method was applied using a panel of 12 judges. Linear scales of 15 cm were employed by the panelists to evaluate appearance, color intensity (visual), turgor and presence of off flavors.

Experimental Design and Statistical Analysis

The preliminary tests were carried out following a completely randomized design with three replicates (n = 3). For

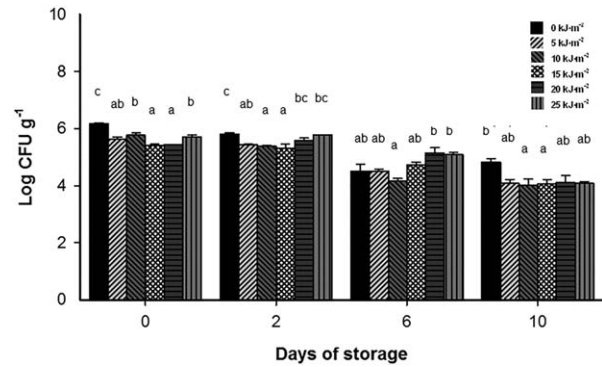


FIG. 1. EFFECT OF UV-C LIGHT (0–25 kJ/m⁻²) ON *E. COLI* ATCC 35218 INOCULATED IN ARUGULA LEAVES STORED AT 5C FOR 10 DAYS. The vertical bars represent the standard error of the means (n = 3). Small letters indicate significant differences among treatments for each day according to the Tukey test (P ≤ 0.05).

the combined UV-C and modified atmosphere treatments a 2 × 2 factorial arrangement of treatments with two levels of UV-C dose (10 or 15 kJ/m⁻²) and two levels of the factor MAP (Ar- or He-enriched atmosphere) were applied. To determine the effect of UV-C and the nonconventional atmospheres, two-way analysis of variance (ANOVA) with a 95% confidence level was carried out. Statistically significant differences (P ≤ 0.05) among the treatments were analyzed using the Tukey test. Additionally, the Dunnett test was applied to evaluate the multiple comparisons between the treatments and the control (P ≤ 0.05). The Minitab Statistical Software 16.1 for Windows (Minitab Inc., State College, PA) was used for all the statistical analysis.

RESULTS AND DISCUSSION

UV-C Light Preliminary Test

The effect of different UV-C doses on inoculated *E. coli* ATCC 35218 is presented in Fig. 1. The UV-C light significantly reduced the initial inoculated microbial load compared to the untreated control from 6.1 log CFU/g to a range between 5.4 and 5.7 log CFU/g for the different UV-C doses. The *E. coli* counts decreased with the storage time, probably due to the competition with the natural microbiota.

During the 10 days of cold storage, the counts of the 10 kJ/m⁻² UV-C treatment remained significantly lower (P ≤ 0.05) than the untreated control. The highest UV-C doses (20 and 25 kJ/m⁻²) negatively affected the sensory appearance and color during the storage time (data not shown), for that reason the 10 and 15 kJ/m⁻² UV-C doses were selected for the combined treatments.

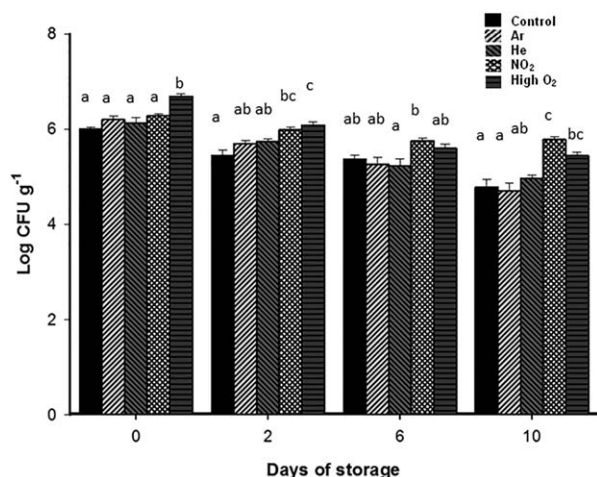


FIG. 2. EFFECT OF NONCONVENTIONAL MODIFIED ATMOSPHERES ON *E. COLI* ATCC 35218 INOCULATED IN ARUGULA LEAVES AND STORED AT 5C FOR 10 DAYS

The vertical bars represent the standard error of the means ($n = 3$). Small letters indicate significant differences among treatments for each day according to the Tukey test ($P \leq 0.05$).

