ORIGINAL ARTICLE

Inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, and *Bacillus cereus* in roasted grain powder by radio frequency heating

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Abstract

Aims: The objective of this study was to evaluate the antimicrobial effects of radio frequency (RF) heating and the combination treatment of RF heating with ultraviolet (UV) radiation against foodborne pathogens in roasted grain powder (RGP).

Methods and Results: Foodborne pathogens inoculated on RGP were subjected to RF heating or RF-UV combination treatments. After 120 s of RF heating, 4·68, 3·89 and 4·54 log reductions were observed for *Escherichia coli*, *Salmonella* Typhimurium and *Bacillus cereus* vegetative cells respectively. The combined RF-UV treatment showed synergistic effects of over 1 log unit compared to the sum of individual treatment for *E. coli* and *S.* Typhimurium, but not for *B. cereus* vegetative cells because of their high UV resistance. Germinated *B. cereus* cells were not significantly inactivated by RF heating (<1 log CFU per gram), and increased heat resistance compared to the vegetative cells was verified with mild heat treatment. The colour of RGP was not significantly affected by the RF or RF-UV treatments.

Conclusions: Applying RF heating to grain-based food products has advantages for the inactivation of *E. coli* and *S.* Typhimurium in RGP.

Significance and Impact of the Study: The results of the present study could be used as a basis for determining the treatment conditions for inactivating *E. coli* and other foodborne pathogens such as *S.* Typhimurium and *B. cereus* in RGP.

Introduction

In recent years, the consumption of grain-based food products such as roasted grain powder (RGP), which is called 'sunsik' in Korean, has increased because consumers are concerned about health and nutrition. However, multistate outbreak from grain-related foods was reported in the United States (Russo *et al.* 2013). Moreover outbreaks of *Escherichia coli* and *Bacillus cereus* from RGP were reported by the Korean press, and there have been difficulties involved in controlling micro-organisms in powdered foods (Ha and Kang 2014). It is difficult to

eliminate foodborne micro-organisms from low water activity foods that exhibit an increased tolerance to heat and other treatments (Beuchat *et al.* 2013). Several alternative methods have been suggested, such as ultraviolet (UV)-C light, gaseous ozone and infrared heating to control powdered foods. However, these treatments have some limitations. For example, the application of UV-C light took a significantly long time to inactivate microbes to an approximately 4-log reduction of *Salmonella enterica* serova Typhimurium in flour powder (Condón-Abanto *et al.* 2016). Following 1·0 ppm ozone treatment in flaked red pepper, *B. cereus* (vegetative form) and

E. coli were decreased by 1·5 and 2·0 logs respectively (Akbas and Ozdemir 2008), which was not enough to apply in low-water activity food processing. During infrared heating, the colour values of a paprika powder surface decreased significantly due to particle agglomeration (Staack et al. 2008).

Radio frequency (RF) heating is a novel heating technology involving the use of electromagnetic energy at frequencies between 1 and 300 MHz (Ha et al. 2013). Among these frequencies, only specific frequencies (13·56, 27·12 and 40·68 MHz) are permitted for domestic, industrial, scientific and medical applications to avoid interfering with communication systems (Piyasena et al. 2003). RF generates heat inside of food due to molecular friction and space charge displacement in response to the application of an alternating electrical field. This technology has great potential for achieving rapid and uniform heating by delivering thermal energy quickly into every part of a food product (Zhao et al. 2000). Thus, RF heating can be used in various food industries to replace conventional heating for solid and semi-solid foods, which have low thermal conductivities.

Combinations with other technologies, known as hurdle technology, could be an alternative to sole use of RF heating. Combined treatments could improve food safety and extend the bacteriological and the sensory shelf life of food products (Khan et al. 2017). Ultraviolet radiation has been widely combined with other technologies because it is oriented towards non-thermal technology and is safe for use in foods (Kim et al. 2019). Because UV radiation causes damage to microbial DNA, it can be combined with other technologies such as mild heat, near-infrared heating, hydrogen peroxide and ozone (Hadjok et al. 2008; Selma et al. 2008; Ha and Kang 2014; Cheon et al. 2015). However, published data of combination treatments using RF heating with UV radiation has been limited.

Bacillus cereus is a Gram-positive aerobic or facultatively anaerobic spore-forming pathogen (Kim et al. 2020). Food poisoning by B. cereus is frequently associated with the consumption of grain-based foods (Drobniewski 1993). Most of the research on using thermal or non-thermal processing for inactivating B. cereus spores has focused on experiments under liquid states, not in solids, such as powdered food products. For example, several decontamination methods, including high pressure with nisin and modified tyndallization with carbon dioxide injection or germinant addition, have been applied to inactivate B. cereus spores in liquid foods (Black et al. 2008; Løvdal et al. 2011; Kim et al. 2012a). However, there was no research about controlling B. cereus spores in powdered food products through RF heating after the germination step.

In this study, the efficacy of RF heating and its combination with UV-C treatment on RGP was evaluated. Non-pathogenic E. coli and foodborne pathogens such as S. Typhimurium and B. cereus (vegetative form) were used to identify the bactericidal effect. The RGP quality was evaluated using colour values (L^*, a^* and b^*). Moreover the germination conditions of B. cereus spores were optimized and the heat resistance of the germinated cells was compared with those of vegetative cells.

