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Antimicrobial efficacy of cold plasma treatment against food-borne pathogens on various foods

Jin-Young Han[1,2,5,6], **Won-Jae Song**[1,2,5,6], **Sangheum Eom**[4], **Seong Bong Kim**[4] and **Dong-Hyun Kang**[1,2,3]

[1] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea
[2] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do 25354, Republic of Korea
[3] Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea
[4] Plasma Technology Research Center, National Fusion Research Institute, Gunsan-si, Jeollabuk-Do 54004, Republic of Korea
[5] Food Science and Biotechnology Major, Division of Bio-convergence, College of Convergence and Integrated Science, Kyonggi University, Suwon, 16227, Republic of Korea

E-mail: kang7820@snu.ac.kr

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Abstract

The objective of this study was to assess the antimicrobial effects of atmospheric cold plasma decontamination treatment on foodborne pathogens on various foods. The study employed a 30 l chamber using surface dielectric barrier discharge as a plasma source applying an atmospheric pressure and ambient gases. The inactivation rates of three foodborne pathogens, *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes*, were examined in fresh vegetables, fruits, nuts, and powdered food samples in response to cold plasma treatment; several strains of each pathogen were evaluated. The hydrophobicity and surface roughness of selected samples were examined using the water contact angle and non-contact three-dimensional surface profiling measurements, respectively. Samples were then inoculated with the food pathogens and treated in cold atmospheric plasma for up to 20 min. As the treatment time increased, different levels of microbial reduction were observed among the samples and pathogens. Surface roughness was negatively correlated with the inactivation rate. Even surfaces showed higher microbial reduction. Taken together, these results indicate that surface roughness is an important factor for the antimicrobial efficacy of cold plasma treatment.

Keywords: cold plasma, foodborne pathogen, fresh produce, surface hydrophobicity, surface roughness

(Some figures may appear in colour only in the online journal)

1. Introduction

Recently, public demand for fresh produce has increased dramatically [1], as many reports have claimed that raw or minimally processed fresh produce has beneficial effects on human health [2, 3]. However, as the consumption of raw or minimally processed fresh produce has increased, the number of foodborne pathogen outbreaks has also surged [4–6]. Minimally processed or raw fresh produce can become contaminated during harvest or transport, making it a potential carrier for foodborne pathogens [7]. In recent years, there has been an increase in the number of foodborne outbreaks

[6] These authors contributed equally to this work

reported globally, due mostly to *E. coli* O157:H7, *L. monocytogenes,* and *S.* spp [8, 9], these outbreaks have been linked to lettuce, sprouted seed, melon, tomatoes, red pepper, basil, and mixed ready-to-eat salads [9–12].

To prevent foodborne illnesses, chlorine-based sanitizers are widely used in the fresh food industry to remove surface contaminants from produce [13]. In practice, the sanitation dose requires ranges from 50 to 200 mg l^{-1}. However, chlorine-based sanitizers produce highly objectionable odors during processing and tend to leave a residue [14]. There is also public concern over the formation of chlorinated carcinogenic compounds in water with this treatment approach, given that it has also demonstrated only a limited ability to inactivate foodborne pathogens [15]. In this respect, non-thermal cold plasma, a thermodynamically nonequilibrium state of ionized gas, could be an emerging approach for surface decontamination of fresh produce [16].

Cold plasma technology continues to gain popularity as a nonthermal processing technology [17]. Cold plasma treatment is a simple, noninvasive surface decontamination technique; the technology does not require complex conditions compared with treatments such as heat treatment, which deteriorates the biochemical quality of fresh produce [18, 19].

Cold plasma has a non-uniform energy distribution, which resulted in the production of multiple reactive species from many chemical reactions under atmospheric conditions [20, 21]. Reactive species consist of reactive nitrogen species (RNS, nitric oxide, peroxynitrite, etc), reactive oxygen species (ROS, ozone, singlet oxygen, etc) and hydrogen peroxide or OH radicals [21].

