Impact of Ozone-Based Postharvest Treatment on the Quality and Shelf Life of Radish (*Raphanus sativus* L.) Microgreens

Efectos del tratamiento poscosecha con ozono en la calidad y la vida útil de microgreens de rabanito (*Raphanus sativus* L.)

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Abstract

Microgreens are young vegetable seedlings that have garnered significant attention due to their high concentrations of health-promoting phytochemicals. However, their highly perishable nature presents a significant challenge for postharvest storage. Among the various preservation technologies available, ozone treatment applied to microgreens-an innovative and environmentally sustainable method-has not been extensively studied. This study evaluated the effect of ozone-based sanitization on the shelf life and quality of radish microgreens. Conventional washing treatments using chlorinated water and tap water were compared to ozonated water. During refrigerated storage, key quality parameters were systematically monitored, including fresh weight loss, electrolyte leakage, color changes, and microbial counts. Ozonated water effectively reduced the initial aerobic mesophilic bacterial populations, with no statistically significant differences compared to conventional chlorine treatment. Furthermore, ozone treatment had minimal impact on color, and the weight loss remained below 1%. Although tissue wilting was observed, it was significantly less severe than that associated with chlorine treatment. These findings suggest that ozonated water is a promising alternative to conventional postharvest treatments for enhancing the shelf life and microbiological safety of ready-to-eat microgreens.

Keywords
micro-scale vegetables • *Raphanus sativus* • ozonated water • sanitization • storage

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Resumen

Los microgreens son plántulas jóvenes de hortalizas reconocidas por sus altas concentraciones de fitoquímicos beneficiosos para la salud. Sin embargo, su naturaleza altamente perecedera representa un desafío significativo para su almacenamiento poscosecha. Entre las diversas tecnologías de conservación disponibles, el tratamiento con ozono aplicado a microgreens-un método innovador y ambientalmente sostenible- continúa escasamente investigado. Este estudio evaluó el efecto de la sanitización con ozono sobre la vida útil y la calidad de los microgreens de rabanito. Se compararon tratamientos convencionales de lavado con agua clorada y agua de red frente al uso de agua ozonizada. Durante el almacenamiento refrigerado, se monitorearon sistemáticamente parámetros de calidad como pérdida de peso fresco, pérdida de electrolitos, cambios de color y recuentos microbiológicos. El agua ozonizada redujo eficazmente las poblaciones iniciales de bacterias mesófilas aerobias, sin diferencias estadísticamente significativas respecto del tratamiento convencional con cloro. Además, el tratamiento con ozono tuvo un impacto mínimo sobre el color, y la pérdida de peso se mantuvo debajo del 1%. Aunque se observó marchitamiento tisular, su severidad fue significativamente menor que la asociada al tratamiento con cloro. Estos resultados sugieren que el agua ozonizada es una alternativa prometedora a los tratamientos poscosecha convencionales para mejorar la vida útil y la seguridad microbiológica de los microgreens listos para consumir.

Palabras clave

micro-hortalizas • *Raphanus sativus* • agua ozonizada • sanitización • almacenamiento

Introduction

Microgreens, the edible seedlings of various vegetable and herb species, exhibit a short growth cycle, typically lasting between 10 to 15 days. After this period, the stem, cotyledons, and the first pair of true leaves are consumed. Microgreens popularity has increased in recent years, driven by growing consumer interest in healthy eating (15). Numerous studies have underlined the high concentrations of bioactive compounds in microgreens, with several health benefits-such as anticancer and antioxidant properties-linked to their consumption (23). However, due to their young and tender nature, microgreens are highly perishable and have a limited postharvest shelf life.

Several factors influence postharvest preservation of vegetables, including temperature, humidity, gas composition during storage, packaging materials, and washing and sanitizing methods. Chlorine is commonly used as a sanitizer in the food industry. However, its use raises concerns regarding environmental contamination and the potential carcinogenic effects of its gaseous byproducts and degradation products (8, 25). In this context, various alternative physical and chemical technologies have been developed for postharvest applications (1). Among these, ozone is notable for its potent antimicrobial properties and its ability to decompose spontaneously into non-toxic byproducts (4, 30).

