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Mandarin preservation by microwave-powered cold plasma treatment

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ABSTRACT

Cold plasma treatment (CPT) was investigated as a nonthermal method for inhibiting *Penicillium italicum* and improving storability of mandarins (*Citrus unshiu* Marc.). Whole mandarin fruits or the peels were treated with cold plasma at 0.7 kPa using a microwave CPT system. The treatment variables were plasma-forming gases, plasma generation power, and treatment time. Nitrogen (N_2)-CPT at 900 W for 10 min, resulted in the highest inhibition of *P. italicum* (84% reduction in disease incidence), significantly increased the total phenolic content and antioxidant activity of mandarin peel after the treatment ($p < 0.05$), but did not significantly affect CO_2 generation, weight loss, content of soluble solids, titratable acidity, pH, ascorbic acid concentration (flesh), or surface color during storage at 4 and 25 °C. These results demonstrate the potential for CPT application as a postharvest technology for preserving mandarins, increasing the total phenolic content and antioxidant activity of mandarin peel.

1. Introduction

The mandarin is one of the most popular fruits in Eastern Asia, including China, Japan, and Korea (Hong, Lee, & Kim, 2007). The flesh and peels of mandarins are rich in biologically active compounds, including β-cryptoxanthin, vitamins, flavonoids, and other phenolic acids (Ma et al., 2008; Sugiura, Ohshima, Ogawa, & Yano, 2006; Wang, Chen, Guo, Abbasi, & Liu, 2016). However, mandarins are harvested for a limited period of time, generally from late autumn to middle winter (Hong et al., 2007), and are therefore kept in a long-term storage for controlled market release (Thompson, 2010).

Major commercial loss of mandarins occurs due to *Penicillium* species-causing diseases, including postharvest blue mold disease and green mold disease (Obagwu & Korsten, 2003; Palou, Usall, Smilanick, Aguilar, & Viñas, 2002). Postharvest blue mold disease, which accounts for ~26% of total disease in mandarins, is caused by *Penicillium italicum*. Therefore, the growth control of *P. italicum* is important for mandarin preservation (Ko & Kim, 1996). Some methods have been pioneered to inhibit *Penicillium* spp. in citrus fruits, including those using hot water, ultraviolet, ethanol, acetaldehyde, and ethyl formate vapor (Ben-Yehoshua, Rodov, Kim, & Carmeli, 1992; Schirra & D'hallewin, 1997; Yuen, Paton, Hanawati, & Sjem, 1995).

To prevent mandarins from spoiling due to microorganisms (*e.g.*, *Penicillium* spp.) during postharvest storage, the fruits are drenched, sprayed, or wax-coated with postharvest fungicides, such as phenylphenate, thiabendazole, and imazalil (Schirra, D'Aquino, Palma, Angioni, & Cabras, 2008; Smilanick, Margosan, Mlikota, Usall, & Michael, 1999). However, the use of synthetic fungicides is unpopular in some countries due to public perception (Boonkorn et al., 2012). Heat treatments, including hot water brushing, hot water dipping, and hot air treatment, are also used (Fallik et al., 1999), but heat can cause discoloration and weight loss in mandarins (Hong et al., 2007). Thus, a new intervention technology is needed to prevent microbial spoilage and maintain quality during postharvest storage of mandarins.

In recent years, cold plasma treatment (CPT) has attracted a great deal of attention as a non-thermal food preservation method for microbial decontamination of fruits or vegetables (Misra, Keener, Bourke, Mosnier, & Cullen, 2014; Yong et al., 2015), minimizing nutrient destruction and sensory property degradation (Ramazzina et al., 2015; Rosset, Noel, & Morelli, 2007). Atmospheric pressure cold plasma (CP) jet treatment at 18 kV using argon–oxygen mixed gas reportedly inactivated *Salmonella* on strawberries by 1.7–2.3 log CFU/sample (Ma et al., 2015). Ziuzina, Patil, Cullen, Keener, and Bourke (2014) reported that atmospheric dielectric barrier discharge CP at 80 kV for 5 min inactivated *Escherichia coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes* on cherry tomatoes by 3.5, 3.8, and 4.2 log CFU/tomato. Song et al. (2015) reported that microwave CPT using nitrogen gas at 400 W reduced the numbers of *E. coli* O157:H7 on lettuce by ~90%, without influencing its sensory or storage quality properties, including weight loss, color, ascorbic acid concentration, and antioxidant activity. However, little research has focused on CP decontamination of citrus fruits, and there have been no previous reports regarding the effectiveness of CPT for preservation of mandarins. The present study was performed to study the effects of CPT against *P. italicum* inoculated into mandarins and the physicochemical properties of mandarins, including CO_2 generation and weight loss rate of whole fruit, soluble solid contents, titratable acidity, maturity, pH, and ascorbic acid contents of

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flesh, color of the fruit surface, and total phenolic compounds and antioxidant activities of both flesh and peel, during storage at 4 and 25 °C.

2. Materials and methods

2.1. Materials

Satsuma mandarins (*Citrus unshiu* Marc.) were harvested from a commercial orchard in Seogwipo in Korea, in December 2015 when the fruits were commercially mature. The fruits were coated with wax when purchased. All fruits screened for experiments were uniform in size and free of physical injury and signs of infection. On the day of experiment, screened fruits were lightly rubbed with 70% (v/v) ethanol-soaked towels to remove wax on the fruit surface and then washed using running tap water and distilled water. Washed fruits were air-dried on a clean bench (HB-402; Hanbaek Co., Ltd., Bucheon, Korea) for 1 h.

