Hort. Environ. Biotechnol. 56(3):324-329. 2015.
DOI 10.1007/s13580-015-0141-y

ISSN (print) : 2211-3452
ISSN (online) : 2211-3460

Research Report

UV-C Treatments Enhance Antioxidant Activity, Retain Quality and Microbial Safety of Fresh-cut Paprika in MA Storage

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Received November 18, 2014 / Revised February 21, 2015 / Accepted May 25, 2015

Abstract. Fresh-cut paprika was treated with 245 nm UV-C irradiations of 7, 15, and 28 $kJ·m^{-2}$, packaged using a laser ablation breathable film with permeability of 20,000 mL $O_2·m^{-2}·day^{-1}·atm^{-1}$, and was stored in general distribution temperature at 8°C. The paprikas were evaluated antioxidant activity and internal quality, reducing microbial populations, modifying composition of CO_2, O_2 and ethylene content in packages. Fresh-cut paprika irradiated with 15 $kJ·m^{-2}$ UV-C has shown the lowest fresh weight loss and highest overall quality during MA storage. The gas composition (CO_2 and O_2 concentrations) of UV-C 15 $kJ·m^{-2}$ treatment showed the closest conditions to CA conditions of paprika. All UV-C treatments presented higher ethylene concentration in package as compared to those of the control as well as NaOCl treatment, because the ethylene content was elevated in response to abiotic stress, possibly by the treatment of UV-C in paprika. After 7 days of storage at 8°C, the antioxidant activity (DPPH activity), total phenolic compound and vitamin C contents of fresh-cut paprika were maintained highest by UV-C 15 $kJ·m^{-2}$ treatments, followed by 7 $kJ·m^{-2}$ and 28 $kJ·m^{-2}$. The microbial population was reduced by UV-C treatment on the 7 days of MA storage, with the fungal incidence rate lowest in 15 $kJ·m^{-2}$ and 28 $kJ·m^{-2}$ UV-C treatment and total bacterial numbers showed the lowest in UV-C 15 $kJ·m^{-2}$ treatment representing lower than 6.0 log $CFU·g^{-1}$ which was the reference limit for microbial contamination. These results suggest that the application of UV-C 15 $kJ·m^{-2}$ on fresh-cut paprika represents the most favorable results of gas composition in package, antioxidant capacity (e.g., vitamin C contents) and sterilization of fungi and bacteria during MA storage, and extending the shelf life.

***Additional key words*:** DPPH activity, ethylene, fungal incidence rate, gas composition, total bacterial number, total phenolic compound, vitamin C

Introduction

The paprika was cultivated since the late 1990s in Korea and has becoming one of the most important vegetable due to export to Japan. The area of cultivation of paprika has increased to 430 ha and productivity has increased to 50,642 tons at 2012 in Korea (MAFRA, 2013). Paprika has been a popular horticultural crop among consumers partly due to high bioactive substance contents. Specially, the yellow paprika which was used in this study is known to be rich in carotenoid, flavonoid, phenolic substance, and vitamin C (Howard et al., 2000).

Paprika is generally hard to cultivate and to build a marketable fruitsin Korea, because it is very sensitive to temperature and needs consistent moisture, and it shows higher incidence of physiological disorder than other vegetables. An export paprika has been higher ratio of unmarketable fruits such as off shapes and sizes, because imported country wanted to certain grades of fruits size, under this condition still paprika has been great portion of wasted fruits and this fruits can be processed into a fresh-cut product (Yoo et al., 2010). Recently, life style was changed such as increasing of single-person households and double-income families, this living styles are following are sult of in creasing the fresh-cut products because most of people need convenience of saving time for cook and sometimes need to eat just on time.

Fresh-cut products can be contaminated easily when exposed to microorganisms during process and distribution, therefore they must be sterilized. Agricultural products are sterilized by chlorine water, electro-analyzed water, hot water, and calcium solutions using dipping methods (Das and Kim, 2010; Nimitkeatkai and Kim, 2009).

