



Effect of cold atmospheric pressure plasma-activated water on the microbial safety of Korean rice cake

Jin-Young Han^{a,b,1}, Won-Jae Song^{a,b,1}, Joo Hyun Kang^c, Sea C. Min^c, Sangheum Eom^d, Eun Jeong Hong^d, Seungmin Ryu^d, Seong bong Kim^d, Sangwoo Cho^e, Dong-Hyun Kang^{a,b,*}

^a Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul, 08826, Republic of Korea

^b Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, 25354, Republic of Korea

^c Department of Food Science and Technology, Seoul Women's University, 621, Hwarangro, Nowon-gu, Seoul, 01797, Republic of Korea

^d Plasma Technology Research Center, National Fusion Research Institute, Gunsan-si, Jeollabuk-Do, 54004, Republic of Korea

^e Pulmuone Co., Ltd., B1, Yonsei Engineering Research Park, Yonsei Univ., 150, Yonsei-ro, Seodaemun-gu, Seoul, 03722, Republic of Korea

ARTICLE INFO

Keywords:

Cold atmospheric plasma
Plasma activated water
Microbial safety
Foodborne pathogen
Korean rice cake

ABSTRACT

We evaluated the effect of plasma-activated water (PAW) on the populations of foodborne pathogens and food-spoilage microorganisms on Korean rice cake. PAW was produced by treating distilled water with two atmospheric dielectric-barrier discharges (51.7 W, 14.4 kHz, air discharge) for 20 min. The inactivation effect of PAW on indigenous microorganism and inoculated *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes*, *Penicillium chrysogenum* and *Candida albicans* on Korean rice cake were measured and their effect on the color and texture values of the samples were determined. PAW treatment for 20 min reduced the numbers of the three foodborne pathogens by about 2.0 log CFU/g. PAW treatment for 20 min reduced the numbers of total aerobes, *P. chrysogenum*, and *C. albicans* by 2.78, 1.97, and 1.00 log CFU/g, respectively. The antimicrobial effect of PAW was not significantly different from 0.2 ppm of sodium hypochlorite solution, which is used in Korean rice cake production. PAW treatment did not affect the color, texture, or pH of Korean rice cake. Therefore, PAW treatment can be used as an alternative method to reduce safety risk and spoilage of Korean rice cake without deteriorating its quality.

1. Introduction

Rice cake, which is made of rice flour, is a traditional Korean food consumed during traditional festivals and ceremonies. Rice cakes may also contain beans, red beans, honey, sesame, pine nuts and pumpkin. Rice cake was formerly consumed as a snack, but is now considered a meal by many people in the Republic of Korea (ROK). Therefore, efforts have focused on developing functional rice cakes by adding, for example, yacon powder (Lee & Shim, 2010), sunflower seeds (Lim, Kang, & Kim, 2008), broccoli powder (Cho, 2009) and rosemary powder (Gwon & Moon, 2009).

Generally, rice cakes are believed to be safe from microbial contamination because they are steamed, boiled, or fried at a high temperature. However, there were six foodborne outbreaks due to rice cake in the ROK in 2000 (Park et al., 2001), and coliform contamination of

rice cake has been reported (Jeong et al., 2012; Lee & Jang, 2008). Thus, efforts have focused on ensuring the microbiological safety of rice cake. Lee, Lee, and Rhim (2000) reported that treatment with a combination of 1% lactic acid and 1% chitosan reduced the total microbial count of white rice cake by 2.5 log CFU/g. In the case of food quality, however, the combination treatment of lactic acid and chitosan reduced overall acceptability of rice cake significantly ($P < 0.05$). Sensorial test was evaluated by panels consisted of 20 members from the Department of Food Engineering, Mokpo National University, and scores were obtained by sensory attributes using the following 5-point scoring method: 5, very good; 4, good; 3, fair; 2, poor; 1, very poor. Kang, Park, Lee, and Kum (2013) showed that grapefruit seed extracts inhibit the growth of microorganisms on rice cake during 30 days storage at room temperature (25 °C). However, grapefruit seed extracts reduced sensory properties (taste and odor) of rice cake. Sensory evaluation was

* Corresponding author. Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Seoul National University, Gwanak-ro 1, Gwanak-gu, Seoul, 08826, Republic of Korea.

E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

¹ These authors contributed equally to this work.

conducted by 30 panelists (researchers of Korean Food Research Institute) by using 9-point hedonic scales: 9, very good; 1, very poor.

Gas-discharge-generated non-thermal plasma triggers reactive species formation. Furthermore, plasma-generated reactive oxygen species (ROS; e.g., ozone, atomic oxygen, superoxide, peroxide, and hydroxyl radical), reactive nitrogen species (RNS; e.g., nitrite, nitrate, and nitric oxide), ultraviolet (UV) radiation, and charged particles exert bactericidal effects (Graves, 2014; Guo, Huang, & Wang, 2015; Scholtz, Pazarova, Souskova, Khun, & Julak, 2015). Non-thermal plasma inactivates microorganisms by damaging DNA (via UV), degrading proteins and lipids (via ROS/RNS), and disrupting membranes (via charged particles) (Liao et al., 2017; Misra, Tiwari, Raghavarao, & Cullen, 2011).

Plasma activated water (PAW) is produced by plasma discharge in or above the surface of water (Ma et al., 2015). The reactive species in PAW, including hydrogen peroxide, ozone, and superoxide and hydroxyl radicals, mediate its bactericidal effect. Xu, Tian, Ma, Liu, and Zhang (2016) reported that treatment with PAW for 10 min reduced bacteria and fungi on button mushroom to about 1 log CFU/g. Xiang et al. (2019) reported that aerobic bacteria, yeast, and mold populations on mung bean sprout were reduced by 2.32 and 2.84 log CFU/g, respectively, by PAW treatment for 30 min.

