

# SAFETY OF READY-TO-EAT WATERCRESS USING ENVIRONMENTALLY FRIENDLY SANITIZATION METHODS

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## ABSTRACT

Chlorine-based washing systems have been widely used by the fresh-cut industry. However, there is much interest in developing safer and more environmentally friendly sanitization methods. Watercress was immersed in sodium hypochlorite (100 mg/L), hydrogen peroxide (167 mg/L) or citric acid (10 g/L) or exposed to ultraviolet C (UV-C) light (6 or 18 kJ/m<sup>2</sup> by exposure for 3 or 7 min, respectively), and stored in modified atmosphere packaging at 5C and 95% relative humidity. The respiration rate, packaging gas composition, color, microbiological growth (psychrotrophic and mesophilic microorganisms, Enterobacteriaceae, mold and yeasts), antioxidant capability, polyphenol content and sensory quality were measured. The different treatments did not affect the respiration rate (average 22 mg CO<sub>2</sub>/kg·h) after 14 days. Psychrotrophic bacteria reached approximately 7 log<sub>10</sub> cfu/g, regardless of the treatment, after 14 days. UV-C decreased mesophilic counts, while H<sub>2</sub>O<sub>2</sub> reduced Enterobacteriaceae. UV-C exposure increased the antioxidant capability, which was maintained throughout the storage. None of the treatments affected the color parameters and sensory quality. All of the treatments analyzed, especially UV-C, may be useful for maintaining watercress quality.

## PRACTICAL APPLICATIONS

Increasing popularity of nontraditional vegetables has created a new opportunity for innovation in the development of horticultural minimally processed fresh products. This industry is interested in developing safer and more environmentally friendly sanitization methods to replace chlorine-based washing systems. This study focused on the assessment of immersion in three chemical-sanitizer solutions (sodium hypochlorite, hydrogen peroxide and citric acid) or the exposure to ultraviolet C (UV-C) light in combination with modified atmosphere packaging and refrigerated storage (5C and 95% relative humidity). The evaluated disinfection methods resulted as or more effective at reducing microbial growth than chlorine washing. The UV-C treatments improved the functional quality of watercress by increasing the total antioxidant capability and total polyphenol content. The implementation of low cost sanitization methods such as the tested alternative sanitizers or UV-C light may be useful for maintaining the overall quality and extending the shelf life of ready-to-eat watercress and other vegetables.

## INTRODUCTION

Changes in dietary habits, which are linked to growing concern for the incidence of cardiovascular diseases and cancer, have led to an increase in the attractiveness of individual or mixed fresh-cut leafy vegetables to consumers looking for healthy and convenient meals. This is the case for several species of the Brassicaceae family, such as watercress (*Nasturtium officinale*), which have become popular in the past few years because they deliver high concentrations of health-promoting, bioactive phytochemicals (Martínez-Sánchez *et al.* 2008). Watercress originated in Europe, where it is normally sold and consumed in fresh vegetable salads, soups and a variety of other recipes (Gill *et al.* 2007).

Many outbreaks of gastroenteritis have been associated with the consumption of raw vegetables and fruits (Franz and van Bruggen 2008). Plants can become contaminated with enteric pathogens both externally and internally. Semenov *et al.* (2010) showed that watercress was very susceptible to colonization by *Escherichia coli* O157:H7 and *Salmonella enterica*. These pathogens grew prolifically on/in the shoots and less so on/in the roots. Therefore, in fresh-cut processing, the reduction of pathogenic microorganisms is an important concern to ensure consumer food safety.

Chlorine-based washing systems have been widely used by the majority of fresh-produce manufacturers to reduce the microbial contamination of fresh-cut vegetables with good efficiency (Sapers *et al.* 2001). However, there is much interest in developing safer and more effective sanitizers for fruits and vegetables. Several alternative disinfectants (including hydrogen peroxide, organic acids and ozone) have been tested to reduce bacterial populations in vegetables (Allende *et al.* 2006; Silveira *et al.* 2008; López-Gálvez *et al.* 2009). Hydrogen peroxide ( $H_2O_2$ ) is considered environmentally friendly because its sole reaction products are water and oxygen (Koivunen and Heinonen-Tanski 2005) and  $H_2O_2$  has been shown to reduce native and pathogenic microorganisms on whole produce (Artés *et al.* 2007; Sapers *et al.* 2008; Silveira *et al.* 2008).

In addition, there are several physical methods that could be used for food decontamination. One method is ultraviolet C (UV-C) radiation in a wavelength range that varies between 200 and 280 nm, which is considered lethal to most types of microorganisms because UV-C affects DNA replication (Bintsis *et al.* 2000; Char *et al.* 2010). UV-C irradiation has been proven to reduce respiration rates and microorganism development and to delay senescence and ripening in different whole and fresh-cut fruits and vegetables, such as lettuce, baby spinach, watermelon, melon, grape berries and mushrooms (Lamikanra *et al.* 2005; Allende *et al.* 2008; Artés-Hernández *et al.* 2010; Escalona *et al.* 2010; Jiang *et al.* 2010; Fava *et al.* 2011; Manzocco *et al.* 2011). Furthermore, UV-C has also been shown to elicit a

range of biochemical responses in fresh produce ranging from induction of antifungal enzymes to the formation of phytoalexin compounds (Guan *et al.* 2012). However, little information is available on the response of watercress to postharvest application of UV-C and to the use of chemical alternatives to chlorine. Because of its functional value, the potential of watercress in the fresh-cut industry is huge, particularly if suitable disinfectant methods are found to reduce microbial growth and extend shelf life, which is the aim of this study.