The germicidal effect of UV lights have been reported on a lot of crops, and recent researches of UV light have been reported that it does not only have sterilization effect but it also increases bioactive substance contents in several crops (Erkan et al., 2008; González-Aguilar et al., 2007; Shama, 2007) such as peppers (Promyou and Supapvanich, 2012; Rodoni et al., 2012), and UV light was also proved a secondary effect of preventing the chilling injuries on pepper (Vicente et al., 2005).

The MAP (Modified Atmosphere Packaging) enables to extend a shelf life of agricultural products in room and optimal storage temperature (Kader, 2002). Fresh-cut paprika of storing in proper MAP condition has been proven to increase a shelf life (González-Aguilar et al., 2004), and itwas maintained as better overall quality and microbial safety in MA (Modified Atmosphere) storage when applied by heat and calcium solutions (Das and Kim, 2010). Many crops enhanced shelf life and microbial safety by combined treatments of UV and MAP conditions (Allende and Artés, 2003; López-Rubira et al., 2005).

In this study, the effects of UV-C treatment on the microbial control, antioxidant quality, and internal quality of fresh-cut paprika were evaluated during MA storage.

Materials and Methods

Plant materials

A yellow paprika 'Score' cultivar was grown in hydroponic systems at Gangwon province (37.45°N, 128.54°E) in Korea from August to November 2012. Concentrations of macro elements in nutrient solution for paprika cultivation included NO_3-N 110 mg·L^{-1}, P 50 mg·L^{-1}, K 140 mg·L^{-1}, Ca 160 mg·L^{-1}, Mg 45 mg·L^{-1}, and S 60 mg·L^{-1}. Nutrient solution was supplied to plants by dripping irrigation system. The EC (electrical conductivity) and pH of applied nutrient solution was 2.5 to 3.0 dS·m^{-1} and 5.5-6.5, respectively.

Sample preparation

The paprika fruits were processed into pieces by width at 3-5 mm and length at 5 cm by sharp sterile knives. The chlorination was treated with 100 mg·L^{-1} chlorine water (NaOCl, pH 7.0) for 3 minutes and control was washed in distilled water only. The UV-C treatment on processed paprika with 245 nm wavelength was done by a UV-C lamp (Spectronics, ENF 240C/FE, USA) 10cm distance between the lamp and sample for 5 to 15 minutes to make the UV-C dose of 7, 15 and 28 kJ·m^{-2}. The processed fresh-cut paprika were packaged 50 g each in a laser ablation breathable film (9 cm × 9 cm) which has oxygen penetrate rate at 20,000 mL O_2·m^{-2}·day^{-1}·atm^{-1} in 8°C. It is the normal distribution temperature that does not cause chilling injuries for 7 days (Choi et al., 2013).

Quality measurement

Five replicates of fresh-cut paprika packages were weighted at the 1st, 3rd, and 7th day of storage and the weight loss was expressed as percentage of initial weight. Overall quality was measured by sensory evaluation, fungi incidence, water-soaking, and other factors. The values were categorize into 5 scores for 5 groups (5 = very fresh, quality at time of harvest; 4 = fresh, no fungi, little water-soaking; 3 = moderate, no fungi; 2 = freshness declined, trace of fungi on segment; and 1 = decayed, non-edible), and the limit of marketable quality was set at 3 score. Firmness of fresh-cut paprika was measured using a penetrometer (DFT-01, TR snc, Forli, Italy) with a 5 mm diameter probe.

Gas composition and Ethylene content

The carbon dioxide and oxygen concentrations in each package headspace were measured by a gas analyzer (Check mate 9900, PBI Dansensor, Ringsted, Denmark). The ethylene was measured by a gas chromatograph (GC 2010 Shimadzu, Shimadzu Corporation, Japan) equipped with BP 20 Wax column (30 m × 0.25 mm × 0.25 μm, SGE analytical science, Australia) and a flame ionization detector (FID). The detector and injector operated at 127°C along with the ovens were at 50°C, and carrier gas (N_2) flow rate was 0.67 mL·s^{-1} (Choi et al, 2011).

