Combined Effect of UV-C and Ozone on Bioactive Compounds and Microbiological Quality of Fresh-Cut Rocket Leaves

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Abstract The separate application of UV-C (25 kJ/m², 380 s) and O₃ gaseous (2.5 mg/L for 10 min) treatments and of their combination (25 kJ/m² UV-C with 2.5 mg/L O₃) were studied to evaluate the effect of combined treatments of UV-C and O₃ on microbial counts, bioactive profile and sensory changes of fresh-cut rocket leaves throughout shelf life to determine whether these treatment have additive or synergistic. The separate application of UV-C and O₃ and of their combination UV-C + O₃ did not affect the sensorial quality, total chlorophyll contents, phenolic compound nor antioxidant capacity of fresh-cut rocket leaves. However, these treatments controlled better the growth of epiphytic microbes than untreated samples. The UV-C treatment had better effect at reducing microorganisms present and not significant differences were found as to the combined treatment. Therefore, applying the combined treatment UV-C + O₃ had neither synergistic nor additive effect in the extension of the fresh-cut rocket leaves shelf life.

Keywords: UV-C, ozone, synergism, bioactive compounds, quality, rocket


1. Introduction

In recent years, the consumption of fresh-cut vegetables has increased enormously due to its freshness and its ease of use, reducing the time of preparation of meals [1,2]. In addition, these products contain different phytochemicals and antioxidants that prevent some diseases [3].

The rocket (Eruca sativa Mill.), belongs to the Brassicaceae family and originated from the Mediterranean region. Its leaves and stems are consumed in salads and are distinguished by their unique and slightly spicy flavor [4]. Brassicaceae vegetables are beneficial for health because they contain many biologically active molecules such as sulfur and nitrogen compounds, which make the rocket more convenient for a healthy eating [5].

During the preparation of fresh-cut vegetables, are applied unit operations such as washing, peeling and cutting, what cause degradative changes limiting their shelf life [1,6]. These physical injuries during processing stimulate respiratory intensity, ethylene production and loss of nutrients from the cells, so fresh-cut vegetables those more susceptible to browning and microbial contaminations compared to whole products [2]. Therefore, it is necessary to find postharvest technologies to improve and prolong the conservation of these products.

In recent times, treatments with UV-C radiation emerged as an alternative for chemical disinfection and reduction of vegetative microorganisms in food [1]. It has been demonstrated that UV-C radiation can be applied in fresh products for the disinfection and reduction of microbial development without affecting its quality [1,7,8].

In this way, studies carried out by several authors have shown that UV-C application can maintain quality and extended the shelf life of various vegetables such as minimally processed broccoli, fresh-cut green pepper, fresh-cut paprika, fresh-cut Chokanan mango and Josephine pineapple [9,10,11]. In addition, the advantages of these treatments with UV-C radiation is that they use low cost equipment, easy to use and do not require large safety equipment [1].

Besides, other authors reported that treatments with low doses of UV-C of 0.25 to 8.0 kJ/m², induce an activation of the plant of primary and secondary compounds that control the reactive oxygen species (ROS), that may can promote the improvement the nutritional quality and health benefits [12,13,14]. For example, several authors reported that treatments with UV-C caused the synthesis of phenolic compounds in different vegetables such as tomato [15,16], peeled garlic [17] and mango Chokanan and pineapple Josephine [11].
Currently, ozone (O₃) is another postharvest technology that has a high interest to be used as a disinfectant agent against human pathogens [18]. The food industries use ozone as a disinfectant agent (approved by US-FDA [19]) due to its rapid decomposition to oxygen, which reduces concerns about the toxic waste it could cause [20]. The antimicrobial action of ozone can be due to the oxidation of the cellular vital components which causes the inhibition of the microbial development or indirectly through the defense mechanisms as a response of the plants [21]. Several studies have been carried out on the antimicrobial capacity of ozone and its effects on the quality of different products such as papaya [18], lettuce and spinach [20], fresh-cut melon [22] and fresh-cut apple [23]. In addition, other study has shown that gaseous ozone can have a positive effect on changes in the content of antioxidant components, e.g., flavonoids and other phenolic compounds [24,25].