Materials and methods

Experimental design

First, RF heating was applied to inactivate E. coli, S. Typhimurium and B. cereus (vegetative form) inoculated on RGP. Second, UV was sequentially combined with RF (RF followed by UV) to increase the pathogen inactivation efficacy (for E. coli, S. Typhimurium and vegetative B. cereus cells), and colour change of RGP was observed. Finally, germination condition for B. cereus spore was optimized and resistance of the germinated B. cereus spore was determined and compared with that of the vegetative cells.

Bacterial strains

Escherichia coli (ATCC 10536, ATCC 25922 and B/4), S. Typhimurium (ATCC 19585, ATCC 43971 and DT 104) and B. cereus (ATCC 10876, ATCC 13061 and ATCC 14579) were obtained from the Bacterial Culture Collection of Seoul National University (Seoul, South Korea). Working cultures were streaked onto tryptic soy agar (TSA; Difco, BD, Sparks, MD), incubated at 37°C for 24 h, and stored at 4°C.

Preparation of pathogen inoculum

All the strains of E. coli, S. Typhimurium and B. cereus were inoculated individually into 5 ml of tryptic soy broth (TSB; Difco) and incubated at 37°C for 24 h. Overnight culture (1 ml) from each strain of E. coli and S. Typhimurium was spread onto three TSA plates to create a bacterial lawn, followed by incubation at 37°C for 24 h. Ten milliliter of 0·2% peptone water (PW; Difco) was added to each plate, and cell suspensions were made after rubbing the agar surface with a sterile swab (3M Pipette Swab; 3M Health Care, St. Paul, MN) to remove cells. For B. cereus, the overnight culture (1 ml) of each strain was inoculated into two 250 ml conical flasks containing 50 ml of TSB to increase the cell number, and the samples were incubated at 37°C for 24 h. Each cell suspension was collected by centrifugation at 4000 g for

20 min at 4°C and washed three times with 0·2% PW. The final pellets were resuspended in 0·2% PW and combined to make a mixed culture cocktail containing approximately equal numbers of cells from each strain of *E. coli*, *S.* Typhimurium and *B. cereus* (*c.* 10^{11}–10^{12} CFU per ml).

Sample preparation and inoculation

Roasted grain powder which is also called 'Misugaru' in Korean, was purchased from a local grocery store (Seoul, South Korea). For inoculation, 5 ml of culture cocktail was added in drops to 250 g samples inside sterile low-density polyethylene bags (268 × 273 mm). The inoculated samples were thoroughly mixed by hand massaging for 10 min to produce a homogeneous dispersal of inoculum throughout the RGP. The inoculated RGP samples were then immediately used in each experimental trial.

RF heating and combination treatment with UV radiation

Radio frequency heating was conducted with a previously described (Jeong *et al.* 2019) RF heating system (Seoul National University, Seoul, South Korea; Dong Young Engineering CO. Ltd, Gyeongnam, South Korea). An RF electric field with a frequency of 27·12 MHz was generated between two parallel-plate electrodes (30·0 × 35·0 cm; 0·6 cm thick) spaced 8 cm apart. For the RF heating treatment, 25 g of inoculated RGP was placed in a polypropylene (PP) jar measuring 6·3 cm in diameter and 6·1 cm deep (NALGENE 2118-0004; Thermo Scientific, Hudson, NH), which was placed on the centre of the bottom electrode. RF heating was applied to each prepared sample, and they were heated up to 120°C to maximize the pasteurization efficiency while maintaining the product quality. During RF treatment, a fibre optic temperature sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a temperature signal conditioner (TMI-4; FISO Technologies Inc.) was used to measure the real-time temperatures in the samples during RF heating. The sensor was inserted directly into the centre of the RGP located in the middle, and the temperature was recorded at 2 s intervals. Approximate mean temperature of RGP was 88·6, 101·6, 111·0 and 119·3°C after 60, 80, 100, 110 and 120 RF heating respectively.

UV radiation for combination treatment

The combined RF heating and UV radiation treatment was conducted sequentially. UV experiments were performed in a UV radiation apparatus consisting of two banks of germicidal lamps (254 nm, G6T5, Sankyo Denki, Japan). These UV lamps were located in the ceiling and bottom of the radiation chamber and were turned on for at least 10 min to stabilize them. Each RF-treated sample was spread to a thickness of 0·07 mm on a 25 × 25 cm PP film located at the middle of the two lamps. The reaction time for UV treatment was 3 min, and the corresponding UV dose was 1·90 kJ m^{-2} as calculated by multiplying the irradiation time by the intensity of the UV lamp. The UV intensity was measured using a spectrometer at a 253·7 nm wavelength (AvaSpec-ULS2048-USB2-UA-50, Avantes, The Netherlands).

Bacterial enumeration

To enumerate the pathogens, 25 g of treated RGP was transferred immediately into a sterile stomacher bag containing 225 ml of 0·2% pre-cooled PW in a 4°C refrigerator (detection limit, 1 log CFU per gram) and homogenized for 2 min with the stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1-ml aliquots of the sample were serially diluted in 9-ml blanks of 0·2% PW, and 0·1 ml of sample or diluent was spread-plated onto selective media, with xylose lysine deoxycholate agar (XLD; Difco) for the enumeration of *S.* Typhimurium and mannitol-egg yolk-polymyxin agar (MYP; Difco) for the enumeration of *B. cereus*. In addition, 1 ml of sample or diluent was dispensed onto Petrifilm (3M Petrifilm Coliform Count Plates; 3M Korea Ltd) to enumerate the *E. coli*. All the agar plates and Petrifilms were incubated at 37°C for 24 h. After that, the black colonies on the XLD, the pink–red colonies with a white precipitate formed on the MYP, and the blue colonies with bubbles on the Petrifilm were counted.

