EFFECT OF THE COMBINED TREATMENT OF UV-C LIGHT AND MODIFIED ATMOSPHERE PACKAGING ON THE INACTIVATION OF *ESCHERICHIA COLI* INOCULATED WATERCRESS

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Received for Publication May 19, 2014
Accepted for Publication August 5, 2014
doi:10.1111/jfpp.12378

ABSTRACT

Minimally processed watercress is an alternative to increase vegetable consumption. However, at almost all stages from field production to processing the vegetables could be contaminated with pathogenic and spoilage bacteria. UV-C light can be effective in controlling bacterial growth, but limited information is available for watercress sanitation and storability. This study reports the effect of UV-C light (0–25 kJ/m²) on the quality of minimally processed watercress inoculated with a nonpathogenic *Escherichia coli* (ATCC 35218), and stored under active modified atmosphere (5% O₂–10% CO₂) during 12 days at 5°C. The application of UV-C treatments reduced *E. coli* counts between 2.6 and 4.4 log cfu/g. Moreover, the application of 15–25 kJ/m² increased the phenolic content and caused slight changes in the visual appearance and color. Therefore, high UV-C doses could be an effective tool to decrease bacterial growth and to extend shelf life of minimally processed watercress.

PRACTICAL APPLICATIONS

This technology represents an economic alternative compared to other sanitation techniques because of the low costs of the infrastructure and maintaining activities. There are no legal restrictions for UV-C application. The beneficial effects on fruit and vegetable quality make this technology an interesting alternative to extend the postharvest shelf life of minimally processed products. Nowadays, the food market is challenging an increasing demand for safer products elaborated under strict environmental and friendly standards. In this sense, the UV-C technology could be an alternative to chemical sanitation that is being questioned because of the formation of potentially toxic compounds. The successful application depends on the selection of doses that delay microbial growth without causing detrimental effects. Before commercial implementation, a systematic research on the effects of UV-C light in several species is required by evaluating and optimizing the effects of this technology on the overall quality of different vegetables.

INTRODUCTION

Fruit and vegetables are positioned in the market by their recognized functional properties providing substances such as vitamins, minerals, fiber, antioxidant compounds, glucosinolates and phytosterols (Slavin and Lloyd 2012).

The new demands for different food products, the changes in lifestyles and need to reduce the time to prepare healthy meals have led to increase minimally processed (MP) fruit and vegetable demand (Oms-Oliu et al. 2010). Fruits and vegetables processed by washing, cutting, rinsing and packaging are well known as MP products, which can
be eaten directly by the consumers. In general, these products keep a freshly appearance for 7 to 14 days under cold storage (Rojas-Graü et al. 2009). In order to reduce mechanical damage caused by minimal processing, cold temperatures and modified atmosphere packaging (MAP) techniques are used. These technologies may delay spoilage of the product caused by microorganism and elevated metabolic activity (Gil et al. 2006). Enzymes and substrates, separated in different cellular organelles inside the cells lose their compartmentalization after cutting allowing chemical reactions among enzymes and substrates. One of these processes is polyphenol oxidase (PPO)-mediated browning, which is exacerbated in processed commodities. These MP products are not free from contamination; for this reason, it is important to assure the product quality (Gil et al. 2009). Also, during cutting and processing, the product surface is exposed to air and possible contamination with bacteria, yeasts and molds. Improper handling during the industrial process of these products increases the chance of contamination with pathogens such as Escherichia coli, Listeria, Salmonella and Yersinia (Harris et al. 2003). Sodium hypochlorite (NaClO) is usually applied as sanitizing treatment to decrease microbial contamination on vegetables. However, it could react with organic matter resulting in chlorine gas, chloramines and trihalomethane formation with potential carcinogenicity effects (Artés et al. 2009).