So, postharvest treatments with UV-C and O₃ could be a technological alternative for the decontamination of the surface of fresh-cut rocket leaves, maintaining their general quality [26]. On the other hand, Martínez-Hernández et al. [27] reported that the combined application of sanitizing treatments could have a synergistic effect that allows a greater microbial reduction in the products. However, up to date, no studies have been reported on the combined effect of postharvest technologies with UV-C and O₃ on the quality of fresh-cut rocket leaves.

Therefore, the objective of this work was to investigate the effect of combined treatments of UV-C and O₃ on microbial counts, bioactive profile and sensory changes of fresh-cut rocket leaves throughout shelf life to determine whether these treatment have additive or synergistic.

2. Materials and Methods

2.1. Plant Material and Chemicals

The rocket was obtained from a local farmer in Santiago del Estero, Argentina. Upon harvest, leaves without stems were transported to laboratory and stored at 5°C in a clean room. The next day, leaves at 5-6°C were minimally processed in a disinfected area at 16°C. The chemicals were obtained from Merck Química (Buenos Aires, Argentina).

2.2. Sample Preparation, Treatments and Storage Conditions

In the experiments, defect-free rocket leaves were used, i.e. yellowish, physically damaged, dehydrated or cut leaves were discarded. Upon selection, they were washed with tap water (5°C) for 1 min, drained on a stainless-steel mesh, sliced in portions of about 20 mm using a stainless-steel knife and eventually re-washed for 2 min at 5°C. The water remaining over the cut leaves was centrifuged with a manual centrifuge and the dried leaves submitted to the separate sanitizing treatments with UV-C and O₃ and that with both combined. The following treatments were applied: T₁ (control): 0; T₂ (O₃): 2.5 mg/L for 10 min O₃; T₃ (UV-C): 25 kJ/m² UV-C (380 s) of UV-C dose (the exposure time was calculated according to the radiation intensity); T₄ (UV-C + O₃): combination of T₂ + T₃. Both the UV-C doses and O₃ gaseous concentrations were selected in accordance with the results of preliminary experiments carried out by the authors with minimally processed rocket leaves [26,28].

UV-C treatment was applied in a reflective stainless-steel chamber equipped with 6 unfiltered germicidal lamps (254.7 nm, TUV 36W/G36, Philips, Amstardam, The Netherlands), on its topside and 6 at its bottom. A constant light source of 254 nm was used (intensity of radiation 0.066 kW) and applied upon the samples for different exposure times according to the test. The UV-C radiation dose was measured with a digital radiometer (Cole-Parmer Instrument Company, Vernon Hill, IL). For O₃ treatment was used a 1 g/h ozone generator (Bio3 Ozone Generator, TDZ-1 model, Uruguay). The ozone concentration within the chamber was recorded via an ozone analyzer (Gas Alert Extreme O₃-BW Technology, Honeywell, Canada). These UV-C and O₃ equipment’s used in this work is fully described in Gutiérrez et al. [26].

In every treatment, 70 g of cut leaves were placed in polypropylene (PP) trays of 600mL capacity which were thermally sealed at the top with a bi-oriented polypropylene film of 35 μm in thickness to generate a passive modified atmosphere packaging (MAP). The O₂ and CO₂ transmission rates at 20°C and 90 % RH was 5,000mL/m²/d/atm and 18,000mL/m²/d/atm, respectively, and the water vapor transmission rate was 110 g/m²/d/atm (data provided by INTI, Argentina). All the trays were stored at 5°C. For each treatment and sampling time, three replicates were prepared. Analyzes of the different parameters were performed on days 1, 4, 8 and 12 of shelf-life.

2.3. Respiration Rate and Gas Composition within Packages

A closed system was used to determine the respiratory rate (RR) of the cut rocket. Samples of 30 g were placed for triplicate in 750mL glass bottles at 5°C. After closing the bottles, the increase in CO₂ was monitored for 1 h. With a gas-tight syringe, 1mL of sample was removed from the headspace gases and analyzed in a gas chromatograph (GC SRI 8610C, USA). The GC was equipped with a thermal conductivity detector (150°C), oven (80°C), injector (150°C) and Poropack-Q 80/100 column. The carrier gas was He (20mL/min). For each treatment and evaluation period, three repetitions were performed.