Korean rice cake production includes several washing process with water containing NaOCl to ensure its microbial safety. However, washing with chlorinated water can generate toxic by-products such as trihalomethanes and chloramines (Richardson et al., 1998). Thus, PAW may be useful for washing rice cakes, as an alternative to the current use of chemical sanitizer (Thirumdas et al., 2018).

To assess the feasibility of PAW as an alternative to chlorinated water, we evaluated the antimicrobial activity of PAW against food-borne pathogens, fungi, and yeasts on Korean rice cake (*tteokbokki tteok*). We also investigated the physicochemical properties of PAW and its effects on the pH and color of rice cake.

2. Materials and methods

2.1. Bacterial strains

Three strains of each of *Escherichia coli* O157:H7 (ATCC 35150, 43889, and 43890), *Salmonella* Typhimurium (ATCC 19585, 43971, and 700408) and *Listeria monocytogenes* (ATCC 15315, 19111, and 19115) were obtained from the bacteria culture collection of Seoul National University (Seoul, Korea). Stock cultures were prepared by mixing 0.7 ml of tryptic soy broth (TSB; BD, Sparks, MD, USA) cultures (24 h, at 37 °C) with 0.3 ml of sterile 50% glycerol, and were stored at -80 °C. Working cultures were streaked onto tryptic soy agar (Difco, BD), incubated at 37 °C for 24 h, and stored at 4 °C.

The food-spoilage fungus *Penicillium chrysogenum* (ATCC 18226) was obtained from the Korean Culture Center for Microorganisms (KCCM). Fungal working cultures were streaked onto yeast mold agar (YM agar; Difco, BD) adjusted to pH 3.0 with lactic acid (Daejung Chemical & Metal, Korea) incubated at 25 °C for 5 days, and stored at 4 °C.

The food-spoilage yeast *Candida albicans* (ATCC 10231) was obtained from the KCCM. Stock cultures were prepared by mixing 0.7 ml of a yeast mold broth (YM broth; Difco, BD) cultures (48 h, at 25 °C) with 0.3 ml of sterile 50% glycerol, and were stored at -80 °C. Working cultures were streaked onto YM agar (pH 3.0), incubated at 25 °C for 48 h, and stored at 4 °C.

2.2. Preparation of cell suspensions

The *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* strains were cultured in 5 ml of TSB at 37 °C for 24 h, harvested by centrifugation at 4,000 × g for 20 min at 4 °C, and washed three times with sterile 0.2% peptone water (PW; BD). The pellets were resuspended in

sterile 0.2% PW to approximately 10⁸–10⁹ CFU/ml. Subsequently, suspensions of each strain of the three pathogens were combined to produce a mixed culture cocktail. The final population of inoculated pathogens were approximately 6.3–6.8 log CFU/g.

A suspension of *P. chrysogenum* was prepared using glass beads (425–600 μm; Sigma-Aldrich Corp., St. Louis, MO, USA) in 0.1% Tween 80 (Sigma-Aldrich Corp.). Three grams of glass beads and 20 ml of 0.1% Tween 80 solution were transferred onto YM agar (pH 3.0) and the agar medium was vigorously agitated for 2 min using the Spindle apparatus to detach microorganisms from the surface (Kim, Kim, & Kang 2015, 2016). Finally, the fungal suspension was transferred to a sterile 50 ml centrifuge tube. The final population of inoculated *P. chrysogenum* were approximately 5.70 log CFU/g.

C. albicans was cultured in 5 ml of YM broth at 25 °C for 48 h, harvested by centrifugation at 4000 × g for 20 min at 4 °C, washed three times in sterile 0.2% PW, and resuspended in sterile 0.2% PW. The final population of inoculated *C. albicans* were approximately 4.86 log CFU/g.

2.3. Sample preparation and inoculation

Experiments were performed using commercially processed Korean rice cake (*tteokbokki tteok*) purchased at a local grocery store (Seoul, ROK) and stored in a refrigerator at 4 °C. About 80 g of Korean rice cake samples (ten pieces) were aseptically transferred onto aluminum foil. For inoculation, 1 ml of culture was applied over the entire rice cake surface using a sterile glass spreader, and samples were dried for 2 h in a biosafety hood at room temperature (22 ± 1 °C).

2.4. PAW preparation and treatment

The plasma device consisted of a powered electrode, ground electrode, and dielectric plate (1 mm Al₂O₃) between two electrodes and was used to generate a surface dielectric barrier discharge (SDBD) at 8 kV, a frequency of 14.4 kHz, and a power of 51.7 W. To produce PAW, 1 L of distilled water (DW) was exposed to two SDBDs in atmospheric air for 20 min (Fig. 1).

Both uninoculated (initial population of total aerobes were 7.2 log CFU/g) and pathogen-inoculated Korean rice cake (80 g) was treated with 1 L of PAW or sterile DW (control) for up to 40 min using a MaXshake OB-2 Digital Orbital Shaker (Daihan Scientific, Gangwon-do, ROK) at 195 rpm. In order to compare its sanitizing effect, we also applied 0.2 ppm sodium hypochlorite, the same concentration as in the water used to wash Korean rice cake, with same PAW treatment conditions.