## MATERIALS AND METHODS

### Plant Material and Growing Conditions

Watercress (*N. officinale*) was grown in a floating-tray system at the Más Vida S.A. Company located in Comuna Calera de Tango, Región Metropolitana, Chile. The work was carried out using winter watercress with a length of 0.20–0.30 m. The leaves were hand harvested 40–60 days after sowing, placed in bags and immediately transported in a portable cooler box to the Center of Postharvest Studies (CEPOC) of the Faculty of Agricultural Sciences of the University of Chile. The leaves were air pre-cooled to 5°C in darkness at 95% relative humidity. The next day, the leaves were minimally processed in a disinfected room at 8°C as described below.

### Sample Preparation, Treatments and Storage Conditions

Stems with characteristics that are unsuitable for consumption (lignified or spoiled stems) were cut off using sharp, disinfected knives. Prior to disinfection, the watercress was washed with tap water at 5°C for 1 min to remove foreign material. Then, the watercress leaves were subjected to one of the following disinfection treatments: 167 mg/L  $H_2O_2$  (Merck, Darmstadt, Germany), 10 g/L citric acid (Merck) or 100 mg/L NaOCl, adjusted to pH 6.0 with citric acid (Merck), which was used as the control treatment. These sanitizers were dissolved in 5 L of water per kilogram of product for 3 min.

UV-C doses were applied using an experimental radiation chamber (Model G-636, KBM Company, Santiago, Chile), which consisted of a metallic box (1.28 m × 0.56 m × 0.60 m) with six unfiltered germicidal emitting lamps ( $\lambda = 254$  nm) distributed at the top and bottom of the chamber and arranged to irradiate a central grid that would support the samples, which were located 0.21 m from the lamps. Different UV-C doses (6 and 18 kJ/m<sup>2</sup>) were applied by altering the exposure time (3 and 7 min, respectively). The fluence rate of the lamps at the level of the samples was 0.344 kJ/s·m<sup>2</sup> as measured with a Radiometer VLX 254 (Vilber Lourmat, France).

The watercress leaves (40 g) were packed in plastic bags (0.20 cm × 0.25 m) provided by the Cryovac Sealed Air Corporation (Duncan, SC). The film permeability at 20C was 3.080 mL O<sub>2</sub>/m<sup>2</sup>·d·atm and 9.240 mL CO<sub>2</sub>/m<sup>2</sup>·d·atm (data provided by the supplier). Five replicates of each treatment for each determination day (a total of 20 bags per treatment) were prepared and stored at 5C for a maximum of 14 days.

### Respiration Rate Analyses

The respiration rate was evaluated according to the methodology described by Char *et al.* (2012). Watercress (approximately 40 g) was placed in airtight glass containers (approximately 1,000 mL capacity) fitted with a lid containing a silicone septum through which gas samples (10 mL) were taken with a plastic syringe 3 h after hermetic sealing. Three replicates of each treatment were evaluated. The increase in the CO<sub>2</sub> content in the headspace was monitored using a gas chromatograph (Hewlett Packard 5890 Series II, Palo Alto, CA) equipped with a thermal conductivity detector (Hewlett Packard). The temperatures of the injector, oven and detector were 50, 50 and 200C, respectively. The carrier gas was helium (Indura, Santiago, Chile) at a pressure of 50 psi. As a standard, 10% CO<sub>2</sub> (Indura) was used. The respiratory rate was expressed in units of milligram of CO<sub>2</sub> per kilogram hour. Measurements were performed in triplicate on days 0 (2–4 h after processing), 1, 4, 7 and 14 after processing.

### Gas Composition within Packages

Changes in O<sub>2</sub> and CO<sub>2</sub> levels in the plastic bags were monitored throughout the shelf life with a portable handheld gas analyzer (model Check Point I, PBI-Dansensor, Ringsted, Denmark). Gas samples were taken from the sealed plastic bags through a silicone septum with a plastic syringe. Five replicates of each treatment were evaluated. The equipment was calibrated by taking samples of atmospheric air (0% CO<sub>2</sub> and 21% O<sub>2</sub>). The values were expressed as the percentage of O<sub>2</sub> and CO<sub>2</sub>.

### Color Measurement

The color of the watercress leaves was determined using a Minolta CR-300 photocolormeter (Konica Minolta, Chiyoda, Tokyo), with an aperture diameter of 8 mm. Three replicates of each treatment were evaluated; 10 leaves from each replicate were measured. The photocolormeter was previously calibrated with a white calibration plate ( $Y = 94.3$ ,  $x = 0.3142$ ,  $y = 0.3211$ , illuminant C and 0° observer) by measuring the  $L$ ,  $a^*$  and  $b^*$  parameters in the CIE scale and calculating the hue angle =  $\arctg\ b^*/a^*$  and chromaticity =  $[(a^*2 + b^*2)^{1/2}]$ .

### Microbial Analyses

Three randomized samples of 10 g each for each treatment were homogenized in 90 mL of sterile 0.1% peptone-buffered water (Merck) in a sterile stomacher bag with a Colworth Stomacher 400 (model Easy Mix, AES Chemunex, Bruz, France) for 1 min. Serial dilutions for each replicate (three samples) were prepared in 0.1% peptone solution as needed for plating three serial dilutions (a total of nine dishes per treatment). On days 0, 1, 7 and 14, psychrotrophic and mesophilic bacteria, molds, yeast and Enterobacteriaceae were quantified. The following media and incubation conditions were used for the enumeration of particular microbial groups: plate count agar (Merck) for psychrotrophic and mesophilic aerobic bacteria was incubated at 5 and 37C for 7 and 2 days, respectively; potato dextrose agar for yeast and mold was incubated at 22C for 2 and 7 days, respectively; and violet red bile dextrose (Merck) overlaid with the same medium and incubated at 37C for 1 day was used for Enterobacteriaceae. Microbial counts were expressed as log<sub>10</sub> cfu/g (colony-forming units per gram of sample). The microbial quality of the product was evaluated by following the Chilean microbial legislation for fruits and vegetables for fresh consumption. According to this legislation, the maximum microbial limit is 5.7 log<sub>10</sub> cfu/g for mesophilic bacteria and 4.7 log<sub>10</sub> cfu/g for Enterobacteriaceae.