The bactericidal mechanism of the cold plasma is still elusive, although reactive species showed major bactericidal agents [22]. The oxidative damage of intracellular components, DNA or cell membrane leads to death. Cell membrane lipids showed high vulnerability to reactive species due to their sensitivity to reactive species [23, 24] Chain reactions occurred lipid peroxidation, cause diffusion of reactive species in the cell, ultimately to cell apoptosis [25].

Numerous studies have investigated cold plasma treatment application to various commodities [26–31]; however, it has been difficult to compare the efficacy of this technique, as the devices used to produce the cold plasma, as well as the experimental conditions, differ considerably among the studies. Additionally, samples exposed to different treatments have different inactivation rates, as evidenced by various investigations [19, 32, 33] of similar apple samples. This study suggests investigating a decontamination efficacy of SDBD by applying identical treatment system to various food samples.

The objective of this study was to verify the antimicrobial effects of cold plasma treatment against foodborne pathogens in fruits, vegetables, nuts, and powdered food and identify its correlations with surface properties. Here, the cold plasma was generated using surface dielectric barrier discharge (SDBD).

2. Material and methods

2.1. Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S. Typhimurium* (ATCC 19585, ATCC 43971, and ATCC 700408), and *L. monocytogenes* (ATCC 15315, ATCC 19114, and ATCC 19115) were obtained from the Bacterial Culture Collection of Seoul National University (Seoul, South Korea). Stock cultures were kept frozen at −80 °C in 0.7 ml of tryptic soy broth (TSB) (Becton Dickinson Difco, Sparks, MD, USA) and 0.3 ml of 50% (vol/vol) glycerol. Working cultures were streaked onto tryptic soy agar (TSA) (BD Difco), incubated at 37 °C for 24 h and stored at 4 °C.

2.2. Preparation of pathogen inoculum

For the inoculum preparation, each strain of the three pathogens was cultured in 5 ml of TSB at 37 °C for 24 h, centrifuged at 4000 g for 20 min at 4 °C, and washed three times with sterilized 0.2% peptone water (BD Difco Bacto, Sparks, MD, USA). The final pellets were resuspended in 0.2% sterile peptone water, corresponding to approximately 10^8–10^9 CFU ml^{-1}. Three strains of *E. coli* (final concentration: ca. 10^7 CFU ml^{-1}) were used in the cold plasma treatment experiments. Subsequently, suspended pellets of each strain of pathogens were combined to produce mixed culture cocktails (nine strains in total). These mixed culture suspensions (final concentration: ca. 10^7 CFU ml^{-1}) were used in the experiments to determine the pathogen inactivation rate.

2.3. Preparation of samples and inoculation

Food samples were divided into four groups: vegetables, fruits, nuts, and powdered foods. Samples were selected to provide a variety of surface properties in terms of surface roughness and hydrophobicity.

Vegetables (cherry tomato, eggplant, leek, carrot, perilla leaf, cabbage, spinach, lettuce, and romaine lettuce), fruits (papaya, apple, honeydew, kiwi, peach, fig, and cantaloupe), nuts (walnut, almond, pine nut, hazelnut, and pistachio), and powdered foods (black pepper, red pepper, mixed grains, powdered infant formula, and flour) were purchased from a local market in Seoul, South Korea. The fresh produce was stored at 4 °C, and nuts and powdered foods were stored at 24 ± 2 °C. All inoculated samples were used immediately in each experiment.

Vegetables and fruit surfaces were cut into 5 cm × 2 cm pieces, and the surface of the produce was wiped using clean tissue paper (Kimtech Science Wipers, Yuhan-Kimberly Inc. Seoul, South Korea) to remove the juice. Prepared samples were placed on aluminum foil inside a laminar-flow biosafety hood. The samples were inoculated with 0.1 ml of a culture cocktail, by depositing droplets with a micropipette in 10 locations. After inoculation, samples were dried again in the laminar-flow biosafety hood for 1 h at 24 ± 2 °C.