2.5. Evaluation of the physicochemical properties of PAW

To measure pH, oxidation-reduction potential (ORP), and electrical conductivity, 1 l of DW was exposed to two SDBD in atmospheric air for 0 (control), 5, 10, 15, or 20 min pH was measured using a FiveEasy™ Plus pH meter (Mettler Toledo, Schwerzenbach, Switzerland), and the ORP and electrical conductivity were measured using a multimeter (K5000-CP; iSTEK, Seoul, ROK).

2.6. Measurement of the color values, pH and firmness of Korean rice cake

After 10 and 20 min of treatment with PAW or DW with shaking at 195 rpm, the color, pH, and firmness of Korean rice cake were assayed. The CIELAB color values (L*, a*, b*) of Korean rice cake were measured using a Chroma Meter CR-400 colorimeter (Minolta Camera Co., Osaka, Japan). To determine the pH, treated Korean rice cakes were cut at 2 mm thickness and immersed in sterile DW at 4 °C for 30 min. The pH of the DW was measured using a pH meter. The firmness of Korean rice cake was measured using a TA-XT2i Analyzer with a 3-mm-diameter cylindrical probe (Stable Microsystem Co, Ltd, Surrey, UK) at three

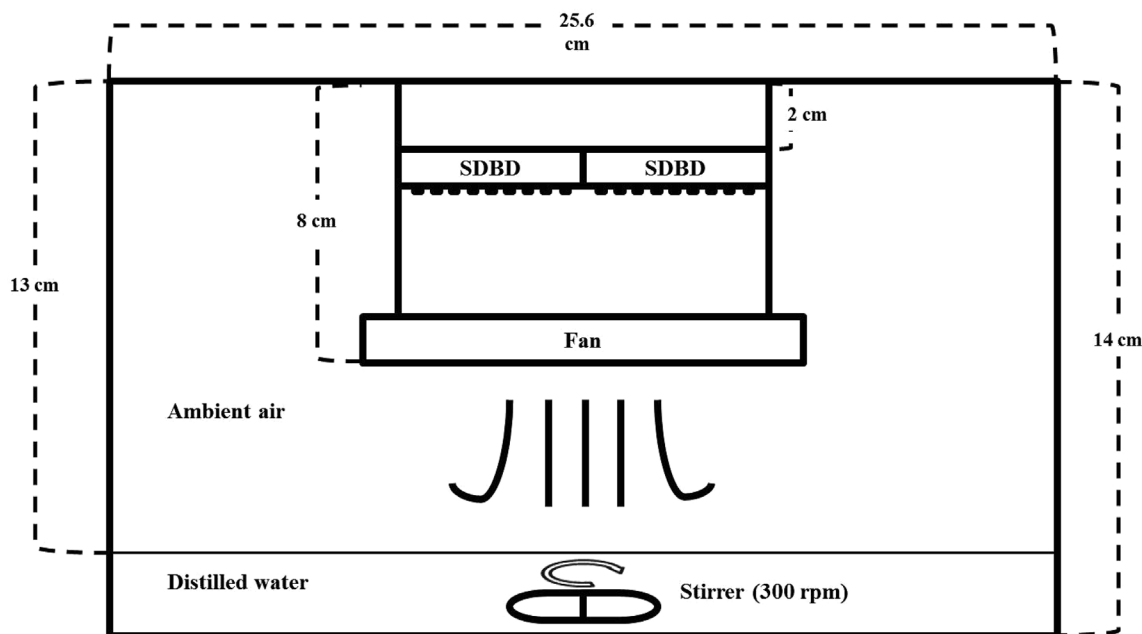


Fig. 1. Schematic of the experimental system used to produce PAW at Seoul National University (Seoul, Republic of Korea).

locations. The operating parameters, pre-test speed, test speed, post-test speed and compression strain, were 2.00 mm/s, 1.00 mm/s, 2.00 mm/s, and 40%, respectively.

2.7. Statistical analysis

The data were subjected to one-way analysis of variance and Duncan's multiple-range test using Statistical Analysis System software (SAS Institute, Cary, NC, USA) to identify significant differences ($P < 0.05$) in mean values. Microbial counts were \log_{10} -transformed before analysis.

3. Results

3.1. Effects of PAW on microorganisms on Korean rice cake

The effect of PAW and DW on foodborne pathogens and food-spoilage microorganisms on Korean rice cake are shown in Tables 1 and 2. Treatment with PAW for 10, 20, 30 and 40 min the number of reduced *E. coli* O157:H7 by 2.01, 2.01, 2.03, and 2.03 log CFU/g, respectively. Treatment with DW for 10, 20, 30 and 40 min reduced the number of *E. coli* O157:H7 by 0.82, 1.27, 1.13, and 1.00 log CFU/g, respectively (Table 1). The reductions in the number of *S. Typhimurium* and *L. monocytogenes* were similar to that in the number of *E. coli* O157:H7.

Treatment with PAW for 10, 20, 30, and 40 min reduced the total aerobic count by 1.62, 2.76, 2.35, and 2.62 log CFU/g, respectively.

Table 1

Inactivation of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* on Korean rice cake by cold atmospheric PAW and sterile DW.