### Antioxidant Capability

The antioxidant capability of watercress leaves was measured using the ferric reducing ability of plasma (FRAP) method (Benzie and Strain 1996). For the extraction, 1 g of watercress leaves frozen in liquid N<sub>2</sub> was used. Three replicates of each treatment were evaluated. The leaves were ground in a mortar to a fine powder. Then, 9 mL 50% ethanol was added until a uniform mixture was obtained, which was filtered with cheesecloth. Subsequently, the filtered extract was centrifuged at 20,000 × g for 45 min at 4C (Hermle Labortechnik GmbH, Wehingen, Germany) and filtered again using Whatman paper No. 2. A 20 μL aliquot of the extract was mixed with 900 μL FRAP reactant and 80 μL Milli-Q water. Quantification was performed in a UV-Vis spectrophotometer (model T70, PG Instruments Ltd., Leicestershire, U.K.) at  $\lambda = 593$  nm. A calibration curve was obtained using Trolox as a standard. The results were expressed in units of milligram equivalents of Trolox per gram of fresh weight.

### Total Polyphenol Content

The total phenolic content was determined with the Folin-Ciocalteu reagent and the method previously described

(Singleton and Rossi 1965). For the extraction, 1 g watercress leaves was ground in a mortar with 9 mL 50% methanol. The extract was centrifuged at  $10,000 \times g$  for 30 min at 4°C. The supernatant was decanted and filtered using Whatman paper No. 2. A 500  $\mu\text{L}$  aliquot of the extract was mixed with 5 mL (1:100 volumen) of Na-K tartrate solution (2.7% w/v) plus sodium carbonate (2% w/v) in NaOH (1 N) solution and incubated for 15 min at 20°C. After the incubation, 1 mL of Folin-Ciocalteu reagent (1:1 v/v, diluted with Milli-Q water) was added, and the sample was incubated 1 h at 20°C in darkness. Three replicates of each treatment were evaluated. The total polyphenols were measured by absorption at  $\lambda = 765 \text{ nm}$  using the spectrophotometer described earlier. The total phenolic content was expressed in units of milligram of gallic acid equivalents per gram of fresh weight.

### Sensory Evaluation

For the sensory evaluation, the descriptive quantitative analysis method was applied with a semitrained panel of 12 judges. A linear scale of 15 points was employed by the semitrained panel to evaluate appearance, color and turgor. Subsamples of three replicates of each treatment were evaluated on each determination day.

### Statistical Analysis

The experiment followed a completely randomized design ( $n = 3$ ). Infostat Estudiantil, version 2011 (Universidad de Córdoba, Córdoba, Argentina), was used for the analysis of variance and least significant difference tests ( $P \leq 0.05$ ) to compare means.

## RESULTS AND DISCUSSION

### Respiration Rate

As a general trend, the respiration rates were higher on day 0 than during storage time (Fig. 1). On day 0, the watercress disinfected with  $\text{H}_2\text{O}_2$  and citric acid exhibited the highest respiration rates (64 and 70  $\text{mg CO}_2/\text{kg}\cdot\text{h}$ , respectively). From that time forward, the respiration rate began to decline until the fourth day of storage, at which time the respiration did not significantly changed until the end of the storage period. During this period, no significant differences between the treatments were observed.

The effects of UV-C radiation on the metabolic behavior of the products are variable and depend on the product considered. In addition, UV-C-treated broccoli (doses from 4–14  $\text{kJ}/\text{m}^2$ ) had a reduced respiration rate and delayed tissue damage (Costa *et al.* 2006). Moreover, some researchers found an increase in the respiration rate of radiated

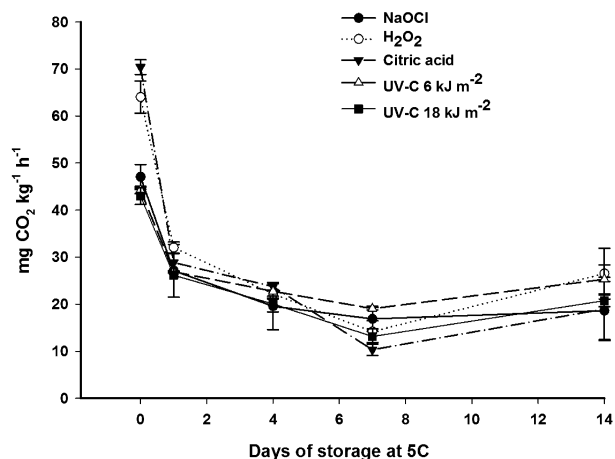


FIG. 1. RESPIRATION RATE OF FRESH-CUT WATERCRESS STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT 5°C DURING 14 DAYS

Vertical bars represent standard error of the means ( $n = 3$ ).

baby spinach and watermelon cubes (Lamikanra *et al.* 2002; Artés-Hernández *et al.* 2010; Escalona *et al.* 2010). According to Silveira *et al.* (2008), different chemical sanitizers, including  $\text{H}_2\text{O}_2$  (50 mg/L), did not affect the respiration rate of fresh-cut “Galia” melon stored for 10 days at 5°C.