There is great interest in the development of novel sanitation techniques, such as UV-C radiation. The sanitizing effect of UV-C radiation is attributed to the formation of pyrimidine dimers that distort the double helix of DNA by blocking replication process of bacteria, viruses and other pathogens. Also, the DNA helix composition could determine the microbial sensitivity, and the ability to repair the damaged DNA (Gayán et al. 2011). Sensitivity to UV-C radiation significantly varies within microorganisms being gram-negative more sensitive than gram-positive bacteria. The different sensitivity has been attributed in part to variation in cell envelopes, which would result in different penetration of the germicide radiation. Microorganisms may differ in their ability to repair damaged DNA. UV-C has been evaluated to delay senescence in commodities like tomato, lettuce and spinach (Baka et al. 1999). However, excessive UV-C doses on the products could alter the permeability of the plasma membrane by increasing the leakage of amino acids and carbohydrates and other small nutritive molecules that would favor microbial growth (Guerrero-Beltrán and Barbosa-Cánovas 2004). Treatments with UV-C light offer several advantages to the food industry. It leaves no residue, has no legal restrictions, it is lethal against a large number of microorganisms and has a low cost (Bintsis et al. 2000). Guan et al. (2012) showed that UV-C doses of 0.45–3.15 kJ/m² resulted in 0.67–1.13 log cfu/g reduction of E. coli O157:H7 on mushroom stored for 21 days at 4C. Erkan et al. (2001) observed that the UV-C light reduced microbial contamination and deterioration of sliced Italian squash (Cucurbita pepo L., cv. Tigress) during storage at 5 and 10C. Also a pretreatment of 2.27 kJ/m² reduced aerobic plate counts and Enterobacteriaceae in organic pepper strips for 12 days at 5C (Artés et al. 2006). In lettuce “Oak leaf” MP, it was found that doses of 1.18, 2.37 and 7.11 kJ/m² were effective at reducing the microorganisms causing deterioration after 10 days at 5C, although the dose 7.11 kJ/m² caused softening and browning of tissues after 7 days (Allende et al. 2006). Furthermore, treatments using UV-C light as an alternative technology would be able to reduce the ripening rate and activate natural defense responses to increase the shelf life of fruit and vegetables (Cuvì et al. 2011).

Watercress is demanded by the consumer because of typical mustard flavor and attractive fresh appearance (Gonçalves et al. 2009). Little technical information related to sanitation and storability of this vegetable is published. Therefore, the purpose of this study was to evaluate the UV-C light effect on quality parameters of MP watercress inoculated with E. coli.

**MATERIALS AND METHODS**

**Vegetal Material**

To carry out this research, hydroponic watercress (Nasturtium officinale R. Br.) leaves from a local commercial company, located in Calera de Tango, Región Metropolitana, Chile, was used as raw material. The culture system was a floating root hydroponic system. The stocking density was three to five seeds per cell and seedlings (along with the substrate) were transplanted into hydroponic system when they reached an average of 8 cm in height, after approximately 25 days. The period from transplanting to harvest was 30 days and the collection was made manually and with sharp scissors at late afternoon.

**Processing**

Once the raw material was received from crop field, it was stored in a dark chamber at 5C and 90% relative humidity for 12 h. Damaged leaves and lignified or spoiled stems were removed or cut off using sharp disinfected knives. All the vegetal material was washed with tap cold water, followed by a second washing with sodium hypochlorite (50 mg/L, 5C, pH 6.5, 1 min; Clorox, Santiago, Chile). Leaves were rinsed out with sterile water to remove any residual of sodium hypochlorite on the leaves. Finally, leaves were drained on
a stainless steel mesh for 3 min and 1 min of hand centrifugation to remove the excess of water.

**E. coli Inoculation**

*E. coli* inoculum (ATCC 35218, Manassas, VA) preserved at 80°C was suspended in a conical flask with 50 mL trypticase soy broth (Merck Chemicals, Darmstadt, Germany) with 0.6 g/mL yeast extract (Merck Chemicals). It was incubated at 37°C for 24 h with constant stirring until a final concentration of 9 to 10 log cfu/mL.