Antioxidant activity and internal quality

Antioxidant activity was measured by DPPH (α, α-diphenyl-β-picrylhydrazyl) method. The paprika samples were grinded at 4°C in 2.0 mL of absolute ethanol with mortar and pestle. A 0.5-mL aliquot was mixed with a 0.25 mL of 0.5 mM DPPH ethanol solution and 0.5 mL of 100 mM acetate buffer (pH 5.5). After standing for 30 min, the absorbance of the mixture was measured at 517 nm (Abe et al., 1998). Phenolic compound were measured using Folin & Ciocalteu's phenol reagent. Garlic acid was the standard material and was measured at 765 nm by UV-spectrophotometer (S-3100, SCINCO, Seoul, Korea) (Kang and Saltveit, 2003). Vitamin C content was analyzed by HPLC (Waters 486, Waters, Milford, USA) using ZORBAX Eclipse XDB-C18 (4.6 cm × 250 mm, 5 μm, Agilent, Santa Clare, USA) column (Kim et al., 2011).

Microbial population

Fugal incidence was determined by counting the number of infected pieces in each packagein 1st, 3rd, 7th, 10th, and 14th day of storage. The Fugal incidence rate was expressed as percentage of infected pieces of total fresh-cut pieces. A 20 g sample was removed from each treatment and placed into a stomacher bag with 180 mL of 0.1% peptone water at 7th day of storage. This mixture was pummeled in a Stomacher (Powermixer, B&F Korea, South Korea) at high speeds (300 rpm) for 120 sec. Each sample was then blended and serial dilutions were made with the sample blends. Total bacterial number was determined using 3M Aerobic Count Plate Petri film (3M Co., St Paul, MN, USA) according to the manufacturer's instructions. Enumeration of colonies was performed after incubation at 35°C for 48 h. Colony counts were calculated as $CFU \cdot g^{-1}$ and then converted into log value for statistical analysis.

Results and Discussion

Although there was no significant difference in fresh weight loss of fresh-cut paprika among the treatments of chlorine water and UV-C irradiations compared with the control, it was found to be the lowest in 15 $kJ \cdot m^{-2}$ UV-C treatment (Fig. 1). Such inhibitory effects of the UV-C treatment on fresh weight loss were demonstrated by Cuvi et al. (2011) in red pepper. It was reported that UV-C treatment might hinder membrane dysfunctions thereby inhibiting ion leakage and minimizing fresh weight loss during their storage (Promyou and Supapvanich, 2012).

Further, in the present study, this research was found that all treatments represented less than 0.5% of fresh weight loss during 7 days storage at 8°C. This might be also contributed by the package using alaser ablation breathable film with 20,000 $mL \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$ oxygen permeability. The film we used in the study previously demonstrated less than 1% of fresh weight loss in which paprika fruits were stored in modified atmosphere packages (MAP) at 7°C for 15 days followed by room temperature storage for additional 7 days (Choi et al., 2011). Even the allowable range of fresh weight loss of paprika fruits is 4% for fresh products (Kays and Paull, 2004), hence the treatments for fresh-cut products of

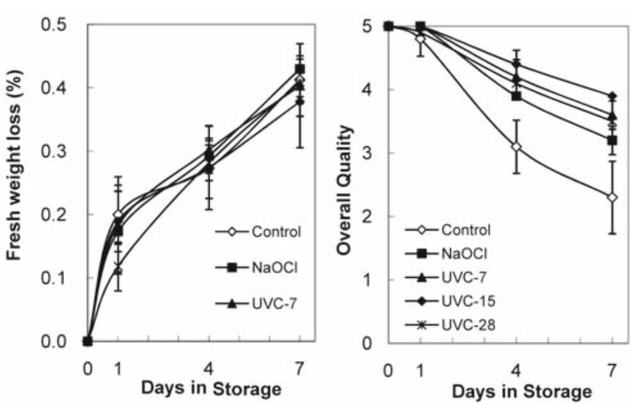

Fig. 1. Changes in fresh weight loss and overall quality of fresh-cut paprika treated 100 $mg \cdot L^{-1}$ NaOCl, and different dose of UV-C lights (7 $kJ \cdot m^{-2}$, 15 $kJ \cdot m^{-2}$, and 28 $kJ \cdot m^{-2}$) before packaging with a laser ablation breathable film (20,000 mL $O_2 \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$) in 8°C storage. The paprika fruits ('Score', Seminis) were grown in hydroponic systems from August to November 2012 in Gangwon province (37.45 °N, 128.54 °E). Vertical bars represent ± SD of means (n = 5). Overall quality was assessed as 5 = excellent, 4 = good, 3 = moderate and marketable, 2 = poor, and 1 = very poor.

paprika given in the study did not result in degradation of marketability owing to fresh weight loss.