Treatment time (min)	<i>E. coli</i> O157:H7		<i>S. Typhimurium</i>		<i>L. monocytogenes</i>	
	PAW	DW	PAW	DW	PAW	DW
10	2.01 ± 0.15 Aa	0.82 ± 0.24 Ab	2.12 ± 0.55 Aa	1.13 ± 0.46 Aa	1.98 ± 0.59 Aa	0.77 ± 0.14 Ab
20	2.01 ± 0.26 Aa	1.27 ± 0.49 Aa	2.08 ± 0.53 Aa	1.15 ± 0.15 Ab	2.02 ± 0.49 Aa	1.06 ± 0.24 Ab
30	2.03 ± 0.41 Aa	1.13 ± 0.21 Ab	2.09 ± 0.35 Aa	1.31 ± 0.53 Aa	2.07 ± 0.37 Aa	1.09 ± 0.29 Ab
40	2.03 ± 0.41 Aa	1.00 ± 0.55 Ab	2.08 ± 0.54 Aa	1.02 ± 0.57 Ab	2.17 ± 0.27 Aa	1.08 ± 0.31 Ab

Means ± standard deviation. Means with the same upper-case letter in the same column and lower-case letter in the same row per microorganism are not significantly different ($P > 0.05$).

Treatment with DW for 10, 20, 30, and 40 min reduced the total aerobic count by 1.49, 1.55, 1.61 and 1.66 log CFU/g, respectively (Table 2). Treatment with PAW for 10, 20, 30 and 40 min reduced the number of *C. albicans* by 0.86, 1.00, 1.07 and 1.00 log CFU/g, respectively, and treatment with DW for 10, 20, 30, and 40 min by 1.06, 1.07, 0.92, and 1.28 log CFU/g, respectively (Table 2). The reduction in the number of *P. chrysogenum* was similar to that of the foodborne pathogens. Treatment with PAW and DW for 10–40 min reduced the number of *P. chrysogenum* by 1.63–2.04 and 0.57–0.84 log CFU/g, respectively.

The magnitude of antimicrobial activity followed the order PAW > NaOCl > DW. There was no significant difference ($P > 0.05$) in the antimicrobial effect of PAW and NaOCl on *S. Typhimurium*, total aerobes, and *C. albicans* (Figs. 2 and 3). Also, PAW, DW, and NaOCl exerted similar effects on the number of *C. albicans* on Korean rice cake ($P > 0.05$).

Table 3 shows the effect of exposure to PAW on the number of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* on Korean rice cake for up to 10 min. Treatment with PAW for 2.5 min exerted similar effects on these three microorganisms ($P > 0.05$). Also, PAW induced a greater reduction in the number of these microorganisms as compared to DW.

3.2. Physicochemical properties of PAW and effect of PAW on the quality of Korean rice cake

The changes in pH, ORP, and electrical conductivity of PAW are shown in Table 4 and those in the color values, pH, and firmness of

Table 2Number of total aerobes, *C. albicans*, and *P. chrysogenum* on Korean rice cake treated with cold atmospheric PAW and sterile DW.

Treatment time (min)	Total aerobic count		<i>C. albicans</i>		<i>P. chrysogenum</i>	
	PAW	DW	PAW	DW	PAW	DW
10	1.62 ± 0.51 Aa	1.49 ± 0.28 Aa	0.86 ± 0.17 Aa	1.05 ± 0.47 Aa	1.63 ± 0.27 Aa	0.58 ± 0.14 Ab
20	2.77 ± 0.33 Ba	1.55 ± 0.24 Ab	1.00 ± 0.31 Aa	1.06 ± 0.35 Aa	1.97 ± 0.19 Aa	0.61 ± 0.09 Ab
30	2.35 ± 0.40 Ba	1.61 ± 0.48 Aa	1.07 ± 0.01 Aa	0.91 ± 0.34 ABa	1.85 ± 0.35 ABa	0.76 ± 0.14 Ab
40	2.62 ± 0.42 Ba	1.66 ± 0.31 Ab	1.00 ± 0.24 Aa	1.28 ± 0.27 Ba	2.04 ± 0.27 Ba	0.84 ± 0.21 Ab

Means ± standard deviation. Means with the same upper-case letter in the same column and lower-case letter in the same row per microorganism are not significantly different ($P > 0.05$).

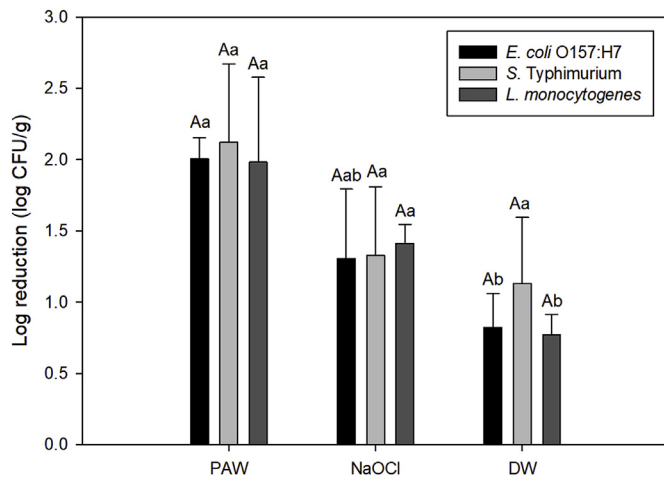


Fig. 2. Inactivation of *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* on Korean rice cake by treatment with PAW, NaOCl or sterile DW for 10 min. Bars labeled with different uppercase letter in the same treatments and lowercase letters within same microorganisms represent significant differences ($P < 0.05$).

