



# Evaluation of cold plasma treatments for improved microbial and physicochemical qualities of brown rice

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## ABSTRACT

This study evaluated the microbial and physicochemical characteristics of brown rice (BR) treated with cold plasma. Cold plasma was generated in a plastic container (250 W, 15 kHz, ambient air) and the cold plasma was applied to BR samples for periods of 5, 10 and 20 min. When BR samples were inoculated with *Bacillus cereus*, *Bacillus subtilis*, and *Escherichia coli* O157:H7, a 20 min plasma treatment resulted in a reduction in bacterial counts by approximately 2.30 log CFU/g. The pH of the BR decreased slightly after the 5 min plasma treatment. BR with hunter color L\* showed an increase in pH, and the a\* and b\* values decreased as a result of the plasma treatment. The  $\alpha$ -amylase activity and water uptake rate increased significantly ( $p < 0.05$ ), while hardness decreased significantly ( $p < 0.05$ ). The results of this study indicate that cold plasma treatments can improve the microbial quality of BR and produce slight changes to the physicochemical quality of BR.

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## 1. Introduction

Rice is a staple food for nearly half of the world's population and is the second leading cereal in terms of production volume. Asia produces almost 90% of the world's total production of rice (Thirumdas, Deshmukh, & Annapure, 2015). In Korea, rice is consistently considered the country's most valuable food crop, and its production level as a milled rice crop was approximately 4300 kilotons in 2012 and 2013, cumulatively, making it the largest produced food crop (USDA, 2014). Per the regulations of the World Trade Organization (WTO), which was established in 1995, the rice market in Korea was opened in 2015 without a special protective tariff for different countries (Chung, Kim, Lee, & Kim, 2015).

With an increase in rice production and rice imports, it is necessary to study both rice storage and safety. Park, Kim, Park, and Kim (2012) reported that aging during storage results in numerous changes in the chemical and physical properties caused by

microorganisms present in rice. These microorganisms change the pasting properties, color, flavor, and composition of rice, which in turn affects the cooking and eating quality of rice. Due to its ubiquitous distribution in nature, *Bacillus cereus* spores are frequently found in a wide range of foods. Rice-based products and farinaceous foods, such as rice bread and noodles, are frequently contaminated and studies have found that these products often contain *B. cereus* spores (Ha, Kim, & Ha, 2012). Recently, studies have shown that contamination of rice-based food products with *Bacillus subtilis* can underlie food-borne diseases in humans (Fangio, Roura, & Fritz, 2010). Major food-borne pathogens found in food also include *Escherichia coli*, which has increased worldwide over recent years (Bell & Kyriakides, 1999).

Thermal treatment can effectively inactivate pathogens, but this often induces side effects in the sensory, nutritional, and functional properties of foods, especially in fresh products (Deng et al., 2007). To overcome these disadvantages, non-thermal methods, such as chemical treatment, ultraviolet, ionizing radiation, and high pressure processing have been developed. However, these technologies also have some drawbacks, including the high cost of application,

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requirements for specialized equipment, generation of undesirable residues, extended processing times, and lower efficiencies (Yun et al., 2010). Several studies have reported that, although these methods can inhibit surface contamination, they often require novel equipment and produce chemical changes that may cause unacceptable detrimental effects to the food products (Baskaran et al., 2007; Latou, Mexis, Badeka, & Kontominas, 2010).

Cold plasma is known for its excellent antimicrobial and surface engineering properties in a range of fields, including the biomedical, textile, and polymer industries (Laroussi, 2005; Stone & Barrett, 1962). The ability to generate non-thermal plasma discharges at atmospheric pressure makes the decontamination process easier and less expensive. Recently, substantial efforts have been made to develop plasma-based inactivation methods of microorganisms. Cold plasma has been already described as a possible decontamination technology on fruits and vegetables including cucumber, carrot and pear slices experimentally contaminated by *Salmonella* (Wang et al., 2012). Reductions of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* counts have also been reported for apples and lettuce (Misra, Tiwari, Raghavarao, & Cullen, 2011). Atmospheric pressure plasma jet was effectively reduced biofilms on collagen casing, polypropylene, and polyethylene terephthalate (Kim et al., 2015). However, the plasma treatment may also result in changes in sensory property of food products which should be overcome (Kim et al., 2015; Yong et al., 2015). Thirumdas, Sarangapani, and Annapure (2015) showed that there are several fields in the food-processing sector where cold plasma can be successfully applied to food products.