Numerous studies have reported the use of ozonated water for preserving minimally processed vegetables, including celery (30), asparagus (11), broccoli (7), spinach (18), as well as carrots and lettuce (21). For ready-to-eat products, microgreens undergo washing and sanitizing processes. Current research on washing and disinfection technologies for postharvest microgreens preservation has evaluated chlorine at various concentrations (13, 27) and its combination with citric (28) or ascorbic acid (6, 20). However, sanitizers based on novel, environmentally friendly technologies have not been widely explored for microgreens preservation. The use of ozonated water to extend the postharvest shelf life of microgreens is a promising approach that, to our knowledge, remains unexplored.

The objective of this study was to compare ozonated water with conventional treatments and assess their effects on quality of radish microgreens during postharvest storage. We measured quality parameters like weight loss (%), electrolyte leakage (%), color change, aerobic mesophilic bacterial counts, and mold and yeast counts.

Materials and Methods

Plant Material and Harvest

Radish (*Raphanus sativus* L.) microgreens were cultivated in a growth chamber under controlled temperature conditions (24 ± 2°C) with artificial LED lighting. The seeds were sown in trays filled with a commercial substrate composed of peat, coconut fiber, and perlite (Cocomix, Ing. Carluccio). Germination occurred in the dark, after which the trays were exposed to light and irrigated daily with tap water. The microgreens were harvested 12 days after sowing using disinfected scissors.

Washing Treatments, Storage, and Experimental Setup

Following harvest, the microgreens were divided into four groups and subjected to the following washing treatments: chlorinated water (100 mg L^{-1} NaClO) (19, 27), ozonated water (0.16 mg L^{-1} O_3) (30), tap water, and an unwashed control. Chlorinated water was prepared by dissolving commercial bleach (58 g L^{-1} Cl) in tap water. Ozonated water was generated by introducing gaseous ozone through a gas diffuser submerged in a container of tap water. Ozone was produced using an ozone generator (Pura® HMB2), and its concentration was monitored with a pH/ORP controller (Walfront Model PH-803W). Each group of microgreens was immersed in its respective sanitizing solution for 5 minutes. For the chlorinated water treatment, a subsequent 1-minute rinse with tap water was performed. After washing, the microgreens were centrifuged for 3 minutes using a manual centrifuge. Three replicates of 30 grams per treatment were stored in PET plastic containers with lids. All treatments were kept refrigerated in the dark at 8 ± 1°C for 12 days. Samples were collected on days 0, 6, and 12 of storage to evaluate postharvest quality parameters.

Quality Parameters

Weight Loss

The weight of each container was recorded using an analytical balance (Denver APX-200) at the beginning of storage (day 0) and during storage on days 2, 6, 8, and 12. Weight loss was expressed as a percentage (%) of the initial weight, calculated by determining the weight difference between the initial and final weights for each evaluation day (21).

Electrolyte Leakage

Electrolyte leakage, an indicator of tissue deterioration, was assessed following the procedure outlined by Xiao *et al.* (2014a), with modifications. Three-gram samples were periodically collected from each container and shaken with 90 mL of distilled water for 15 minutes. The electrical conductivity of the solution (μS cm^{-1}) was measured using a conductivity meter (Hanna HI 8733). Electrolyte leakage values were expressed as a percentage of the total electrolyte content, which was determined using the same procedure on a sample that had been previously frozen at -20°C for 24 hours and thawed at the time of measurement.

Color Determination

Color changes were measured using an 8 mm-aperture colorimeter (Konica Minolta Chroma Meter CR-400), which was calibrated to a standard white tile (Y 93.5, x 0.3114, y 0.3190). The CIELAB color space coordinates were recorded in quintuplicate for each sample on the transparent surface of the container. Measurements were taken at random locations on each sample to obtain color data from all parts of the microgreens, including both cotyledons and stems. The parameters a* (redness/greenness), b* (yellowness/blueness), and L* (lightness, ranging from 0 = black to 100 = white) were recorded (9). Hue Angle and Chromaticity were calculated from the a* and b* values using the following formulas:

$$\text{Hue Angle } (h°) = \tan^{-1}(b*/a*) + 180° \quad (5)$$

$$\text{Chromaticity } (C)* = \sqrt{(a^2 + b^2)} \quad (15)$$

The Hue Angle (h°) denotes the color tone and is expressed on a circular scale, where 0°/360° corresponds to red, 90° to yellow, 180° to green, and 270° to blue (29). Chromaticity reflects the overall color intensity or saturation, with brighter colors (*i.e.*, less white or black) exhibiting higher C* values.

