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Responses of phytonutrients and tissue condition in persimmon and cucumber to postharvest UV-C irradiation

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ABSTRACT

Postharvest UV-C irradiation is expected to be a widely applicable sterilization technology for fruit and vegetables. In this study, the effects of UV-C irradiation on phytonutrients and tissue conditions in persimmon and cucumber were investigated. UV-C having a peak wavelength of 253.7 nm was irradiated on their surface (12.9 W m^{-2}) for 0 to 15 min. The UV-C irradiation was not effective for the enhancement of phytonutrients (polyphenol, β-carotene, ascorbic acid, chlorophyll) in persimmon and cucumber. However, some responses of tissue condition to UV-C irradiation appeared. Some persimmon fruit became blackened during storage, and the transfer of tannin from the parenchyma to the epidermal tissue was observed in the fruit. We also evaluated the factors responsible for the color change in the fruits. In addition, electrical impedance was measured, and electrical properties were obtained by equivalent circuit analysis. These properties reflected the tissue conditions. In particular, the extracellular resistance of UV-C-irradiated cucumber exhibited clear differences from non-irradiated samples.

1. Introduction

The quality of fruit and vegetables is maintained according to their storage conditions and postharvest treatments. Low temperature and high humidity are basic approaches to suppress the respiration rate in fruit and vegetables (El-Ramady et al., 2015; Liamnimitr et al., 2018). Additionally, gas environment control, such as MA packaging and CA storage, is a popular technique for reducing respiration during storage (Daş et al., 2006). Microbial control is also important for suppressing the decay of fruit and vegetables. However, chemical disinfection is generally not preferred because consumers are nervous about chemical residues on agricultural products. Because such concerns do not exist with regard to physical treatments, hot water and steaming treatments have been studied over the past decade. Heat treatments cause heat transfer to microorganisms by heat conduction and convective heat transfer, causing the microorganisms to reach a fatal temperature (Uchino, 2013). The temperature rise denatures and deactivates microbial proteins and enzymes. In addition, biomolecules are oxidized and decomposed by the heat. Thus, biological activity in the microorganisms cannot be maintained (Uchino, 2013). Heat treatments contribute not only to decay control but also to the quality control of fruit and vegetables (McDonald et al., 1999). To date, several studies have reported ripening inhibition and quality improvement using heat treatments on fruit and vegetables such as apple, grapefruit, potato, and tomato (Fallik, 2004; McDonald et al., 1999; Porat et al., 2000; Ranganna et al., 1998; Smith and Lay-Yee, 2000). On the other hand, heat treatment can cause the deterioration of tissue and texture quality before obtaining a sufficient bactericidal effect (Uchino, 2013).

Recently, electromagnetic irradiation has been focused on as a physical disinfection technique, with the specific use of infrared (IR) and ultraviolet (UV) irradiation (Trivittayasil et al., 2016, 2014). IR irradiation directly raises the temperature of microbial cells by radiant heat transfer without a heating medium such as air, water, or steam. Thus, IR sterilization can be performed efficiently with less energy than other heat treatments (Uchino, 2013). IR treatment is also regarded as a heat method, and it has some problems such as drying and heat damage of the fruit and vegetables during treatment (Trivittayasil et al., 2014; Uchino, 2013).

UV rays are electromagnetic waves in the wavelength band from about 1 nm to 400 nm. These wavelengths are further classified into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm) (Rastogi et al., 2010). UV-C is commonly used for sterilization because the absorption band of nucleic acids has a peak around 260 nm, which coincides with the wavelength band of UV-C. When UV-C is used to

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irradiate pyrimidine bases (thymine, cytosine, and uracil) present adjacent to each other in the same chain, a dimer is formed between the two nucleotide monomers, and then the replication function of the nucleic acid is lost (Uchino, 2013). UV-C irradiation has been put to practical use as a sterilization technique for the surface of fruit due to its high bactericidal effect.

The bactericidal effect by UV-C has been demonstrated and its mechanism clarified. UV-C also induces biological stress in plants and defense mechanisms of plant tissues with the consequent production of phytoalexin compounds (Jiang et al., 2010). Phytoalexin accumulation could be accompanied by other inducible defenses such as cell wall modifications, defense enzymes, and antioxidant activity (González-Aguilar et al., 2007). Thus, we expected that postharvest UV-C treatment is useful for enhancing antioxidant phytonutrients including polyphenol, ascorbic acid, β-carotene and chlorophyll. Actually, Costa et al. (2006) demonstrated that UV-C irradiation of 4–14 kJ m^{-2} on broccoli inhibited chlorophyll decomposition and tissue degradation. Jiang et al. (2010) also indicated inhibition of softening and enhancement of antioxidant capacity in mushroom by UV-C irradiation of 4 kJ m^{-2}. In addition, Liu et al. (2012) reported that total polyphenol content and antioxidant capacity in tomato increased by UV-C irradiation of 4 and 8 kJ m^{-2}. Now that UV-C is being established as a practical sterilization technique, we must evaluate the influences from variety aspects and clarify both the positive and negative aspects of treatment. In this study, we evaluated changes occurring inside persimmon and cucumber. The former is commonly eaten as a mature fruit and the latter as an immature vegetable-fruit. Changes in the content of characteristic phytonutrients in each of them were measured. Additionally, the responses of tissues to UV-C irradiation were investigated by microscopy and impedance measurements.

