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Microbial decontamination of onion powder using microwave-powered cold plasma treatments

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ABSTRACT

The effects of microwave-integrated cold plasma (CP) treatments against spores of *Bacillus cereus* and *Aspergillus brasiliensis* and *Escherichia coli* O157:H7 on onion powder were investigated. The growth of *B. cereus*, *A. brasiliensis*, and *E. coli* O157:H7 in the treated onion powder was assessed during storage at 4 and 25 °C, along with the physicochemical and sensory properties of the powder. Onion powder inoculated with *B. cereus* was treated with CP using helium as a plasma-forming gas, with simultaneous exposure to low microwave density at 170 mW m^{-2} or high microwave density at 250 mW m^{-2}. High microwave density-CP treatment (HMCPT) was more effective than low microwave density-CP treatment (LMCPT) in inhibiting *B. cereus* spores, but induced the changes in the volatile profile of powder. Increase in treatment time in HMCPT yielded greater inhibition of *B. cereus* spores. Vacuum drying led to greater inhibition of spores of *B. cereus* and *A. brasiliensis* than hot-air drying. HMCPT at 400 W for 40 min, determined as the optimum conditions for *B. cereus* spore inhibition, initially reduced the numbers of *B. cereus*, *A. brasiliensis*, and *E. coli* O157:H7 by 2.1 log spores/cm^2, 1.6 log spores/cm^2, and 1.9 CFU/cm^2, respectively. The reduced number of *B. cereus* spores remained constant, while the number of *A. brasiliensis* spores in the treated powder increased gradually during storage at 4 and 25 °C and was not different from the number of spores in untreated samples by the end of storage at 4 °C. The *E. coli* counts in the treated powder fell below the level of detection after day 21 at both temperatures. HMCPT did not affect the color, antioxidant activity, or quercetin concentration of the powder during storage at both temperatures. The microwave-integrated CPTs showed potential for nonthermal decontamination of onion powder.

1. Introduction

Onion (*Allium cepa* L.) is one of the most commonly consumed vegetables (Sellappan and Akoh, 2002). Onions are recognized for their various biological activities, such as antioxidant and antibacterial effects, which are mediated by their contents of sulfur, phenolic, and selenium compounds (Arnault and Auger, 2006; Sallam et al., 2004; Tang and Cronin, 2007). The flavonoids present in onion include quercetin and anthocyanin, the major compounds responsible for the antioxidant activity of onion (Iqbal and Bhanger, 2007). Powdered onion is widely distributed and used as a spice and a food in the majority of countries (Do et al., 2004).

Foodborne pathogens have been found in powdered food with low water activity (Ha and Kang, 2013; Kim et al., 2014). Microbial contamination of powder ingredients, including pepper powder, is frequently the cause of outbreaks of foodborne infection (Centers for Disease Control and Prevention (CDC), 2010). Sagoo et al. (2009) reported that some dried spices and herbs sampled during production and at retail stores contained high counts of *Bacillus cereus* ($\geq 10^5$ CFU/g) and/or *Escherichia coli* ($\geq 10^2$ CFU/g). The various toxins excreted by fungi are major risk factors for mycotoxicoses in human and animals (Reddy et al., 2010). *Aspergillus brasiliensis* causes postharvest disease in agricultural products (Li et al., 2013), produces fumonisin B_2 and ochratoxin A (Ouf et al., 2014), and is one of the most common fungi contaminating foods.

Generally, γ-irradiation, superheated steam, and ultraviolet (UV) have been used for microbial decontamination of powdered food products such as spice and dry ingredients (Schweiggert et al., 2007). However, food products treated by irradiation or those

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containing irradiated ingredients are not popular in some countries due to public perception and regulations (Stefanova et al., 2010). Superheated steam treatment of powdered spice is also effective in reducing microbial contamination of powdered products but may cause noticeable deterioration of its sensory and nutritional properties (Barbieri et al., 2004). UV treatment is generally recognized as an ineffective method for decontaminating microorganisms in powder products (Song et al., 2015; Taylor et al., 1995). Thus, a new method of decontaminating powdered products, which minimizes effects on their inherent sensory and nutritional properties, has been sought.

Cold plasma (CP) treatment (CPT), a nonthermal food preservation method (Niemira, 2012a), has the potential to inactivate microorganisms in powdered products without altering their sensory properties (Hertwig et al., 2015; Kim et al., 2014). Microorganisms are inactivated by the chemical interaction of reactive species in CP, including excited oxygen (O_2) and nitrogen (N_2), reactive oxygen species, electrons, ions, and free radicals, with cell membranes by damaging the membranes and internal cellular components by UV photons and by production of lesions on the cell surface (etching) due to radical bombardment (Lee et al., 2015; Niemira, 2012b).

Corona discharge, dielectric barrier discharge, plasma jet, and microwave discharges are commonly used to generate CP (Toshifuji et al., 2003). Microwave CP discharges are formed by electromagnetic waves with frequencies exceeding hundreds of megahertz (Scholtz et al., 2015). Microwave-induced plasma produces more energetic electrons than do lower-frequency sources (Iza et al., 2007; Kwon et al., 2012). Combined treatment with argon plasma and a microwave power density of 4.21 W/cm^3 for 30 min reduced *E. coli* numbers on a polypropylene tube by 6.29 log (Purevdorj et al., 2002).

