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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 α-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.

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_2, O_3, OH, NO, and NO_2 (Laroussi, 2009). Schutze et al. (1998) reported that the density of charged species with low-pressure plasma discharge is around 10^8–10^{13} cm^{-3}. 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, Jeongeup, 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, Jeongeup, 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 10^8 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, Behsnilian, & Schubert, 1996).

$$\mathrm{Log}\frac{N}{N_0} = -\frac{t}{D}$$

t = time
N = the number of colonies per unit volume at time t
N_0 = the number of colonies per unit volume at the time t_0 ($t_0 = 0$)

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

(Spectrophotometer CM-3500d; Konica Minolta Sensing, Inc., Osaka, Japan). The Hunter color values, L* (lightness), a* (redness), and b* (yellowness) was determined. The instrument was calibrated with a standard black and white plate before analysis. The Hunter values were monitored by a computerized system using SpectraMagic software (Konica Minolta Sensing, Inc.) and the measurements were performed in triplicate.

2.6. Assay of α-amylase

Activity of α-amylase by following the method described by Chen et al. (2016). One gram of BR was ground in 10 mL of 0.1 M acetate buffer, pH 4.75, homogenized for 30 min, and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was used for the enzyme assay. Enzyme extract (0.25 mL) was taken and diluted to 1 mL. One milliliter of a 5% starch solution was added and this was incubated for 60 min at 37 °C. Two milliliters of DNS regent (1% dinitrosalicylic acid in 0.2 M sodium hydroxide and 30% sodium potassium tartrate) were added and kept this solution in a boiling water bath for 5 min. After cooling in water, absorbance was measured at 540 nm (Infinite M200, TECAN, Inc., Zürich, Switzerland). For the controls, 1 mL double-distilled water was used in place of the enzyme extract. A standard graph was calculated from the standard curve and expressed as mg glucose/g fresh weight. All of the measurements were conducted in triplicate.

2.7. Water uptake

BR (2 g) was soaked in test tubes containing 20 mL distilled water in an incubator (25 °C). After soaking for 6 h, analyses were performed at an interval of 1 h, excess water was drained off and the contents were transferred to filter paper (Whatman No. 2; Amersham, Buckinghamshire, UK) to remove surface moisture. The samples were then weighed and the water uptake ratio was calculated per the procedure of Singh, Kaur, Singh Sodhi, and Singh Sekhon (2005).

2.8. Texture analysis

The texture of the BR was analyzed using testXpert II, a texture analyzer from Zwick Roell (Ulm, Germany). The software used was Texture Expert Exceeds. A cylindrical probe sms P/4 was used for measuring the texture. The force was measured in terms of compression (N). The instrument was calibrated with a 50 kg load cell. The test speed was 2 mm/s and the probe was allowed to compress 20% of the strain into the sample.

2.9. Statistical analyses

The data were analyzed using SPSS for software (ver. 18.0; SPSS Inc., Chicago, IL, USA). The statistical analyses were performed using one-way analysis of variance (ANOVA). When significant differences were detected, the differences among the mean values were determined by performing the Duncan's multiple comparison test at a confidence level of $p < 0.05$. Mean values and standard errors of the mean are reported.

3. Results and discussion

3.1. Microbial analyses

The initial amount of total aerobic bacteria on the BR was 4.10 log CFU/g (Fig. 1). Using the cold plasma treatment, this amount was significantly reduced ($p < 0.05$) to 3.49, 3.19 and 2.70 log CFU/g after being performed for 5, 10 and 20 min, respectively.