2.2. CPT system

CPT was performed with the SWU-2 (Seoul Women's University, Seoul, Korea) as described previously by Kim, Lee, and Min (2014). SWU-2 consists of a microwave generator, cooling system, treatment chamber, gas mass flow rate controller, vacuum pump, and parameter controller. The magnetron (Magnetron 2M246; LG electronics Inc., Seoul, Korea) in the microwave generator produces a 2.45-GHz wave discharge operated at a power level of 50–1000 W. Mandarin or mandarin peel samples were located at 24 cm from the center bottom in the treatment chamber. The microwave density at the sample position was predicted to be 0.25 $W \cdot m^{-2}$ by simulation using COMSOL Multiphysics (COMSOL 4.4; COMSOL, Inc., Palo alto, CA, USA) (Fig. 1). The electromagnetic wave (2.45 GHz, 900 W, TE10 mode) was interpreted by Maxwell's equation using the radio frequency module of COMSOL Multiphysics. The treatment chamber is made of stainless steel and has dimensions of 43 cm (width) × 37 cm (height) × 40 cm (length) (Fig. 1). The plasma-forming gas is controlled by a gas mass flow rate controller and flows at a maximum rate of 20 standard liters/min. The pressure in the chamber can range from 500 to 30,000 Pa and is adjusted by a vacuum valve.

2.3. Microbial inoculation and CPT

P. italicum, isolated from Satsuma mandarin (*Citrus unshiu* Marc.), (KACC 40826) was provided by the Rural Development Administration-Genebank Information Center (Jeonju, Korea). The culture was prepared according to Tao, Fan, Jia, and Zhang (2014). The strain was kept at −80 °C and thawed on ice before use. Potato dextrose agar (PDA; Difco, Detroit, MI, USA) and potato dextrose broth (PDB; Difco) were used as growth media. Frozen stock culture was streaked on PDA and incubated at 25 ± 2 °C for 5 days. After culture, the surface of the PDA agar was gently scraped after applying an aqueous solution of Tween 80 (0.1 mL/100 mL) onto the agar surface; the content obtained by scraping was centrifuged at 7000 × *g* at 22 °C for 2 min and suspended in 0.1% (w/w) peptone water. The suspension was diluted with 0.1% peptone water to produce the desired inoculum concentration (~6.0 log spores/mL). The spore concentration was determined using a hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Konigshofen, Germany). *P. italicum* was inoculated on mandarin peel according to the method of Boonkorn et al. (2012). Briefly, the peels of mandarin fruits were cut into circular pieces (diameter: 4 cm, weight: 1.5 g, thickness: ~3 mm) using a sterilized circular knife. Flesh was not attached with the peel. Each peel sample was punctured once with a sterile needle to a depth of approximately 1 mm. A droplet (20 μL) of *P. italicum* conidia suspension (~6.0 log spores/mL) was spotted into a puncture. Inoculated peels were dried for 1 h in a clean bench and treated with CP using different types of plasma-forming gas, treatment power, and treatment time. The treatment conditions for each type of gas were as follows. For nitrogen (N_2)-CPT, the gas flow rate, treatment pressure, plasma generation power, and treatment time were 1000 $mL \cdot min^{-1}$, 0.7 kPa, 400, 650, and 900 W, and 2, 5, and 10 min, respectively; for

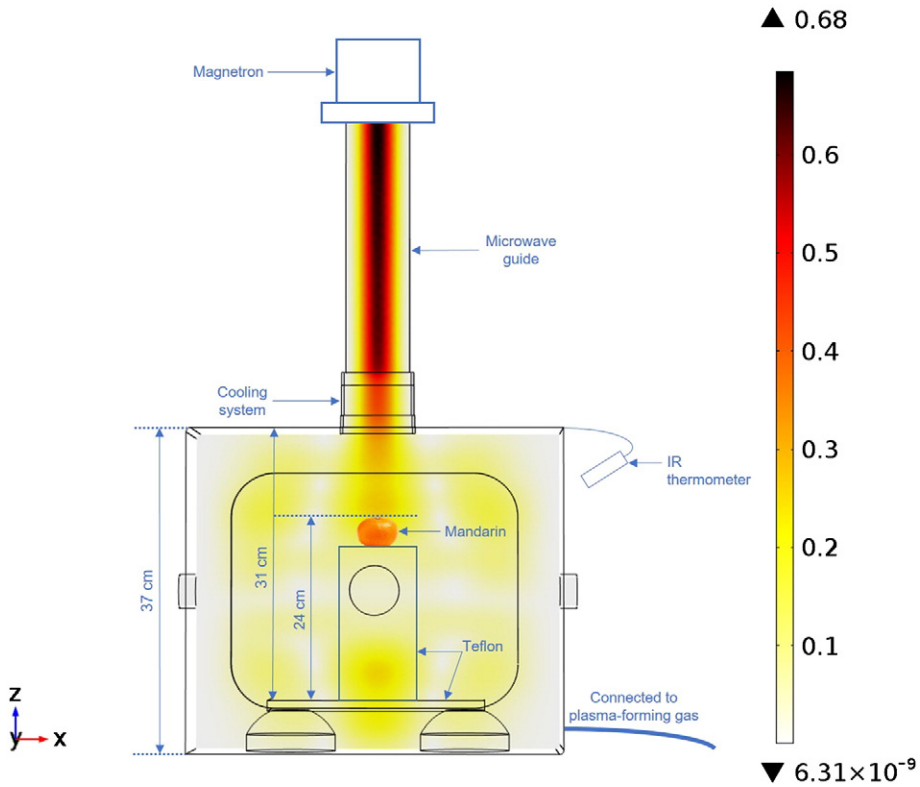

Fig. 1. Schematic diagram of the cold plasma treatment chamber and the simulated microwave power density distribution in the chamber.