Nuts were sorted to remove any damaged shells and kernels before being used for experiments. For surface inoculation, 6 ml of prepared culture cocktail was added to 100 g samples inside sterile stomacher bags (Labplas, Inc. Sainte-Julie, Quebec, Canada), and then mixed thoroughly by hand for 1 min. The inoculated samples were dried for 24 h inside the biosafety hood (24 ± 2 °C) with the fan running until the moisture content and water activity of the samples equaled those of non-inoculated samples. Moisture content was determined using a

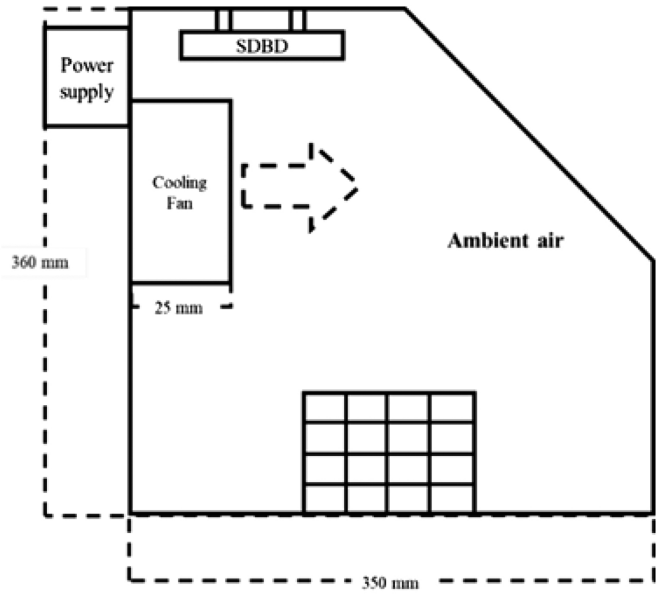

Figure 1. Schematic diagram of the experimental cold plasma system at Seoul National University (Seoul, Republic of Korea).

halogen moisture analyzer (HB43-S, Mettler Toledo, Columbus, OH) and water activity was measured using a water activity meter (AQUALAB 4TE, Meter group, US). The final cell concentration was 10^6–10^7 CFU g^{-1}.

Powdered food inoculation involved using 2 ml of a culture cocktail that was added to 100 g of the sample contained in sterile high-density polyethylene bags (size: 300 mm × 450 mm). The inoculated samples were mixed thoroughly by hand by massaging the bag for 5 min to produce a homogeneous dispersal of inoculum throughout the powdered food. The powdered food samples were then dried for 2 h inside a biosafety hood (24 ± 2 °C) with the fan running until the moisture content and water activity of the sample equaled that of non-inoculated samples. The final cell concentration was 10^5–10^6 CFU g^{-1}.

2.4. Cold plasma system and treatment

Figure 1 shows the experimental setup, which consisted of a cold plasma SDBD system, a power supply, a cooling fan, and a 30 l chamber. The size of the SDBD component was 77 mm × 50 mm (W × D), with a gap between electrodes of 3 mm; the gap was filled with an Al_2O_3 dielectric. In the study, cold plasma system was used under ambient air supplied 220 V AC with 50 Hz. The plasma is generated homogeneously on the SDBD surface by applying a sinusoidal AC voltage of 8 $kV_{p\text{-}p}$ (peak to peak) at 14.4 kHz. The power and power density of the SDBD were 51.7 W and 71.5 W cm^{-3}, respectively. A cooling fan was located 6 cm below the SDBD to cool the system and circulate air inside the chamber. The fan had dimensions of 120 mm × 120 mm × 25 mm (W × D × H) and operated at 2300 revolutions per minute. The chamber was constructed from polyvinyl chloride with dimensions of 345 mm × 350 mm × 360 mm (W × D × H). Prepared samples were placed on a stainless steel treatment grid (height: 75 mm). The grid was positioned in the center of the chamber. For the pathogen inactivation study, inoculated food samples were treated with cold plasma SDBD for up to 20 min.