Colour measurement

The colours of the untreated, RF and UV combination-treated RGP were measured using a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan). The values of the L^* (lightness), a^* (redness) and b^* (yellowness) were used to quantify the colour attributes and indicate the lightness, redness and yellowness of the samples respectively.

Germination and inactivation of *B. cereus* spores

For the germination of the *B. cereus* spores, 25 g of inoculated RGP was placed in a PP jar measuring 6·3 cm in diameter and 6·1 cm deep (NALGENE 2118-0004; Thermo Scientific, Hudson, NH), which was placed in the constant temperature and humidity chamber (D-TH31; Dice Labtech, Kyunggi-do, Korea) under various conditions. The temperatures were 80 and 90°C, the

relative humidity values were 30, 50 and 70%, and the duration times were 1, 2, 3 and 4 h. Then, the samples were placed in the low temperature incubator (IL-11; Jeio Tech, Daejeon, Korea) at 30°C for 1 h. RF heating was applied to each prepared sample, and they were heated to approximately 120°C to inactivate the germinated cells in the RGP. Because the germinated cells were not significantly inactivated by RF heating, the heat resistance of the vegetative and germinated cells was verified with mild heat treatment. For the mild heat treatment, the water bath was set to 60, 70 or 80°C. Twenty-five gram of RGP inoculated with vegetative cells or B. cereus spores for each sample was placed in a PP jar. The samples inoculated with spores were placed in the constant temperature and humidity chamber at 90°C and 70% for 4 h followed by a cooling-down period at 30°C in the low temperature incubator for 1 h. Then, 25 g of samples inoculated with vegetative cells or spores and with treatments were transferred into the sterile stomacher bag containing 225 ml of 0·2% PW and homogenized for 2 min with the stomacher. After homogenization, 1-ml aliquots of the samples inoculated with vegetative cells or spores were dispensed into 9-ml blanks of 0·2% PW in a water bath preheated to 60, 70 or 80°C. Then, 1-ml aliquots of the samples were enumerated immediately by serial dilution.

Enumeration of B. cereus spores

To enumerate the B. cereus spores, 25 g of treated RGP was transferred immediately into the sterile stomacher bag containing 225 ml of 0·2% PW that had been precooled in a 4°C refrigerator (detection limit, 1 log CFU per gram) and homogenized for 2 min with a stomacher. After homogenization, 1-ml aliquots of the samples inoculated with B. cereus spores were dispensed into 9-ml blanks of 0·2% PW in an 80°C -water bath (BW-05G; JEIO TECH) for 20 min to eradicate all the vegetative B. cereus cells. Then, 1-ml aliquots of the samples were serially diluted in 9-ml blanks of 0·2% PW, and 0·1 ml of sample or diluent was spread-plated onto a selective medium, mannitol-egg yolk-polymyxin agar (MYP; Difco), which was used to enumerate the B. cereus. All the agar plates were incubated at 37°C for 24 h. After that, pink–red colonies with a zone of white precipitate formed on the MYP.

Statistical analysis

All the experiments were performed in triplicate. Data were analysed by analysis of variance using the Statistical Analysis System (SAS Institute, Cary, NC), and the separation of means was performed using Duncan's multiple range test at a probability level of $P < 0.05$.

Results

Heating rate and pathogen inactivation of RGP during RF heating

The average temperature histories of the RGP during RF heating at a constant frequency of 27·12 MHz are shown in Fig. 1. The temperature rapidly rose as the treatment time increased, and the temperature of the RGP reached c. 120°C after 120 s of RF heating. The populations (log CFU per gram) of E. coli, S. Typhimurium and B. cereus in the RGP after RF heating are shown in Table 1. The pathogen populations were reduced as the treatment time increased. The reductions in E. coli, S. Typhimurium and B. cereus were 4·68, 3·89 and 4·54 log CFU per gram respectively after 120 s of RF heating.

Combination treatment of RF heating and UV-C radiation

Radio frequency heating was combined with UV radiation to increase the pathogen inactivation efficacy. The survival numbers of E. coli, S. Typhimurium and B. cereus cells in the RGP after RF heating, UV radiation and the sequential application of both technologies are represented in Fig. 2. The UV radiation treatment (for 2 min) alone does not lead to significant ($P > 0.05$) reductions in E. coli, S. Typhimurium and B. cereus. RF heating for 100 and 120 s followed by UV radiation exhibited 4·48 and 5·39 log reduction in E. coli respectively. In addition, reductions of 3·98 and 4·76 log units were observed in S. Typhimurium after sequential RF-UV combined treatment for 100 and 120 s respectively.

Colour values of untreated, RF and RF-UVC-treated samples

The colour values of the RGP after RF treatment and combined RF-UV treatment are shown in Table 2. The L^*, a^* and b^* values of the RF-UV-treated RGP were not significantly different ($P > 0.05$) from those of the non-treated samples. The L^*, a^* and b^* values of RGP were 73·37–75·07, 3·92–4·08 and 20·12–20·65 respectively.