The leaves were inoculated with *E. coli* nonpathogenic strain by spraying culture in aseptic conditions. An adaptation period of 24 h under dark and cold temperature (5°C) conditions was allowed to settle down *E. coli* inoculums before treatment applications. The inoculation was performed reaching a concentration of about 8 to 9 log cfu per gram of leaves.

**UV-C Treatments**

Inoculated watercress leaves were subjected to six different doses of UV-C light (0 to 25 kJ/m²). The application was made at a station chamber 1.28 × 0.56 × 0.60 m (Model G-636, KBM Company, Santiago, Chile) equipped with type C ultraviolet lamps (TUV 36 W/G36 T8, Philips, Amsterdam, the Netherlands) arranged in two rows, with three lamps each one, at the top and the bottom of the chamber. The chamber had a central stainless steel mesh equidistant at the center (0.24 m) of the lamps, to support the samples, causing little interference with radiation coming from the bottom lamps set. The different UV-C doses were applied by modifying the exposure time. The emission rate of the lamps at the level of the samples was 0.344 kJ/s/m² and it was measured with a Radiometer VLX 254 (Vilber Lourmat, Torcy, France) at both sides of the steel mesh.

**Packaging and Active Modified Atmosphere**

After the application of different doses of UV-C light, 100 g of watercress were packed in polyethylene bags (San Jorge Packaging Company, Santiago, Chile), with dimensions of 0.25 × 0.2 m and permeability of 6.000 mL/m²/day for O₂ and 19.000 mL/m²/day for CO₂ at 23°C and 1 atm.

To provide an active modified atmosphere, each bag was injected with a gas concentration of about 5 of O₂ and 10% CO₂, filling the rest of package with N₂ (85%). Thereafter, the bags were then sealed (Impulse Sealer Tew Equipment Co., Taipei, Taiwan). Five replicates of each treatment for each evaluation day were prepared and stored at 5°C up to 12 days.

Gas evolution of O₂ and CO₂ inside the packages was determined by means of a portable gas analyzer (Checkpoint, GDP Dansensor, Rongsted, Denmark). All samples were analyzed to check that proper MAP conditions were achieved. Results were expressed as percentages of O₂ and CO₂.

**Color**

Leaf color was measured with a compact tri-stimulus colorimeter (Konica Minolta CR-300 Chroma Meter, Tokyo, Japan). The parameter values were registered as lightness (L*, where 0 = black, 100 = white), chroma (C* = [a*² + b*²]¹/²) and hue angle (H*ab = 180- tan⁻¹ b*/a*) (McGuire 1992). Color was measured on 10 leaves per package, on the adaxial side.

**Survival of Nonpathogenic *E. coli* Strain**

Samples of 10 g were homogenized in 90 mL of sterile 0.1% peptone buffered water (Merck Chemicals) in a sterile stomacher bag with a Colworth Stomacher 400 (model Easy Mix, AES Chemunex, Bruz, France) for 30 s. Serial dilutions for each replicate (three samples) were prepared in 0.1% peptone buffer as needed for plating three serial dilutions. Samples were taken on days 1, 4, 8 and 12 of storage. The survival and propagation analysis of the inoculated *E. coli* were experimentally conducted by plating serial dilutions in eosin methylene blue agar (Merck Chemicals), incubated at 37°C for 2 days. Microbial counts were expressed as log cfu/g.

**Sensory Analysis**

Sensory analysis was applied under a descriptive quantitative analysis method with a panel of 12 judges, which recognize quality and features of fresh watercress, all of them defined as regular consumers. A linear scale was used to evaluate visual appearance and color intensity, where a judge panel adjudicated to watercress leaves different values from the best to the worst samples. Zero points were adjudicated to the worst product, with clear manifestation of decay and quality loss. Samples were randomized into white plates with three-digit code for identification.