2.5. Microbiological analysis

At selected time intervals, all treated samples were immediately used for bacterial enumeration. Treated vegetable and fruit samples were transferred into sterile stomacher bags (Labplas Inc.) containing 100 ml of buffered peptone water (BPW). Nuts and powdered foods were transferred into sterile stomacher bags containing 90 ml of BPW (detection limit, 10 CFU g^{-1}) and homogenized for 2 min with a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of samples were 10-fold serially diluted in 9 ml blanks of BPW, and 0.1 ml of sample or diluent was spread-plated onto selective media: Sorbitol MacConkey agar (SMAC; Difco), xylose lysine desoxycholate agar (XLD; Difco), and Oxford agar base with Bacto Oxford antimicrobial supplement (OAB; Difco), for the enumeration of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes*, respectively. Where low numbers of surviving cells were anticipated, 1 ml of undiluted sample was equally distributed onto four plates to lower the detection limit. All agar media were incubated at 37 °C for 24 h before colonies were counted. The decontamination effect of cold plasma treatment was quantified by the log reduction, log (N0/N), where N0 is the initial number of inoculated cells on the untreated food surfaces, and N is the number of viable cells remaining after treatment.

2.6. Surface properties measurement

2.6.1 Surface hydrophobicity measurement. The hydrophobicity of the produce was evaluated using water contact angle measurements. The water contact angle was measured by the sessile drop method using a theta optical tensiometer (Theta Lite, Biolin Scientific, Sweden) equipped with a camera. Small drops (3 μl) of distilled water were deposited onto the produce surfaces described above, using a microliter syringe and a 0.5 mm diameter needle at room temperature (24 ± 2 °C). Contact angle measurements were conducted for no more than 20 s, to avoid changes in the test surfaces. Ten data points were taken for each sample (n = 10).

2.6.2 Surface roughness measurement. Scanning interferometry was used to quantify sample surface roughness. Samples were mounted onto a noncontact three-dimensional surface profiler (Nano View-E1000, Nanosystem, Daejeon, Korea), which was used to measure the surface roughness of the scan area (125 × 95 μm^2) via a 10 × objective lens. Average roughness values for the surface topography were acquired using NanoMap software (version 2.5.17.0, Nanosystem, Daejeon, Korea) from five randomly chosen scan areas; here, R_a represents the arithmetic mean deviation of the absolute ordinate values within a sampling length, and R_q is defined as the sum of the largest profile peak height and the largest profile valley depth within a sampling length [34].