Nonconventional Modified Atmospheres Preliminary Test

The gas composition of arugula nonconventional-atmosphere packages changed during storage due to arugula respiration and the film permeability. As expected, O₂ concentrations decreased from 40% to 20–26% in Ar, He and N₂O-enriched atmospheres, and from 80 to 58% in the high O₂ atmosphere (data not shown). The CO₂ levels increased in all MA packages from approximately 0 to 20–23%. The concentrations of Ar, He, N₂O and N₂ were maintained constant in their initial values during all the storage time.

With regard to the effect of the nonconventional atmospheres on the inoculated *E. coli*, it was observed that Ar and He-enriched atmospheres were as effective as the control in air to decrease the counts during storage (Fig. 2). High O₂ and N₂O did not inhibit microbial growth, showing even higher microbial counts than the control. The Ar and He-enriched atmospheres were selected to evaluate the effect of the combination with UV-C light.

Combined UV-C and Nonconventional Modified Atmospheres Test

The UV-C doses (10 or 15 kJ/m⁻²) and He and Ar-enriched modified atmospheres were selected from the results of the preliminary tests. The selected UV-C doses and MAP conditions were chosen on the basis of getting the highest microbiological growth delay. The tested combined treatments are described in Table 1 and the results of the determinations measured in these systems are commented below.

TABLE 1. TREATMENTS OF THE FACTORIAL DESIGN PLUS THE CONTROL APPLIED TO ARUGULA LEAVES

Treatment	UV-C (kJ/m ⁻²)	MAP
Control	0	Air (21% O ₂ + 79% N ₂)
10 + Ar	10	55% Ar + 40% O ₂ + 5% N ₂
10 + He	10	55% He + 40% O ₂ + 5% N ₂
15 + Ar	15	55% Ar + 40% O ₂ + 5% N ₂
15 + He	15	55% He + 40% O ₂ + 5% N ₂

MAP, modified atmosphere packaging.

Modified Atmosphere Gas Composition

The CO₂, O₂, Ar and He levels determined within the packages over time are shown in Fig. 3. The Ar and He levels (55 ± 2%) remained constant throughout the entire experiment; as well as the O₂ and CO₂ levels in the control perforated bags. In all of the MAP treatments the O₂ levels decreased from approximately 40% to a range of 27–22% and the CO₂ levels increased from 0 to a range of 13–16% during the storage at 5C, mainly due to arugula respiration and the permeability of the film.

The type of gas used to generate the modified atmosphere (Ar or He) significantly affected the evolution of O₂ and CO₂ concentrations that were attained since the sixth day of the experiment. The O₂ consumption was significantly higher in Ar- than in He-enriched atmosphere ending the experiment with values between 22.5–22.7 and 25.4–26.7%, for the 10 + Ar, 15 + Ar, 10 + He and 15 + He, respectively. A similar effect was reported by Char *et al.* (2012) who mentioned that the Ar-enriched atmosphere produced the higher increase of CO₂ (8–10%) compared to the He-enriched atmosphere (6–8%). This difference could be due

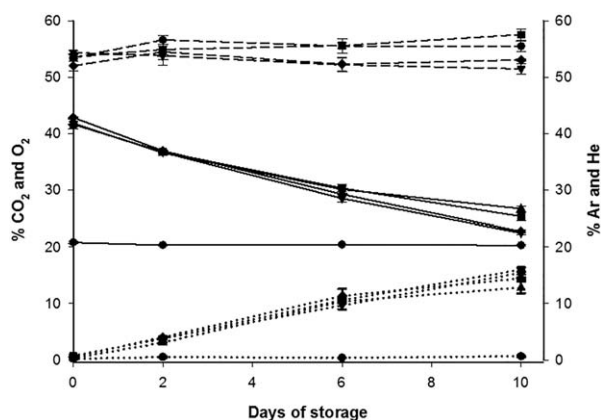


FIG. 3. GAS COMPOSITION OF THE HEAD SPACE OF THE ARUGULA TREATED WITH DIFFERENT DOSES OF UV-C PACKAGED IN NOBLE GAS-ENRICHED MODIFIED ATMOSPHERES (Ar AND He) AND STORED AT 5C FOR 10 DAYS

(---) Ar and He; (—) O₂; (...) CO₂; (●) control; (▼) 10 + Ar; (■) 10 + He; (◆) 15 + Ar; (▲) 15 + He. The vertical bars represent the standard error of the means ($n = 3$).