Germination and heat resistance of B. cereus spores

The survival of the vegetative cells and spores of B. cereus in RGP during RF heating is shown in Fig. 3. Significant differences in the survival of two forms of vegetative cells and spores are shown for all the treatment times. At 120 s, there was a significant difference (3·9-log reduction) between the reduction of two forms of B. cereus. The germination rates (%) of B. cereus
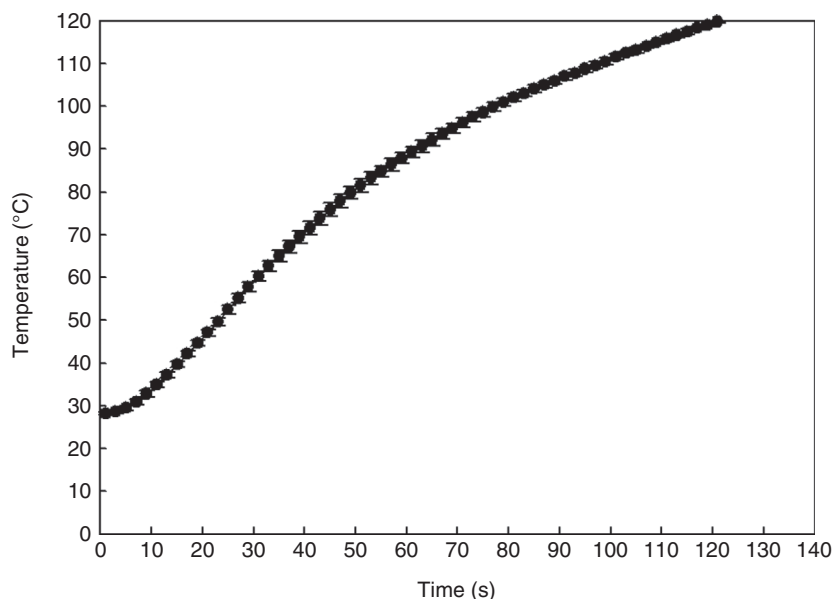
Figure 1 Average temperature-time history for the centre of the RGP during RF heating. The results are the means from three experiments, and the error bars indicate standard deviations

Table 1 Populations (log CFU per gram) of *Escherichia coli*, *Salmonella* Typhimurium and *Bacillus cereus* in RGP after radiofrequency heating*

Pathogen	Treatment time (s)					
	0	60	80	100	110	120
E. coli	7·17 ± 0·05 A	5·21 ± 0·40 B	4·40 ± 0·36 C	3·87 ± 0·12 D	2·86 ± 0·13 E	2·49 ± 0·20 E
S. Typhimurium	7·50 ± 0·16 A	5·99 ± 0·26 B	5·06 ± 0·29 C	4·74 ± 0·22 C	3·86 ± 0·02 D	3·61 ± 0·16 D
B. cereus	7·78 ± 0·11 A	3·60 ± 0·05 B	3·49 ± 0·23 BC	3·48 ± 0·18 BC	3·29 ± 0·18BC	3·24 ± 0·24 C

Values followed by the same letters within the row are not significantly different ($P > 0.05$).

*Means ± standard deviations from three replications.

spores at various temperatures and humidity values are shown in Fig. 4. First, the *B. cereus* spore germination rate was identified at a fixed temperature (90°C) with three different relative humidity values (30, 50 and 70%). For 1 h in that situation, there was no significant change in the form of the spore, but a 99·41% germination rate was achieved in the 90°C and 70% RH chamber after 4 h as shown in Fig. 4a. Second, the germination rate was compared at a fixed relative humidity (70%) with the rates recorded at 80 and 90°C. Even after 4 h, only a 63·9% germination rate was achieved at 80°C as shown in Fig. 4b.

Discussion

In this study, *E. coli*, *S.* Typhimurium and *B. cereus* (vegetative cell) inoculated on RGP were inactivated effectively by RF heating. Previous studies also reported that