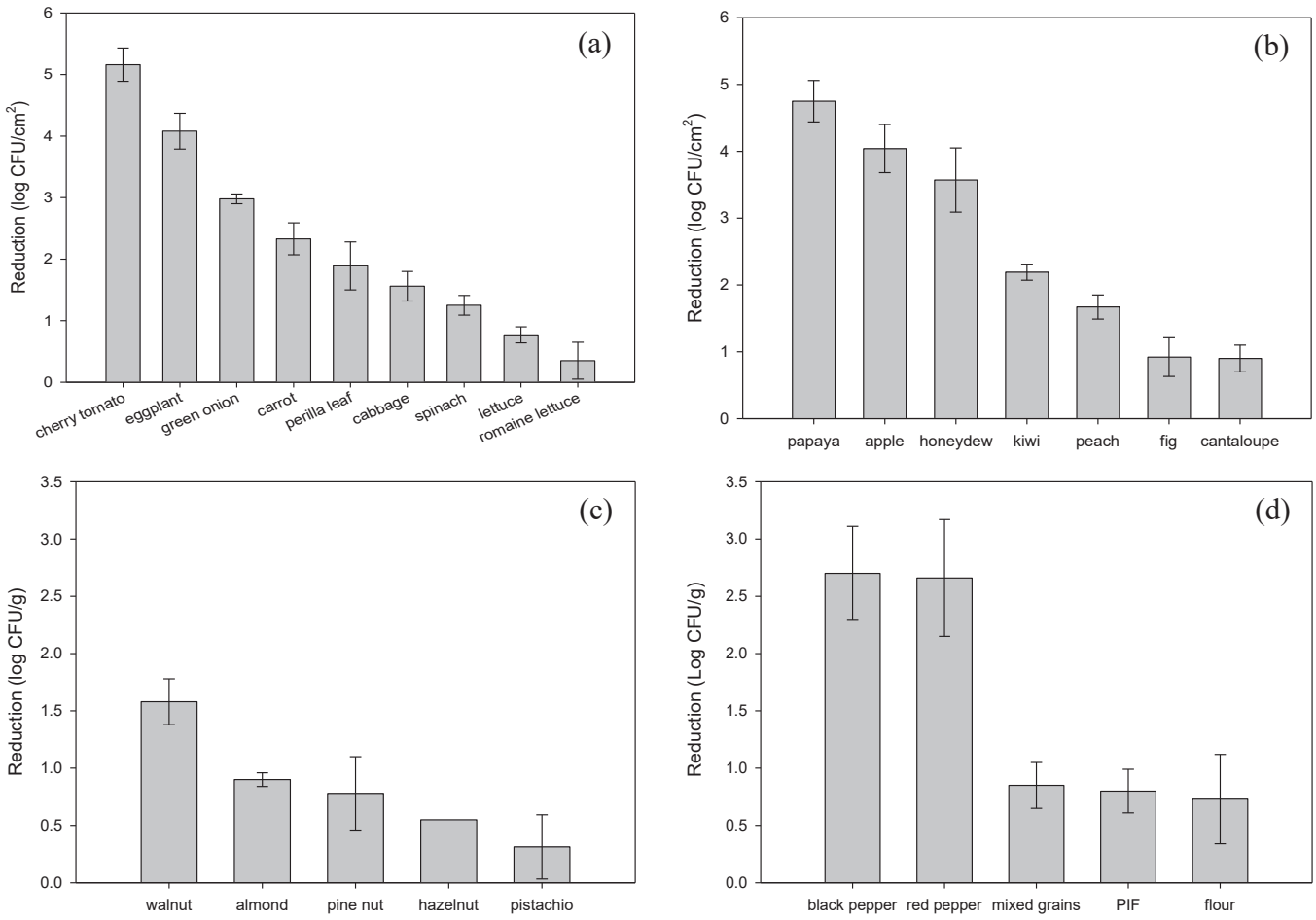

Figure 2. Microbial reduction of *E. coli* O157:H7 in (a) vegetables, (b) fruits, (c) nuts, and (d) powdered foods after 20 min cold plasma treatment. The reduction is presented in log (N_0/N) CFU cm^{-2} or g. N_0 refers to the initial population of inoculated pathogens, and N represents the population remaining after the treatment. Error bars represented a standard deviation. PIF: powdered infant formula.

2.7. Statistical analysis

The inactivation experiments were replicated three times and error bars represented a standard deviation. Texture and surface roughness analyses were replicated five times. Data were analyzed by analysis of variance using Statistical Analysis System software (SAS Institute, Cary, NC, USA) and the separation of means by Duncan's multiple range test, at a probability level of $p < 0.05$. Correlation coefficients between surface properties and the log reduction of pathogens were calculated using SPSS statistical software (version 25, IBM Corp., Armonk, NY, USA).

3. Results

3.1. Screening food samples suitable for cold plasma treatment

Figure 2 shows the log reduction of *E. coli* O157:H7 inoculated on various food sample surfaces after 20 min cold plasma treatment. Different levels of inactivation were observed among samples.

Concerning vegetables (figure 2(a)), cherry tomatoes and eggplants subjected to 20 min-cold plasma treatment showed the highest decontamination rates. Inoculated *E. coli* O157:H7 on cherry tomatoes and eggplant were reduced to 4.08 and 5.16 log CFU cm^{-2}, respectively. Less than a log reduction of *E. coli* O157:H7 was observed on lettuce and romaine lettuce surfaces compared with the other vegetables after the 20 min cold plasma treatment.

With regard to fruit (figure 2(b)), the log reduction of the pathogen increased gradually in the following order: cantaloupe, fig, peach, kiwi, and honeydew. Significantly higher inactivation was achieved in apple and papaya, with more than 4.04 and 4.74 log reduction.

Among nuts (figure 2(c)), the decontamination efficacy of selected samples showed no more than 2 log reduction. Walnut showed the highest inactivation rate (1.58 logs reduction) compared with the other nuts.