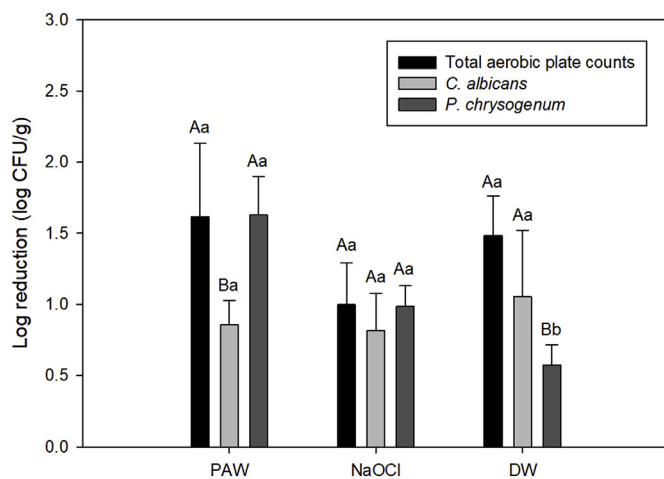


Fig. 3. Effects on the number of total aerobes, *C. albicans*, and *P. chrysogenum* on Korean rice cake with PAW, NaOCl, or sterile DW for 10 min. Bars labeled with different uppercase letter in the same treatments and lowercase letters within same microorganisms represent significant differences ($P < 0.05$).

Korean rice cake in Table 5. The pH of PAW initially decreased and the rate of reduction decreased with increasing treatment duration. In contrast, the ORP and the electrical conductivity of PAW increased with increasing treatment duration. Neither PAW nor DW significantly affected the color values, pH, or firmness of Korean rice cake ($P > 0.05$).

4. Discussion

Non-thermal plasma has been used to inactivate microorganisms on food. Kilonzo-Nthenge, Liu, Yannam, and Patras (2018) reported that treatment with atmospheric cold plasma for 180 s reduced the number of *S. Typhimurium* ATCC 13311 and *E. coli* ATCC 25922 on Golden Delicious apples by 4.8 and 5.3 log CFU/cm², respectively. Yannam, Estifae, Rogers, and Thagard (2018) showed that treatment with high-voltage electrical plasma (30 kV; frequency, 40 Hz) reduced the number of *E. coli* in tangerine juice by > 5 log CFU/ml without affecting product quality. Moreover, after 5 min of treatment with atmospheric cold plasma, the cell density in *L. monocytogenes* and *Pseudomonas fluorescens* biofilm cultured on lettuce at 15 °C for 48 h was reduced to below the limit of detection (1.0 log CFU/ml), which decreased by > 6.0 and > 4.5 log CFU/ml, respectively (Patange et al., 2019).

Compared to gas plasma, PAW results in a lesser reduction in product quality and is easier to apply. Indeed, loss of quality of food, such as surface topography changes due to etching and degradation of bioactive compounds, is minimized by the use of PAW treatment (Thirumdas et al., 2018). In this study, treatment of Korean rice cake with PAW did not affect the color values, pH, and firmness; moreover, PAW is easier to apply than conventional chemical treatments (Thirumdas et al., 2018). Also, PAW is more cost-effective and environmentally friendly than chlorine-based products, which may leave carcinogenic residue (Ma et al., 2015). We found that PAW had similar or better antimicrobial activity than 0.2-ppm NaOCl. Moreover, the residual reactive species dissolved in PAW can be used as a source of nitrite for sausage production (Jung et al., 2015), to enhance seed germination (Naumova, Maksimov, & Khlyustova, 2011), and to accelerate plant growth (Takahata et al., 2015).

Korean rice cake typically does not require sanitization because its production involves thermal treatment (steaming, frying, or boiling). However, there have been foodborne outbreaks due to contaminated rice cake. Park and Ha (2018) reported that *E. coli* is capable of replicating in *garaetteok* (a Korean traditional rice cake) during refrigeration at 10 °C. Prior attempts to ensure the microbial safety of Korean rice cake have involved addition of substances with antimicrobial activity; e. g., green tea and rosemary leaf powders (Lee, Gwon, Kim, & Moon, 2009), curry powder (An, 2009), *Smilax china* L. root extract (Ko et al., 2012) and plantain (Kim, Oh, Jung, & Han, 1999). Lee, Kim, and Paik (2011) reported that use of 100% CO₂ atmosphere suppressed microbial growth during storage of Korean traditional rice cake (*backseolgi*). We found that Korean rice cake was contaminated a high level of aerobic bacteria. Based on its antimicrobial activity, PAW can be used to sanitize or extend shelf-life of Korean rice cake.

The sensitivities of microorganisms to resist interventions vary. Because of the barrier function of their thick cell wall (Zuizina, Patil, Cullen, Keener, & Bourke, 2014), gram-positive bacteria are typically more resistant to sanitization methods and stresses than gram-negative bacteria. Cho, Kim, Kim, Song, and Rhee (2016) reported that gram-positive bacteria are more resistant to marination than gram-negative bacteria. Also, the level of resistance differs among gram-negative

Table 3
Inactivation of foodborne pathogens on Korean rice cake by cold atmospheric PAW and sterile DW for up to 10 min.

Treatment time (min)	<i>E. coli</i> O157:H7		<i>S. Typhimurium</i>		<i>L. monocytogenes</i>	
	PAW	DW	PAW	DW	PAW	DW
2.5	1.57 ± 0.41 Aa	1.12 ± 0.52 Aa	1.97 ± 0.27 Aa	0.93 ± 0.13 Ab	1.68 ± 0.59 Aa	0.94 ± 0.19 Aa
5	1.80 ± 0.07 Aa	0.85 ± 0.48 Ab	2.03 ± 0.39 Aa	1.13 ± 0.20 Ab	1.95 ± 0.55 Aa	0.93 ± 0.40 Ab
7.5	1.86 ± 0.62 Aa	1.26 ± 0.33 Aa	2.14 ± 0.29 Aa	0.82 ± 0.29 Ab	1.85 ± 0.57 Aa	0.89 ± 0.46 Aa
10	1.91 ± 0.16 Aa	0.82 ± 0.24 Ab	2.19 ± 0.26 Aa	1.13 ± 0.46 Ab	1.90 ± 0.16 Aa	0.77 ± 0.14 Ab

Means ± standard deviation. Means with the same upper-case letter in the same column and lower-case letter in the same row per microorganism are not significantly different ($P > 0.05$).