**Methanol-Soluble Phenolics**

The watercress leaves were frozen at −80°C until analysis. After that, about 1 g of frozen watercress was taken for the extraction of phenolic compounds and was homogenized.
with methanol-water solution (50% v/v). The solution was homogenized for 2 min using an Ultra Turrax (IKA, T18 Basic, Königswinter, Germany) at 13,500 rpm. The mixture was centrifuged at 10,000 × g in a thermoregulated centrifuge (Hermle Z 326K, Hermle Labortechnik, Wehngen, Germany) at 4°C for 15 min. The supernatant was recovered and filtered through filter paper Whatman No. 2 (Sigma-Aldrich Chemicals, St. Louis, MI). Samples were analyzed according to Singleton and Rossi (1965), using the Folin–Ciocalteu reagent. The absorbance was measured at 660 nm with an UV-Vis spectrophotometer (T70 PG Instruments, Ltd., Leicester, U.K.). The results were expressed as µg of gallic acid equivalents per g fresh weight (µg GAE/g fw).

Antioxidant Capacity by FRAP

The antioxidant capacity of watercress leaves was based on the ability of the extracts to reduce Fe³⁺ to Fe²⁺ by ferric reducing ability of plasma (FRAP) method. This measurement was performed according to Benzie and Strain (1996) with some modifications.

For extract preparation, the sample was frozen with liquid nitrogen and crushed in a mortar to a fine powder. Then, 4.5 mL of ethanol : water (4:1) was added and filtered through filter paper Whatman No. 2 (Sigma-Aldrich Chemicals). Subsequently, the filtrate was centrifuged for 30 min at 10,000 × g at 4°C (326K Z, Hermle Labortechnik).

Watercress extracts (40 µL) were mixed with 900 µL of FRAP solution (300 mmol/L pH 3.5 of acetate buffer, ferric chloride hexohydrous 20 mmol/L) and TPTZ [2, 4, 6-(tri(2-pyridyl-s-triazine))] 10 mmol/L in 0.2 M HCl, and 60 µL of milli Q-water for reaction. Absorbance was measured at 593 nm until stable readings were reached. The results were expressed as µg Trolox equivalents per g of fresh weight (µg Trolox Equiv/g fw).

Statistical Analysis

Data were evaluated with analysis of variance. Significant differences between treatments were analyzed by comparing mean values by Tukey test (P ≤ 0.05). MINITAB statistical software v.15 (Addlink Software Científico, S.L., Barcelona, Spain) was used for statistical analysis.

RESULTS AND DISCUSSION

Modified Atmosphere

Active modified atmosphere is usually applied in leafy vegetables to achieve the beneficial effects of moderate O₂ and CO₂ concentration corresponding to 5% of O₂ and 10% CO₂. Similar gas concentration has been previously suggested by Allende and Artés (2003) in Lollo Rosso lettuce and spinach cv. Emilia (Artés-Hernández et al. 2009). Throughout the storage period, the concentration of CO₂ increased from 8–10% to values between 11.5 and 15.9%, with no significant differences among treatments (Fig. 1). The tendency of O₂ concentration was to decrease, from 7–9% to the final values of 3.2–5.3%, without significant difference among treatments. Results were explained by the product respiration and the gas diffusion rate across the film (Allende and Artés 2003). The UV-C light doses did not affect gas concentration within package during cold storage. Nevertheless, several reports have shown that higher UV-C doses activate multiple biological processes in vegetables, including stimulation of respiratory activity causing a higher CO₂ production (Allende et al. 2006). The proper selection of the packaging material allowed reaching desired final atmosphere composition for conservation of watercress under cold storage.

