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Microbial decontamination of red pepper powder by cold plasma

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ABSTRACT

Effects of the microwave-powered cold plasma treatments (CPTs) on the inhibition of microorganisms in red pepper powder, including *Aspergillus flavus* and *Bacillus cereus* spores, were investigated. Combinations of heat treatment with CPT were investigated for the inhibition of *B. cereus* spores on the powder. The number of *A. flavus* was reduced by 2.5 ± 0.3 log spores/g by the CPT with nitrogen at 900 W and 667 Pa for 20 min. CPT at 900 W and 667 Pa for 20 min inhibited naturally occurring total aerobic bacteria in the red pepper powder by approximately 1 log CFU/g. *B. cereus* spores were inhibited (3.4 ± 0.7 log spores/g reduction) only when heat treatment at 90 °C for 30 min was integrated with the CPT using a helium-oxygen gas mixture at 900 W. Fermi's model and Weibull model adequately described the inhibition of *A. flavus* on the red pepper powder by the CPT. The changes in treatment temperature and water activity were less than 5.0 °C (initial temperature: 23.8 °C) and 0.22, respectively, and were affected by both treatment power and time ($P < 0.05$). The CPTs have demonstrated the potential to reduce the microbial counts of red pepper powder and other powder products.

1. Introduction

Red pepper (*Capsicum annuum* L.) is cultivated worldwide and consumed fresh or in dried powdered form as a food ingredient (Rico et al., 2010). Red pepper powder is primarily used to impart a bright red color with a pungent taste and enhance the flavor of many processed food products (Akbas and Ozdemir, 2008; Rico et al., 2010). Red pepper powder is of agricultural origin and therefore is generally highly contaminated by microorganisms before any decontamination processes (Buckenhuskes and Rendlen, 2004; Oularbi and Mansouri, 1996). The use of contaminated red pepper powder can result in rapid spoilage of the foods to which the powder was applied. Pathogenic microorganisms, including *Aspergillus flavus*, *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus* are often present in red pepper powder (Aydin et al., 2007; Buckenhuskes and Rendlen, 2004).

Fumigation with ethylene oxide, irradiation, steam heat sterilization, and ultraviolet (UV) treatments are used to decontaminate undesirable microorganisms in red pepper powder (Schweiggert et al., 2007). Fumigation with ethylene oxide, the technique used for the longest period, effectively inhibits microbes. However, its use is prohibited in many countries due to carcinogenicity (Fowles et al., 2001; Schweiggert et al., 2007). Gamma irradiation at 2–7 kGy has been shown to effectively decontaminate various spices (Farkas, 1998). However, owing to public fear and the legal regulation for the products treated by irradiation or those containing any irradiated ingredients, irradiated red pepper powder is unpopular in some countries. An alternative method is thermal treatment using superheated steam. However, this method is expensive and causes the powder to undergo undesirable sensory and nutritional changes (Moisan et al., 2001a). Although steaming is effective for decontamination, the treatment is usually applied before grinding of dried pepper. Thus, the product can be re-contaminated during grinding (Schweiggert et al., 2007) and requires an additional decontamination step prior to packaging. UV lamps have been installed in many powder production lines but do not inhibit the growth of microbes effectively during production (Fine and Gervais, 2004; Sharma and Demirci, 2003). The ineffectiveness of the UV radiation could be due to the lack of penetration, the strong dependence on the distance from the UV source, and/or significant absorption of irradiation by glass and plastics, which can result in non-homogeneous microbial decontamination (Song et al., 2010).

CPT, which generates plasma by gas excitation with electron discharge without a marked temperature increase, has been investigated as a non-thermal food preservation method (Niemira, 2012; Perni et al., 2008). Plasma consists of highly energetic species that break covalent bonds and initiate various chemical reactions. These species include UV photons, electrons, positive and negative

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ions, free radicals, and excited or non-excited molecules and atoms, which in combination can inhibit microorganisms, more effectively (Fernandez et al., 2011; Song et al., 2010). Diffusion of the reactive species through cell membranes can cause severe damage by reacting with macromolecules, such as membrane lipids, proteins, and nucleic acids. Electrons, ions, and free radicals can also cause surface erosion and localized lesions in the cell membrane; these result in inhibition of microorganisms. The formation of surface erosion and localized lesions may also facilitate penetration of the reactive species into cells, which can enhance microbial inhibition (Gallagher et al., 2007). The UV also can cause DNA modifications and consequent improper cell replication (Bolshakov et al., 2004). Oxidation of cell membranes and amino acids due to reactive oxygen and nitrogen species, including atomic oxygen, ozone, hydroxyl, nitric oxide, and nitrogen dioxide, is also an important mechanism of microbial inhibition mediated by cold plasma (Laroussi and Leipold, 2004).

Although several studies have demonstrated the microbial inhibition effects of CPTs, little is known about their effect on microorganisms in foodstuffs, particularly powder food products. Thus, the objectives of this study were to (1) investigate the microbial inhibition effects of CPT on the inhibition of naturally-occurring aerobic microorganisms in red pepper powder and *A. flavus* and *B. cereus* spores inoculated on the powder using a microwave-powered CPT system; (2) optimize treatment conditions for the inhibition of *A. flavus*; (3) evaluate deterministic models of *A. flavus* inhibition by CPT; and (4) assess the effect of CPT integrated with heat treatment against *B. cereus* spores.