Total Aerobic Mesophilic Bacterial Count (AMB)

To assess microbial quality, five-gram pooled samples per treatment were periodically taken in sterile stomacher bags (BPS-750, Microclar) and homogenized for 5 minutes with 50 mL of sterile peptone water (3, 12). Serial dilutions of these suspensions were plated in duplicate on Plate Count Agar. The plates were incubated for 24 hours at 30°C, after which the colony-forming units (CFUs) were counted. Results were expressed as log CFU g^{-1} (19).

Total Yeast and Mold Count (Y&M)

Using the same suspensions described for the total aerobic mesophilic bacterial count, serial dilutions were plated in duplicate on Potato Dextrose Agar. The plates were incubated for 48 hours at 22°C, and the CFU count was subsequently performed. Results were expressed as log CFU g^{-1} (19, 27).

Statistical Analysis

For each parameter evaluated, three replicates were analyzed per treatment on each sampling day during storage. Results were expressed as means ± standard deviation. Statistical analyses were performed using Infostat V.2020 software. Data were subjected to analysis of variance (ANOVA) with general and mixed linear models. Mean values for treatments, as well as their interactions, were compared using Duncan's Multiple Range Test (DGC) ($p \leq 0.05$).

Results

Weight Loss

The weight of radish microgreens was influenced by storage time in interaction with the washing treatments (table 1). Weight loss reached approximately 0.97% by the end of the experiment (day 12).

NS, *, and ** denote non-significant or significant effects at $p \leq 0.05$, and $p \leq 0.01$, respectively.

WL: weight loss, EL: electrolyte leakage, L*: lightness, C*: chroma, h°: hue angle, AMB: aerobic mesophilic bacteria, Y&M: yeast and mold.

NS, *, y ** indican efectos no significativos o significativos para $p \leq 0,05$, y $p \leq 0,01$, respectivamente.

WL: pérdida de peso, EL: pérdida de electrolitos, L*: luminosidad, C*: croma, h°: ángulo de tono, AMB: bacterias aerobias mesófilas, Y&M: mohos y levaduras.

Table 1. ANOVA for weight loss, electrolyte leakage, lightness, chroma, hue angle, aerobic mesophilic bacterial counts, and yeast and mold counts of radish microgreens stored at 8°C for 12 days.

Tabla 1. ANOVA de la pérdida de peso, pérdida de electrolitos, luminosidad, croma, ángulo de tono, recuento de bacterias aerobias mesófilas y recuento de mohos y levaduras de micro-hortalizas de rabanito almacenadas a 8°C durante 12 días.

Source of Variance	WL (%)	EL (%)	L*	C*	h°	AMB	Y&M
Wash treatment (W)	NS	**	*	NS	NS	NS	**
Storage time (T)	**	**	**	**	**	**	**
T x W	*	**	NS	NS	*	*	NS

Significant differences in weight loss were observed among the washing treatments only during the later stages of storage, starting from day 8 onwards (figure 1, page 203). On days 8 and 12, weight loss in the ozone-treated and unwashed control was significantly greater than in the chlorine and tap water treatments. The differences between the unwashed/ozone treatments and the chlorine/tap water treatments were 0.10% on day 8 and 0.17% on day 12.

Electrolyte Leakage

Figure 2 and table 1 (page 202), show the effect of washing treatments * storage time on electrolyte leakage in radish microgreens. All treatments exhibited a significant increase in electrolyte leakage over time, which was visualized as an increased tissue wilting. Moreover, electrolyte loss increased differentially depending on the treatment, which is confirmed by the significant treatment × storage time (T × W) interaction. Tap water resulted in higher leakage compared to chlorine, and chlorine showed more leakage than ozone.

Vertical bars represent ± standard error. Significant differences between wash treatments (within the same time point) according to DGC Test ($p \leq 0.05$) are indicated with different lowercase letters above the plots.