The partial pressures of the gases (O₂ and CO₂) were recorded inside the packages on the day of processing and on days 1, 4, 8, 12 of storage at 5°C, for which a gas analyzer (PBI Dansensor, Check-Point, Ringsted, Denmark) was used. For each treatment and evaluation period, three repetitions were determined.

2.4. Sensory Evaluation

An eight-membered trained panel (aged between 24-64) performed the sensory evaluation of the samples on days 1, 4, 8 and 12 of storage using a 9-point scale for overall visual quality and decay as follows: 9 = excellent, 7 = good, 5 = acceptable (limit of acceptability), 3 = poor and 1 = extremely poor. Color and odor were evaluated using a 5-point scale, that is, 5 = full characteristic of the product, 3 = acceptable (limit of acceptability) and 1 = no characteristic, as indicated by Gutiérrez et al. [26].
2.5. Microbiological Analysis

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

2.6. Chlorophyll and Carotenoid Content

Both chlorophyll and carotenoids were determined in accordance with Gutiérrez et al. [29]. To perform the chemical determinations, samples (10 g) of each treatment per replicate were frozen at-80°C and stored until the measurements were taken on the different days of evaluation. An 0.4 g frozen rocket sample was triturated using 15 mL of an acetone:water (80:20, v: v) solution. The samples were then centrifuged for 15 min at 5,000 x g at 4°C. Their absorbance was measured at 663.2, 646.8 and 470 nm using an UV-visible spectrophotometer (JASCO, model V-630, Japan). To determine the total chlorophyll and carotenoid contents, equations described by Lichtenthaler [30] and the results expressed in mg per 100 g of fresh weight (fw) were used.

2.7. Total Phenolic Compounds

The phenolic compounds were extracted according to the procedure described by Gutiérrez et al. [29]. The 4 g frozen rocket samples were homogenized with 20 mL of methanol and centrifuged at 6,000 x g at 4°C for 15 min being the supernatant used as an extract of each sample. The total phenolic contents were determined using the Folin-Ciocalteau reagent as described by Singleton [31]. The absorbance of each sample was measured after an hour of incubation at 25°C in darkness using an UV-visible spectrophotometer (JASCO V-630) at 765 nm. The results were expressed as chlorogenic acid equivalents (ChAEq) in mg/g fw. All the measurements were made in triplicates.

2.8. Antioxidant Capacity

The antioxidant capacity of the samples was assessed through the method described by Brand-Williams et al. [32], namely 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging. A 150 μL aliquot of the sample extract described above was added to 2,850 μL of 0.1 mM DPPH solution dissolved in ethanol. After one hour in the dark, the absorbance of the sample at 515 nm was measured using a spectrophotometer (JASCO V-630, UV-vis). A calibration curve was depicted using the Trolox solution. The results were expressed as Trolox equivalents (TE) in mg/g fw. All the measurements were made in triplicate.

2.9. Statistical Analysis

The data were statistically analyzed through ANOVA using the 2011 Infostat software (UNC-Argentina). All the experiments were conducted in triplicate. The data were presented as means ± SD. The significant differences were compared against the least significant difference (LSD) at a significance level of 0.05.

3. Results and Discussion

3.1. Respiration Rate and Gas Composition within Packages

After the initial stress induced by the minimal processing, the RR of the control samples was 457 mL CO₂/kg/h at 5°C Figure 1. Initially, the O₃ treatment inhibited significantly the RR in respect of the control and the UV-C and UV-C+O₃ treatments. No significant difference was found between UV-C and UV-C+O₃ treated samples and control. Then, the RR of all the treated rocket leaves samples decreased significantly (P < 0.05) along the 12 day storage at 5°C. The UV-C treated samples registered RR similar to the combined treatment with UV-C + O₃ for the first four days, showing values higher that the control and the O₃ treated samples. However, at the end of storage all the treatments presented similar RR values without significant differences.

The increase in the RR of the fresh-cut rocket leaves produced by the UV-C radiation agrees with studies by Martínez-Hernández et al. [27] on broccoli treated with 1.5, 4.5, 9.0 and 15.0 kJ/m² UV-C doses and by Artés-Hernández et al. [33] on fresh-cut watermelon treated with 1.60, 2.80, 4.80 and 7.20 kJ/m² UV-C doses.