Air plasma is an excellent source of reactive oxygen-based and nitrogen-based species, such as O, O<sub>2</sub>, O<sub>3</sub>, OH, NO, and NO<sub>2</sub> (Laroussi, 2009). Schutze et al. (1998) reported that the density of charged species with low-pressure plasma discharge is around 10<sup>8</sup>–10<sup>13</sup> cm<sup>-3</sup>. Furthermore, Chen et al. (2016) and Sarangapani, Devi, Thirundas, Annapure, and Deshmukh (2015) have attempted to identify an efficient plasma system that is optimally suited to maintain rice quality characteristics after plasma treatment. However, there have been no reports on potential microbial (such as *E. coli* and *B. cereus*) reduction in commercial brown rice (BR) after plasma treatment.

Therefore, the objective of this study is to evaluate the microbial safety and possible physicochemical quality changes of commercial BR following application of cold plasma.

## 2. Materials and methods

### 2.1. Sample preparation and plasma application

BR (*Oryza sativa* cv. *Chindeul*), harvested in the Kyungpook province in South Korea 1 day prior to the experiment, which was stored in a refrigerator at 4 °C. The plasma apparatus was used by Kim et al. (2015). Optimum conditions such as treatment time and input power of cold plasma and that condition was used in previous and preliminary study (data not shown). Briefly, a cold dielectric barrier discharge (DBD) plasma source was constructed using a rectangular, parallel-piped, plastic container (137 × 104 × 53 mm). The actuator was made of copper electrodes and a polytetrafluoroethylene sheet was attached to the inner walls of the container. A bipolar square-waveform voltage at 15 kHz to one electrode while the other electrode was grounded. Plasma was generated inside the container with an input power of 250 W. BR was placed in a petri dish at the bottom of the container and the distance between the sample and the plasma generator was 20 mm. The sample was treated with the atmospheric pressure plasma source for 5, 10 and 20 min.

### 2.2. Microbial analysis

The prepared sample (5 g) was mixed for 2 min in a sterile Stomacher bag containing 45 mL of sterile saline solution (0.85%) using a Stomacher BagMixer® 400 (Interscience Co., Saint Nom, France). Total plate count agar was prepared for counting the total number of aerobic microbes (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37 °C for 48 h, and the colony forming units (CFU) per gram were counted at a dilution of 30–300 CFU per plate.

### 2.3. Inoculation test

For the inoculation test, the packed samples were exposed to an irradiation dose of 30 kGy (point source AECL, IR-79; MDS Nordion, Ontario, Canada) using a cobalt-60 irradiator at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongseup, Korea.

Three pathogens, *Bacillus cereus* (KCTC 3624), *B. subtilis* (KCTC 1682), and *E. coli* O157:H7 (KCCM 40406), were used in this study. The pathogens were obtained from the Korean Collection for Type Cultures (KCTC, Jeongseup, Korea) and the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). *B. cereus* and *B. subtilis* were cultivated to mid log phase in nutrient broth (Difco Laboratories), and *E. coli* O157:H7 was cultivated to mid log phase in tryptic soy broth (Difco Laboratories), all at 37 °C for 48 h. The cultures were then centrifuged (3000 rpm for 10 min at 4 °C) using a refrigerated centrifuge (model VS-5500; Vision Scientific Co., Seoul, Korea). The resulting pellet was washed twice with sterile saline solution (0.85%) and re-suspended in the same saline solution. The viable cell density was measured to be approximately 10<sup>8</sup> CFU/mL.

Inoculated samples were plasma-treated and then mixed in a sterile stomacher bag as described above. Serial dilutions were prepared with the sterile saline solution. The media used for recording the growth of *B. cereus* and *B. subtilis* was nutrient agar (Difco Laboratories), and the media used for *E. coli* O157:H7 was tryptic soy agar (Difco Laboratories). Incubation of the plates and colony counting were done as explained above.

In addition, D-value (decimal reduction time; the exposure time required to inactivate 90% of a population) is calculated using the following equation (Haas, Behnsilian, & Schubert, 1996).

$$\log \frac{N}{N_0} = -\frac{t}{D}$$

t = time

N = the number of colonies per unit volume at time t

N<sub>0</sub> = the number of colonies per unit volume at the time t<sub>0</sub> (t<sub>0</sub> = 0)

### 2.4. pH

The pH was measured using a pH meter (Model 750; iSTEC, Seoul, Korea). About 1 g of each sample was added to 10 mL of distilled water, homogenized for 30 s, and the pH was then measured. Calibration was performed using standard buffers provided by the manufacturer at pH 4, 7, and 10 at room temperature.

### 2.5. Color

BR was poured into a petri dish and the color of the BR was evaluated using a Color Difference Meter System

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