Las barras verticales representan ± error estándar. Las diferencias significativas entre los tratamientos de lavado (dentro del mismo día de muestreo) según la Prueba DGC ($p \leq 0{,}05$) se indican con letras minúsculas diferentes sobre los gráficos.

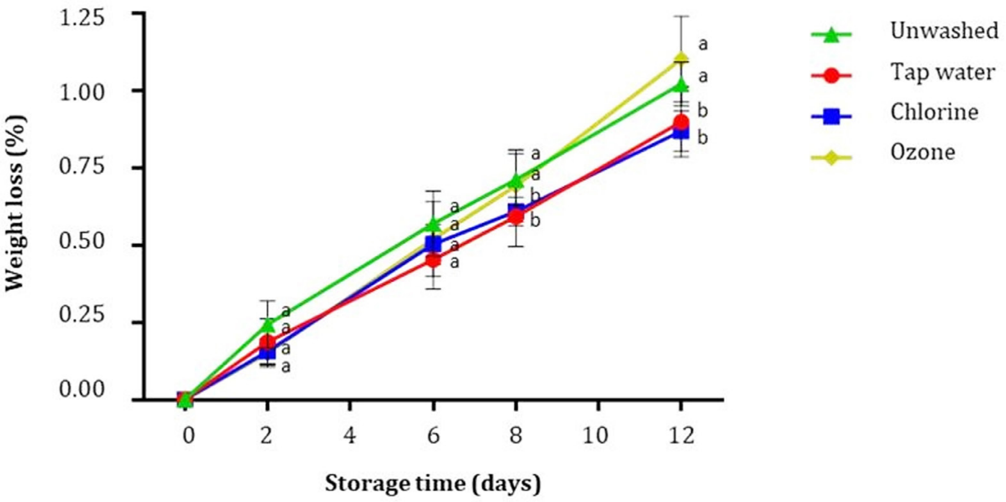


Figure 1. Effect of the washing treatments on weight loss (%) of radish microgreen during 8°C storage (n=3).

Figura 1. Efecto de los tratamientos de lavado sobre la pérdida de peso (%) en micro-hortalizas de rabanito durante el almacenamiento a 8°C (n=3).

Vertical bars represent ± standard error. Significant differences between wash treatments (within the same time point) according to DGC Test ($p \leq 0.05$) are indicated with different lowercase letters above the plots.

Las barras verticales representan ± error estándar. Las diferencias significativas entre los tratamientos de lavado (dentro del mismo día de muestreo) según la Prueba DGC ($p \leq 0{,}05$) se indican con letras minúsculas diferentes sobre los gráficos.

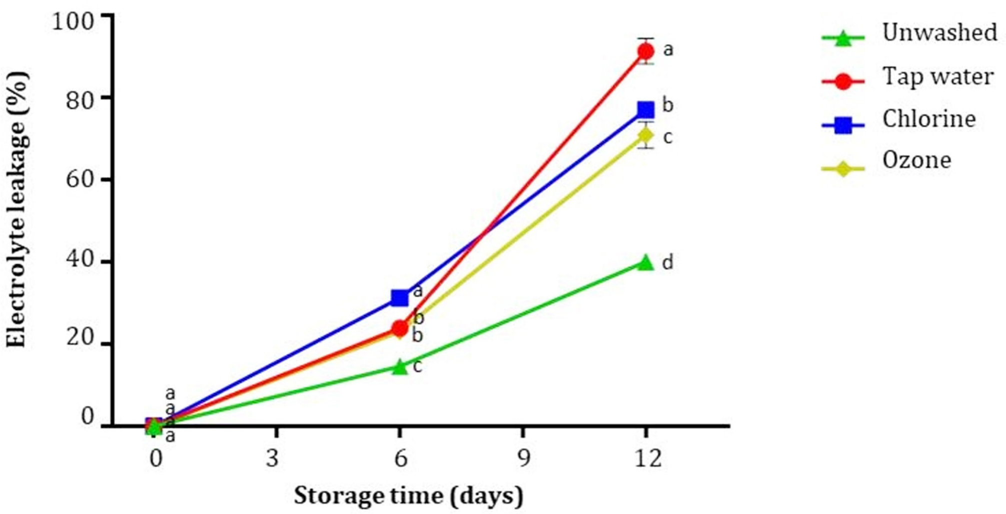


Figure 2. Effect of the washing treatments on electrolyte leakage (%) of radish microgreen during 8°C storage (n=3).