In addition, these results with O₃ treatments agreed with that of Ong et al. [18] who reported that the papaya fruit, when treated with concentrations of O₃ lower than 5 μL/L, presented slower RR with respect to the control. In the same way, Tzortzakis et al. [34] also reported that the RR of tomato fruits stored at 13°C did not suffered significant changes in atmospheres with low levels of ozone.

Figure 1. Respiration rate within packages of fresh-cut rocket leaves treated with O₃ and UV-C, and their combination, during storage up to 12 days at 5°C. Data represent mean values of three replicates (n = 3 ± SD).
The changes that were registered of the partial pressures of the \( O_2 \) and \( CO_2 \) gases during storage inside the MAP packages are showed in Figure 2. Due to the respiratory activity of the vegetable, \( O_2 \) levels decreased while those of \( CO_2 \) increased both in the control and in the treatments [26,27]. From day-1 to day-12 storage, the partial pressures of the gases at equilibrium for all samples were 7-8 kPa \( O_2 \) and 3-4 kPa \( CO_2 \) at 5°C.

During the first four days of storage at 5°C, both UV-C and \( UV-C+O_3 \) treatments registered higher \( CO_2 \) levels with respect to the control sample and the \( O_3 \) treatment. The different doses of UV-C radiation used could cause these differences in the results [8]. However, from day-8 until the end of storage no significant differences were found among the control and treated samples. These results agree with those observed in RR as significant differences are not observed for all the treatments at the end of the conservation period. These results indicated that the separate treatments with UV-C and \( O_3 \) and that with both combined had no influence on the RR of fresh-cut rocket leaves.

These results with UV-C light coincide with those reported by Tomás-Callejas et al. [35], who reported that \( UV-C \) treatment (4.54 kJ/m\(^2\)) in fresh-cut Tatsoi baby leaves showed higher levels of \( CO_2 \) and lower \( O_2 \) through storage at 5°C. Previous work also reported that treatment with \( O_3 \) did not produce a higher RR with respect to chlorine treatments [26,36].

The results obtained in this work using \( UV-C \) agree with Tomás-Callejas et al. [35] who found after 11 days of storage at 5°C, a significant reduction in the overall sensory quality of baby leaves. On the other hand, Jemni et al. [38] reported that a combined treatment of \( UV-C \) and \( O_3 \) helped date fruits (cv. Deglet Nour) save good color after 30 days at 20°C with respect to the control.

3.2. Sensory Evaluation

The products are accepted in the market if they satisfy the consumers, therefore it is vital to evaluate the sensory and organoleptic characteristics of the vegetables to which the practical innovations are applied [34, 37]. Their visual quality, color, and odor were the most affected sensory parameters during the 12 day storage at 5°C. In all the treatments a decrease of these parameters was observed during the storage at 5°C, although they did not present scores below the acceptability limit due to the fact that they did not present undesirable symptoms Table 1. At the end of storage, only were significant differences found among the \( O_3 \) and \( UV-C+O_3 \) treated samples.

According to these results of the sensory evaluation, it could be concluded that the treatments with \( UV-C \), \( O_3 \) or the combination of both, did not affected the general sensory attributes of the fresh-cut rocket leaves during 12 days at 5°C.

The results obtained in this work with \( O_3 \) treatments agree with those of Ali et al. [37], who found that papaya fruits treated with different concentrations of \( O_3 \) (2.5 and 3.5 mg/L) showed high scores on sensory attributes after 12 days of storage at room temperature. Olmez and Akbas [36] also reported that lettuce treated with \( O_3 \) showed better scores on sensory attributes with respect to washing with hypochlorite at day 9.

In turn, the results obtained in this work using \( UV-C \) agree with Tomás-Callejas et al. [35] who found after 11 days of storage at 5°C, a significant reduction in the overall sensory quality of baby leaves. On the other hand, Jemni et al. [38] reported that a combined treatment of \( UV-C \) and \( O_3 \) helped date fruits (cv. Deglet Nour) save good color after 30 days at 20°C with respect to the control.