When it comes to the overall quality, the control represented the lowest score 3 and it was lost the marketability after 4th day of storage. The score 3.9 for the overall quality was shown to be highest in 15 $kJ \cdot m^{-2}$ UV-C treatment and followed UV-C 7 $kJ \cdot m^{-2}$, UV-C 28 $kJ \cdot m^{-2}$, and NaOCl treatment on the 7th day of the storage (Fig. 1). The UV-C 28 $kJ \cdot m^{-2}$ treatment prevented fungi growth compared to that of UV-C 15 $kJ \cdot m^{-2}$ treatment which was found to be the most effective dose for the overall quality, yet there were not only noticeable water-soaking induced by leakage from paprika tissue but also 'pitting' phenomena noted in some samples, indicating that the dose UV-C radiation might be too high. Choi et al. (2013) addressed the fruits skin pitting might be resulted when excessive UV radiation was provided. And Vicente et al. (2005) demonstrated that excessive UV-C application lowered the suppressive potency against spoilage in pepper.

In addition to the water-soaking, fungal incidence is another detrimental factor for overall quality. Fungi started to grow on 4th day, and the fungal incidence rate was increased 60% on the 10th day of storage in the control fresh-cut, but NaOCl and UV-C 15 $kJ \cdot m^{-2}$ of treated fresh-cut paprika exhibited 18 and 7% of fungal incidence rate on 10 days of storage. It was found to be less than 10% of fungal incidence on 14 days of storage after UV-C 15 $kJ \cdot m^{-2}$ and 28 $kJ \cdot m^{-2}$ treatments (Fig. 2). Therefore, it is likely that treatment of UV-C higher than 15 $kJ \cdot m^{-2}$ treatment might be required for fresh-cut paprika storage. Previously, the signs of spoilage, such as tissue maceration, softening, dehydration, and juice leakage, was shown to be most effective in which the 20 $kJ \cdot m^{-2}$ UV-C was radiated in fresh-cut of bell peppers, compared to that of the UV-C 10 $kJ \cdot m^{-2}$ treatment (Rodoni et al., 2012).

The UV-C 15 $kJ \cdot m^{-2}$ treatment demonstrated the most drastic changes in the atmospheric compositions in packages under the storage conditions. Overall, the levels of carbon dioxide and oxygen were 4 and 10% in UV-C 15 $kJ \cdot m^{-2}$ treatment, respectively; to note, the oxygen level was less than 3% on 7 days of the storage (Fig. 3). The maximum allowable range of carbon dioxide for paprika storage is 2% while the CA conditions indicate 2-5% ranges for both oxygen and carbon dioxide (Kader, 2002); the gas composition of UV-C 15 $kJ \cdot m^{-2}$ treatment showed the most close to CA conditions of paprika.

Regarding the ethylene content, all UV-C treatments were higher compared to those of the control as well as NaOCl treatment (Fig. 3). This result might be because the ethylene content was elevated in response to abiotic stress, possibly caused by the treatment of UV-C in paprika. Similarly, Tiecher et al. (2013) also indicated that the ethylene content was increased more than 2 folds when tomato was treated with UV-C.