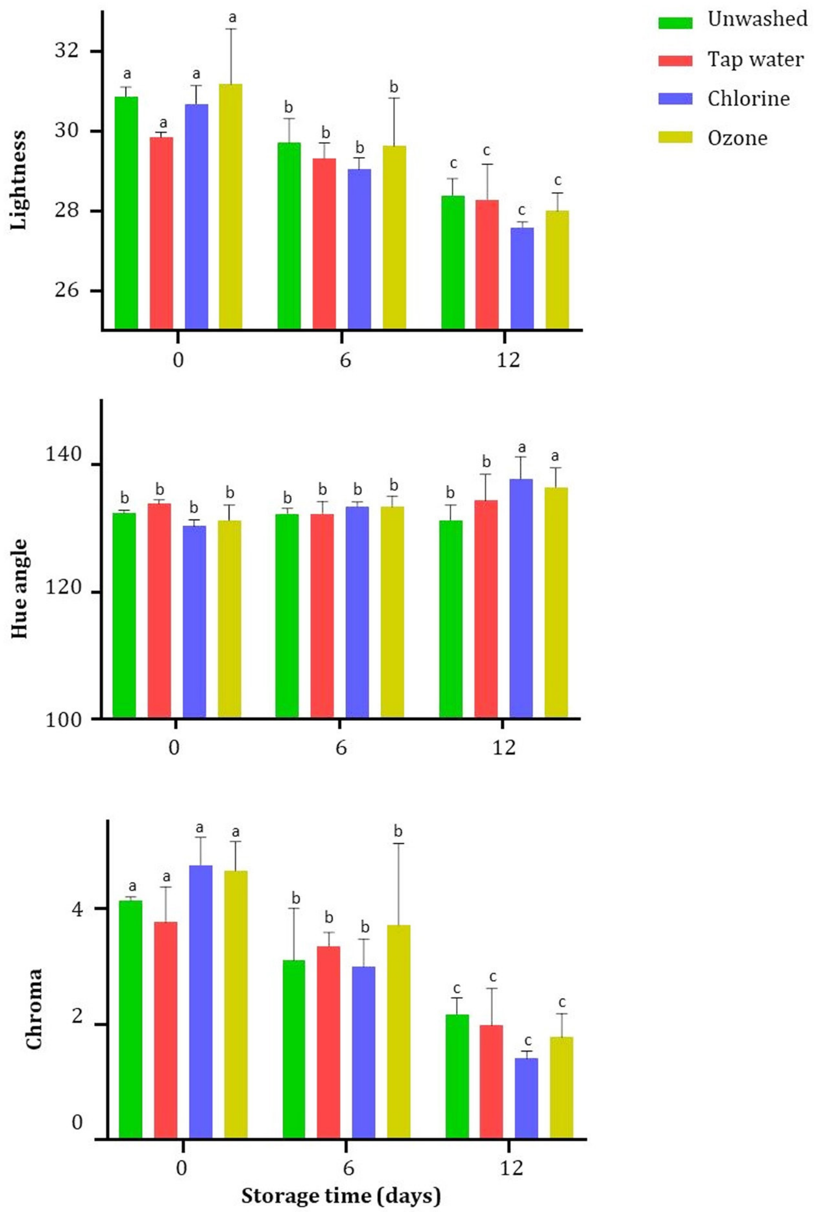
Figura 2. Efecto de los tratamientos de lavado sobre la pérdida de electrolitos (%) en micro-hortalizas de rabanito durante el almacenamiento a 8°C (n=3).

The unwashed control exhibited the lowest electrolyte loss compared to all washing treatments, this difference becoming more pronounced as storage time progressed.

Among the treatments involving wetting, by the end of the storage period, ozone treatment significantly reduced electrolyte leakage compared to chlorine and tap water. Tap water treatment, which involved wetting without disinfection, resulted in the greatest electrolyte loss compared to the other treatments.