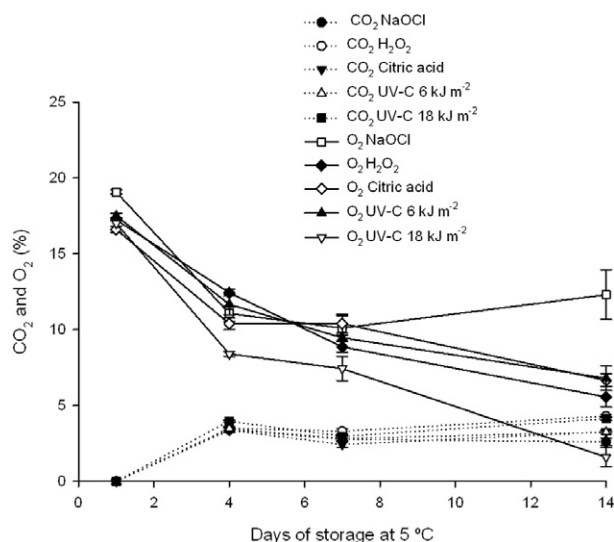
### Gas Composition within Packages

The plastic bag described above with 40 g of watercress decreased  $\text{O}_2$  content during storage and reached values of 4.27–10.3% after 14 days of storage (Fig. 2). As expected, the  $\text{CO}_2$  concentration increased during storage, reaching levels of approximately 2.6 and 4.13% after 14 days of storage. The increasing  $\text{CO}_2$  concentrations inside the bag at day 14 indicated that steady state had not yet been reached in the modified atmosphere packaging.

The changes in the  $\text{O}_2$  and  $\text{CO}_2$  concentrations inside the packages of watercress subjected to different disinfection treatments were similar during storage, indicating that the respiration rate was not influenced by the washing solution, as was shown previously for fresh-cut lettuce and arugula leaves (Martínez-Sánchez *et al.* 2008; López-Gálvez *et al.* 2009).

### Color Parameters

The watercress color parameters are shown in Fig. 3. The luminosity ( $L$ ) values decreased during storage; the values were approximately 51 on day 1 and fell to 49 on day 14. Only the watercress disinfected with  $\text{H}_2\text{O}_2$  differed statistically from the other treatments at all evaluation times, with values of 48 and 46 for day 1 and day 14, respectively, which were the lowest values among the treatments (Fig. 3A). No



**FIG. 2.** GAS COMPOSITION ( $O_2$  AND  $CO_2$ ) OF FRESH-CUT WATERCRESS STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT 5°C DURING 14 DAYS

Vertical bars represent standard error of the means ( $n = 3$ ).

significant differences in the hue angle were found between the treatments. This parameter was only affected by the time of storage and decreased during the experiment from 129 (day 1) to 118 (day 14) on average for all of the treatments (Fig. 3B). The chroma was not affected by the factors considered in this experiment (disinfection method and storage period) and exhibited initial values of approximately 18 and final values of approximately 34 (data not shown). As with other physicochemical parameters, the effect of UV-C radiation on the color of the plant products was fairly variable.

### Microbiological Growth

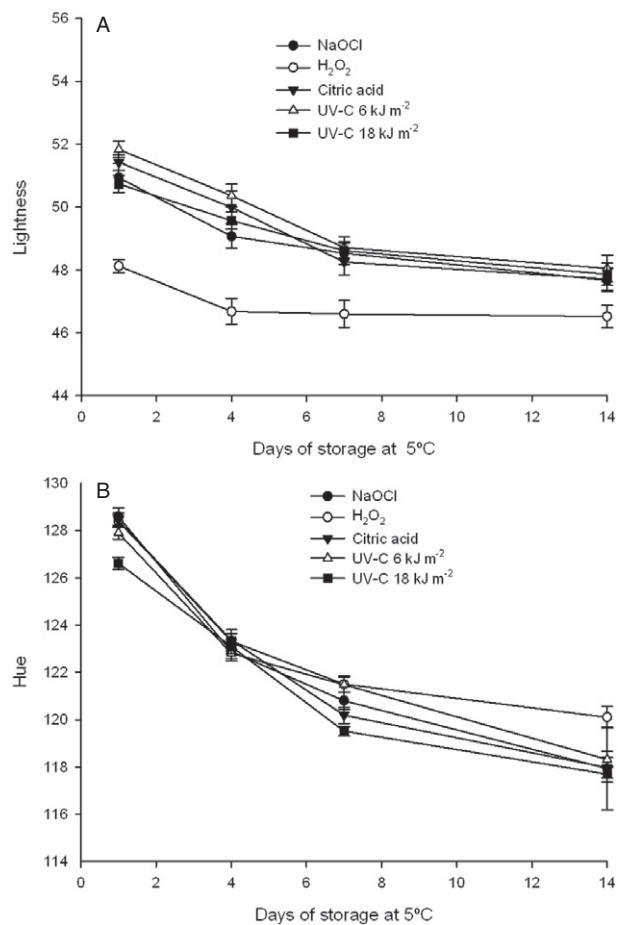
The disinfection methods reduced the psychrotrophic load by approximately 2 log units/g (Fig. 4A). UV-C radiation and  $H_2O_2$  were more effective than chlorine and citric acid. However, the initial differences between the disinfection methods were not maintained over time. After 10 days of storage at 5°C, the psychrotrophic load of the watercress was approximately 7 log units/g, with no difference between the treatments.

Mesophilic growth was affected by the disinfection methods, in contrast to the effect on psychrotrophic growth. The initial microbial load was reduced by approximately 1 log unit/g (Fig. 4B), and UV-C radiation and  $H_2O_2$  were again the most effective. The UV-C radiation effect was maintained until the end of the storage period, at which time a reduction of 1 log unit/g compared with NaOCl was

observed. No differences were observed between the UV-C radiation doses, and both were equally effective against mesophilic growth.