Table 1. Visual quality score (A), color score (B) and odor score (C) of fresh-cut rocket leaves treated with \( O_3 \) and \( UV-C \), and their combination, during storage up to 12 days at 5°C

<table>
<thead>
<tr>
<th>Sensory parameter and treatments</th>
<th>Days at 5°C</th>
<th>1</th>
<th>4</th>
<th>8</th>
<th>12</th>
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<tr>
<td><strong>Visual quality score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td></td>
<td>8.95</td>
<td>8.50</td>
<td>6.91</td>
<td>6.52</td>
</tr>
<tr>
<td>( O_3 )</td>
<td></td>
<td>8.95</td>
<td>8.60</td>
<td>7.11</td>
<td>6.65</td>
</tr>
<tr>
<td>( UV-C )</td>
<td></td>
<td>8.80</td>
<td>8.32</td>
<td>6.77</td>
<td>6.27</td>
</tr>
<tr>
<td>( UV-C+O_3 )</td>
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<td>8.97</td>
<td>8.45</td>
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<td></td>
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<tr>
<td>Control</td>
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<td>4.62</td>
<td>3.80</td>
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</tr>
<tr>
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<td>4.75</td>
<td>3.90</td>
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</tr>
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<td>4.55</td>
<td>3.65</td>
<td>3.42</td>
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<tr>
<td>( UV-C+O_3 )</td>
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<td>4.95</td>
<td>4.75</td>
<td>3.67</td>
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<tr>
<td><strong>Odor score</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
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<td>4.95</td>
<td>4.25</td>
<td>3.80</td>
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<tr>
<td>( O_3 )</td>
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<tr>
<td>( UV-C+O_3 )</td>
<td></td>
<td>4.95</td>
<td>4.12</td>
<td>3.55</td>
<td>3.12</td>
</tr>
</tbody>
</table>

Mean values in the same row with different superscript letters or in the same column for same type sample with different lowercase letters were significantly different according to LSD test at \( P < 0.05 \).

3.3. Microbiological Profile

To evaluate the efficacy of these treatments as to the initial reduction and microbial growth during storage at 5°C and to compare them to the traditional method that uses chlorine disinfection, a treatment with hypochlorite sodium (NaClO) (100 mg/L, 2 min) was included. Such a treatment is usually employed in the food industry.

The control samples showed an initial microbial load for total mesophilic aerobic bacteria (TMAB) of 5.7 log CFU/g Figure 3A. This count was reduced up to 0.8 and 1.1 log CFU/g with the NaClO and \( O_3 \) treatments, respectively. In turn, the \( UV-C \) and the combined \( UV-C+O_3 \) treatments were even more effective than the remainder since they reduced the load to 1.3 and 1.4 log CFU/g respectively, with respect to the control. The antimicrobial effect of the applied treatments was
maintained during the first 8 days at 5°C compared to the control. However, on day 12, treatments with UV-C and UV-C + O₃ showed higher reductions in TMAB in comparison with the control and O₃ treatment, and no significant differences between them were found.

The control samples showed an initial microbial load for psychrophilic counts of 6.1 log CFU/g Figure 3B. The treatment with NaClO showed 0.92 log CFU/g reduction while the O₃, UV-C and UV-C + O₃ treatments led to reductions in the count of 1.20, 1.37 and 1.22 log CFU/g respectively related to the control. The sanitizing treatments inhibited the psychrophilic growth in a similar way to the TAM counts, achieving a bacteriostatic effect during the first 8 days at 5°C. However, at day 12, contrary to TMAB counts, the O₃ treatment presented the lowest psychrophilic count (P < 0.05) compared to the control and the other treatments with no significant difference among them.

As to enterobacteria, the initial count was 4.7 log CFU/g Figure 3C. The treatment with NaClO showed a reduction of the initial load of enterobacteria in 0.3 log CFU/g while the treatments O₃, UV-C and UV-C + O₃ produced reductions ranging from 1.07 to 0.67 log CFU/g in comparison with control. The antimicrobial effects of the sanitizing treatments lasted the first 8 days at 5°C. However, at the end of storage, the UV-C treatment was the only maintaining the lowest count compared to the control and the other treatments.