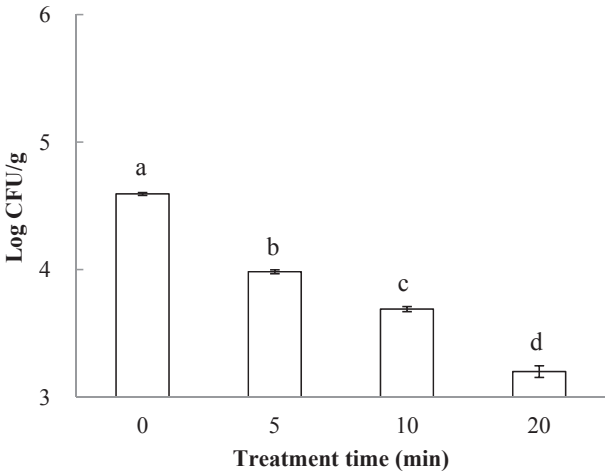

Fig. 1. Inactivation of total aerobic bacterial count with cold plasma, [a-e] Different letters indicate significant differences among the treatments ($p < 0.05$).

Sarrias, Valero, and Salmeron (2003) studied the effects of electron beam irradiation on *B. cereus* reduction, and found a reduction from 3.40 log CFU/g to undetectable levels using 3.2 kGy in unhusked rice, and from 1.43 log CFU/g to undetectable levels with 1.1 kGy in husked rice. Kim, Lee, Park, and Rhee (2013) reported that a combination treatment of fermented ethanol (30%) and SC-CO_2 (44 °C, 200 bar, 30 min) can significantly improve the microbiological safety of rice and rice-based products, simultaneously resulting in beneficial changes to the overall quality of the rice.

When inoculated, *B. cereus* and *B. subtilis* were initially loaded at levels of 4.29 and 4.23 log CFU/g, respectively. The application of cold plasma reduced the number of microorganisms to 1.30 and 1.29 log CFU/g for *B. cereus* and *B. subtilis*, respectively (Fig. 2). *E. coli* O157:H7 bacteria were initially loaded at levels of 5.26 log CFU/g. After cold plasma treatment for 5, 10, and 20 min. *E. coli* O157:H7 bacteria were significantly reduced by 4.23, 3.26, and 2.96 log CFU/g, respectively (Fig. 2). The calculated D-values of this plasma against *B. cereus*, *B. subtilis* and *E. coli* O157:H7 were 15.43, 15.49, and 8.70 min on the BR, respectively. Thus, longer time was required to inactivate *B. cereus* and *B. subtilis* than *E. coli* O157:H7 using plasma. This difference among pathogens may be due to microbiological parameters (e.g. Gram positive or negative). Gram-negative bacteria such as *E. coli* O157:H7 possess a unique outer membrane in their cell envelope and could be more vulnerable than Gram-positive bacteria such as *B. cereus* and *B. subtilis*, which have a thick peptidoglycan structure on the outside of the cell that is resistant to chemical changes. Lee et al. (2012) reported that the outer membrane of Gram-negative bacteria exhibited structural damage following exposure to cold plasma, whereas Gram-positive bacteria did not show the same degree of morphological changes.

This DBD can create specific types of reactive oxygen species (ROS), such as oxygen atoms, ozone, metastable oxygen molecules, peroxide, superoxide, and hydroxyl radicals, all of which are bactericidal. These ROS have strong oxidizability and are prone to interact with the bacteria cells (Birmingham, 2004; Yun et al., 2010). Ma, Zhang, Shi, Xu, and Yang (2008) reported that ROS play a central role in the inactivation of microorganisms treated with plasma. Suhem, Matan, Nisoa, and Matan (2013) showed that cold atmospheric plasma treatments appeared to be inhibitory against *Aspergillus flavus* both in an agar medium and in BR cereal bars. Hury, Vidal, Desor, Pelletier, and Lagarde (1998) reported that oxygen, H_2O_2 and CO_2-based plasmas were more effective than