Color

Washing effects on color were evaluated by considering the coordinates of lightness (L*), chroma (C*), and hue angle (h°) (table 1, page 202). No significant effects of the washing treatments were observed for C* or h°. Storage time had a significant effect on all three color parameters, with a significant treatment × time interaction for h° (figure 3).



Vertical bars represent ± standard error. Significant differences between wash treatments (within the same time point) according to DGC Test ($p \leq 0.05$) are indicated with different lowercase letters above the plots.

Las barras verticales representan ± error estándar. Las diferencias significativas entre los tratamientos de lavado (dentro del mismo día de muestreo) según la Prueba DGC ($p \leq 0,05$) se indican con letras minúsculas diferentes sobre los gráficos.

Figure 3. Effect of the washing treatments on color coordinates of radish microgreen during 8°C storage (n=3).

Figura 3. Efecto de los tratamientos de lavado sobre las coordenadas de color en micro-hortalizas de rabanito durante el almacenamiento a 8°C (n=3).

Lightness values, regardless of the treatment, decreased approximately 8% by the end of storage, from around 30 to 28.

Hue angles exhibited minimal variation under the tested conditions. Significant increases in h° were only observed on day 12 in microgreens washed with chlorine and ozone. However, this variation was less than 3% and visually imperceptible (figure 4).

Figure 4. Image of radish microgreens subjected to different washing treatments during 12 days of storage at 8±1°C.

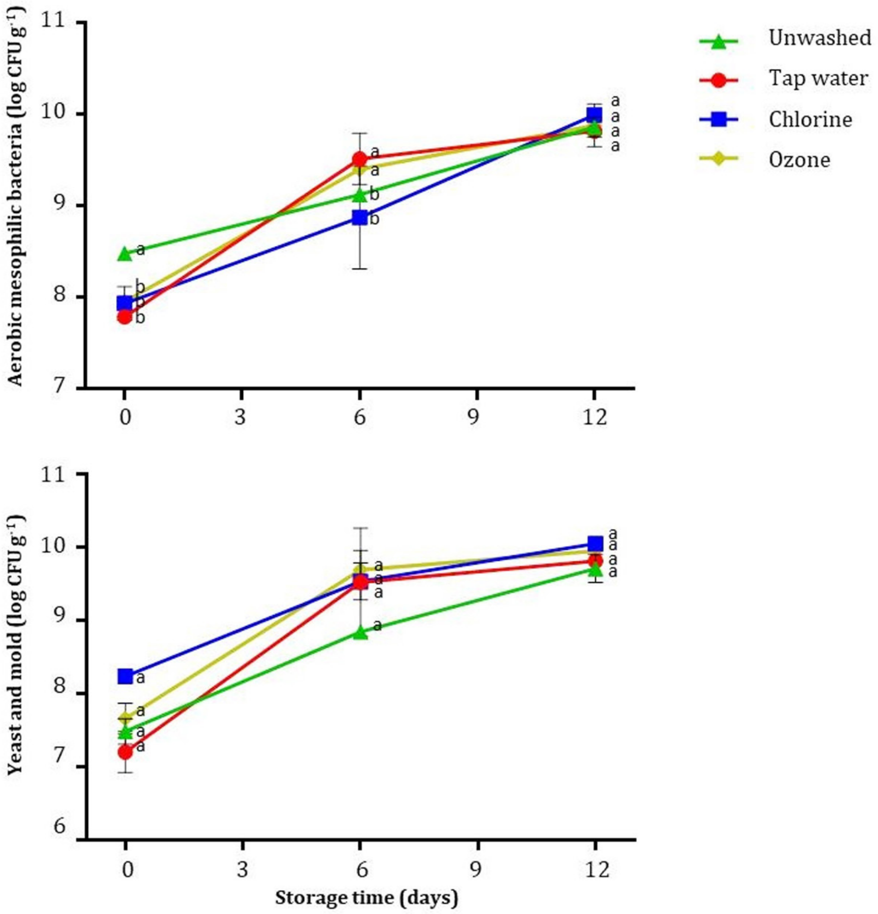
Figura 4. Imagen de micro-hortalizas de rabanito sometidas a diferentes tratamientos de lavados almacenadas a 8°C durante 12 días.

Chroma values decreased significantly in all samples as storage time progressed, from an average of 4.3 to 1.8. Chroma was the most affected color parameter by time, with a reduction of over 50%.