Both mold and yeast were significantly inhibited by applying the sanitizing treatments though such effect was smaller than those produced upon bacteria Figure 3D. The NaClO and O₃ treatments led to an initial reduction of molds and yeasts in approximately 0.34-0.59 log CFU/g, respectively, compared to the control (5.09 log CFU/g). A stronger inhibitory effect occurred when the UV-C and UV-C + O₃ treatments were applied since they showed reductions of 0.76 and 0.87 log CFU/g respectively. At the end of storage, the UV-C, O₃ and UV-C + O₃ treatments kept the mold and yeast counts reductions compared to the control and NaClO.

These results of the use of UV-C treatments agree with those of other vegetables in publications of other authors. Gogo et al. [14] reported that UV-C treatments (1.7 kJ/m²) in amaranth leaves stored at 20°C significantly reduced the counts of mesophiles and yeasts after being applied, whereas in the mold counts, the control and UV-C treatments showed significant differences throughout the storage. Tomás-Callejas et al. [35] also reported that after being treated with UV-C, the microbial growth in Tatsoi baby-leaves was significantly reduced. In similar way, Martínez-Hernández et al. [39] reported that treatment with UV-C reduced the initial microbial counts of broccoli and its effect was higher in the counts of mesophiles, yeasts and molds.

The reduction in microbial counts observed in this work could be attributed to the germicidal effect of UV-C radiation causing denaturation of the DNA and/or the response of vegetable defense system to UV-C treatment [14,40]. In fresh-cut products it is being used as an alternative to conventional chlorine washes, UV-C radiation due to its germicidal effect [41].

With respect to the results with ozone, these agree with Olmez and Akbas [36] who found that treatments with O₃ (2 ppm-2 min) in lettuce caused a decrease in the counts of mesophiles, psychrotrophic and enterobacteria in approximately 1.5, 1.1 and 1.5 log CFU/g, respectively. Yeoh et al. [42] also reported that treatment with O₃ (9.2 ± 0.2 μL/L) during 10, 20 and 30 min in fresh-cut papaya produced a reduction in the psychophilic count. In addition, Horvitz and Cantalejo [43] observed in fresh red peppers treated with O₃ (0.7 mg/L) during 1, 3 and 5 min a reduction in the counts of yeasts, molds, mesophilic and psychrotrophic bacteria, while the treatment with 20 mg/L chlorinated water was not as effective.

On the other hand, Jemni et al. [38] found that dates fruits treated with the combination of 0.55 mg/L O₃ + 6.22 kJ/m² UV-C produced the most effective reduction in the microbial load compared to that involving only 6.22 kJ/m² UV-C, which shows a synergistic effect between them. In some European countries, specific microbiological criteria have been adopted for minimally processed fruits and vegetables, such as Spain, where the legislation establishes that the maximum acceptable limit for the total viable count is 7 log CFU/g. It is worth mentioning that different research studies report on the antimicrobial effect of disinfectant treatments immediately after being applied, however it is also important to maintain this microbial reduction during storage.

In this work, the fresh-cut rocket leaves shelf-life was limited by the development of psychrophilic microorganisms. The microbial counts could be maintained below 7 log CFU/g up to day 8 of storage at 5°C with the separate and combined application of UV-C and O₃ sanitizing treatments whereas shelf-life expectancy for control samples was lower than 6 days. In summary, the combined treatment UV-C + O₃ did not produce a greater reduction of the microbial load with respect to the separate treatments of UV-C and O₃.

Therefore, with the application of the combined treatment of UV-C and O₃, not synergistic or additive effect was observed with respect to the treatments applied separately.

![Figure 3](image-url)
3.4. Chlorophyll and Carotenoid Contents

The initial total chlorophyll content was similar in all treatments, ranging from 92.5 to 98.7 mg/100g fw Table 2, with chlorophyll a contributing with 72-78% of the total and chlorophyll b with 28-22% to it (data not shown). Every treatment followed the same behavior concerning the total chlorophyll content throughout shelf-life at 5°C; it decreased the first four days of storage and kept constant up to the end of storage. After 12 days, the total chlorophyll values were between 70.6 and 72.7 mg/100g fw, so they decreased by 24-27% of the initial values regardless the treatment applied, without significant differences between all the treatments. Gogo et al. [14] reported that UV-C radiation could cause a stress which would induce a decrease in the content of chlorophylls and carotenoids during storage. The data obtained in this work agreed with those reported by Tomás-Callejas et al. [35] who found that treatment with UV-C (4.54 kJ/m²) in Tatsoi baby leaves did not cause any significant changes in the total chlorophyll content for 11 days at 5°C. On the other hand, Gogo et al. [14] reported that vegetable amaranth and leaves of African Solanaceae treated with 1.7 and 3.4 kJ UV-C/m², presented a decrease in the chlorophyll content until four days of storage at 5°C.