The firmness of fresh-cut paprika were investigated in

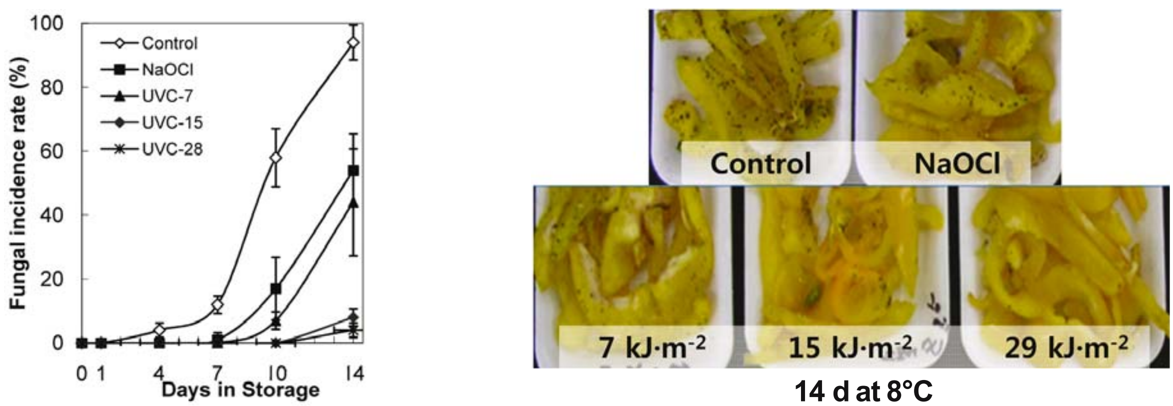

Fig. 2. The fungal incidence rate (left) and appearance (right) of fresh-cut paprika treated 100 mg·L^{-1} NaOCl, and different dose of UV-C lights (7 kJ·m^{-2}, 15 kJ·m^{-2}, and 28 kJ·m^{-2}) before packaging with a laser ablation breathable film (20,000 mL O_2·m^{-2}·day^{-1}·atm^{-1}) during the storage at 8°C. The paprika fruits ('Score', Seminis) were grown in hydroponic systems from August to November 2012 in Gangwon province (37.45 °N, 128.54 °E). Vertical bars represent ± SD of means (n = 5).

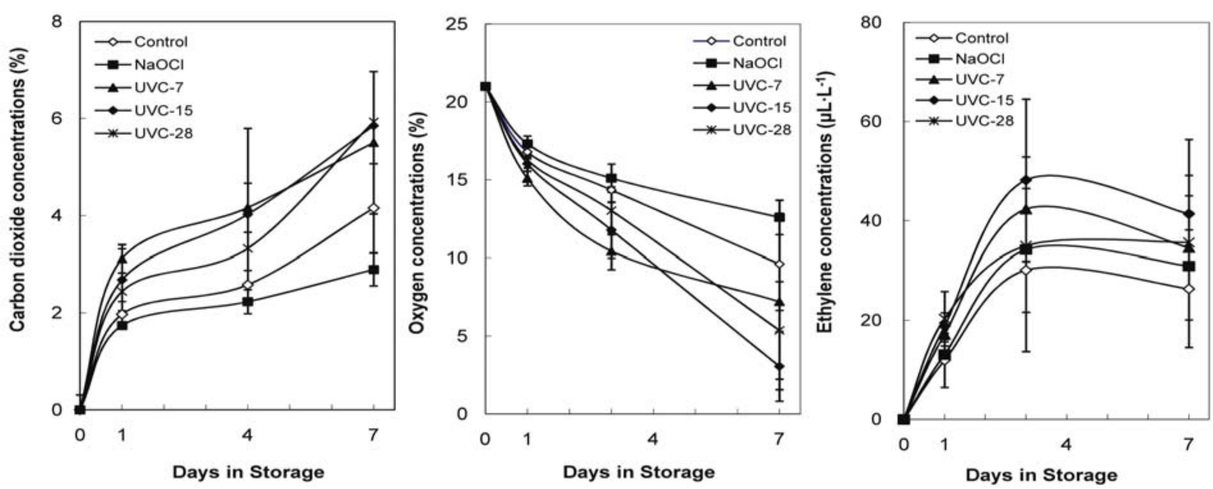

Fig. 3. Changes of carbon dioxide, oxygen and ethylene concentrations in laser ablation breathable film (20,000 mL O_2·m^{-2}·day^{-1}·atm^{-1}) packages contained fresh-cut paprika treated 100 mg·L^{-1} NaOCl, and different dose of UV-C lights (7 kJ·m^{-2}, 15 kJ·m^{-2}, and 28 kJ·m^{-2}) in 8°C storage. The paprika fruits ('Score', Seminis) were grown in hydroponic systems from August to November 2012 in Gangwon province (37.45 °N, 128.54 °E). Vertical bars represent ± SD of means (n = 5).