Table 4
Physicochemical properties of cold atmospheric PAW.

Treatment time (min)	pH	Oxidation reduction potential (mV)	Electrical conductivity ($\mu\text{S}/\text{cm}$)
0	7.08 ± 0.09 A	224.58 ± 9.78 A	109.14 ± 1.32 A
5	3.53 ± 0.13 B	251.23 ± 11.69 B	113.91 ± 1.50 A
10	2.86 ± 0.09 BC	414.55 ± 21.46 C	290.89 ± 21.40 B
15	2.58 ± 0.06 C	469.37 ± 10.52 D	608.51 ± 5.49 C
20	2.48 ± 0.02 C	507.23 ± 5.12 E	910.24 ± 10.79 D

Means ± standard deviation. Means with the same letter in the same column are not significantly different ($P > 0.05$).

bacterial taxa. Song et al. (2014) reported that the D-values of *S. Typhimurium* on black and red pepper were greater than those of *E. coli* O157:H7 when treated with gamma irradiation. However, *E. coli* O157:H7 was more resistant to carvacrol and caprylic acid than *S. Typhimurium* (Chung, Cho, & Rhee, 2018; Kim & Rhee, 2015). The antimicrobial efficacy of plasma differs according to the target microorganism. Ziuzina, Patil, Cullen, Keener, and Bourke (2014) reported that *L. monocytogenes* showed more resistance to atmospheric cold plasma than *E. coli* and *S. Typhimurium*. In this study, *C. albicans* showed the highest level of resistance to PAW, in agreement with the report of Klämpfl et al. (2012) that *C. albicans* exhibited a higher level of resistance to PAW than *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The resistance of *C. albicans* to PAW could be due to the chitin and cellulose fibrils in a polysaccharide matrix in its cell wall. *C. albicans* is capable of altering composition and/or thickness of its cell wall to resist the effects of antimicrobials (Klämpfl et al., 2012). In addition, the genome of *C. albicans* has a large number of introns (non-coding regions) and barrier function of the nuclear membrane protects against DNA damage. Moreover, specialized organelles, such as unique ribosomes, may enable *C. albicans* to resist the effects of biocides (Klämpfl et al., 2012).

The pH, ORP, and electrical conductivity of PAW affect its antimicrobial activity. Oehmigen et al. (2011) reported that pH is a determinant of the antimicrobial activity of PAW. Nitrate, nitrite, and hydrogen peroxide, which are generated during the production of PAW, exert a bactericidal effect at $\text{pH} \leq 3.0$. The ORP value can be used to quantify the concentrations and activities of ROS; a higher ORP value indicates a higher ROS concentration (Zhang et al., 2013). Electrical conductivity, i.e., the number of active ions, plays an important role in the inactivation of microorganisms by PAW (Tian et al., 2015). In this

Table 5
Color values, pH and firmness of Korean rice cake treated with cold atmospheric PAW and sterile DW.

Treatment	L*	a*	b*	pH	Firmness (N)
Control	76.03 ± 0.52 A	-1.34 ± 0.11 A	6.27 ± 0.29 A	6.75 ± 0.07 A	37.09 ± 3.11 A
DW 10 min	76.05 ± 0.76 A	-1.51 ± 0.11 A	6.39 ± 0.22 A	6.86 ± 0.27 A	35.84 ± 6.42 A
DW 20 min	76.75 ± 0.19 A	-1.54 ± 0.07 A	6.00 ± 0.26 A	6.75 ± 0.14 A	36.33 ± 3.36 A
PAW 10 min	77.19 ± 1.45 A	-1.44 ± 0.18 A	6.03 ± 0.98 A	6.73 ± 0.15 A	36.14 ± 3.13 A
PAW 20 min	76.31 ± 0.94 A	-1.51 ± 0.05 A	5.93 ± 0.65 A	6.84 ± 0.18 A	35.54 ± 5.17 A

Means ± standard deviation. Means with the same letter in the same column are not significantly different ($P > 0.05$).

study, we treated DW with cold atmospheric plasma for 20 min to produce PAW with a pH of 2.48, ORP of 507.23 mV, and electrical conductivity of 910.24 $\mu\text{S}/\text{cm}$. These values are lower (pH) or higher (electrical conductivity) than or similar (ORP) to those of the PAW used by Ma et al. (2015). In that study, treatment with PAW (activated for 20 min) for 5–15 min reduced the number of *S. aureus* on strawberry by 2.0 log CFU/g.

In conclusion, we evaluated the feasibility of PAW for sanitization of Korean rice cake. Treatments with PAW (generated by exposure for 20 min to two SDBDs for 10 min) reduced the numbers of foodborne pathogens, total aerobes, and *P. chrysogenum* on Korean rice cake by ~2 log CFU/g without affecting the pH or color values. Treatment with PAW for 2.5 min also reduced the number of pathogens by ~2.0 log CFU/g. Therefore, PAW has the potential for use by the Korean rice-cake industry. However, further studies are needed to determine the shortest treatment duration that reduces the number of microorganisms to ~2.0 log CFU/g. PAW treatment inactivated microorganisms on rice cake without altering its pH or color properties, and so has potential for microbial decontamination of rice and possibly other cooked rice-based ready-to-eat products.