Color

Lightness (L*). After 8 days at 5°C, watercress leaves treated with 10 kJ/m² had a lower lightness value (L* = 37.8) compared with the other treatments (L* = 45 to 50). At the end of storage (day 12), there were no significant differences among treatments. Generally, a high value of L* could be associated with a light green color. Overall, this shows that lightness was unaffected by the UV-C treatments (Fig. 2A).

Chroma (C*). After 8 days at 5°C, untreated UV-C watercress leaves had a higher chroma values than other
treatments, which showed values between 27 and 31. A high value of chroma must be related to color intensity that would indicate that the UV-C light delayed the color changes. In this investigation, there were no significant differences (p ≤ 0.05) among the applied treatments at the end of storage (day 12; data not shown). Artés-Hernández et al. (2009) did not find initially significant differences between UV-C-treated shredded spinach and control samples stored for 13 days at 5°C.

Nonpathogenic E. coli Strain Survival

The day after processing, the untreated inoculated leaves of watercress presented an initial count of 8.6 log cfu/g, which was significantly higher than the treated UV-C samples (6.9 to 7.2 log cfu/g; Fig. 3). A slight reduction in all UV-C treatments compared with control was found on day 4. This would indicate an effect of UV-C light reducing survival and proliferation of inoculated E. coli. On day 8, the doses of 20 and 25 kJ/m² had significant reduction in counts (reaching 3.3 log cfu/g reductions) compared with other treatments. The same trend was followed after 12 days showing that UV-C doses had a significant delay in microbial growth. These reductions were higher than previously reported by Yaun et al. (2004) that applied UV-C doses inhibitory effect of UV-C light on chlorophyll breakdown by modifying activity rates of enzymes involved in this process as chlorophyllase, chlorophyll degrading peroxidase and Mg-dechelatase, keeping chlorophyll for long periods of storage (Funamoto et al. 2002; Costa et al. 2006; Mahdavian et al. 2008; Chairat et al. 2013). Also, the modified atmosphere condition with low O₂ and moderate CO₂ levels reached during this study would help to decrease the color changes of the leaves (Artés et al. 2009). Color is one of the most important characteristics of watercress leaves, and it is the major problem in postharvest because of rapid senescence, which is reflected in the yellowing of the leaves, resulting from the degradation of chlorophyll (Hinojosa et al. 2013).
from 9 to 24 kJ/m² on lettuce leaves reaching log reductions of *Salmonella* spp. and *E. coli* O157:H7 (2.65 and 2.79, respectively). The effectiveness of UV-C light depends on the structure and topography of the surface area where it is applied. Bacterial sensitiveness to UV-C varies within species and also among the different strains of the same species (Guerrero-Beltrán and Barbosa-Cánovas 2004; Escalona et al. 2010).

**Sensory Quality**

**Visual Appearance.** The UV-C light did not affect visual appearance of leaves during 12 days of storage compared with control (Fig. 4A). Similar results have been found with doses of 6 and 18 kJ/m² on MP watercress packaged on passive modified atmosphere and storage for 7 days at 5C and 90% relative humidity. According to Hinojosa et al. (2013), 18 kJ/m² had a slight effect on visual appearance, getting lower scores than the samples treated with 6 kJ/m² after 14 days at 5C. Artés-Hernández et al. (2009) did not find visual appearance differences between UV-C-treated shredded spinach and control during 13 days at 5C. Even though significant differences were not detected among UV-C light and control (*p* ≤ 0.05), the decreased scores during the storage could be more related to the slight water condensation and the colonization of microorganisms on the surface of leaves.

**Color Intensity.** The longest color preservation was observed on leaves treated with lowest UV-C doses, similar to control (Fig. 4B). Apparently, doses higher than 15 kJ/m² could favor the green color losses after 4 days although this was not observed by instrumental measurements (Fig. 2). High UV-C doses could increase free radical production and senescence of plant tissues as was reported by Allende and Artés (2003). These authors found in chopped lettuce that 7.1 kJ/m² after 10 days at 5C caused browning and texture damages. It was described that a progressive solubilization of the pectin layers in the vegetal cell wall could be stimulated by UV-C (Pombo et al. 2009). The visual appearance on food is a primary consideration that consumers take into account. Color has a key role in the choice of food and acceptability (Rico et al. 2007). If an MP vegetable shows an unacceptable visual quality, a potential consumer will not experience the other attributes such as taste, texture and smell, and will not be interested to buy or consume the product (Martínez-Sánchez et al. 2008).