The applied disinfection methods yielded a significant reduction in Enterobacteriaceae with respect to the untreated raw material; in watercress washed with citric acid, the reduction was greater than 2.5 log units/g (Fig. 4C). Upon further storage, only  $H_2O_2$ -treated watercress differed statistically, with a reduction of approximately 1 log unit/g compared with the other treatments.

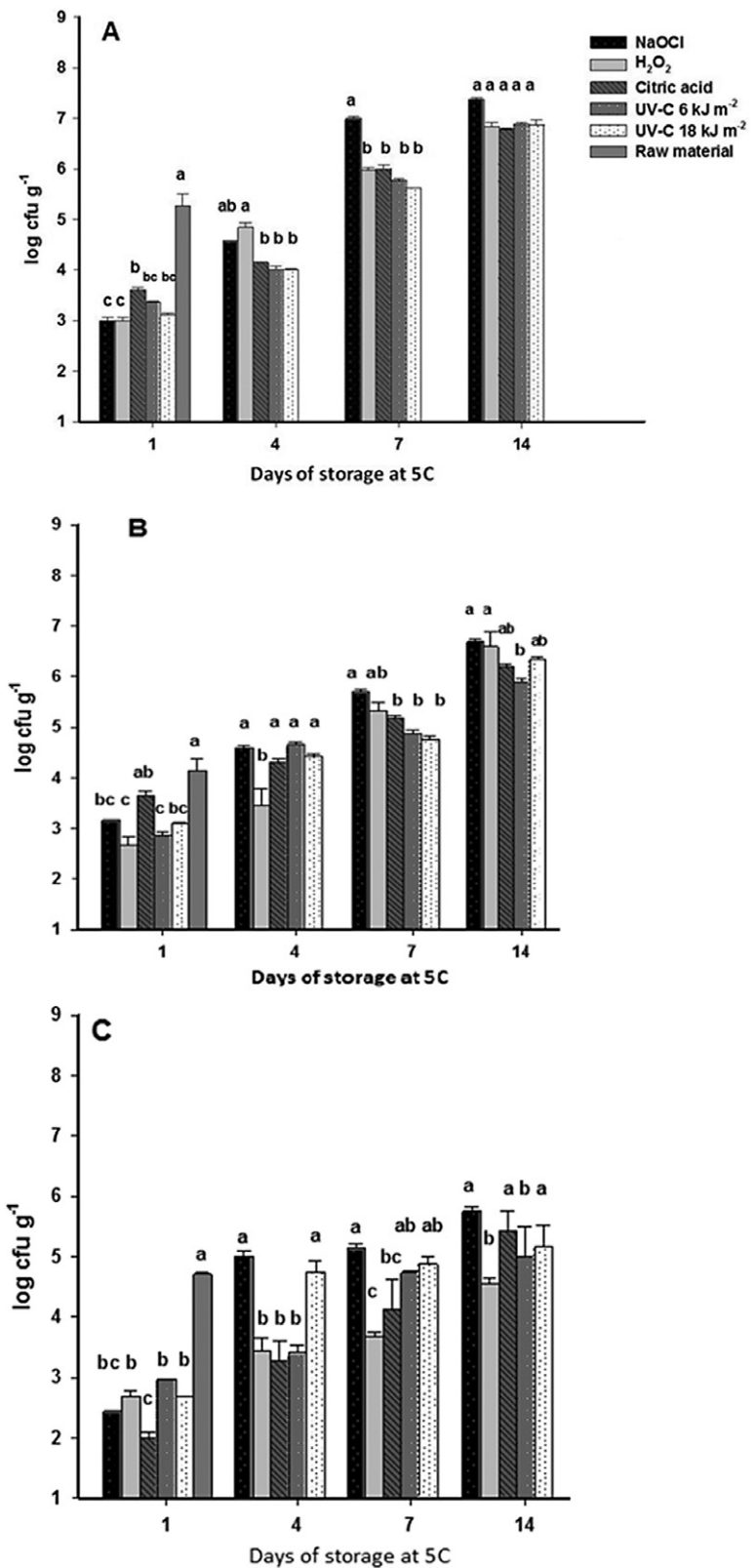
The different disinfection methods reduced the mold and yeast population on the watercress leaves from 2.1  $\log_{10}$  cfu/g (raw material) to a range of 2 to  $<1 \log_{10}$  cfu/g, the higher reduction corresponding to the citric acid treatment (data not shown). This reduction was maintained during the storage period, and after 14 days, the mold and yeast populations for all the treatments remained  $<2 \log_{10}$  cfu/g.



**FIG. 3.** COLOR PARAMETER OF FRESH-CUT WATERCRESS STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT 5°C DURING 14 DAYS. (A) LIGHTNESS ( $L^*$ ); (B) HUE

Vertical bars represent standard error of the means ( $n = 30$ ).





These results confirm the decontamination efficiency of UV-C treatments and H<sub>2</sub>O<sub>2</sub> as reported in other studies, indicating the ability of these treatments to promote higher microbial stability during storage, thereby leading to a longer shelf life.

UV-C radiation was used to decontaminate fresh-cut melon cubes immediately after processing, and this treatment affected the growth of microbial populations during subsequent storage, leading to a 2 log reduction in both the total viable count and Enterobacteriaceae, the counts of which remained 2 log units lower than that of the untreated sample during 14 days of storage at 6C (Manzocco *et al.* 2011).