Specific author contributions

Jin-Young Han and Won-Jae Song; Designed and performed experiments, analyzed data and co-wrote the paper.

Joo Hyun Kang and Sea C. Min; performed total aerobes microbial experiment, physicochemical properties of the PAW analyses and discussion of the paper.

Sangheum Eom, Eun Jeong Hong, Seungmin Ryu, Seongbong Kim; performed brief discussion, provided PAW system.

Sangwoo Cho; performed brief discussion, provided PAW system and experiment samples (Korean rice cake).

Dong-Hyun Kang; Corresponding author of the manuscript, designed experiments, performed discussion, data analyzing and co-wrote the paper.

Declaration of competing interest

No conflicts of interest to declare.

Acknowledgments

This work was supported by R&D Program of ‘Plasma Advanced Technology for Agriculture and Food (Plasma Farming)’ through the National Fusion Research Institute of Korea (NFRI) funded by the Government funds. This work was also supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) through the High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (318026-03).

References

- An, J. W. (2009). Properties of rice cake for *Toppokki* with curry powder. *Korean Journal of Food and Cookery Science*, 25, 467–473.
- Cho, K. R. (2009). Quality characteristics of *Seolgiddok* added with broccoli (*Brassica oleracea* var. *italica* Plen.) powder. *The Korean Journal of Food and Nutrition*, 22, 229–237.
- Cho, T. J., Kim, N. H., Kim, S. A., Song, J. H., & Rhee, M. S. (2016). Survival of foodborne pathogens (*Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*) in raw ready-to-eat crab marinated in soy sauce. *International Journal of Food Microbiology*, 238, 50–55.
- Chung, D., Cho, T. J., & Rhee, M. S. (2018). Citrus fruit extracts with carvacrol and thymol eliminated 7-log acid-adapted *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes*: A potential effective natural antibacterial agents. *Food Research International*, 107, 578–588.
- Graves, D. B. (2014). Reactive species from cold atmospheric plasma: Implications for cancer therapy. *Plasma Processes and Polymers*, 11, 1120–1127.
- Guo, J., Huang, K., & Wang, J. (2015). Bactericidal effect of various non-thermal plasma agents and the influence of experimental conditions in microbial inactivation: A review. *Food Control*, 50, 482–290.
- Gwon, S. Y., & Moon, B. (2009). The quality of characteristics of *sulgidduk* prepared with green tea or rosemary powder. *Korean Journal of Food and Cookery Science*, 25, 150–159.
- Jeong, S. H., Choi, S. Y., Cho, J. I., Lee, S. H., Hwang, I. G., Na, H. J., et al. (2012). Microbiological contamination levels in the processing of Korea rice cakes. *Journal of Food Hygiene and Safety*, 27, 161–168.
- Jung, S., Kim, H. J., Park, S., Yong, H. I., Choe, W. H., Jeon, H. J., et al. (2015). The use of atmospheric pressure plasma-treated water as a source of nitrite for emulsion-type sausage. *Meat Science*, 108, 132–137.
- Kang, H. J., Park, J. D., Lee, H. Y., & Kum, J. S. (2013). Effect of grapefruit seed extracts and acid regulation agents on the qualities of *Topokkidduk*. *Journal of the Korean Society of Food Science and Nutrition*, 42, 948–956.
- Kilonzo-Nthenge, A., Liu, S., Yannam, S., & Patras, A. (2018). Atmospheric cold plasma inactivation of *Salmonella* and *Escherichia coli* on the surface of golden delicious apples. *Frontiers in Nutrition*, 5, 120.
- Kim, D. K., Kim, S. J., & Kang, D. H. (2015). Comparison of a four-section spindle and stomacher for efficacy of detaching microorganisms from fresh vegetable. *Journal of Food Protection*, 78, 1380–1386.
- Kim, S. J., Kim, D. K., & Kang, D. H. (2016). Evaluation of micro-organism-detaching efficacy from meat samples by spindle or stomacher treatment and quality analysis of suspensions. *Journal of Applied Microbiology*, 120, 946–954.
- Kim, K. H., Oh, S. T., Jung, H. O., & Han, Y. S. (1999). Shelf-life extension of noodle and rice cake by the addition of plantain. *Korean Journal of Food and Cookery Science*, 15, 68–72.
- Kim, S. A., & Rhee, M. S. (2015). Use of caprylic acid to control pathogens (*Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium) in apple juice at mild heat temperature. *Journal of Applied Microbiology*, 119, 1317–1323.
- Klämpfl, T. G., Isbary, G., Shimizu, T., Li, Y. F., Zimmermann, J. L., Stolz, W., et al. (2012). Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. *Applied and Environmental Microbiology*, 78, 5077–5082.
- Ko, Y. J., Kim, J. Y., Kim, E. J., Kim, E. J., Seol, H. G., Park, G. H., et al. (2012). Treatment of *Smilax china* L. root extract for improvement of storage stability of *Mang-gae* rice cake. *Korean Journal of Food Preservation*, 19, 167–172.
- Lee, S. Y., Gwon, S. Y., Kim, S. J., & Moon, B. K. (2009). Inhibitory effect of commercial green tea and rosemary leaf powders on the growth of foodborne pathogens in laboratory media and oriental-style rice cakes. *Journal of Food Protection*, 72, 1107–1111.