**Methanol-Soluble Phenolics**

On day 1, there were no significant differences among treatments with average values of 2.7 μg GAE/g fw. On days 4 and 8, all treatments reached similar values between 1.6 and 2.5 μg GAE/g fw. On day 12, treatments between 15 and 25 kJ/m² had significantly higher mean values of 2.4 μg GAE/g fw than other doses (Fig. 5). Methanol-soluble phenolic compounds are substances involved in maintaining the antioxidant capacity of the tissues. UV-C light has a significant effect to increase the methanol-soluble phenolic content; it could stimulate the production of phenylalanine ammonia-lyase and the formation of phytoalexins (phenolic compounds; Cuvi et al. 2011). The results obtained in this study showed that control treatment had reduced methanol-soluble phenolic content along storage period similar to treatment of 5 kJ/m². After day 4, methanol-soluble phenolic content increased from 1.5 to 3.0 μg GAE/g fw for those treated with UV-C doses higher than 5 kJ/m², particularly those with 20 kJ/m² that presented a
final content of 2.8 μg GAE/g fw at day 12 of storage. Studies of Ruiz et al. (2010) found in cabbage leaves treated with UV-C light a significant increase in polyphenol content than the control after 2 and 9 days at 6C. It must be considered that high doses of UV-C light could exert visible damage to the surface of vegetables and the extent of this phenomenon depends on the stage of maturity and cultivar treated (Allende and Artés 2003). As mentioned earlier, visual appearance and color intensity could be affected by UV-C when the doses exceed 10 kJ/m². Based on this result, it could be suggested that UV-C doses ≤ 15 kJ/m² could preserve the sensorial quality but does not necessarily help to improve the functional quality of vegetables along cold storage.

Antioxidant Capacity by FRAP

Throughout the cold storage, no significant differences among treatments were found. All treatments along the assay period showed antioxidant capacity in the range of 0.12 to 0.2 μg Trolox equivalents/g fw (Fig. 6). In general, samples (with and without UV-C light exposition) showed a steady decline until day 4. This response could be due to the processing operations which cause oxidative stress in cell membrane. Artés-Hernández et al. (2009) did not find significant differences in antioxidant capacity on spinach leaves treated or untreated with UV-C packed in modified atmosphere and stored at 5C. In the same study, the general trend was to decrease over time, and after 13 days, the treated samples showed values significantly lower than the control. However, Ruiz et al. (2010) determined that the UV-C-treated cabbage leaves had a significantly higher antioxidant capacity than the control after 2 and 9 days at 6C.

CONCLUSIONS

Results suggest that the proposed UV-C doses and modified atmosphere may be useful as innovative techniques to the MP industry maintaining a fresh visual appearance of MP watercress. High UV-C doses reduced and had a bacteriostatic effect on E. coli loads reaching reductions of 3.3 log on microbial counts, allowing the commercialization of watercress until 12 days at 5C.

In addition, promising results were observed for phenolic content of vegetables exposed to high UV-C doses (15 to 25 kJ/m²). However, along with an increase in phenolics, a slight decrease in the sensory quality of watercress was noticed. Our results confirm that UV-C could be a valuable tool for the MP industry in order to guarantee the safety of food products.

ACKNOWLEDGMENTS

The authors thank to CONICYT-CHILE (FONDECYT Project 1120274 and Postdoctoral Research Project 3130460) for financial support to this study and the collaboration of Dr. Bustamante.

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