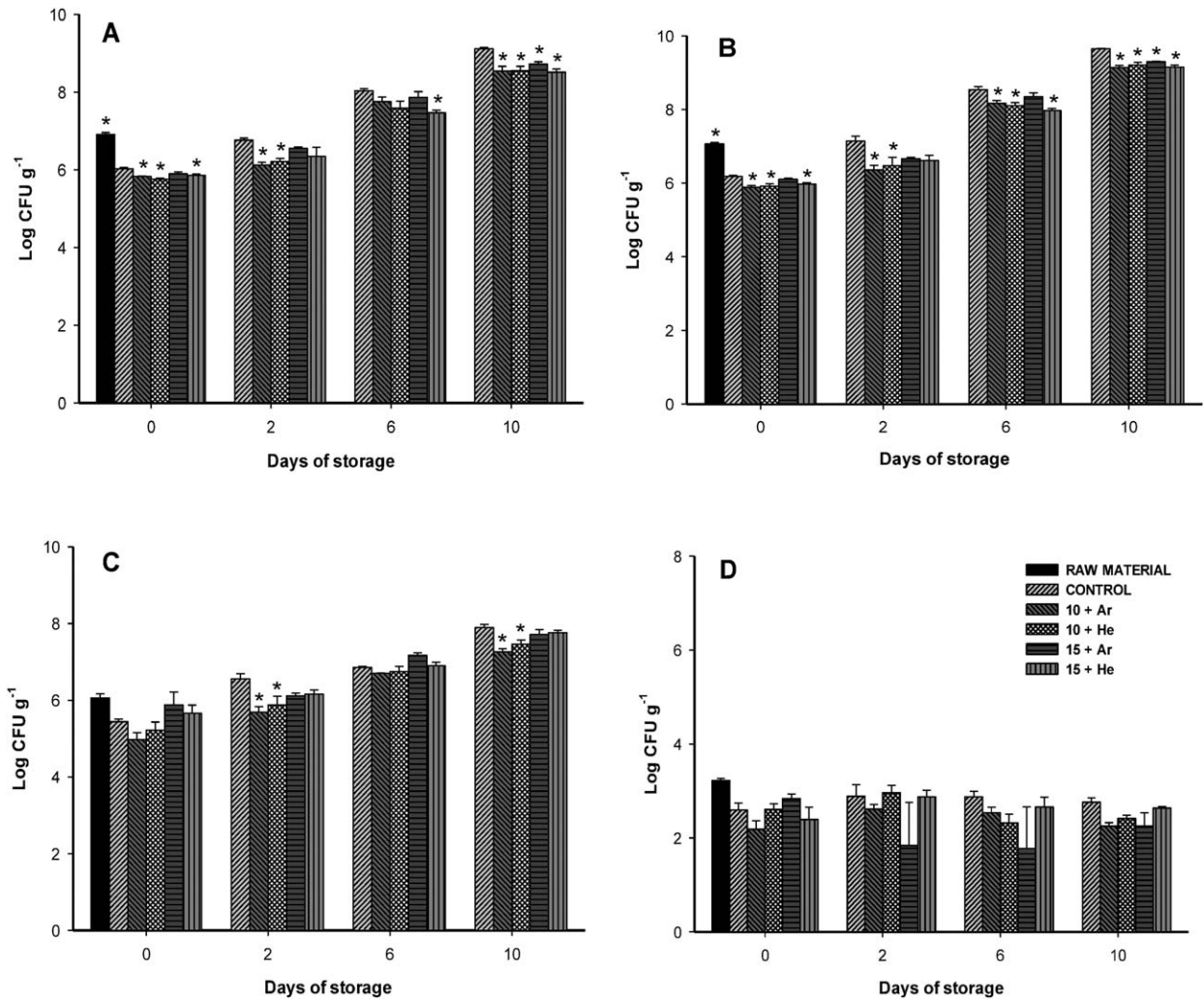


FIG. 4. MICROBIAL GROWTH OF ARUGULA LEAVES TREATED WITH DIFFERENT DOSES OF UV-C, PACKAGED IN NOBLE GAS-ENRICHED MODIFIED ATMOSPHERES AND STORED AT 5C FOR 10 DAYS (A) Total aerobic mesophilic bacteria; (B) Psychrotrophic bacteria; (C) Enterobacteriaceae; and (D) Yeasts and molds. The vertical bars represent the standard error of the means ($n = 3$). The (*) indicate significant differences between the treatments and the control according to Dunnett test ($P \leq 0.05$).

to the different gas solubility in the vegetal tissue and the gas permeability through the plastic film.