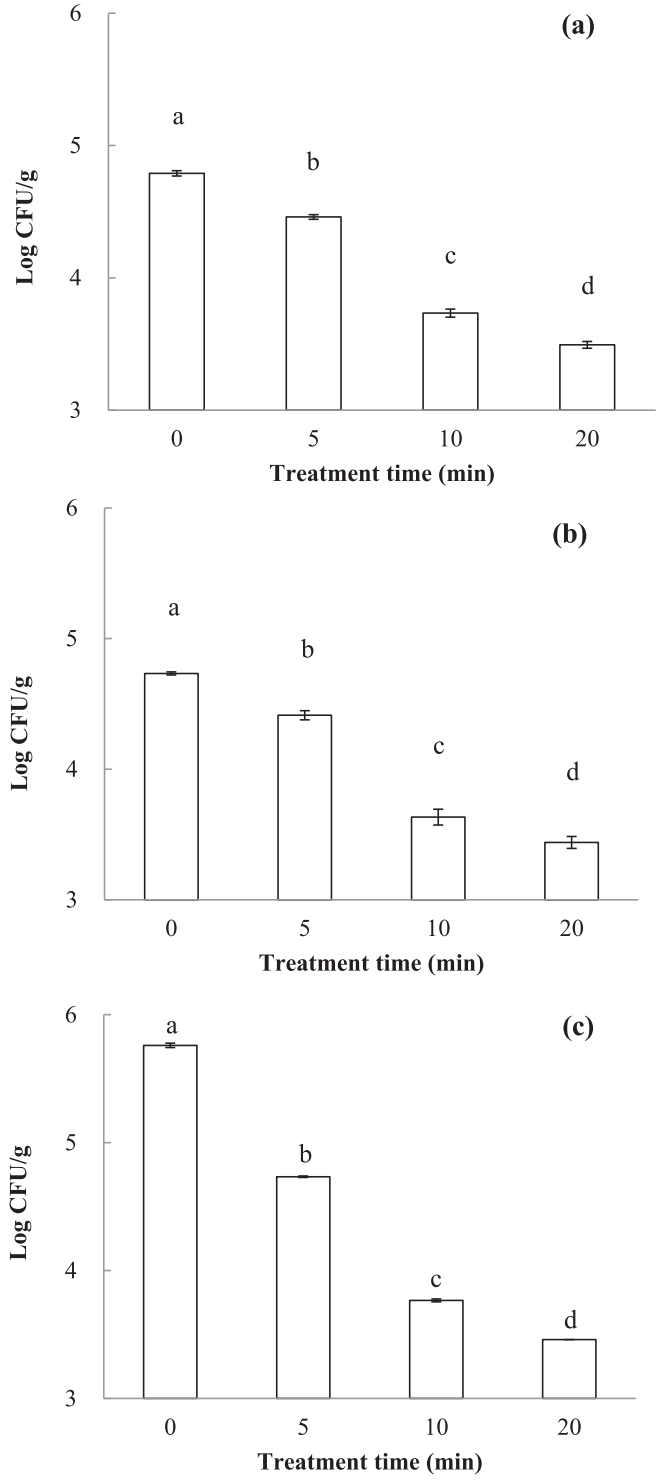

Fig. 2. Inactivation of microorganisms inoculated on agar plates with cold plasma: (a) *Bacillus cereus*, (b) *Bacillus subtilis*, (c) *Escherichia coli* O157:H7; [a-e] Different letters indicate significant differences among the treatments ($p < 0.05$).

argon plasma. Namely, oxygen-based plasma destroys microorganisms via slow combustion with the oxygen atoms and oxygen-containing radicals present in the plasma (Kim et al., 2013). This result suggests that adding oxygen could increase the inactivation rate due to increased production of active radicals (Gweon, Kim, Moon, & Choe, 2009).

Table 1
Hunter color values of brown rice treated with cold plasma.

	Treatment time (min)				SEM[a]
	0	5	10	20	
L	56.89 ± 0.07d	57.77 ± 0.08b	57.40 ± 0.08c	58.28 ± 0.12a	0.072
a	5.69 ± 0.07a	5.50 ± 0.01b	5.32 ± 0.03c	5.52 ± 0.08b	0.044
b	25.24 ± 0.14a	25.00 ± 0.03b	24.75 ± 0.08c	24.89 ± 0.09bc	0.074

a–d Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard error of the mean (n = 3).