2. Materials and methods

2.1. Sample preparation

Persimmon (*Diospyros kaki* L., cv. Fuyu) and cucumber (*Cucumis sativus* L., cv. Kanari) were harvested from a local farm in Fukuoka prefecture, Japan. Injury-free persimmon and cucumber of the same size and shape were selected for the experiments. Initial moisture contents were 83.6% and 96.8% for persimmon and cucumber, respectively. They were treated with UV-C irradiation immediately after transfer to the laboratory. Then, they were stored for up to 14 d (persimmon) and 10 d (cucumber) in the dark at 25 °C and 15 °C, respectively. The relative humidity was about 60%. Under these conditions, physiological changes were accelerated without being affected by light.

A UV-C lamp with a peak wavelength of 253.7 nm (GL-6, Toshiba Lighting & Technology) was used as the light source. In order to stabilize the irradiation intensity from the UV-C lamp, the lamp was turned on for 5 min before the irradiation treatment. One persimmon or one cucumber was set under the light. The distance between the lamp and the object was adjusted to 50 mm, and the intensity on the irradiated side was 12.9 W m^{-2}. The irradiation times of UV-C were set from 0 to 15 min. The object was placed so that the axis was perpendicular to the irradiation direction of the lamp. The persimmon and cucumber were stored within 2 h after the irradiation, and all the measurements were conducted within 5 h after the storage.

2.2. Phytonutrients

2.2.1. Polyphenol content

Total polyphenol content in persimmon fruit (g kg^{-1}) was determined using the Folin–Ciocalteu method (Kanaya, 2006; Luthria et al., 2006). The flesh and peel of the fruit were separated, and each was freeze dried using a freeze dryer (FDU-1200, EYELA). The dried sample was weighed accurately (200 mg) and mixed with 10 mL of 80% (v/v) ethanol in a test tube. To inactivate polyphenol oxidase, the mixture was heated in a water bath at 80 °C for 3 min. After cooling in iced water for 5 min, the sample solution was sonicated for 30 min and centrifuged for 10 min (20 °C, 1670 × *g*). Then, the supernatant was filtered and collected. The residue was mixed with the precipitate in the tube. Resuspension in 10 mL of ethanol, sonication, centrifugation, and filtration were repeated. The first and second filtrates were mixed, and the sample solution was adjusted to 20 mL. Next, 0.1 mL of the sample solution was mixed with 7.9 mL of distilled water, and 0.5 mL of Folin–Ciocalteu reagent was added to the mixture. After standing for 8 min, 1.5 mL of 20% sodium carbonate solution was added, and the mixture was reacted for 1.5 h. Absorbance of the solution at a wavelength of 725 nm was measured using a spectrophotometer (V-530, JASCO), and total polyphenol concentration in the solution (mg 100 m L^{-1}) was calculated from the calibration curve prepared in advance. Moreover, the value was converted to total polyphenol content per fresh weight of persimmon fruit (g kg^{-1}). The measurement was performed on 5 individual samples for each condition.

2.2.2. L-ascorbic acid

The L-ascorbic acid content per fresh weight of cucumber (g kg^{-1}) was determined using an RQ-flex reflectometer (Merck) (Orikasa et al., 2014). A 10 g portion of the cucumber was homogenized with 30 mL of 5% metaphosphoric acid solution. The mixture was filtered, and the liquid phase was used to analyze the ascorbic acid concentration. The measurement was performed on 5 individual samples for each condition.

2.2.3. β-carotene and chlorophyll

β-carotene and chlorophyll contents were determined in accordance with the method of Nagata and Yamashita (1992) with minor modifications. Peel (0.5 g) and flesh (1.0 g) of persimmon and peel (1.0 g) of cucumber were homogenized with 15 mL of extracting solvent (acetone:hexane = 2:3, v/v). The sample solution was centrifuged, and absorbance of the supernatant at 453, 505, 645, 663, and 750 nm was measured using a spectrophotometer. The concentrations of carotene and chlorophyll in the solution were calculated using the following equations:

$$C_{car} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452 A_{453} \quad (1)$$

$$C_{chl} = 1.6711\ A_{645} + 0.671\ A_{663} \quad (2)$$

where C_{car} and C_{chl} are the concentrations of carotene and chlorophyll, respectively, in the sample solution (mg 100 m L^{-1}), and A_{453}, A_{505}, A_{645}, and A_{663} were the values obtained by subtracting the absorbance at 750 nm from the absorbance at each wavelength (subscript indicates each wavelength). The concentrations were converted to content per fresh weight (g kg^{-1}). The measurement was performed on 5 individual samples for each condition.

2.3. Weight loss

Changes in the weight of the persimmon and cucumber during storage were measured using an electronic balance (EK-1200i, A&D). Values were divided by the initial weight and expressed as weight loss (%). The measurement was performed on 5 individual samples for each condition.

2.4. Color

The surface color was evaluated by the $L^*a^*b^*$ color system using a chromameter (CR-200, Minolta). For each, two points on both the irradiated and non-irradiated sides were randomly measured. For persimmon, data obtained from every measuring points was expressed as a^* and b^* plots against L^*. Additionally, to simplify the figure, all the treated samples were expressed together. For cucumber, two measured values obtained from the irradiated side were averaged and used as the

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