Microbial Growth

The total mesophilic bacteria count for the raw material was 6.9 log CFU/g. Just after processing, the counts of all the treatments were reduced between 0.9 and 1.1 log units. During the 10 days of cold storage the population increased reaching final counts of approximately 8.5 log CFU/g showing significant differences only between the treatments and the control (Fig. 4A). The same trend was observed for the psychrotrophic bacteria, which had an initial load of 7.1 log CFU/g and was reduced to a range between 5.9 and 6.1 log

CFU/g after processing. In the same way, significant differences were observed only with the control (Fig. 4B). Similar initial reductions of total mesophilic and psychrotrophic bacteria have been reported for MP spinach leaves treated with UV-C doses from 4.5 to 11.4 kJ/m⁻² and stored at 5C.

The raw material had an initial load of enterobacteria of 6.1 log CFU/g, which was reduced to values between 5.0 and 5.9 log CFU/g after the combined UV-C and MAP treatments. In all the treatments, including the control, there was a progressive and proportional growth during the evaluation period (Fig. 4C). The factor UV-C dose significantly affected the counts, resulting in the most inhibitory the 10 kJ/m⁻² dose. In contrast, no effect was observed due to the atmosphere factor (Table 2). Apparently, high doses of

TABLE 2. EFFECT OF UV-C AND NONCONVENTIONAL MODIFIED ATMOSPHERES ON ENTEROBACTERIA COUNTS OF ARUGULA STORED AT 5C FOR 10 DAYS

Time (day)	Enterobacteria (log CFU/g)			
	0	2	6	10
<i>UV-C (D)</i>				
10	5.10a	5.79a	6.72a	7.37a
15	5.77b	6.14b	7.04b	7.74b
<i>Atmosphere (A)</i>				
Ar	5.43	5.90	6.94	7.49
He	5.44	6.02	6.82	7.62
<i>Interaction (D × A)</i>				
10 + Ar	4.98	5.69	6.70	7.27
10 + He	5.22	5.88	6.75	7.46
15 + Ar	5.88	6.11	7.18	7.71
15 + He	5.66	6.16	6.90	7.76
<i>Significance</i>				
<i>D</i>	*	*	*	*
<i>A</i>	NS	NS	NS	NS
<i>D × A</i>	NS	NS	NS	NS

* Indicates significant differences between the treatments according to Tukey test ($P \leq 0.05$).

Values for each factor followed by the same small letter are not significantly different ($P \leq 0.05$) according to the Tukey test.

NS, no significant differences among treatments.

UV-C could increase the superficial damage on the vegetable leaves making the nutrients available for microbial growth. In this sense Escalona *et al.* (2010) mentioned that the UV-C dose–response relationship could be biphasic, in which low doses stimulated beneficial reactions while high doses resulted detrimental on the quality of baby spinach leaves.

The initial mold and yeast count of the raw material was 3.2 log CFU/g, after processing the counts decreased between 1 and 0.4 log U (Fig. 4D). In general, the mold and yeasts counts remained constant in the range between 1.8 and 2.8 log CFU/g, and no significant differences among treatments were observed during the 10 days of storage.

The UV-C factor was the most significant on reducing the microbial growth. However, in addition to the dose-effect discussed above, it is also important to consider that the type of vegetable that is being treated by UV-C light affects its microbiological efficiency (Sastry *et al.* 2000). In this sense, UV-C ranging from 1.5 to 24 kJ/m⁻² was more effective in reducing *Salmonella* spp. or *E. coli* O157:H7 populations inoculated on the surface of apples (with no wax application) than in lettuce due to the topography of the samples (Yaun *et al.* 2004).

