Master's Thesis of Agricultural Science

Application of radio-frequency heating for inactivation of foodborne pathogen in grain-based food products

곡물 소재 식품의 식중독균 제어를 위한 고주파 가열의 활용

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석사학위논문

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지도교수 강동현

이 논문을 석사학위 논문으로 제출함

2018 년 8 월

서울대학교 대학원 농생명공학부

정 기 옥

정기옥의 석사 학위논문을 인준함

2018 년 8 월

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ABSTRACT

The objectives of this study were, (i) to evaluate the efficacy of RF heating for inactivating food-borne pathogens, including *Salmonella* Typhimurium and *Staphylococcus aureus* on rice by different milling degrees, (ii) evaluate the antimicrobial effects of RF heating and the combination treatment of RF heating with ultraviolet (UV) radiation against foodborne pathogens, including *Escherichia coli* and *S.* Typhimurium in roasted grain powder, and (iii) evaluate conditions for the germination of *Bacillus cereus* spores in roasted grain powder. RF heating is a useful heating technology because heat is generated inside foods by applying an alternating electric field between two electrodes. Thus, RF heating can achieve rapid and uniform heating in foods. RF heating can be applied to control pathogens in grain-based food products without affecting product quality. Effects of milling degrees of rice was important in RF heating, so decreasing the milling degree of rice made the heating rate faster in general. Also, roasted grain powder was subjected to conventional convective heating and RF heating after inoculated with *E. coli*

and *S.* Typhimurium. The heating rate of RF heating was about 7.5 times as fast as that of conventional convective heating. The pathogen inactivation rate in roasted grain powder slowed down at last few seconds, so the RF-UV combined treatment was needed. The RF-UV combined treatment showed synergistic effects over 1 log unit. The combination treatment of RF heating with other technology can be an alternative to conventional heating treatments. In addition, it is difficult to control *B. cereus* spore in many rice-based products, especially in dried and powdered foods. Thus, modified tyndallization could be applied to roasted grain powder inoculated with *B. cereus* spore, but there are various considerations for conditions of spore germination. Roasted grain powder needed to be treated by modified tyndallization with additional germinant or other treatments. Therefore, application of RF heating for grain-based food products has many advantages of inactivating foodborne pathogens and there are many considerations which have significant effects on the performance of RF heating for controlling foodborne pathogens.

Keywords: **radio-frequency heating, food-borne pathogens, milling degrees, UV-C radiation, *Bacillus cereus* spore**
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I. INTRODUCTION

In recent years, consumption of grain-based food products like roasted grain powder called ‘sunsik’ in Korean has increased because consumers have been concerned about health and nutrition. Rice is a common ingredient in various grain-based food products. Also, rice is one of the most important cereals and consumed as a staple food in many places in the world. Generally, rice is consumed as a whole kernel of white rice treated by dehulling and polishing rough rice. After that, rice is sold in different kinds of milling degrees that is one of the important factors affecting physiochemical properties of rice.

However, outbreaks of *Salmonella* Typhimurium, *Staphylococcus aureus*, and *Bacillus cereus* in grain-related foods have been reported steadily in America in last 20 years. Especially, outbreaks of *Escherichia coli* and *B. cereus* in roasted grin powder were reported by the Korean press, and there are difficulties of controlling microorganisms in powdered foods. Furthermore, it is hard to eliminate foodborne microorganisms in low-a_w foods exhibiting increased tolerance to heat and other treatments (Beuchat et al., 2013). There were several alternative methods such as UV-C light, gaseous ozone, and infrared heating to control powdered foods. However, it took long time to inactivate about a 4-log reduction of *S*. Typhimurium in flour powder by UV-

C light (Condón-Abanto et al., 2016). When flaked red pepper was treated with 1.0 ppm ozone concentration for 360 min, *B. cereus* and *E. coli* were decreased by 1.5 and 2.0 log numbers, respectively (Akbas and Ozdemir, 2008). During infrared heating, the colour value decreased significantly on the surface of paprika powder due to particle agglomeration (Staack et al., 2008).

For the above reasons, it is necessary to develop new pasteurization technologies for grain-based food products, especially for powdered foods. Radio-frequency (RF) heating which involves the use of electromagnetic energy at frequencies between 1 and 300 MHz is a novel heating technology. Among these frequencies, only particular frequencies (13.56, 27.12, and 40.68 MHz) are permitted for domestic, industrial, scientific, and medical applications so as not to interfere with communication systems (Piyasena et al., 2003). RF generates heat inside of food due to molecular friction and space charge displacement in response to an applied alternating electrical field. This technology has huge potential for achieving rapid and uniform heating by delivering thermal energy quickly into every part of food products (Marra et al., 2009; Zhao et al., 2000). Thus, RF heating can be used in various food industries replacing conventional heating for solid and semi-solid food which have low thermal conductivities.

The effectiveness of RF heating treatment for pasteurization of roasted grain powder was less than that of rice. Combinations with other technologies, known as hurdle technology, could be an alternative to overutilization 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 (UV) radiation was selected because it is oriented to non-thermal technology and safe for use in foods. Also, UV radiation can cause damage to microbial DNA, so it was recommended for use in combination with other treatments such as mild heat, near-infrared heating, hydrogen peroxide, and ozone (Cheon et al., 2015; Ha and Kang, 2014; Hadjok et al., 2008; Selma et al., 2008). Thus, hurdle effect of RF heating with UV radiation was newly investigated in this study.

