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Effects of processing parameters on the inactivation of *Bacillus cereus* spores on red pepper (*Capsicum annum* L.) flakes by microwave-combined cold plasma treatment

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ABSTRACT

The efficacy of microwave-combined cold plasma treatment (MCPT) for inactivating *Bacillus cereus* spores contaminating red pepper (*Capsicum annum* L.) flakes was investigated. The effects of red pepper drying method, particle size, and water activity (a_w) were also evaluated at two levels of microwave power (1700 and 2500 W/cm^2). The inactivation effect of MCPT was higher at higher microwave power. Spore reduction was more effective with vacuum-dried red pepper than far-infrared-dried flakes. A significantly higher level of spore reduction was observed with the red pepper sample with a smaller surface to volume ratio when one surface (exterior surface) was inoculated ($p < 0.05$). Spore reduction by MCPT at high microwave power increased from 1.7 to 2.6 log spores/cm^2 when the a_w of flake increased from 0.4 to 0.9 ($p < 0.05$). MCPT did not change the color of red pepper flakes. MCPT demonstrated potential as a microbial decontaminating technology for red pepper flakes.

1. Introduction

Red pepper (*Capsicum annum* L.) is cultivated worldwide, and is widely used as a food or a food ingredient (Rico et al., 2010). It is known to lend a characteristic flavor and pungency to food. This dried spice has long been used in food mixtures, salad dressings, instant soups, frozen pizzas, and many other convenient foods (Arslan and Özcan, 2011). Although the use of red pepper is prevalent in the food industry, contamination of red pepper by pathogens is a food safety concern. Red pepper, an agricultural plant, is easily exposed to mesophilic bacteria (Rico et al., 2010; Schweiggert et al., 2007). Indeed, many studies have reported the presence of pathogens in red pepper powder, for example, *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus* (Banerjee and Sarkar, 2003; Buckenhiiskes and Rendlen, 2004). Red pepper contaminated by these bacteria may cause food-borne illnesses, especially if it is consumed without undergoing microbial decontamination (Kim et al., 2012; Rico et al., 2010).

A recent study reported that most dried red pepper was contaminated by *B. cereus* (Choo et al., 2007). *B. cereus* is a Gram-positive bacteria that occurs naturally in food and soil (Imatake et al., 2009). It belongs to pathogenic groups along with *B. anthracis* among *Bacillus* species (Ash et al., 1991). Ingesting food contaminated by *B. cereus* or its toxins can result in health concerns, including diarrhea, vomiting, abdominal cramps, and nausea (Rajkovic, 2014). *B. cereus* forms spores that are resistant to thermal and drying treatments, and can thus survive cooking and storage (Berthold-Pluta et al., 2015; Rajkovic, 2014). Therefore, the control of *B. cereus* and its spores in food material, including red pepper, is an important issue in food safety. Various measures, including fumigation, irradiation, and heat treatment, have been used to control pathogenic microorganisms in food such as red pepper (Fine and Gervais, 2004; Moisan et al., 2001; Schweiggert et al., 2007). However, these methods are associated with several issues, including health concerns, public fear, property (e.g., color) deterioration, and low efficacy.

Cold plasma treatment (CPT) is a non-thermal process that uses plasma (ionized gases) generated by gas excitation with released electrons (Niemira, 2012; Perni et al., 2008; Selcuk et al., 2008). Plasma is the source of highly reactive agents that include UV photons, electrons, ions, free radicals, and excited or non-excited molecules and atoms (Fernández et al., 2012; Song et al., 2009). These plasma-derived species can break covalent bonds and thereby cause chemical reactions, contributing to the destruction of pathogen cell membranes without thermal activity (Fernández et al., 2012; Song et al., 2009). Helium (He) has been used as a plasma-forming gas, and was reported to

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produce stable plasma at the broadest levels of pressure and plasma-generation power of microwave-powered CPT, compared with nitrogen gas and nitrogen-oxygen mixtures at different ratios (Kim et al., 2014). This suggests that He is a favorable gas for forming plasma in microwave-powered CPT for research.

Microwave irradiation has been widely used in food processing for the purposes of cooking, thawing, baking, and microbial inactivation (Tang, 2015). In particular, its inactivation effects on pathogenic microbes have received a great deal of attention for the pasteurization or sterilization of food (Tang, 2015). Microbial inactivation by microwave treatment is achieved by heating of cellular materials by the vibration of dipolar molecules (such as water) induced by electric energy (magnetic fields). Several studies also support the non-thermal effects of microwave treatment for microbial destruction (Khalil and Villota, 1986; Khalil and Villota, 1989; Kozempel et al., 1998). However, the underlying mechanism for the nonthermal microbial inactivation of microwave treatment has not yet been revealed and thus the controversy over the existence of the effect of nonthermal microwave treatment has not been settled (Bhattacharjee and Delsol, 2014). The effect of microwave treatment on microbial inactivation can allow for a more efficient process if it is combined with other intervention technologies (Kim et al., 2017).

In the present study, a novel combined treatment of microwave and cold plasma was investigated for the inactivation of *B. cereus* spores. The effects of drying method, particle size, and water activity (a_w) of red pepper flakes, and the microwave power of the treatment, were assessed in terms of their effects on the efficacy of *B. cereus* spore inactivation.