RF heating can be applied effectively to control foodborne pathogens in low water activity foods such as red and black pepper (Kim et al. 2012b; Jeong and Kang 2014), raw shelled almonds (Jeong *et al.* 2017), pistachios (Jeong *et al.* 2019) and cumin seeds (Chen *et al.* 2019). For example, Kim *et al.* (2012b) reported that 50 s RF heating resulted in maximum 4·29 log reduction of *E. coli* O157:H7 or *S.* Typhimurium in black pepper and> 5 log reduction in red pepper without colour change. Similarly, Jeong *et al.* (2017) indicated that 40 s RF heating achieved 3·7-, 6·0- and 5·6-log reductions in *S.* Enteritidis, *S.* Typhimurium and *S.* Senftenberg respectively without changes in colour and degree of lipid oxidation. Although RF heating was effective at controlling the pathogens in the RGP, 5 log reductions were not observed for all three pathogens in the present study even at the maximum treatment time. Therefore, RF heating was combined with UV-C irradiation.
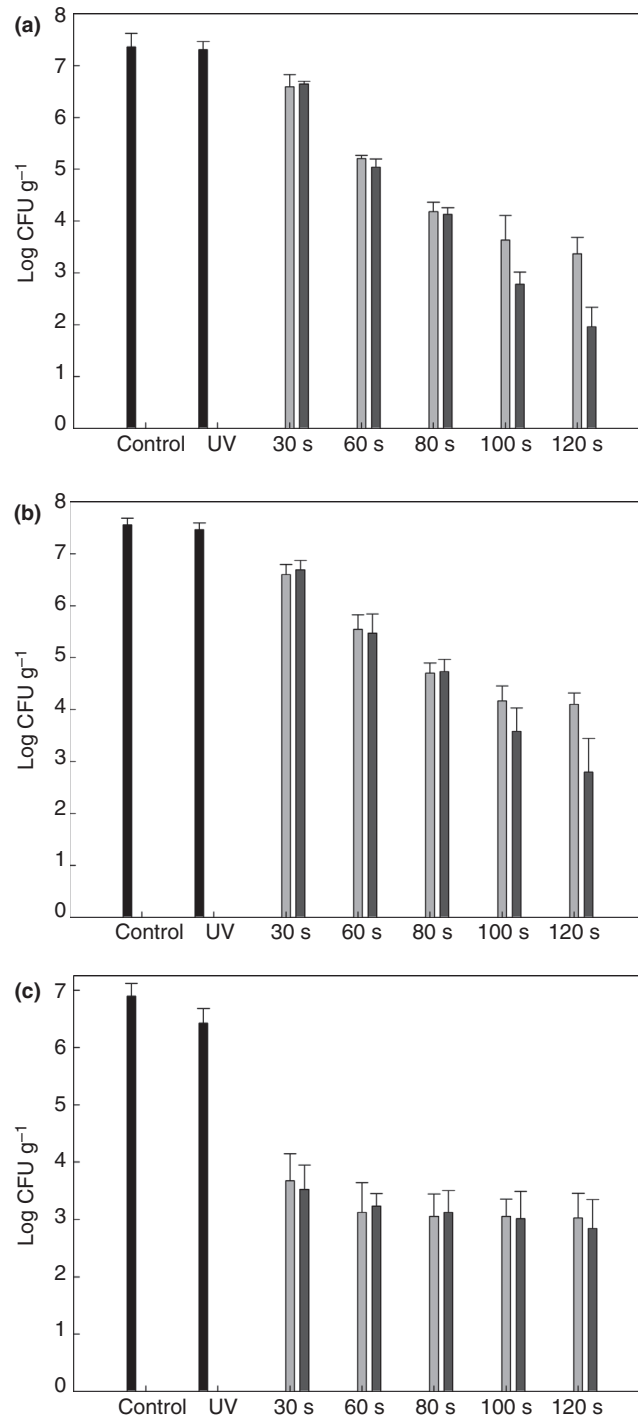
Figure 2 Survival (log CFU per gram) of (a) *Escherichia coli*, (b) *Salmonella* Typhimurium and (c) *Bacillus cereus* in RGP during RF heating, UV radiation and the combined application of both technologies. Sole UV was applied for 120 s while RF and RF-UV were applied for 30, 60, 80, 100 and 120 s. The results are the means from three experiments, and the error bars indicate the standard deviations. Results for control and UV were represented as black while those of RF and RF-UV were represented as grey and dark grey respectively

The sums of the individual RF and UV-C inactivation results were lower than the values obtained from the combined treatments. The log reductions from the

synergistic effects after 120 s, which were calculated by subtracting the sums of the RF and UV reductions from the values obtained during sequential RF-UV treatment,

Table 2 Surface colour values of RF-treated and untreated RGP[†]

Treatment	Colour		
	L*	a*	b*
Control	74·47 ± 0·54 A	4·07 ± 0·05 A	20·12 ± 0·17 A
RF (2 min)	73·37 ± 1·41 A	3·92 ± 0·31 A	20·65 ± 0·74 A
RF (2 min)-UV (3 min)	75·07 ± 0·40 A	4·08 ± 0·20 A	20·47 ± 0·52 A

Colour parameters are L* (lightness), a* (redness), b* (yellowness).
[†]Means ± standard deviations from three replications. Values followed by the same letters within the column are not significantly different ($P > 0.05$).

were 1·40 and 1·31 log units for *E. coli* and *S.* Typhimurium respectively. These results indicate that synergistic effects were observed during the inactivation of *E. coli* and *S.* Typhimurium. Statistically significant differences ($P < 0.05$) between the sums of the RF and UV inactivation and the inactivation values achieved by combination treatment were observed only after a treatment time of 120 s. Significant differences were not observed ($P > 0.05$) between the individual and combined treatment for the inactivation of *B. cereus* vegetative cells even after 120 s treatments. Kim *et al.* (2017) reported that disinfection rate by UV-LED was the greatest for Gram-negative pathogen, followed by Gram-positive pathogen and yeast. The more significant UV resistance of Gram-positive pathogen than that of Gram-negative pathogens indicated by Kim *et al.* (2017) could be an explanation to that no synergistic effect was observed in *B. cereus* in the present study.

In the previous studies, several hurdle combinations for controlling microorganisms in powdered foods were reported. Cheon *et al.* (2015) obtained a synergistic effect

with combined UV-C irradiation and mild heating of *E. coli* O157:H7 and *S.* Typhimurium in powdered red pepper. Hamanaka *et al.* (2011) subjected fresh fig fruits to sequential UV and infrared treatments and achieved 3 log reductions in the fungal counts (30 s of infrared heating followed by 30 s of UV radiation). In this study, it was indicated that RF-UV combination treatment can be used effectively to control pathogen in RGP, but further studies about damaged relationships between the cell wall and membrane with intracellular substances such as nucleic acids are needed to understand the mechanism of the synergistic effect from combined RF-UV treatments.

Colour values of RGP were not affected by RF or RF-UV treatments in the present study. Other studies also reported that the RF heating treatment did not affect the qualities of agricultural products. Gao *et al.* (2010) reported that the RF treatment did not affect almond quality according to measurements such as the peroxide values, fatty acids and kernel colour. Zheng *et al.* (2017) reported that RF-treated corn maintained the moisture content, water activity, protein, starch, ash, fat, fatty acid and colour levels. Even though colour values were not significantly affected in the present study, further study is needed to identify the change of other quality indicators after harsher treatment conditions to control pathogens including injured cells.