Total Aerobic Mesophilic Bacterial Count (AMB)

Aerobic mesophilic bacterial populations increased significantly over time for all sanitization treatments (table 1, page 202). However, a significant interaction between treatment and storage time was observed (figure 5).

The unwashed control showed an initial bacterial load of 8.49 log CFU g^{-1}. All washing treatments equally reduced aerobic mesophilic bacterial counts by approximately 0.6 log CFU g^{-1}. After 6 days of storage, all samples showed an increase in aerobic mesophilic bacteria counts, with the unwashed control exhibiting the slowest growth rate (0.64 log CFU g^{-1}). By day 12, bacterial counts increased significantly across all treatments. Treatments no longer differed significantly from the unwashed control, however, the unwashed and chlorine treatments showed higher growth rates.



Vertical bars represent ± standard error. Significant differences between wash treatments (within the same time point) according to DGC Test ($p \le 0.05$) are indicated with different lowercase letters above the plots.

Las barras verticales representan ± error estándar. Las diferencias significativas entre los tratamientos de lavado (dentro del mismo día de muestreo) según la Prueba DGC ($p \le 0,05$) se indican con letras minúsculas diferentes sobre los gráficos.

Figure 5. Effect of the washing treatments on aerobic mesophilic bacteria and yeast & mold populations of radish microgreen during 8°C storage (n=3).

Figura 5. Efecto de los tratamientos de lavado sobre los recuentos de bacterias aerobias mesófilas y los mohos y levaduras en micro-hortalizas de rabanito durante el almacenamiento a 8°C (n=3).

Total Yeast and Mold Count (Y&M)

Yeast and mold counts were significantly affected by both storage time and washing treatment, although no significant interaction between treatment and time was observed (table 1, page 202).

Regardless of the washing treatment, yeast and mold counts increased significantly during storage. Initial counts were approximately 7.65 log CFU g^{-1}, rising to 9.89 log CFU g^{-1} by the end of storage (figure 5, page 206).

Regarding the effect of washing treatment, tap water and unwashed control samples exhibited lower counts (8.76 log CFU g^{-1}) than chlorine and ozonated water treatments (9.19 log CFU g^{-1}).

Discussion

This study evaluated the effects of different washing treatments on quality and shelf life of radish microgreens during refrigerated storage.

Regarding weight loss, washing treatments showed no significant differences in weight loss until later stages of storage, consistent with findings from other microgreens storage studies (28). After day 8, ozone and unwashed control treatments differed significantly from chlorine and tap water in weight loss. However, the recorded values were low and practically negligible for this parameter. Maximum mean weight loss observed at the end of storage on day 12 was 0.97%. Although these values are lower than those reported in other studies (10), similar results were found in daikon radish microgreens stored in the dark (26). Our experiment was conducted in a domestic refrigerator that remains dark when closed; thus, the results suggest that dark storage may contribute to reducing weight loss. By keeping the stomata closed, transpiration-induced weight loss may be reduced. Therefore, it can be concluded that the postharvest treatments evaluated in our study performed acceptably with respect to weight loss.

Electrolyte leakage is a key indicator of cell membrane damage and subsequent tissue deterioration, which can result from physiological stress or mechanical injury. It is closely associated with postharvest shelf life, as it reflects the extent of senescence in fresh-cut vegetables (10, 13, 15). In radish microgreens, washing treatments significantly increased electrolyte leakage over time. Furthermore, our results are consistent with several studies reporting a sharp increase in electrolyte loss after approximately 6 to 8 days of storage (10, 13, 17). The lower electrolyte leakage observed in the unwashed control compared to the washing treatments aligns with findings in the literature for microgreens (13). The high moisture content in the packages due to washing likely promoted microbial growth, which in turn contributed to tissue damage and increased electrolyte leakage. Among the washing treatments, ozonated water resulted in the lowest electrolyte leakage compared to chlorine and tap water, which agrees with similar studies in lettuce (25). Electrolyte leakage occurs when the integrity of the cell membrane is compromised, often due to oxidation of the phospholipids and unsaturated fatty acids that constitute the membrane. Both chlorine and ozone are oxidizing agents, with ozone being the stronger oxidizer of the two (24). Despite its higher oxidizing potential, ozone at 0.16 ppm caused less tissue damage than chlorine at 100 ppm. The lower ozone concentration may explain the reduced wilting compared to standard chlorine disinfection..