2. Materials and methods

2.1. Red pepper

Fresh red pepper (*Capsicum annuum* L.) was purchased from a local store (Goesan, Nonghyup, South Korea) and the red pepper uniform in color and free from fungal decay was prepared as the lab-scale sample. Briefly, red pepper was washed with running deionized water once and trimmed into pieces (6.0 cm in diameter) using a sterile cheese borer. The cut sample was dried to 12.1 ± 1.7% (w/w) moisture content using the method (conditions) used in the red pepper industry, far infrared drying (7–20 μm, 85 °C, 6 h) or vacuum drying (50 kPa, 85 °C, 2 h). Dried red peppers were further cut into smaller pieces (3.0 × 1.5 cm) and exposed to ultraviolet (40 W) for 20 min per each side (40 min in total) in a laminar flow biohazard hood (SterilGARD; Baker Company, Inc. Sanford, ME, USA) to reduce the background microbial load before inoculation. The total mesophilic aerobic bacteria counts of the sterilized peppers, determined using plate count agar (PCA), fell below the level of detection (< 10 CFU/g).

2.2. Preparation of B. cereus spore inoculum

Three diarrheal strains of *B. cereus*, ATCC 10876, ATCC 13061, and W-1 (Forghani et al., 2015; Kim et al., 2005; Sastalla et al., 2013), were used to prepare a spore inoculum. These strains were received from the Agricultural Biotechnology Culture Collection at Seoul National University (Seoul, South Korea). *B. cereus* spores were prepared according to the method of Finley and Fields (1962). The bacterial cells, which were grown for 24 h at 37 °C in 0.1 ml tryptic soy broth (TSB, Difco, Sparks, MD, USA), were spread onto tryptic soy agar (TSA, Difco). They were then incubated at 37 °C for 7 days for sporulation. The sporulation temperature was selected because it did not lower the amount of sporulation. Cell sporulation was monitored via microscopic analysis until around 80% of the cells were sporulated. Spores on the TSA were harvested using sterile peptone water [2 ml, 0.1% (w/v)] and a sterile loop. A spore suspension pooled from five TSA plates was transferred to a conical tube and incubated in a water bath (80 °C) for 10 min. The pellets of spores of each strain were collected via centrifugation at 3600 × *g* at 4 °C for 20 min, after which the pellets were washed three times and resuspended in 0.1% (w/w) sterile peptone water. A *Bacillus* spore cocktail was prepared by combining spores from each strain in equal proportions to a concentration of approximately 10^8 spores/ml. The cocktail was diluted to the desired inoculum concentration that resulted in the number of *Bacillus* of ~10^6 spores/cm^2 on red pepper in the absence of CPT, which is equivalent to the level used in a previous study (Kim et al., 2014).

2.3. Inoculation

Red pepper cut into pieces 3.0 × 1.5 cm in size was dried, either by far infrared drying or vacuum drying, to the level of a_w 0.5. *B. cereus* spores (10^6 spores/cm^2) were inoculated on the outer (waxed) surfaces of red pepper using a sterile glass sprayer (BT1270S-100, Joylab Co., Seoul, Korea) and then dried in the biohazard hood for 1 h at 22 ± 2 °C. The results from a preliminary study demonstrated higher inactivation of *B. cereus* spores on waxed surfaces than on unwaxed ones. To assess the effect of particle size, spore-inoculated red pepper was further cut into 0.5 × 0.5 cm or 1.5 × 1.5 cm pieces before microwave-integrated CPT.

2.4. Microwave-integrated CPT (MCPT)

CPT was performed with the SWU-2 (Seoul Women's University, Seoul, South Korea), as described previously by Kim et al. (2014). The treatment samples were located in the treatment chamber such that they were exposed to a certain level of microwave at the same time when they were subjected to cold plasma. Low-microwave-power-density cold plasma treatment (LMCPT) was conducted on a Teflon plate (Fig. 1), where the microwave power density of plasma was estimated to be 1700 W/cm^2; high-microwave-power-density cold plasma treatment (HMCPT) was executed above the plate, where the power density was predicted to be 2500 W/cm^2 (Fig. 1). LMCPT can be referred to a general microwave-powered CPT, operated at 900 W with a 2.45-GHz wave discharge (Kim et al., 2014). The microwave density was predicted by a simulation using COMSOL Multiphysics (COMSOL 4.4; COMSOL, Inc., Palo Alto, CA, USA) as previously described by Kim et al. (2017). The plasma-forming gas used in the study was helium (He). The flow rate of the gas was controlled at 1 standard l/min. The plasma generation power, time, and pressure in the treatment chamber were 900 W, 20 min, and 0.7 kPa, respectively. The selection of the plasm-forming gas and those treatment conditions was made based on our previous reports (Kim et al., 2014). During LMCPT or HMCPT, the surface temperatures of red peppers were measured using an infrared thermometer (DT 44L; DIAS Infrared GmbH, Dresden, Germany) calibrated with a thermocouple (Type K, 1.6-mm diameter; Fisher Scientific, Hampton, NH, USA). The set-up for the thermometer was previously reported (Kim et al., 2014).

2.5. Effects of drying method on the inactivation of B. cereus spores in red pepper

The cut red peppers (3.0 × 1.5 cm) were evenly spread on stainless steel trays, and were dried to 13.2 ± 2% using either far-infrared (7–20 μm, 85 °C, 6 h) or vacuum drying (50 kPa, 85 °C, 2 h). The moisture content was measured using a moisture content analyzer (Thermo 163L; BEL Engineering S.R.L., Monza, Italy). The exterior surfaces of dried red pepper were inoculated and then the red pepper was further cut into 0.5 × 0.5 cm sections. The red pepper flakes were treated by either LMCPT or HMCPT. Microbial analysis was conducted after treatment as described below.