Powdered foods (figure 2(d)) had significantly lower ($p < 0.05$) pathogen inactivation. Black and red pepper had significantly higher pathogen inactivation ($p < 0.05$) than the others, with 2.7 and 2.66 log reductions, respectively.

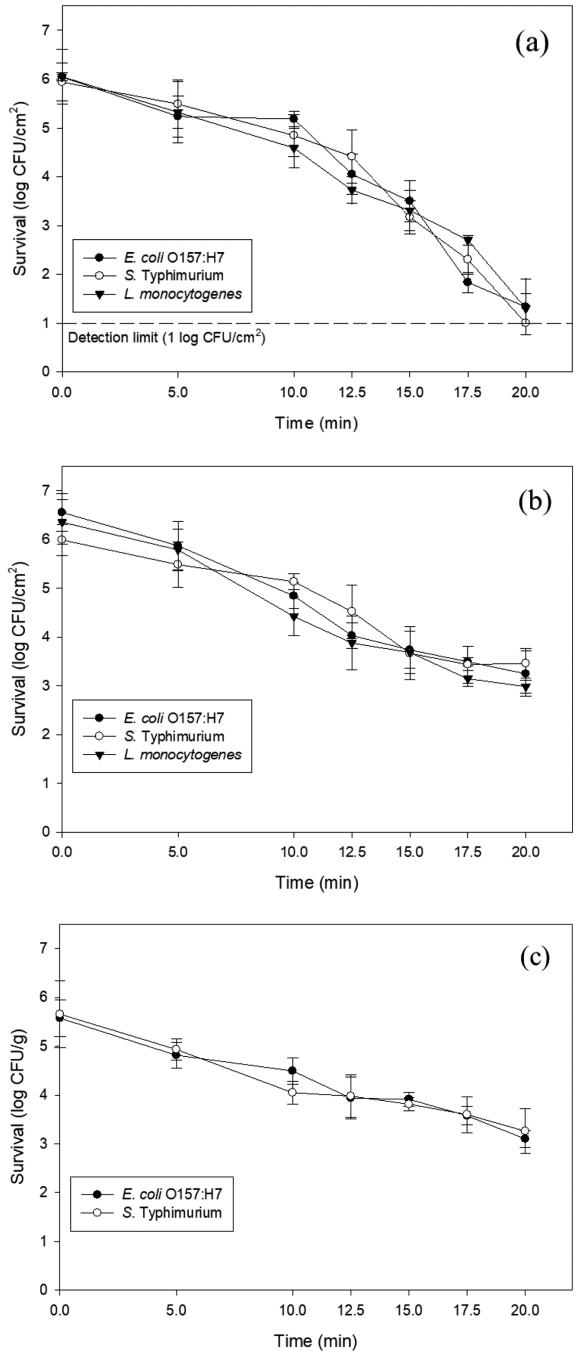

Figure 3. Inactivation of *E. coli* O157:H7 (●), *S. Typhimurium* (○), and *L. monocytogenes* (▼) corresponding to microbial inactivation on (a) apple, (b) cabbage, and (c) red pepper surfaces subjected to cold plasma treatment.

3.2. Inactivation of foodborne pathogens on apple, cabbage, and red pepper surfaces by cold plasma treatment

Figure 3 shows the survival of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* on the surfaces of selected produce (apple, cabbage, and red pepper) after cold plasma treatment. As the treatment time increased, the reduction effect of the treatment also increased. Initial populations of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* on untreated apple surfaces were 6.04, 5.94, and 5.05 log CFU cm^{-2} and those on cabbage were 6.55, 5.99, and 6.35 log CFU cm^{-2}, respectively (figures 3(a) and (b)). The initial populations of *E. coli* O157:H7 and *S. Typhimurium* on red pepper were 5.57 and 5.65 log CFU g^{-1}, respectively (figure 3(c)). The detection limit of samples was 1.00 log CFU cm^{-2} or g. The populations of the three pathogens, *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes*, on apple and cabbage surfaces were reduced to 4.71, 4.94, and 3.65 log CFU cm^{-2} and 3.31, 2.53, and 3.37 log CFU cm^{-2}, respectively, after 20 min of cold plasma treatment. Populations of two pathogens, *E. coli* O157:H7 and *S. Typhimurium*, were reduced to 2.47 and 2.40 log CFU g^{-1} on red pepper surfaces, respectively.