Interestingly, the decontamination effect did not improve when the intensity of the UV-C treatment was increased. Similar results were also observed by Fonseca and Rushing (2006), who detected no additional decrease in the microbial populations of UV-C-treated watermelon cubes when the fluence was increased from 1.4 to 6.9 kJ/m<sup>2</sup> or in UV-C treated fresh-cut spinach when the fluence was increased from 12 to 24 kJ/m<sup>2</sup>. In addition, natural and inoculated microorganisms were reduced by UV-C treatment of fresh-cut spinach (Escalona *et al.* 2010).

Guan *et al.* (2012) showed that UV-C doses of 0.45–3.15 kJ/m<sup>2</sup> resulted in reductions of 0.67–1.13 log<sub>10</sub> cfu/g of *E. coli* O157:H7 inoculated on mushroom-cap surfaces. UV-C radiation also reduced total aerobic plate counts by 0.6–0.9 log<sub>10</sub> cfu/g on the surface of mushrooms. The UV-C effect could be attributed to direct elimination by DNA denaturation, as reported for melons (Lamikanra *et al.* 2005).

H<sub>2</sub>O<sub>2</sub> (50 mg/L) has been shown to be more effective than chlorine against microbial growth, with a reduction of approximately 1 log unit/g of the microbial growth on fresh-cut “Galia” melon (Silveira *et al.* 2008). In the cited study, the author noted that the use of a washing solution of H<sub>2</sub>O<sub>2</sub> (5%) was highly effective in reducing the microbial load of apples and melons prior to cutting, with reductions of 3 log<sub>10</sub> cfu/g, especially when the treatment was combined with vigorous stirring at 50C (Sapers *et al.* 2008).

More recently, it has been reported that citric acid (2%) and H<sub>2</sub>O<sub>2</sub> (2%) were associated with reductions of 1 and 1.5 log units/g in fresh-cut processed baby spinach contaminated with *E. coli* O157:H7 (Huang and Chen 2011).

The inhibitory activities of organic acids and H<sub>2</sub>O<sub>2</sub> have been proposed to be pH dependent (Ruiz *et al.* 2010). Acids have optimal inhibitory activity at low pH because low pH favors the uncharged, undissociated state of the molecule, which is responsible for the bactericidal activity (Huang and Chen 2011). Enteric pathogens such as *E. coli* O157:H7 and *S. enterica* grow better under reduced oxygen conditions in the presence of bacterial competitors. What is more, enteric pathogens, apparently, compete better with other microbes under anaerobic or semi-anaerobic conditions (Semenov

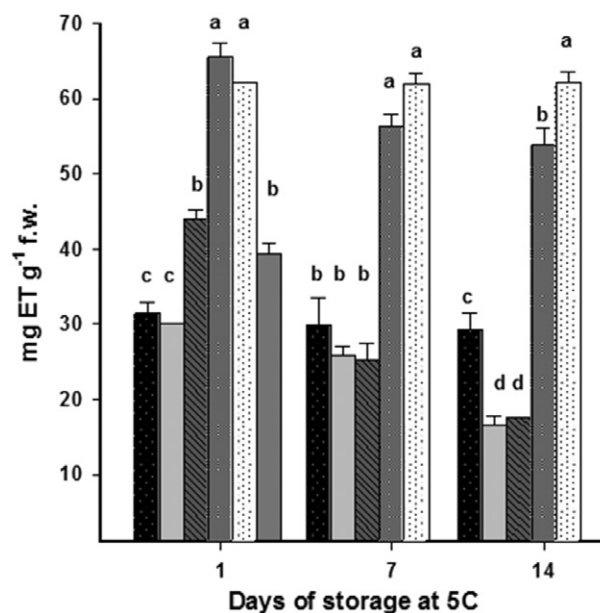
*et al.* 2011). In the present experiments, the suppression of the natural microbial community with the applied treatments followed by storage at CO<sub>2</sub> levels between 4 and 5% and reduced O<sub>2</sub> (5–12%) may allow some enteric pathogens to outcompete other residual bacteria. Therefore, the use of low temperatures during processing, distribution and storage is essential to minimize microbiological risks, as well as to increase the overall quality of the ready-to-eat products.

## Antioxidant Capability

As shown in Fig. 5, UV-C irradiation at 6 and 18 kJ/m<sup>2</sup> resulted in 70 and 79% increases in the antioxidant capability of the raw material. Although the total antioxidant capability decreased during the storage period, UV-C-treated watercress continued to exhibit the highest antioxidant capability.

The most significant differences among the treatments were observed after 14 days of storage. At that time, the irradiated watercress showed a twofold increase in antioxidant capability compared with chlorine washing and a threefold increase compared with H<sub>2</sub>O<sub>2</sub> and citric acid disinfection.

These findings are in agreement with those of Ruiz *et al.* (2010), who also observed a higher antioxidant capability in fresh-cut cabbage exposed to 0.06 and 0.12 kJ/m<sup>2</sup> UV-C doses and storage for 9 days at 6C. In addition, Jiang *et al.*



**FIG. 5.** ANTIOXIDANT ACTIVITY (MG/100 G) OF FRESH-CUT WATERCRESS STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT 5C DURING 14 DAYS

Vertical bars represent standard error of the means ( $n=3$ ). Small letters: values followed by the same small letter are not significantly different ( $P \leq 0.05$ ).

(2010) reported higher total flavonoids and ascorbic acid, which act as antioxidants in plants, and a delayed increase in superoxide anions ( $O_2^-$ ) in shiitake mushrooms exposed to UV-C radiation ( $4 \text{ kJ/m}^2$ ) and stored in modified atmosphere packaging for 15 days at  $1\text{C}$ .

UV-C radiation can affect the antioxidant activity of a variety of vegetables during postharvest storage, such as fresh-cut broccoli (Lemoine *et al.* 2010).