2. Materials and methods

2.1. Red pepper powder

Red pepper powder (*C. annuum* L.) was purchased from a local store. The powder was prepared using the following steps: red pepper harvested in 2012 in Goesan (Korea) was washed in water and cut in half after removing the stalk. The half-cut red pepper was dried for 2 h using a far-infrared dryer at 83 °C and ground into fine powder (>1.7 mm). The powder was exposed to UV (320 W, 10 s) prior to packaging.

2.2. Microbial strains and preparation of inoculum subculture

A. flavus (ATCC 200026, American Type Culture Collection, Manassas, VA, USA) was cultured for 5 days at 20 °C on potato dextrose agar (PDA, Difco, Sparks, MD, USA) acidified with 10% tartaric acid (Sigma–Aldrich, St. Louis, MO, USA). Sterile distilled water (10 mL) containing 0.1% Tween 80 was added to the PDA for growth of *A. flavus*. The PDA surface was gently scraped with an inoculation loop and the content was transferred to a sterile 15-mL tube (SPL Life Science Co., Pocheon, Korea). The tube was shaken vigorously to liberate spores. The shaken suspension was filtered through sterile cloth and spores filtered were enumerated using a hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Konigshofen, Germany). Spore density was adjusted by dilution with 0.1% peptone water (Difco).

B. cereus ATCC 10876, ATCC 13061, and W-1 were obtained from the Agricultural Biotechnology Culture Collection at Seoul National University (Seoul, Korea). *B. cereus* vegetative cells were cultured for 24 h at 37 °C in tryptic soy broth (TSB, Difco), harvested by centrifugation at 10,000 rpm for 2 min, and washed twice with 0.1% (w/v) sterile peptone water. The pellets of each strain were resuspended in 0.1% peptone water, corresponding to approximately 10^8 CFU/mL. The cocktail was prepared by combining equal amounts of each strain, and diluted with 0.1% peptone water to produce the desired inoculum concentration. The method of Finley and Fields (1962) was adopted to prepare *B. cereus* spores. Cell suspensions grown for 24 h at 37 °C in TSB (0.1 mL) were spread onto tryptic soy agar (TSA, Difco) and incubated for 7 days at 37 °C until at least 80% of the cells sporulated, as determined by microscopic examination. Spores were harvested by depositing 2 mL of 0.1% (w/v) sterile peptone water onto the surface of TSA culture plates. Spores were dislodged by gently rubbing with a sterile loop. Pooled suspensions of five plates of each strain were transferred to a 15-mL tube and heated in an 80 °C water bath for 10 min (including a 1-min warm-up time). Heat-treated suspensions were centrifuged at 3600 × *g* at 4 °C for 20 min and washed three times with 0.1% peptone water. The pellet of each strain was resuspended in 0.1% peptone water. A microbial cocktail was prepared by combining each strain in equal proportions to produce an inoculum of approximately 10^8 spores/mL. The cocktail was diluted in 0.1% (w/v) sterile peptone water to produce the desired inoculum concentration.

2.3. Inoculation and sample preparation

Red pepper powder (200 g) in a 250-mL glass bottle was autoclaved for 15 min at 121 °C. The powder (5.0 g) was spread evenly over a 16 cm × 16 cm area of the surface of a Teflon plate (25 cm × 25 cm). A 1.0-g suspension of *A. flavus* or *B. cereus* spores was inoculated onto the powder using a sterile glass sprayer (BT1270S-100, Joylab Co., Seoul, Korea) and then dried in a laminar flow biohazard hood for 1 h at 22 ± 2 °C. Non-autoclaved and non-inoculated powder (5.0 g) was also prepared to evaluate the effect of CPT on naturally occurring aerobic microorganisms.

2.4. Cold plasma treatment system

The SWU-2 CPT system, illustrated in Fig. 1, consists of a microwave generator, a cooling system, a treatment chamber, a gas mass flow rate controller, a vacuum pump, and a parameter controller (Fig. 1). The magnetron (Magnetron 2M246, LG electronics Inc., Seoul, Korea) in the microwave generator produces a 2.45-GHz wave discharge operated at the 50–1000 W power levels. The treatment chamber is of stainless steel and has dimensions of 43 cm (width) × 37 cm (height) × 40 cm (length) with a fused silica (quartz) observation window. Cooling water flows at 0.8 m^3/min. The plasma-forming gas flows at a maximum of 20 slm (standard liter/min), which is controlled by a gas mass flow-rate controller (2 channels, Model 3660, Kojima Instrument Inc., Osaka, Japan). The pressure in the chamber ranges from 500 to 30,000 Pa, adjusted by a vacuum valve (Model 2-way electric ball valve, DongjooAP, Incheon, Korea). The parameter controller monitors and regulates supplied power (treatment power), gas flow rates, and pressure in the treatment chamber (treatment pressure).

2.5. Cold plasma treatments (CPTs)

2.5.1. Determination of the conditions for forming stable plasma

The conditions used for formation of stable plasma for the treatment of red pepper powder were determined for each plasma-forming gas treatment power and pressure. The feed gases for plasma emission were nitrogen (N_2), an N_2-oxygen (O_2) mixture (N_2:O_2 = 99.3:0.7), helium (He), and a He–O_2 mixture (He:O_2 = 99.8:0.2). The formulations for the mixtures of N_2–O_2 and He–O_2 were selected based on the studies of Pintassilgo et al. (2007) and Hong et al. (2009), respectively. The gases were dried and filtered. The power and pressure ranges were 300–900 W and 267–26,680 Pa, respectively. Powder (5.0 g) was evenly spread on the Teflon plate and treated with cold plasma. Plasma was observed through the observation window (Fig. 1).