3.2. Color

The lightness values were higher for the treated samples than for the control samples, while a slight decrease in the yellowness and redness were observed for the treated samples. With an increase in time of plasma treatment the whiteness index was increased from 56.89 to 58.28 (Table 1). A whitening of the rice (an increase of lightness and a decrease of yellowness) is attractive to consumers (Kim et al., 2013). Moisture content is known to correlate with lightness, such that moisture may explain the change in the L* value (Sanabria, Martín-Álvarea, & Carrascosa, 2004). Dutta and Mahanta (2012) found that the lightness value was directly correlated with the extent of gelatinization during parboiling. Thirumdas, Deshmukh et al. (2015) reported that the lightness values, yellowness, and redness of basmati rice increased with increasing plasma treatment time.

3.3. pH

Typically, pH is important in determining a system's decontamination capabilities (Gurol, Ekinci, Aslan, & Korachi, 2012). Following treatment with cold plasma, the pH of the BR decreased slightly (Table 2). However, no difference among the plasma treatment times was observed. The significant decrease ($p < 0.05$) in pH after the indirect plasma treatment may be attributed to acidogenic molecules such as NO_x that are normally generated in air plasma (Soffels, Sakiyama, & Graves, 2008). It was observed a slight decrease in the pH of the BR due to the action of the plasma (Bruggeman et al., 2008). Liu et al. (2010) suggested that this can be attributed to the multistep reactions of the plasma-generated reactive species, including NO_x, O, and O_3, with water at the gas/water interface (the quasi-steady gas cavity surface, as well as on the surfaces of micro droplets of liquids inside the gas cavity).

3.4. α-amylase activity

α-amylase (EC3.2.1.1, 1,4-α-D-glucan glucano-hydrolase, endoamylase), which hydrolyzes starch, glycogen, and related polysaccharides by randomly cleaving internal α-1,4-glucosidic linkages, is an important industrial enzyme (Shaw, Lin, Chen, &

Table 2
pH changes in brown rice following treatment with cold plasma.

Treatment time (min)	pH
0	7.18 ± 0.02a
5	7.11 ± 0.02b
10	7.11 ± 0.01b
20	7.11 ± 0.02b
SEM[a]	0.012

a, b Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean (n = 3).

Table 3
α-amylase activity (U/g) in brown rice following treatment with cold plasma.

Treatment time (min)	α-amylase activity
0	3.49 ± 0.08b
5	4.21 ± 0.37a
10	3.99 ± 0.34a
20	3.90 ± 0.31ab
SEM[a]	0.212

a, b Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean (n = 3).

Table 4
Hardness (N) of brown rice following treatment with cold plasma.

Treatment time (min)	Hardness
0	116.24 ± 6.73a
5	110.28 ± 6.01b
10	107.03 ± 5.47bc
20	104.60 ± 7.56c
SEM[a]	1.767

a–c Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean (n = 27).

Chen, 1995). Briefly, α-amylase enzyme activity is a factor that affects the sweet taste of rice via converted sugars arising from the degradation of starch (Lee, Choi, Hwang, & Song, 2015).

The activity of α-amylase measured in plasma-exposed BR revealed significantly higher values ($p < 0.05$) than the unexposed controls in most stages (Table 3). The activity of α-amylase significantly increased at 5, 10 and 20 min, with a maximum value 1.21-fold greater than the unexposed controls after 5 min of exposure. Chen et al. (2016) reported that α-amylase activity of plasma-exposed germinated BR revealed significantly higher values than the unexposed controls in most stages. The bran layer of BR is a water-resistant material containing fat, fiber, protein, and ash (Khatoon & Gopalakrishna, 2004). However, plasma treatments resulted in the bran layer of the BR developing fissures, an effect that was increased by α-amylase activity. Chen, Chen, and Chang (2012) reported that scanning electron microscopy (SEM) images of the brown rice surface revealed that plasma treatment resulted in the rice bran losing its natural morphology. The bran surface of untreated brown rice is compact and shows the natural morphological structure. After 1 and 2 kV plasma treated, the surface showed a pattern of wide and shallow destruction, while the pattern was narrow and deep on the rice treated at 3 kV. After the plasma treatment the surface of grain showed fissures and depressions, similar type of effect was seen in case of plasma treated brown rice by Thirumdas, Deshmukh et al. (2015).