response to UV-C irradiation; in UV-C 15 kJ·m^{-2} treatment which represented the highest overall quality, maintained the highest firmness while it the lowest in UV-C 28 kJ·m^{-2} treatment (Table 1). It has been reported that UV-C radiation inhibits activities of enzymes, responsible for cell wall degradation and fungi infection which weakens firmness of plant tissues (Cristescu et al., 2002; Stevens et al., 2004). Therefore it is possible to postulate that UV-C treatment may be effective to maintain the firmness of plants. However, Choi (2011) reported that UV treatment on persimmons were resulted lowered hardness of fruits due to the increment of ethylene content, because UV-C treatment acts as stress, the content of ethylene could be elevated hence lowering hardness as ethylene itself induces plant tissue softening.

After 7 days of storage at 8°C, the antioxidant activity (measured via DPPH radical scavenging assay) and vitamin C content of fresh-cut paprika were elevated with UV-C treatments; it was found to be the highest in UV-C 15 kJ·m^{-2} treatment yet rather decreased in UV-C 28 kJ·m^{-2} treatment. Similarly, the total phenolic compounds level was increased by UV-C treatments; to note, it was highest in UV-C 7 kJ·m^{-2} treatment (Table 1). Promyou and Supapvanich (2012) reported that yellow bell peppers treated with UV-C 6.6 kJ·m^{-2} showed an increasing in total carotenoids, total flavonoids contents, and activities of antioxidative enzymes.

The total bacterial numbers were evaluated after 7 days of storage. As results, both UV-C treatments showed low total bacterial numbers compared to the control as well as NaOCl treatment. Specifically, the UV-C 15 kJ·m^{-2} treatment represented lower than 6.0 log CFU·g^{-1} which is the reference limit for microbial contamination (Kim et al., 2009), followed by UV-C 28 kJ·m^{-2} treatment (6.02 log CFU·g^{-1}). Although there

Table 1. Aerobic plate count, DPPH activity, firmness, total phenolic compounds contents, and vitamin C contents of fresh-cut paprika treated 100 mg·L^{-1} NaOCl, and different dose of UV-C lights (7 kJ·m^{-2}, 15 kJ·m^{-2}, and 28 kJ·m^{-2}) before packaging with a laser ablation breathable film (20,000 mL O_2·m^{-2}·day^{-1}·atm^{-1}) at 7th day of storage at 8°C. The paprika fruits were grown in hydroponic systems from August to November 2012 in Gangwon province (37.45 °N, 128.54 °E).

Treatment	Aerobic plate count (log_{10} CFU·g^{-1})	DPPH activity	Firmness (N)	Total phenolics (mg GAE·$100g^{-1}$)	Vitamin C (mg·$100g^{-1}$ FW)
Control	7.65 a[z]	73.2 c	0.43 ab	141 c	126 b
NaOCl	6.89 b	73.9 c	0.44 ab	147 c	134 ab
7 kJ·m^{-2}	6.49 c	74.6 ab	0.44 ab	218 a	133 ab
15 kJ·m^{-2}	5.94 d	82.6 a	0.49 a	194 ab	141 a
28 kJ·m^{-2}	6.08 d	77.0 b	0.35 c	184 b	113 c

[z]Mean separation within columns by Duncan's multiple range tests at p < 0.05.

was a trend showing that antiseptic potency was increased with stronger UV radiation, no difference was noted in between treatments of 28 and 15 kJ·m^{-2} dose. Therefore, it is concluded that appropriate level of UV-C radiation for sterilization might be 15 kJ·m^{-2} dose.

In previous literatures, the optimal dose of UV-C radiation was 7 kJ·m^{-2} in reference to the sterilization of pepper (Vicente et al., 2005), but results herein demonstrated that the highest sterilization potency was shown with UV-C 15 kJ·m^{-2} which is more than 2 folds. This might be due to increased surface area of fresh-cut paprika thereby providing a further likelihood of microbial contamination.

Taken altogether, we demonstrated that application of UV-C 15 kJ·m^{-2} on fresh-cut paprika represents the most favorable results of antioxidant capacity (e.g., vitamin C contents) and sterilization of fungi and bacteria during MA storage thereby extending the shelf life.

Acknowledgement: This study was supported by Bio-industry Technology Development Program(111080-3) of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries.

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