Table 1. Surface properties of various food samples.

	Surface properties		
	Surface roughness (μm)[a]		Hydrophobicity
Food sample	R_a	R_q	Contact angle (°)
Cherry tomato	1.01 ± 0.19 A[b]	1.40 ± 0.34 A	85.76 ± 3.75 C
Apple	1.82 ± 0.61 B	2.41 ± 0.82 A	83.39 ± 3.28 C
Honeydew	1.87 ± 0.13 B	2.49 ± 0.11 A	80.79 ± 4.78 C
Cabbage	5.83 ± 0.64 C	7.90 ± 0.58 B	108.63 ± 4.06 D
Lettuce	7.52 ± 0.27 D	8.57 ± 1.11 B	52.49 ± 6.89 A
Cantaloupe	14.56 ± 0.30 E	18.65 ± 0.64 C	60.94 ± 4.80 B

[a]R_a, arithmetic mean roughness; R_q, root mean squared roughness. [b]Means with different letters within a column are significantly different ($p < 0.05$).

Table 2. Correlation matrix of Pearson coefficients between surface properties and bacterial log reductions.

		Surface properties		
		Surface roughness (μm)		Hydrophobicity
Cold plasma treatment	Bacterial reduction	R_a	R_q	Contact angle (°)
20 min	*E. coli* O157:H7	−0.838*	−0.818*	NS[a]

[a]NS, not significant. *, level of significance at $p < 0.05$.

3.3. Roughness and contact angle of food sample surfaces

Table 1 lists the surface characteristics of the sample surfaces. When the water contact angle exceeds 65°, this could be an indicator of the hydrophobic nature of the specimen [35]. Cabbage had a significantly higher ($p < 0.05$) water contact angle than the other produce. Cherry tomato, apple, and honeydew did not differ significantly ($p > 0.05$) in terms of water contact angle values.

Surface roughness is associated with the crevices and peaks of a particular surface. The R_a and R_q values of cherry tomato were significantly higher ($p < 0.05$) than those for apple and honeydew.

Table 2 lists the correlation coefficients between surface properties and bacterial log reductions. The R_a and R_q values of fresh produce were highly negatively correlated with the

log reduction of *E. coli* O157:H7 after cold plasma treatment. In contrast, there was a weaker correlation between the contact angle and pathogen inactivation rate. Contact angle values were not significantly ($p > 0.05$) correlated with the log reduction of the pathogen after the 20 min cold plasma treatment.

4. Discussions

The purpose of this study was to screen food samples suitable for cold plasma decontamination treatment, to examine the different microbial inactivation processes, and to evaluate the relationship between microbial reduction efficacy and surface characteristics.

The decontamination of fresh produce has been studied extensively using various types of cold plasma systems [27, 31, 36, 37] in the present study, nuts and powdered food decontamination were also evaluated using cold plasma technology. In previous research, a gliding arc AC plasma jet device reduced *E. coli* O157:H7 to 1.34 log CFU ml^{-1}, with *E. coli* O157:H7 C9490 showing the greatest reduction, after a 20 s treatment using a 6 cm spacing [38]. In another study, *B. cereus* and *Cronobacter sakazakii* were reduced to 0.6 and 0.9 log CFU g^{-1} in onion powder and powdered infant formula, using a 20 min cold plasma treatment at 900 W [39].

Nevertheless, no study has compared the inactivation rates among different food groups using the same cold plasma treatment system. Applying an identical system could easily control experiment conditions which were suitable for comparing and determining the decontamination efficacy among different types of food.

In the present study, vegetables and fruits had various inactivation rates; from 0.5 log reduction to 5 logs reduction; cherry tomato, eggplant, papaya, apple, and honeydew have relatively smooth surfaces, and smoothest surfaces had higher decontamination rates. Nuts and powdered products (powdered infant formula and flour) were not appropriate for the current cold plasma treatment, as the inactivation rate was low compared with fresh produce; this was attributed to the surface structure of this food type and its low moisture content.