Sample visual appearance was analyzed by measuring surface color during storage, serving as an indicator of senescence progression. The washing treatments showed no effect on hue angle or chromaticity, aligning with previous studies indicating that disinfectants typically do not alter color (6, 7, 25). In this regard, it is crucial that treatments applied to extend the shelf life of vegetables do not negatively affect their visual quality.

The lightness (L*) values observed were consistent with those reported in other studies on radish microgreens (16). The decrease in L* over time, reflected by tissue darkening, was likely caused by browning (6).

Hue angle values were observed within the 90° to 180° quadrant, indicating yellow to green colors. The h° increases on day 12 in chlorine- and ozone-washed microgreens indicate a shift toward a green-blue hue with reduced yellow. Yellowing due to chlorophyll degradation is a common phenomenon during storage (6, 26). The observed shift from green to blue in our study may be attributed to an incipient browning process. Browning is generally caused by the oxidation of phenolic compounds, leading to the formation of brown pigments such as melanin (10). Given that both chlorine and ozone are oxidizing agents (25), the color change observed may signal the onset of oxidation.

Chroma (C*), which reflects the saturation or intensity of color (16, 26), decreased significantly over time, suggesting a loss of color intensity during postharvest storage. Similar effects have been reported in microgreens of other species, where a general reduction in chromaticity during storage is associated with browning (10).

Regarding the populations of aerobic mesophilic bacteria, differences in the effect of washing treatments were observed depending on storage time. The initial population in the unwashed control was high, typical of leafy vegetables, and slightly above levels reported for other microgreen species (6, 13). Nonetheless, all washing treatments were effective in reducing the bacterial load. Notably, ozonated water at 0.16 ppm was as effective in reducing bacterial counts as chlorine. This result is consistent with studies demonstrating the efficacy of ozonated water on other vegetables, such as fresh-cut celery, cilantro, and broccoli (7, 24, 30). The bacterial rebound after 6 days in washed samples, matching or exceeding unwashed control, aligns with findings from other microgreen studies (27). Washing treatments may promote microbial growth due to residual moisture and tissue damage from postharvest handling (2, 13, 14, 23, 27). Therefore, if any washing treatment were to be applied for ready-to-eat microgreens, it would be advisable to consume them before 6 days of storage. This recommendation aligns with current safety standards for fresh-cut salads, which suggest a shelf life of 5 to 7 days (22).

The initial yeast and mold counts were comparable to those reported in other microgreen species (14, 27, 28). In contrast to the findings for aerobic mesophilic bacteria (AMB), none of the washing treatments reduced the initial Y&M populations compared to the unwashed control. Specifically, chlorine disinfection has been reported to exhibit intermediate sensitivity to yeasts and strong resistance to mold spores. Additionally, bacteria are generally more sensitive to ozone than yeasts and fungi (8). Lower counts in the unwashed control suggest that soaking microgreens, even with sanitizers, may be ineffective against fungal growth. These results are consistent with several studies indicating that washing treatments for microgreens can hinder effective decontamination. Such treatments can compromise product quality and potentially lead to microbial growth rebounds, that exceed those observed in unwashed samples (2, 13, 14, 23, 27).

Conclusions

This study evaluated the effect of aqueous ozone disinfection on the postharvest quality and shelf life of radish microgreens. The results demonstrate that ozonated water at 0.16 ppm was effective in preserving the microgreens during storage at 8°C. The initial load of aerobic mesophilic microorganisms was reduced without altering weight, color, or causing substantial wilting compared to other treatments. Based on these findings, ozone treatment is proposed as a viable alternative for the postharvest preservation of ready-to-eat radish microgreens. In this sense, it would be interesting to further evaluate the effects of different ozone concentrations to determine the optimal dose.

However, given that microgreens have low tolerance to washing processes, it is essential to develop dry disinfection technologies to extend their shelf life. In light of this, further research is needed to explore the potential of gaseous ozone as an alternative disinfection method for microgreens.

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