Table 2. Total chlorophyll, total carotenoids, total polyphenols contents and total antioxidant activity changes of fresh-cut rocket leaves treated with O₃ and UV-C, and their combination, during storage up to 12 days at 5°C

<table>
<thead>
<tr>
<th>Bioactive compounds and treatments</th>
<th>Days at 5°C</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total chlorophyll (mg/100g fw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>93.6 a</td>
<td>80.6 ab</td>
<td>70.7 a</td>
<td>72.7 a</td>
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<td>O₁</td>
<td>98.7 a</td>
<td>82.4 a</td>
<td>73.8 a</td>
<td>72.3 a</td>
</tr>
<tr>
<td>UV-C</td>
<td>93.2 ab</td>
<td>76.2 ab</td>
<td>69.5 a</td>
<td>72.3 a</td>
</tr>
<tr>
<td>UV-C + O₃</td>
<td>92.5 ab</td>
<td>72.1 b</td>
<td>71.0 a</td>
<td>70.6 a</td>
</tr>
<tr>
<td>Total carotenoids (mg/100g fw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.4 a</td>
<td>16.1 a</td>
<td>17.7 b</td>
<td>14.6 a</td>
</tr>
<tr>
<td>O₁</td>
<td>21.6 a</td>
<td>16.9 a</td>
<td>17.9 a</td>
<td>18.2 a</td>
</tr>
<tr>
<td>UV-C</td>
<td>20.2 ab</td>
<td>17.1 a</td>
<td>17.3 a</td>
<td>17.1 a</td>
</tr>
<tr>
<td>UV-C + O₃</td>
<td>21.0 ab</td>
<td>16.3 a</td>
<td>17.1 a</td>
<td>15.7 a</td>
</tr>
<tr>
<td>Total polyphenols content (mg ChAE/g fw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.8 a</td>
<td>1.9 a</td>
<td>1.9 a</td>
<td>1.9 a</td>
</tr>
<tr>
<td>O₁</td>
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<td>1.9 a</td>
<td>1.9 a</td>
<td>1.9 a</td>
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<td>1.9 a</td>
</tr>
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<td>1.8 a</td>
</tr>
<tr>
<td>Total antioxidant capacity (mg TE/g fw)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>2.4 a</td>
<td>2.5 a</td>
<td>2.6 a</td>
</tr>
<tr>
<td>O₁</td>
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<td>2.4 a</td>
<td>2.5 a</td>
<td>2.5 a</td>
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<tr>
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<td>2.4 a</td>
<td>2.4 a</td>
<td>2.5 a</td>
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<td>2.5 a</td>
<td>2.5 a</td>
<td>2.5 a</td>
</tr>
</tbody>
</table>

Mean values in the same row with different superscript letters or in the same column for same type sample with different lowercase letters were significantly different according to LSD test at P < 0.05.

As to ozone, the results of this work agree with those of Karaca and Velioglu [20] who found that the content of chlorophyll was not modified in parsley treated with gaseous O₃ at a concentration of 950 ± 12 μL/L for 20 min. Notwithstanding, Olmez and Akbas [36] reported that ozone treatments caused discoloration in lettuce after being treated.

The initial total carotenoids content in fresh-cut rocket leaves was similar in all treatments, ranging from 20.2 to 21.6 mg/100g fw Table 2. Along storage at 5°C, with the exception of the control and UV-C + O₃ treatments that showed decreases of 32 and 26%, respectively as to their initial value, the total content of carotenoids remained constant. However, non-significant differences were found either among treatments or they respect to the control. Gogo et al. [14] found that UV-C treatments with doses of 1.7 and 3.4 kJ/m² in vegetable amaranth and African Solanaceae leaves caused a decrease in the total carotenoid content during the first 4 days of storage at 5°C. Different authors suggested that the treatments with UV-C radiation synthesize and degrade (photooxidation) the content of chlorophylls and carotenoids [14,44].