Broccoli florets treated with  $8 \text{ kJ/m}^2$  and hot air ( $48\text{C}$  for 3 h) showed a significant increase of approximately 13% in the antioxidant capability immediately after application of the combined treatment. Although the antioxidant capability decreased in both the control and treated samples, the treated sample retained significantly higher antioxidant capability (ranged from 12 to 50%) than the control during 7 days of storage. These authors argue that this phenomenon could be due to the effect of UV-C on vegetable metabolism. UV-C stress produces damage in plant tissues by inducing oxidative stress that can lead to lipid peroxidation, protein denaturation, carbohydrate oxidation and DNA damage. Furthermore, plants respond to this stress by activating defense mechanisms by inducing enzymes that play a role in scavenging reactive oxygen species (ROS) as well as key enzymes related to the biosynthesis of antioxidant compounds, such as flavonoids (Lemoine *et al.* 2010).

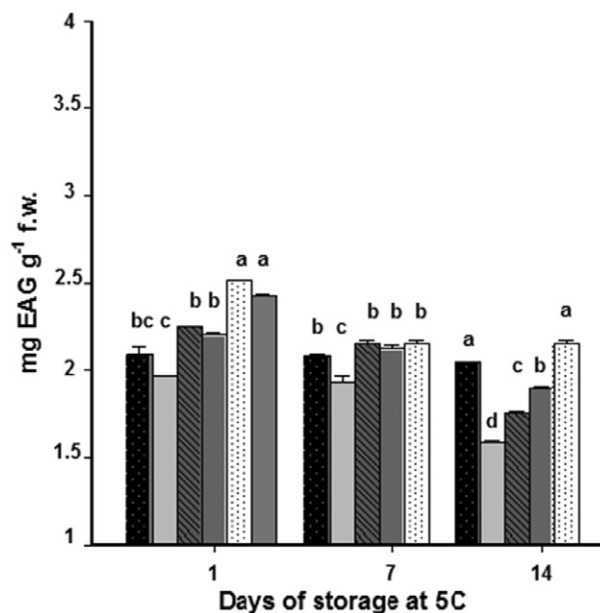
In line with the effects of the other disinfection methods evaluated in this work, Martínez-Sánchez *et al.* (2006) showed that lactic acid ( $20 \text{ mL/L}$ ) markedly reduced vitamin C and glucosinolate content.

The negative effect of  $\text{H}_2\text{O}_2$  on antioxidant compounds was associated with the above effect on vitamin C and glucosinolate content by Zhang *et al.* (2011). These authors mentioned that the exogenous application of  $\text{H}_2\text{O}_2$  ( $51 \text{ mg/L}$  for 12 h) caused moderate stress and thereby induced ROS ( $O_2^-$  and  $\text{H}_2\text{O}_2$ ) and lipid peroxidation in cucumber leaves.

### Total Polyphenol Content

The effect of the different treatments on polyphenol content is shown in Fig. 6. The treatments, with the exception of UV-C, reduced the polyphenol content by approximately 7.4% (citric acid treatment) and 19% ( $\text{H}_2\text{O}_2$  treatment) relative to the raw material. UV-C-treatment preserved the total polyphenol content of the watercress, and the content did not differ statistically from the raw material. The polyphenol content decreased throughout storage. At the end of the storage period, only watercress exposed to the higher UV-C dose differed statistically and had the highest polyphenol content.

The trend in the total polyphenol content is similar to that observed for the antioxidant capability. The effects of UV-C on phenolic content have not been completely eluci-



**FIG. 6.** TOTAL POLYPHENOLS CONTENT (MG/100 G) OF FRESH-CUT WATERCRESS STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT  $5\text{C}$  DURING 14 DAYS

Vertical bars represent standard error of the means ( $n=3$ ). Small letters: values followed by the same small letter are not significantly different ( $P \leq 0.05$ ).