Overall, *E. coli, S. Typhimurium*, and *L. monocytogenes* on selected samples had similar inactivation rates over time. Various studies have shown that Gram-positive bacteria were more resistant to cold plasma treatment that Gram-negative bacteria [17, 18, 40] *E. coli* O157:H7 and *S. Typhimurium* are Gram-negative, with a thinner membrane, compared with Gram-positive bacteria, *L. monocytogenes*, which may have a slightly thicker membrane layer. The thick cell wall may act as a shield against the diffusion of reactive species generated by the cold plasma treatment. In contrast, one study demonstrated the higher sensitivity of Gram-positive *L. innocua* compared with Gram-negative *S. Typhimurium* and *E. coli* on inoculated tomato surfaces [41]. Other comparative studies have also reported a similar vulnerability between Gram-positive and Gram-negative bacteria to cold plasma with regard to decontamination [42, 43]. In the present study, there were no clear differences in the resistance of the Gram-positive and Gram-negative bacteria to regarding the system, process, or medium observed; thus, further study of the susceptibility of bacteria to cold plasma treatment is necessary.

The degree of pathogen reduction varied among samples. Cherry tomato had the highest microbial reduction rate, and in contrast, cantaloupe had the lowest microbial inactivation rate. Cherry tomato was reduced to the detection limit (1 log CFU cm^{-2}) with a 20 min cold plasma treatment, but only a 0.9 log reduction was observed in cantaloupe. This significant difference ($p < 0.05$) in response to cold plasma treatment is associated with the surface properties of the produce sample and its surface roughness. Cantaloupe had higher R_a and R_q values than the other fruits, so pathogens were better able to hide within the crevices of the food surface, thus affecting the decontamination efficacy.

Wang *et al* [44] reported a negative correlation between R_a and decontamination efficacy on metal and fruit surfaces, including apple and cantaloupe. The linear relationship between surface roughness and bacterial reduction implies that an increase in surface roughness provides protection for bacteria from disinfecting agents.

According to Selcuk *et al* [30], wheat, barley, oats, lentil, rye, corn, and chickpea exhibited various inactivation rates to *Penicillium* spp. and *Aspergillus* spp. under a low-pressure cold plasma treatment, using ambient air and sulfur hexafluoride for 5–20 min. They also concluded that the cold plasma feed gas, treatment time, seed surface structure, and hull composition all affected the efficacy of cold plasma treatment. Kwon *et al* [45] observed relationships between microbial reduction and surface roughness using super-heated steam on watermelon and cantaloupe surfaces; they also examined the negative relationship between inactivation efficacy and surface roughness.

According to Bhide *et al* [46], sandpaper with differing degrees of surface roughness (grit) had varying degrees of inactivation ($p < 0.05$) to nalidixic acid-resistant *Enterobacter aerogenes* under treatment with a cold plasma jet. Among the samples, 600 grit sandpaper had the highest microbial reduction, 2 log reduction, whereas 280 grit sandpaper had a 1.5 log reduction. The correlation between microbial reduction and surface roughness may be an important parameter for effective surface decontamination using cold plasma treatment.

5. Conclusion

In summary, cold plasma treatment led to various levels of efficacy, depending on the type of food and its surface properties. The results of this study demonstrated that cold plasma treatment is effective for vegetables and fruits. Bacteria type did not have an effect on the decontamination efficacy of cold plasma treatment. Surface roughness played a more important role in bacterial inactivation than hydrophobicity. Thus, understanding the nature of cold plasma treatment and the characteristics of various food samples to be treated is necessary to ensure effective application and future commercialization of

this process. The results of this study provide insights into suitable food types for which cold plasma decontamination would be most effective.

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ORCID iDs

Jin-Young Han https://orcid.org/0000-0003-4851-7562
Sangheum Eom https://orcid.org/0000-0001-5742-0127
Seong Bong Kim https://orcid.org/0000-0002-0472-8694
Dong-Hyun Kang https://orcid.org/0000-0002-0265-9699

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