Controlling not only the vegetative cells but also the spores in RGP is important. Various germination methods were investigated because germinating the spores before antibacterial treatment is an effective way to control the spores. In the present study, incubation at 90°C with 70% humidity for 4 h is suggested as the optimized condition for *B. cereus* germination even though it is not a normal condition to produce RGP. RF heating until
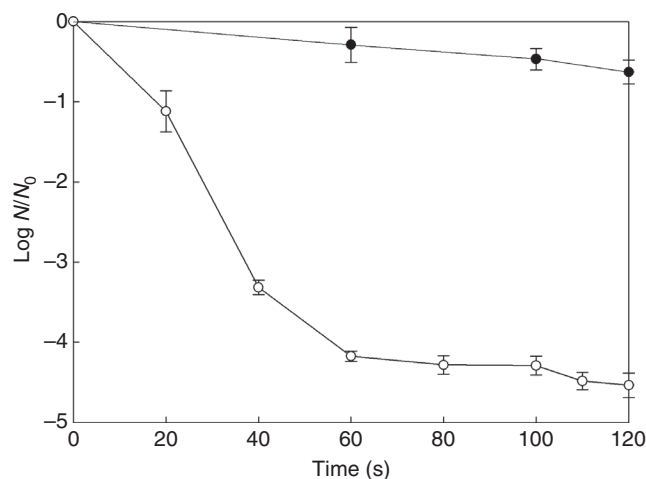
Figure 3 Survival curves for spores (●) and vegetative cells (○) of *Bacillus cereus* in RGP treated with RF heating. The results are the means from three experiments, and the error bars indicate the standard deviations
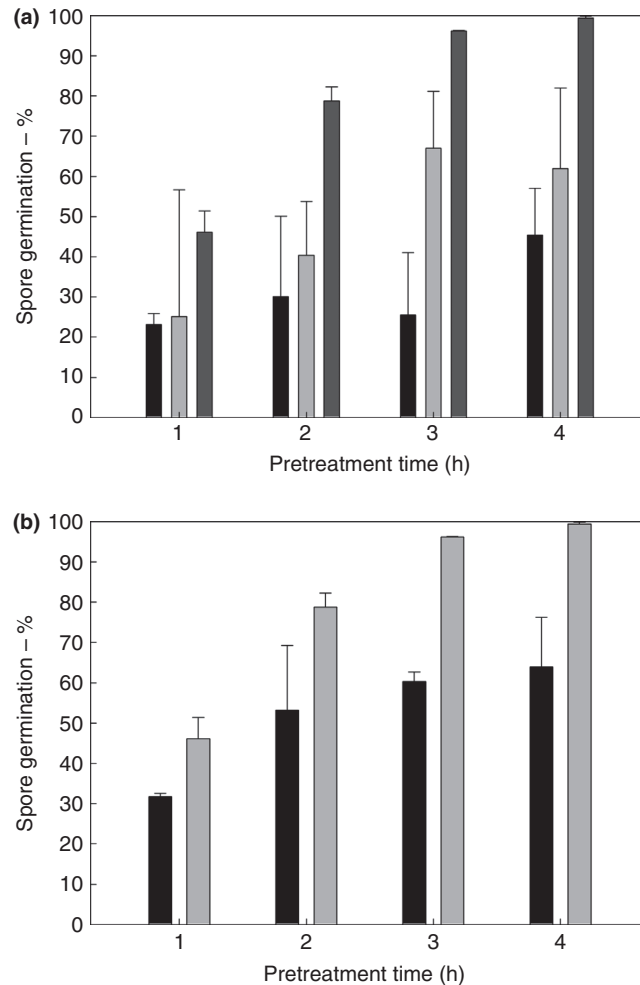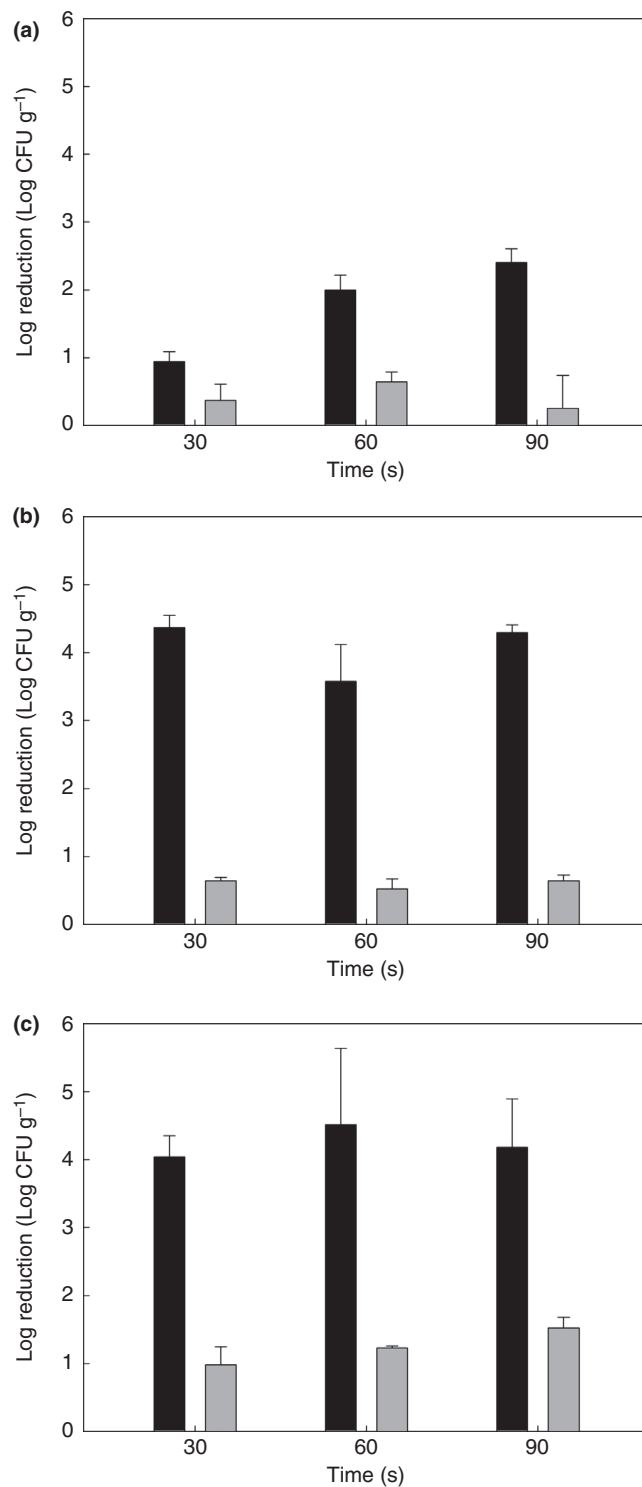
Figure 4 *Bacillus cereus* spore germination rate (%) after storage in a constant temperature and humidity chamber. The results are the means from three experiments, and the error bars indicate the standard deviations. (a) Fixed temperature (90°C) with various humidity values (30, 50, and 70% represented as black, grey and dark grey respectively), and (b) fixed humidity (70%) with various temperatures (80 and 90°C represented as black and grey respectively)