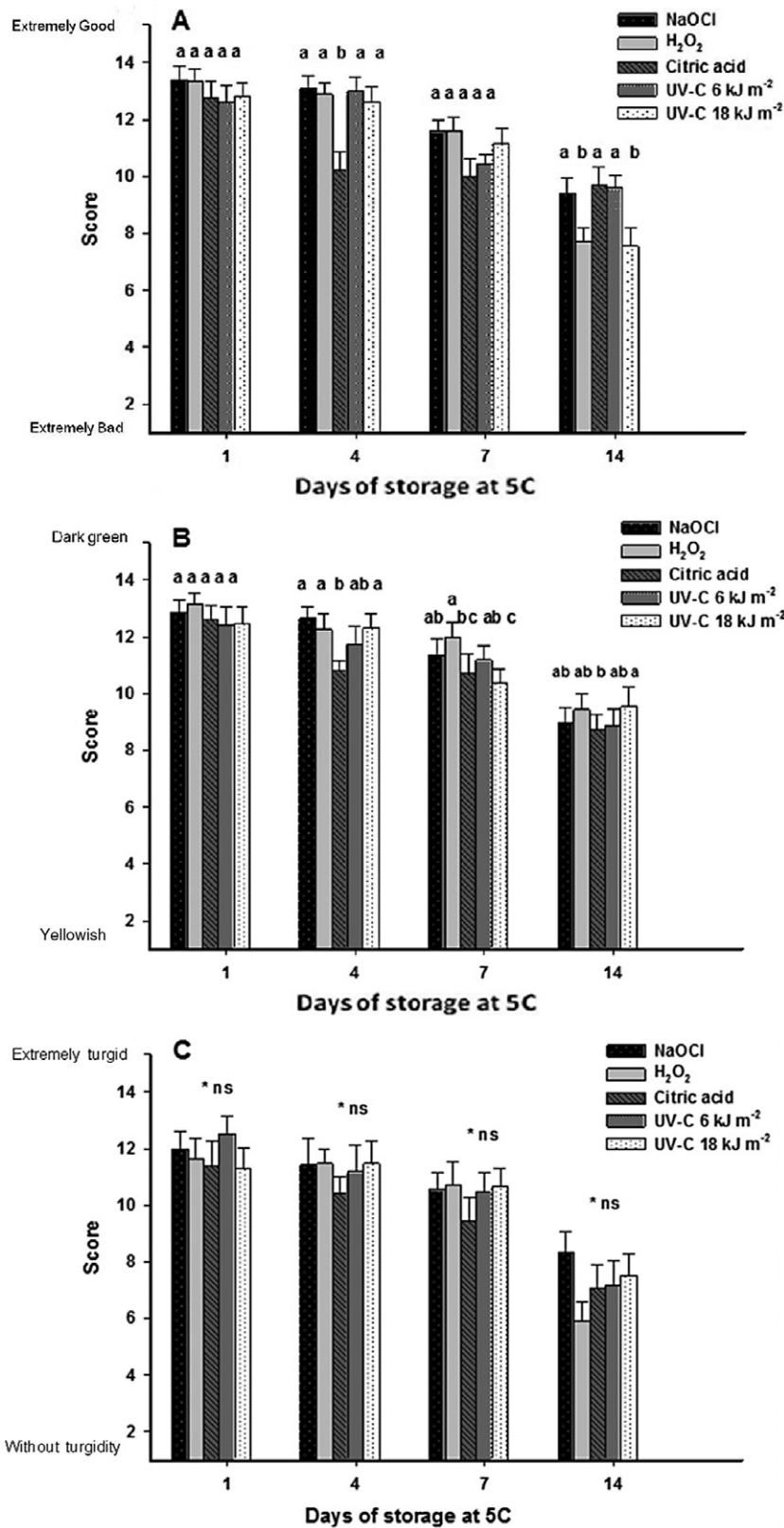
dated. More research is needed in this regard, and the results vary depending on the product investigated and the UV-C dose considered. In this respect, Erkan *et al.* (2008) reported an increase in the phenolic content of strawberry fruit exposed to doses of  $0.43$ – $4.3 \text{ kJ/m}^2$  and stored for 15 days at  $10\text{C}$ . By contrast, Artés-Hernández *et al.* (2010) did not observe an effect of UV-C ( $1.6$ ,  $2.8$ ,  $4.8$  and  $7.2 \text{ kJ/m}^2$ ) on fresh-cut watermelon that was stored for 11 days at  $5\text{C}$ . In a more recent study, the polyphenol contents of button mushrooms treated with UV-C doses of  $0.22$ ,  $0.45$  and  $0.90 \text{ kJ/m}^2$  were similar after 14 days of storage at  $4\text{C}$  (Guan *et al.* 2012).

Previous studies have generally concluded that chemical sanitizers do not affect polyphenol content. Martínez-Sánchez *et al.* (2006) reported that the flavonoid content of arugula leaves was not affected by washing solutions at the time of processing and remained almost constant throughout storage in air. However, glucosinolates were the most affected constituents of rocket leaves; the glucosinolate content decreased 4–33% when samples were stored in air at  $4\text{C}$ , while the decrease was between 60 and 100% in low  $\text{O}_2$  ( $6 \text{ kPa}$ ) + high  $\text{CO}_2$  ( $8 \text{ kPa}$ ) at  $4\text{C}$ .

### Sensory Evaluation

The average scores for the appearance of fresh-cut watercress are presented in Fig. 7A. The disinfection methods did





**FIG. 7.** SENSORY EVALUATION (SCALE 1–15) OF FRESH-CUT WATERCRESS STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT 5C DURING 14 DAYS. (A) APPEARANCE; (B) COLOR; AND (C) TURGOR. Vertical bars indicate the standard error of the means ( $n = 12$ ). Small letters: values followed by the same small letter are not significantly different ( $P \leq 0.05$ ); ns: no significant differences among treatments.

not influence the appearance on day 1, with a score greater than 13. The most significant differences ( $P \leq 0.05$ ) were recorded after 14 days of storage, at which time the lowest values corresponded to watercress disinfected with  $H_2O_2$  (7.5) or the higher UV-C dose (7.2). During storage, the color of fresh-cut watercress degraded slightly, although at the end of the storage period, all of the scores indicated good sensory qualities without significant differences among the disinfection methods (Fig. 7B).

Turgor was not affected by the disinfection methods because no differences were observed among the treatments (Fig. 7C). This parameter decreased from a score 11 to less than score 8 during storage (14 days). Different effects of UV-C radiation on sensory attributes have been described by other researchers. UV-C doses improved the quality of “Red Oak Leaf” lettuce by delaying senescence (Allende *et al.* 2008). According to the literature, the chemical disinfectants evaluated here have different effects on the sensory qualities of products. Lactic acid (20 mL/L) was particularly detrimental to the sensory quality of rocket leaves, while  $H_2O_2$  (300 mL/L) did not affect this parameter (Martínez-Sánchez *et al.* 2006).

## CONCLUSIONS

The evaluated disinfection methods are as or more effective at reducing microbial growth than chlorine washing and are less harmful to the environment. Compared with the other treatments, UV-C irradiation improved the functional quality of watercress by increasing the total antioxidant capability and total polyphenol content. Although citric acid and  $H_2O_2$  were effective in delaying microbial growth, these treatments affected the color of the watercress. However, the sensory qualities of the watercress remained with acceptable scores for all the treatments during 14 days of storage. These results suggest that the studied treatments, especially UV-C, may be useful methods for maintaining watercress quality and extending shelf life.

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