In our study, when cold plasma treatment time increased, the activity of α-amylase decreased slightly. Thus, additional investigations are required to determine the mechanisms that are responsible for these results.

3.5. Water uptake

Significant linear increases in the moisture content of regular BR were observed progressively with increases in the soaking time, and the maximum quantity was 21.42% after 5 h of soaking (Table 5). The hydration curves of BR treated with plasma showed a rapid increase in the water content during the first 3 h of soaking. In addition, BR treated with plasma showed higher water absorption than regular BR for all soaking times, and the maximum value was 24.78% after 5 h of soaking. The water uptake ratio was found to increase, which correlates with a reduction in cooking time (Mohapatra & Bal, 2006). The changes in the microstructure of the rice bran layers caused by the plasma treatment allowed water to easily penetrate the BR kernel (Chen et al., 2012), which resulted in an increased water absorption ratio during treatment and cooking. The effectiveness of the plasma treatment was enhanced by increased treatment time, which is reflected in a further reduction in the optimal cooking time.

3.6. Hardness

The acceptable texture of cooked BR is mostly governed by its consumption as a whole grain. Hardness is an important parameter for the evaluation of cooked BR texture (Ghasemi, Mosavian, & Khodaparast, 2009). Our results showed that the hardness measures of BR after cold plasma treatment were significantly lower than for untreated BR (Table 4). Sarangapani et al. (2015) showed that the cold plasma affected the hardness of parboiled rice and decreased according to plasma power and time of treatment. Thirumdas, Sarangapani, et al. (2015) also reported a similar decrease in hardness. Plasma treatments can be used effectively to reduce the cooking time required for BR (Chen, 2014). Notably, α-amylase activity and the water absorption rate are both deemed to decrease the hardness of BR, which is often avoided despite adding nutritional benefit.

4. Conclusion

In our study, cold plasma is capable of improving the microbial safety of BR against microorganisms such as *B. cereus*, *B. subtilis*, and *E. coli* O157:H7. Briefly, plasma effectively inactivates microorganisms inoculated into BR. Furthermore, plasma treatments can effectively increase water uptake and α-amylase activity while decreasing the pH value of BR. Cold plasma suitably modified the textural properties of BR, leading to reduction in hardness. It can be concluded that the use of cold plasma, a novel technology in whole

Table 5
Water uptake of brown rice following treatment with cold plasma.

Treatment time (min)	Soaking time (h)							
	0.5	1	2	3	4	5	6	SEM [a]
0	5.48 ± 0.87Bg	9.97 ± 0.50Af	14.83 ± 0.29Be	15.78 ± 0.31Cd	21.06 ± 0.28Cc	22.46 ± 0.56Bb	23.61 ± 0.68Ba	0.440
5	8.15 ± 0.58Ag	10.48 ± 0.88Af	16.45 ± 0.54Ae	19.10 ± 0.60Ad	22.55 ± 0.76Bc	25.25 ± 0.54Ab	26.62 ± 0.69Aa	0.544
10	7.13 ± 0.57Ad	11.33 ± 1.04Ac	17.24 ± 1.07Ab	18.27 ± 0.55ABb	25.60 ± 0.73Aa	25.00 ± 0.94Aa	25.92 ± 0.90Aa	0.697
20	7.30 ± 0.28Af	11.01 ± 0.59Ae	16.30 ± 0.99ABd	17.83 ± 0.58Bc	21.63 ± 0.70BCb	24.42 ± 1.00Aa	24.42 ± 0.56Ba	0.582
SEM [a]	0.499	0.640	0.646	0.428	0.530	0.646	0.585	

a–g Different online letters within the same row indicate significant differences ($p < 0.05$).
A–C Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean (n = 3).

grain processing, not only maintained the microbial safety of BR, but also provided a better textural quality. However, before applying this technology to the wider food industry, more detailed studies are recommended to assess other qualities, such as the sensory impact of cooked BR after cold plasma treatment.

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