reaching 120°C was applied to inactivate the germinated cells in RGP after cooling at 30°C for 1 h. However, there was no significant difference (<1 log) in the treated and untreated samples in terms of the *B. cereus* population after germination as shown in Fig. S1. The conditions of *B. cereus* germination were established, but the heat resistance problem remained. Moir (2006) and Ghosh and Setlow (2010) reported that dormant spores return to their vegetative growth cycle during a process that includes germination. From the results, it was presumed that the 90°C and 70% RH storage condition for 4 h was harsh and the pathogen would acquire heat resistance during germination. Therefore, the altered heat resistance was verified by subjecting the vegetative and germinated cells to mild heat using a water bath (Fig. 5). The heat resistance of the germinated cells was remarkably higher

than that of the vegetative cells regardless of the treatment temperature. In particular, the difference was 3·73-log when treating at 70°C for 30 s. These results indicate that the heat resistance could be altered after germination, but further study is needed to clarify the resistance acquisition by the various germination methods because germinated vegetative cell and spore cell were mixed in the present study.

In conclusion, 5-log reductions of *E. coli* and *S.* Typhimurium were achieved in RGP without affecting the product quality as measured by colour by performing RF heating with UV radiation. Although the pilot apparatus used in this study was a batch type, and its capacity was comparatively small, the RF and UV combined processing for powdered foods could be expanded to the industrial scale using it in the form of continuous line processing.

Figure 5 Log reductions for vegetative cells (black) and germinated *Bacillus cereus* spores (grey) in RGP subjected to (a) 60°C, (b) 70°C and (c) 80°C mild heat in the water bath. The results are the means from three experiments, and the error bars indicate the standard deviations

In addition, further study is needed to optimize the conditions of *B. cereus* spore germination in powdered foods. As demonstrated in this study, the germination of *B.* *cereus* spores was possible, but there are many factors to consider due to the potential increased resistance of germinated spores.

Acknowledgements

Conflict of Interest

No conflicts of interest to declare.

References

Akbas, M.Y. and Ozdemir, M. (2008) Effect of gaseous ozone on microbial inactivation and sensory of flaked red peppers. *Int J Food Sci Tech* **43**, 1657–1662.

Beuchat, L.R., Komitopoulou, E., Beckers, H., Betts, R.P., Bourdichon, F., Fanning, S., Joosten, H.M. and Ter Kuile, B.H. (2013) Low–water activity foods: increased concern as vehicles of foodborne pathogens. *J Food Protect* **76**, 150–172.

Black, E., Linton, M., McCall, R., Curran, W., Fitzgerald, G., Kelly, A. and Patterson, M. (2008) The combined effects of high pressure and nisin on germination and inactivation of *Bacillus* spores in milk. *J Appl Microbiol* **105**, 78–87.

Chen, L., Wei, X., Irmak, S., Chaves, B.D. and Subbiah, J. (2019) Inactivation of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 in cumin seeds by radiofrequency heating. *Food Cont* **103**, 59–69.

Cheon, H.-L., Shin, J.-Y., Park, K.-H., Chung, M.-S. and Kang, D.-H. (2015) Inactivation of foodborne pathogens in powdered red pepper (*Capsicum annuum* L.) using combined UV-C irradiation and mild heat treatment. *Food Cont* **50**, 441–445.

Condón-Abanto, S., Condón, S., Raso, J., Lyng, J.G. and Álvarez, I. (2016) Inactivation of *Salmonella* typhimurium and *Lactobacillus plantarum* by UV-C light in flour powder. *Innov Food Sci Emerg* **35**, 1–8.

Drobniewski, F.A. (1993) *Bacillus cereus* and related species. *Clin Microbiol Rev* **6**, 324–338.