Although recently many studies have demonstrated the antimicrobial effects of CPTs, little is known about their effect against microorganisms in powder food products. Microwave-powered CPT was evaluated in this study for decontamination of problematic microorganisms in onion powder and for enhancing its quality after storage. We also hypothesized in this study that the microbial inactivation effect of CPT would be enhanced when the treatment was conducted at a higher microwave power density due to the combined effects of CP and microwaves on microorganisms. Thus, the objectives of this study were to (1) investigate the effects of CPTs at different levels of microwave density on the inactivation of *B. cereus* spores and the volatile profiles of onion powder, (2) investigate the effects of treatment time and CP generation power on the inactivation of *B. cereus* spores, (3) determine the effects of the different drying methods generally used for drying onion powder on the inactivation of *B. cereus* spores and *A. brasiliensis* spores by CPT, and (4) assess the effects of CPT on the safety and storage stability of onion powder by investigating the growth of *B. cereus*, *A. brasiliensis*, and *E. coli* O157:H7 and the changes in antioxidant activity, quercetin content, and color properties of the powder during storage at 4 and 25 °C.

2. Materials and methods

2.1. Onion powder preparation

Fresh onions (*Allium cepa* L.) were purchased from a local store. They were peeled manually, washed in aqueous sodium hydrogen carbonate solution (8 g/100 g), prepared in sterile distilled water, and dried at 23 ± 2 °C for 2 h. The onions were then cut into pieces (12 × 12 mm) using a food dicer (U3A, Emura Food Machine Co., Nagoya, Japan). Cut onion (30 g) was spread evenly on a stainless steel tray and then dried to 8± 2% relative humidity (RH) by either hot-air drying at 100 °C for 8 h using an oven dryer (C-DF, Chang Shin Scientific Co., Seoul, Korea) or vacuum drying at 65 °C and 28 kPa for 8 h using a vacuum dryer (Fisher Isotemp Vacuum Oven Model 281, Fisher Scientific, Pittsburgh, PA, USA). Dried onion was punched into a square of 5 × 5 mm to prepare samples. The moisture content of onion was determined using a moisture content analyzer (i-Thermo 163 L, Bel Engineering Inc., Milan, Italy).

2.2. Microbial strains and preparation of inoculum subculture

B. cereus ATCC 10876, ATCC 13061, and W-1 strains were obtained from the Agricultural Biotechnology Culture Collection at Seoul National University (Seoul, Korea). Spores were prepared according to the method of Finley and Fields (1962). Cultures grown on tryptic soy agar (TSA, Difco) were incubated for 7 days at 37 °C until more than 80% of the cells had sporulated, as determined by microscopic examination. Pooled suspensions were transferred to a 15 mL tube, incubated at 80 °C for 10 min in a water bath (including a 1 min warm-up) and then washed three times with peptone water (0.1 g peptone/100 mL) by centrifugation at 3600 ×*g* at 4 °C for 20 min. Equal volumes of each strain were mixed to prepare a microbial cocktail at ~10^8 spores/mL. The cocktail was diluted to produce a final working concentration of ~10^6 spores/mL using sterile peptone water.

A. brasiliensis (KCCM 160404) was obtained from the Korea Culture Center for Microorganisms (Seoul, Korea) and cultured for 5 days at 20 °C on potato dextrose agar (PDA, Difco) acidified with tartaric acid (Sigma-Aldrich, St. Louis, MO, USA) solution (10 g tartaric acid/100 mL). After culturing, 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 transferred to a sterile 15 mL tube (SPL Life Science Co., Pocheon, Korea). The suspension was filtered through a two-layer sterile cloth, and the spore concentration was determined using a hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Konigshofen, Germany).

E. coli O157:H7 was obtained from the Agricultural Biotechnology Culture Collection at Seoul National University (Seoul, Korea). The strains were grown at 37 °C for 24 h prior to subculturing in TSA twice. After overnight subculturing, the cell pellets were washed three times with peptone water (0.1 g peptone/100 mL) by centrifugation (GyroSpin, Gyrozen Co. Ltd., Seoul, Korea) at 4000 ×*g* for 15 min at 22 °C and suspended in peptone water (0.1 g peptone/100 mL) at a concentration of ~10^9 CFU/mL.

2.3. Microbial inoculation

Areas of onion powder of 0.5 × 0.5 cm were prepared in a 3 × 3 array on a Petri dish and exposed to UV for 30 min to decontaminate background microorganisms. A 1–2 μL inoculum was spotted onto the surface of each piece of powder; 10 μL was applied to all of the nine pieces in total. These inoculated powder samples were dried in a laminar flow biohazard hood (SterilGARD, Baker Company, Inc. Sanford, ME, USA) for 1 h at 22 ± 2 °C before treatment.

2.4. CPT

CPT was performed using the SWU-2 CPT system (Seoul Women's University, Seoul, Korea) described previously by Kim et al. (2014). The treatment samples were placed in position A or B (Fig. 1) for low microwave density CPT (LMCPT) or high microwave density CPT (HMCPT). Positions A and B were 0 and 24 cm above the square Teflon plate (Fig. 1). The microwave power density values at the two positions were estimated to 170 and 250 m W·m^{-2}, respectively, by simulation using COMSOL