According to our results, the atmosphere factor (He or Ar with O₂) did not affect the microbial growth; whereas, some works have reported certain antimicrobial effect. In fact, Robles *et al.* (2010) found that storing mizuna leaves in controlled atmospheres (83% He + 15% CO₂ + 2% O₂ and 98% He + 2% O₂) at 5C for 8 days was more effective for

inhibiting mesophilic bacteria counts when compared to a commercial MA atmosphere (1% O₂ + 20% CO₂ + 79% N₂).

Total Polyphenol Content (TP) and Total Antioxidant Activity

The polyphenol content of the arugula leaves treated with the combination of UV-C and He or Ar atmospheres is presented in Fig. 5A. The treated samples maintained their polyphenol content during the 10 days of storage in the range between 1.42 and 1.89 mg GAE/g (fw) with no significant differences among the treatments. However, the polyphenol content of the untreated control significantly diminished on the tenth day (from 1.91 on the processing day to 1.13 mg GAE/g (fw)).

In the same way, the treatments and the control started with a total antioxidant activity range of 1.07–1.18 mg TE/g (fw) that was maintained by certain treatments until the

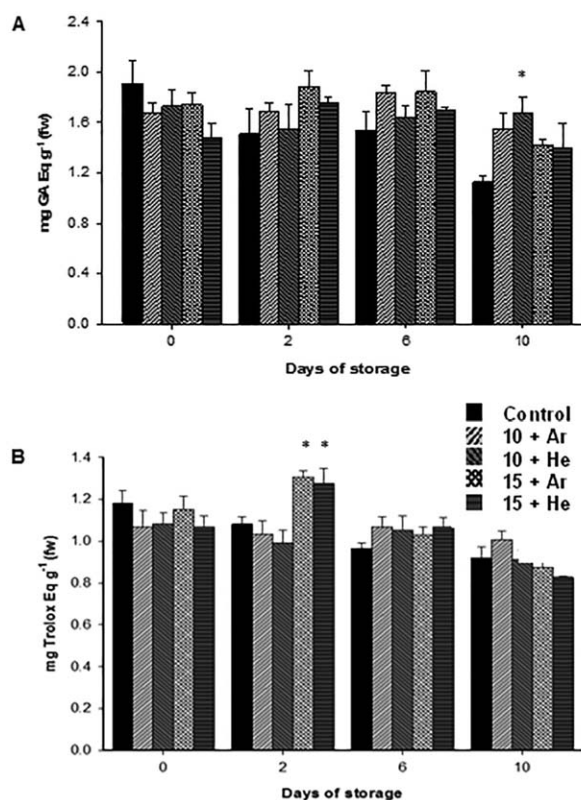


FIG. 5. TOTAL POLYPHENOL CONTENTS (A) AND TOTAL ANTIOXIDANT CAPACITY (B) OF ARUGULA LEAVES TREATED WITH DIFFERENT DOSES OF UV-C (10 and 15 kJ/m⁻²), PACKAGED IN NOBLE GAS-ENRICHED MODIFIED ATMOSPHERES (Ar and He) AND STORED AT 5C FOR 10 DAYS

The vertical bars represent the standard error of the means ($n = 3$). * Indicates significant differences between the treatments and the control according to Dunnett test ($P \leq 0.05$).

TABLE 3. EFFECT OF UV-C AND NONCONVENTIONAL MODIFIED ATMOSPHERES ON THE TOTAL ANTIOXIDANT CAPACITY OF ARUGULA STORED AT 5C FOR 10 DAYS

Time (day)	Total antioxidant capacity (mg TE/g (fw))			
	0	2	6	10
<i>UV-C (D)</i>				
10	1.08 ¹	1.01a	1.06	0.96a
15	1.11	1.29b	1.05	0.85b
<i>Atmosphere (A)</i>				
Ar	1.11	1.17	1.05	0.94
He	1.07	1.13	1.06	0.87
<i>Interaction (D × A)</i>				
10 + Ar	1.07	1.03	1.07	1.01
10 + He	1.08	0.99	1.05	0.91
15 + Ar	1.15	1.31	1.03	0.87
15 + He	1.07	1.28	1.07	0.83
<i>Significance</i>				
<i>D</i>	NS	*	NS	*
<i>A</i>	NS	NS	NS	NS
<i>D × A</i>	NS	NS	NS	NS

* Indicates significant differences between the treatments according to Tukey test ($P \leq 0.05$).