Gao, M., Tang, J., Wang, Y., Powers, J. and Wang, S. (2010) Almond quality as influenced by radio frequency heat treatments for disinfestation. *Postharvest Biol Tech* **58**, 225–231.

Ghosh, S. and Setlow, P. (2010) The preparation, germination properties and stability of superdormant spores of *Bacillus cereus*. *J Appl Microbiol* **108**, 582–590.

Ha, J.-W. and Kang, D.-H. (2014) Synergistic bactericidal effect of simultaneous near-infrared radiant heating and

UV radiation against *Cronobacter sakazakii* in powdered infant formula. *Appl Environ Microbiol* **80**, 1858–1863.

Ha, J.-W., Kim, S.-Y., Ryu, S.-R. and Kang, D.-H. (2013) Inactivation of *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157: H7 in peanut butter cracker sandwiches by radio-frequency heating. *Food Microbiol* **34**, 145–150.

Hadjok, C., Mittal, G. and Warriner, K. (2008) Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *J Appl Microbiol* **104**, 1014–1024.

Hamanaka, D., Norimura, N., Baba, N., Mano, K., Kakiuchi, M., Tanaka, F. and Uchino, T. (2011) Surface decontamination of fig fruit by combination of infrared radiation heating with ultraviolet irradiation. *Food Cont* **22**, 375–380.

Jeong, S.-G., Baik, O.-D. and Kang, D.-H. (2017) Evaluation of radio-frequency heating in controlling *Salmonella enterica* in raw shelled almonds. *Int J Food Microbiol* **254**, 54–61.

Jeong, S.-G. and Kang, D.-H. (2014) Influence of moisture content on inactivation of *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Typhimurium in powdered red and black pepper spices by radio-frequency heating. *Int J Food Microbiol* **176**, 15–22.

Jeong, S.-G., Ryu, S. and Kang, D.-H. (2019) Salt content dependent dielectric properties of pistachios relevant to radio-frequency pasteurization. *Sci Rep* **9**, 1–9.

Khan, I., Tango, C.N., Miskeen, S., Lee, B.H. and Oh, D.-H. (2017) Hurdle technology: a novel approach for enhanced food quality and safety–a review. *Food Cont* **73**, 1426–1444.

Kim, D.-K., Kim, S.-J. and Kang, D.-H. (2017) Bactericidal effect of 266 to 279 nm wavelength UVC-LEDs for inactivation of Gram positive and Gram negative foodborne pathogenic bacteria and yeasts. *Food Res Int* **97**, 280–287.

Kim, H., Kim, H., Bang, J., Kim, Y., Beuchat, L. and Ryu, J.H. (2012a) Reduction of *Bacillus cereus* spores in sikhye, a traditional Korean rice beverage, by modified tyndallization processes with and without carbon dioxide injection. *Lett Appl Microbiol* **55**, 218–223.

Kim, S.-S., Kim, S.-H., Park, S.-H. and Kang, D.-H. (2020) Inactivation of *Bacillus cereus* spores on stainless steel by combined superheated steam and UV-C irradiation treatment. *J Food Protect* **83**, 13–16.

Kim, S.-S., Park, S.-H., Kim, S.-H. and Kang, D.-H. (2019) Synergistic effect of ohmic heating and UV-C irradiation for inactivation of *Escherichia coli* O157: H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in buffered peptone water and tomato juice. *Food Cont* **102**, 69–75.

Kim, S.-Y., Sagong, H.-G., Choi, S.H., Ryu, S. and Kang, D.-H. (2012b) Radio-frequency heating to inactivate *Salmonella* Typhimurium and *Escherichia coli* O157: H7

on black and red pepper spice. *Int J Food Microbiol* **153**, 171–175.

Løvdal, I.S., Hovda, M.B., Granum, P.E. and Rosnes, J.T. (2011) Promoting *Bacillus cereus* spore germination for subsequent inactivation by mild heat treatment. *J Food Protect* **74**, 2079–2089.

Moir, A. (2006) How do spores germinate? *J Appl Microbiol* **101**, 526–530.

Piyasena, P., Dussault, C., Koutchma, T., Ramaswamy, H. and Awuah, G. (2003) Radio frequency heating of foods: principles, applications and related properties—a review. *Cr Rev Food Sci* **43**, 587–606.

Russo, E.T., Biggerstaff, G., Hoekstra, R.M., Meyer, S., Patel, N., Miller, B. and Quick, R. (2013) A recurrent, multistate outbreak of *Salmonella* serotype Agona infections associated with dry, unsweetened cereal consumption, United States, 2008. *J Food Protect* **76**, 227–230.

Selma, M.V., Allende, A., Lopez-Galvez, F., Conesa, M.A. and Gil, M.I. (2008) Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiol* **25**, 809–814.

Staack, N., Ahrné, L., Borch, E. and Knorr, D. (2008) Effect of infrared heating on quality and microbial decontamination in paprika powder. *J Food Eng* **86**, 17–24.

Zhao, Y., Flugstad, B., Kolbe, E., Park, J.W. and Wells, J.H. (2000) Using capacitive (radio frequency) dielectric heating in food processing and preservation–a review. *J Food Process Eng* **23**, 25–55.

Zheng, A., Zhang, L. and Wang, S. (2017) Verification of radio frequency pasteurization treatment for controlling *Aspergillus parasiticus* on corn grains. *Int J Food Microbiol* **249**, 27–34.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Populations of vegetative cells and *B. cereus* spores in the RGP treated with RF heating after pretreatments.