On the other hand, the results obtained with ozone agree with those reported by Tzortzakis et al. [34], who reported that the content of carotenoids (beta carotene, lutein and lycopene) were not affected by treatments with O₃ in tomato fruits.

3.5. Total Phenolic Content

The initial total phenolic content was similar in all the treatments, ranging between 1.8 and 1.9 mg ChAE/g fw Table 2. During the shelf life at 5°C, the phenolic content remained constant in all treatments, with no differences between the control and the treated samples.

These results agree with those reported by Jenni et al. [38], who reported that date fruits (cv. Deglet Nour) immediately after treated with 2.37; 6.22; 8.29 and 12.14 kJ/m² UV-C and 0.55 mg/L O₃ + 6.22 kJ/m² UV-C, did not show significant differences in the phenolic content compared to the control. However, they reported a slight increase in their phenolic content through storage at 20°C, particularly in the control and samples treated with UV-C + O₃. Based on the above, they concluded that the phenolic compounds are stabilized by UV-C radiation. Despite this, UV-C treatments can also be effective to increase the phenolic contents of vegetables [35]. For example, it was reported that UV-C treatments increase the phenolic compounds in wine grapes [45], watermelon [33], Tatsoi baby leaves [35], minimally processed yam slices [1,46] and minimally processed lily bulb [2].

This finding related to ozone treatment agrees with the results obtained by Glowacz and Rees [47], who reported that green peppers exposed to different concentrations of O₃ (0.45, 0.9 and 2 μmol/mol) were not affected in the total phenolic content. Likewise, Glowacz et al. [48] found no significant differences between untreated red peppers and those treated with ozone at concentrations of 0.1 and 0.3 μmol/mol.

3.6. Antioxidant Capacity

The initial total antioxidant activity was similar in all the treatments, ranging from 2.4 and 2.5 mg TE/g fw. As it can be observed for the total phenolic content, the total antioxidant capacity maintained constant through storage in every treatment showing no differences among them Table 2. These results with UV-C radiation agreed with that reported by Jenni et al. [38], who informed that the
total antioxidant activity of Deglet Nour cv. was stabilized with UV-C treatments during storage at 20°C. Jin et al. [49] maintain that the total phenolic content is related to the antioxidant capacity.

In contrast, Tomás-Callejas et al. [35] reported that UV-C treatments (4.54 kJ/m²) in Tatsoi baby leaves significantly increased the total phenolic content and total antioxidant capacity throughout the first four days of storage at 5°C. Huang et al. [2] also reported that UV-C treatments caused an improvement of antioxidant capacity in minimally processed bulbs, due to an increase in total phenolic content. In addition, similar results were observed in the antioxidant activity of mushrooms treated with UV-C [50].

The results of the present work with ozone agree with those obtained by Glowacz and Rees [47] who observed that treatments with O₃ (0.45, 0.9 and 2 μmol/mol) in red chili peppers did not affect their antioxidant capacity. Similarly, Tzortzakis et al. [34] also reported that treatments with O₃ (1 μmol/mol) in tomatoes, did not cause changes in the antioxidant activity of the same for 6 days.

4. Conclusions

The development of psychrophilic microorganisms limited the shelf-life of the fresh-cut rocket leaves until 8 days at 5°C for all the treatments applied. The separate UV-C or O₃ treatments or both combined showed better effect than the traditional washing with NaClO in controlling psychrophilic growth. In addition, these treatments did not affect the bioactive compounds or their main quality attributes during storage at 5°C.

The application of these treatments could be useful tools for disinfection of fresh-cut rocket leaves, maintaining their general quality and prolonging their shelf life. The application of the combined treatment of UV-C and O₃ did not produce a synergistic or additive effect with respect to the treatments applied separately. Therefore, taking into account the costs and processing times at an industrial level, it would be appropriate to apply treatment with 25 kJ UV-C/m².

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References