Values for each factor followed by the same small letter are not significantly different ($P \leq 0.05$) according to the Tukey test.

NS, no significant differences among treatments.

sixth day (Fig. 5B). However, on the second day of storage the UV-C dose effect was significant showing a peak for the 15 kJ/m⁻² UV-C dose combined either with He or Ar atmospheres, with TAA values of 1.28 and 1.31 mg TE/g (fw), respectively. Conversely, the atmosphere factor was not statistically significant (Table 3).

The positive effect on the increase of TAA could be attributed to the wound-induced phenomenon in the phenolic metabolism that may have been caused by the UV-C light, but not directly by the different gases used for the modified atmosphere, according to our statistical results.

Certain studies in cabbage leaves and broccoli florets also reported higher TAA and TP content for UV-C (6–12 kJ/m⁻²) treated samples compared to the untreated control. This effect was observed mainly from the second to the sixth or ninth day of storage, respectively, after which a decrease occurred (Costa *et al.* 2006; Ruiz *et al.* 2010).

A processing effect reducing the initial TP and TAA of minimally processed arugula washed with sodium hypochlorite or hydrogen peroxide and packed in Ar- or He-enriched atmospheres was previously reported (Char *et al.* 2012). The TP and TAA values were restored or even increased by some of the treatments, such as the Ar- and He-enriched atmospheres combined with H₂O₂.

In accordance with our observations which indicated that the atmosphere factor was not significant, Jamie and Salveit (2002) reported that controlled atmospheres containing 2% O₂ and 90% He or 90% N₂ did not delay the accumulation

of phenolics in minimally processed lettuce stored at 7C for 4 days. Conversely, a positive effect of He-, O₂- and N₂-enriched atmospheres on the polyphenol content of red chard was reported by Tomás-Callejas *et al.* (2008). They observed that O₂, He- and N₂-enriched atmospheres increased the TP between 61 and 93% with respect to the initial value.

Glucose Content

The glucose content was maintained in a range between 8.9 and 11.0 mg/g (fw) on the processing day to a range between 12.2 and 14.8 mg/g (fw) after 10 days of storage (Fig. 6). According to the results the combined treatments would not accelerate its catabolism as compared with the untreated control.

Color

The lightness (*L*) of the arugula treated with UV-C and packed with He- or Ar-enriched atmospheres was maintained constant during the 10 days of storage, with no significant differences among treatments (Table 4). Conversely, the *L* of the untreated control increased since the sixth day, which was noticeable by yellowing.

The chroma (*C*) (data not shown) and the hue angle (*H_{ab}*) were also preserved by all the combined treatments during the experiment (Table 4). In contrast, the untreated control presented the highest *C* and the lowest *H_{ab}* from the sixth day until the end of storage.

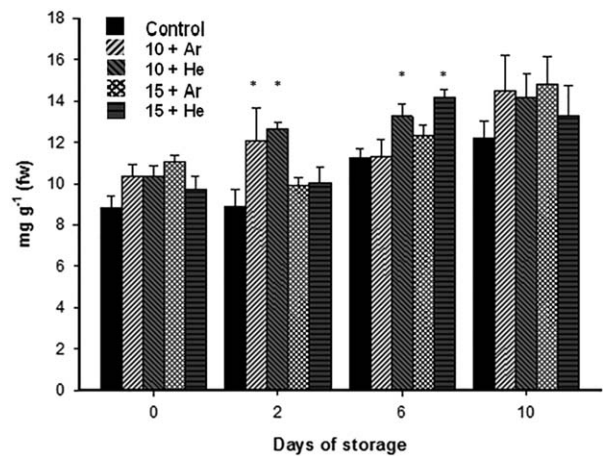


FIG. 6. GLUCOSE CONTENT OF ARUGULA LEAVES TREATED WITH DIFFERENT DOSES OF UV-C (10 and 15 kJ/m⁻²), PACKAGED IN NOBLE GAS-ENRICHED MODIFIED ATMOSPHERES (Ar and He) AND STORED AT 5C FOR 10 DAYS

The vertical bars represent the standard error of the means (*n* = 3).

* Indicates significant differences between the treatments according to Tukey test ($P \leq 0.05$).