- Lee, H. S., & Jang, M. S. (2008). The development of the HACCP plan in Korean rice cake manufacturing facilities. *Korean Journal of Food and Cookery Science*, 24, 652–664.
- Lee, K. A., Kim, K. T., & Paik, H. D. (2011). Physicochemical, microbial, and sensory evaluation of cook-chilled Korean traditional rice cake (*backseolgi*) during storage via various packaging methods. *Food Science and Biotechnology*, 20, 1069–1074.
- Lee, J. W., Lee, H. H., & Rhim, J. W. (2000). Shelf life extension of white rice cake and wet noodle by the treatment with chitosan. *Korean Journal of Food Science and Technology*, 32, 828–833.
- Lee, E. S., & Shim, J. Y. (2010). Quality characteristics of *Sulgidduk* with yacon powder. *Korean Journal of Food and Cookery Science*, 26, 545–551.
- Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X., et al. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control*, 75, 83–91.
- Lim, J. H., Kang, Y. S., & Kim, J. G. (2008). Quality characteristics of *Sulgidduk* supplemented with sunflower (*Helianthus annuus*) seeds. *Journal of the East Asian Society of Dietary Life*, 18, 337–344.
- Ma, R., Wang, G., Tian, Y., Wang, K., Zhang, J., & Fang, J. (2015). Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce. *Journal of Hazardous Materials*, 300, 643–651.
- Misra, N. N., Tiwari, B. K., Raghav Rao, K. S. M. S., & Cullen, P. J. (2011). Nonthermal plasma inactivation of foodborne pathogens. *Food Engineering Reviews*, 3, 159–170.
- Naumova, I. K., Maksimov, A. I., & Khlyustova, A. V. (2011). Stimulation of the germinability of seeds and germ growth under treatment with plasma-activated water. *Surface Engineering and Applied Electrochemistry*, 47, 263–265.
- Oehmigen, K., Winter, J., Hähnel, M., Wilke, C., Brandenburg, R., Weltmann, K. D., et al. (2011). Estimation of possible mechanisms of *Escherichia coli* inactivation by plasma treated sodium chloride solution. *Plasma Processes and Polymers*, 8, 904–913.
- Park, S. Y., & Ha, S. D. (2018). Predictive growth model of the effects of temperature on the growth kinetics of generic *Escherichia coli* in the Korean traditional rice cake product “*Garaetteok*”. *Journal of Food Science & Technology*, 55, 506–512.
- Park, H. O., Kim, C. M., Woo, G. J., Park, S. H., Lee, D. H., Chang, E. J., et al. (2001). Monitoring and trends analysis of food poisoning outbreaks occurred in recent years in Korea. *Journal of Food Hygiene and Safety*, 16, 280–294.
- Patange, A., Boehm, D., Zuzina, D., Cullen, P. J., Gilmore, B., & Bourke, P. (2019). High voltage atmospheric cold plasma control of biofilms on fresh produce. *International Journal of Food Microbiology*, 293, 137–145.
- Richardson, S. D., Thruston, A. D., Caughran, T. V., Collette, T. W., Patterson, K. S., & Lykinds, B. W. (1998). Chemical by-products of chlorine and alternative disinfectants. *Food Technology*, 52, 58–61.
- Scholtz, V., Pazarova, J., Souskova, H., Khun, J., & Julak, J. (2015). Nonthermal plasma – a tool for decontamination and disinfection. *Biotechnology Advances*, 33, 1108–1119.
- Song, W. J., Sung, H. J., Kim, S. Y., Kim, K. P., Ryu, S., & Kang, D. H. (2014). Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in black pepper and red pepper by gamma irradiation. *International Journal of Food Microbiology*, 172, 125–129.
- Takahata, J., Takai, K., Satta, N., Takahashi, K., Fujio, T., & Sasaki, Y. (2015). Improvement of growth rate of plants by bubble discharge in water. *Japanese Journal of Applied Physics*, 54, 01AG07.
- Thirumdas, R., Kothakota, A., Annapure, U., Siliveru, K., Blundell, R., Gatt, R., et al. (2018). Plasma activated water (PAW): Chemistry, physico-chemical properties, applications in food and agriculture. *Trends in Food Science & Technology*, 77, 21–31.
- Tian, Y., Ma, R., Zhang, Q., Feng, H., Liang, Y., Zhang, J., et al. (2015). Assessment of the physicochemical properties and biological effects of water activated by non-thermal plasma above and beneath the water surface. *Plasma Processes and Polymers*, 12, 439–449.
- Xiang, Q., Liu, X., Liu, S., Ma, Y., Xu, C., & Bai, Y. (2019). Effect of plasma-activated water on microbial quality and physicochemical characteristics of mung bean sprouts. *Innovative Food Science & Emerging Technologies*, 52, 49–56.
- Xu, Y., Tian, Y., Ma, R., Liu, Q., & Zhang, J. (2016). Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*. *Food Chemistry*, 197, 436–444.
- Yannam, S. K., Estifae, P., Rogers, S., & Thagard, S. M. (2018). Application of high voltage electrical plasma for the inactivation of *Escherichia coli* ATCC 700891 in tangerine juice. *LWT – Food Science and Technology*, 90, 180–185.
- Zhang, Q., Liang, Y., Feng, H., Ma, R., Tian, Y., Zhang, J., et al. (2013). A study of oxidative stress induced by non-thermal plasma-activated water for bacterial damage. *Applied Physics Letters*, 102, 203701.
- Zuzina, D., Patil, S., Cullen, P. J., Keener, K. M., & Bourke, P. (2014). Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiology*, 42, 109–116.