TABLE 4. Color PARAMETERS OF ARUGULA LEAVES TREATED WITH DIFFERENT DOSES OF UV-C, PACKAGED IN NOBLE GAS-ENRICHED MODIFIED ATMOSPHERES (ARGON AND HELIUM) AND STORED AT 5C FOR 10 DAYS. LIGHTNESS (L) AND HUE ANGLE (H_{ab})

Parameter	Time (day)	Control	10 + Ar	10 + He	15 + Ar	15 + He
L	0	43.7 ± 0.4	42.8 ± 0.2	42.0 ± 0.6	41.4 ± 0.7*	42.0 ± 0.4
	2	43.2 ± 0.3	43.1 ± 0.4	42.9 ± 0.1	42.4 ± 0.6	43.3 ± 0.2NS
	6	44.2 ± 0.3	42.6 ± 0.5	42.3 ± 0.5*	42.2 ± 0.3*	42.8 ± 0.4
	10	44.2 ± 0.3	42.0 ± 0.6*	41.7 ± 0.3*	41.6 ± 0.6*	42.4 ± 0.7
H_{ab}	0	126.6 ± 0.3	127.0 ± 0.2	126.9 ± 0.1	127.2 ± 0.1	127.2 ± 0.3NS
	2	126.1 ± 0.2	126.5 ± 0.3	126.4 ± 0.2	126.4 ± 0.1	126.6 ± 0.2NS
	6	122.5 ± 0.3	123.4 ± 0.1	124.1 ± 0.3*	123.9 ± 0.2*	124.5 ± 0.2*
	10	124.9 ± 0.3	126.8 ± 0.1*	127.4 ± 0.7*	126.8 ± 0.2*	126.9 ± 0.6*

Values represent the mean of three replicates ± standard error.

No significant differences were found among treatments according to the Tukey test ($P \leq 0.05$).

* Indicates significant differences between the treatments and the control according to Dunnett test ($P \leq 0.05$).

NS, no significant differences were found between the treatments and the control according to Dunnett test.

The color parameters of the combined treatments resulted to be significantly different to the untreated control after the sixth day of storage at 5C. This fact demonstrated that the combination of UV-C with He and Ar atmospheres was useful to maintain the color of arugula preventing the yellowing caused by the breakdown of chlorophyll throughout all the proposed storage time.

The increase of L values for the untreated control and for low UV-C doses (4.5 kJ/m^{-2}) in MP spinach leaves was also reported by Artés-Hernández *et al.* (2009). They also observed that UV-C doses of 7.95 and 11.35 kJ/m^{-2} maintained or even reduced L (10–15%), obtaining better results at 5C than at 8C. In opposition, 7–11% increase in L for red chard packed in He, N_2 , N_2O and high O_2 -enriched active MAP (100% initial gas level) was reported by Tomás-Callejas *et al.* (2011b). They observed that the He- and N_2 -enriched atmospheres better preserved the L values than N_2O during cold storage, with the C and H_{ab} values remaining constant during the shelf life.

Sensory Evaluation

The panel did not perceive changes regarding the appearance, visual color intensity, turgor and off flavor of the MP arugula throughout the 10 days of storage at 5C. All the samples were above the acceptable values and no significant difference was seen among the treated samples, and between any of the treatments and the control (data not shown). Additionally, none of the preservation factors or the interaction resulted significant.

The final gas composition (above 50% He or Ar, above 20% O_2 and less than 15% CO_2) probable influenced the good sensory quality of the arugula. Allende *et al.* (2004) reported that the depletion of O_2 (< 1%) and the accumulation of CO_2 (>15%) limited the appearance of MP baby spinach after 7–9 days, probably due to the onset of fermentative metabolism. Additionally, some studies demon-

strated that the presence of Ar or He may result in better sensory quality of leafy vegetables than atmospheres with N_2 (Robles *et al.* 2010; Char *et al.* 2012).

CONCLUSIONS

The combinations of UV-C and the He- or Ar-enriched atmospheres were useful to reduce the microbial proliferation on arugula leaves with respect to the untreated control, while maintaining the polyphenol content and antioxidant activity, color and sensory quality.

The UV-C factor of the combined treatments was the determining factor on reducing the microbial growth and increasing the total antioxidant activity. The highest dose (15 kJ/m^{-2}) induced an increase on the total antioxidant activity; however, the 10 kJ/m^{-2} dose showed a better control of microbial counts.

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