B. cereus is a gram-positive aerobic or facultatively anaerobic spore-forming pathogen. It is a cause of food poisoning, which is frequently associated with the consumption of grain-based foods (Drobniewski, 1993). Most of the research on thermal or non-thermal processing for inactivation of *B. cereus* spore has focused on experiment in liquid state not in solid like powdered food products. In order to inactivate *B. cereus* spore in liquid form of food, several decontamination methods including high pressure with nisin and modified tyndallization with carbon dioxide injection or germinant

addition were evaluated (Black et al., 2008; Kim, Kim, et al., 2012; Løvdal et al., 2011). The classical tyndallization process is a means of sterilization by which food is heated for a period of time on three successive days with intermittent storage at ambient temperature. Furthermore, the modified tyndallization process was adjusted by decreasing the interval of time between heat treatments. However, there was no research about controlling *B. cereus* spore in powdered food products by germination of spores. Therefore, the modified tyndallization was applied to roasted grain powder inoculated with *B. cereus* spore, but there are various considerations for the first heat treatment conditions.

In this study, rice was milled to degrees of 0, 2, 8, and 10% (based on brown rice) for investigating the changes of populations of pathogens and quality treated by RF heating. In addition, the efficacy of RF heating treatment for roasted grain powder was evaluated and roasted grain powder was treated by combination of RF heating and UV-C radiation. Lastly, the conditions of germination of *B. cereus* spores in roasted grain powder were evaluated.

II. MATERIALS AND METHODS

2.1. Radio frequency heating system

The RF heating system (Fig. 1) were composed of a RF heater (Seoul National University, Seoul, South Korea; Dong Young Engineering CO. Ltd., Gyeongnam, South Korea) and a temperature signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada). The 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. The temperature signal conditioner was connected to a computer for control using FISO Commander 2 Control and Analysis Software (FISO Technologies Inc.).

2.2. Experiments of rice by milling degrees influencing the performance of RF heating

2.2.1. Bacterial strains

S. Typhimurium (ATCC 19585, ATCC 43971, and DT 104), and *S. aureus* (ATCC 25923, ATCC 27213, ATCC 29213) were obtained from the Bacterial Culture Collection of Seoul National University (Seoul, South Korea) and were used in the experiments. Stock cultures were kept frozen at -80℃ in 0.7 ml of tryptic soy broth (TSB; Difco Becton, Dickinson, Sparks, MD, USA) and 0.3 ml of 50% (vol/vol) glycerol. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37℃ for 24 h, and stored at 4℃.

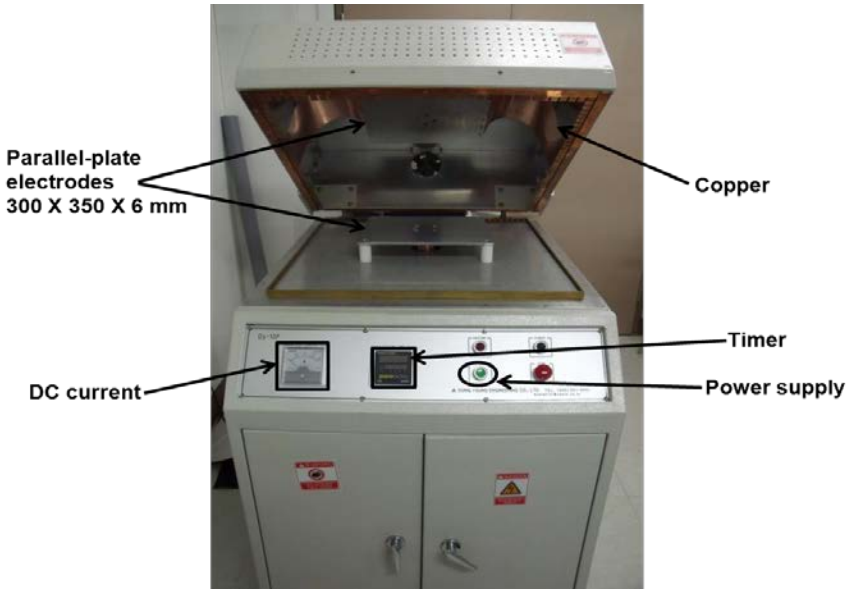


Fig. 1. Radio-frequency (27.12 MHz) heating system at Seoul National University (Seoul, Korea).

2.2.2. Preparation of pathogen inoculum

All strains of *S.* Typhimurium, and *S. aureus* were inoculated individually in 5 ml of TSB and incubated at 37℃ for 24 h. Overnight culture (1 ml) of each strain of *S.* Typhimurium and *S. aureus* was spread onto three TSA plates to make a bacterial lawn. 9 ml of 0.2% peptone water (PW; Difco) was added to each plate, and cell suspensions were made by rubbing the agar surface with a sterile swab (3M pipette swab, 3M Korea Ltd.) to remove cells. Those cell suspensions (ca. 10^{11}-10^{12} CFU/ml) were mixed to make a mixed culture cocktail.

2.2.3. Sample preparation and inoculation

Rice (Kyungsungmiga, Kyung-gi, South Korea) with different milling degrees was purchased from a local grocery store (Seoul, South Korea). For inoculation, 10 ml of culture cocktail was added in drops to 250 g of samples inside sterile. The inoculated samples were thoroughly mixed by hand massaging for 10 min to produce a homogeneous dispersal of inoculum throughout the rice. Then inoculated samples were dried for 1 h in a biosafety hood (22 $\pm$ 2℃) with the fan running until the water content of the sample

was equal to that of the uninoculated sample. The inoculated and dried rice samples were then immediately used in each experimental trial.

2.2.4. RF heating treatment

For the RF heating treatment, 25 g of inoculated rice were place in a polypropylene jar, 4.5 cm in diameter and 4.0 cm deep (NALGENE 2118-0002; Thermo Scientific, Hudson, NH), which was placed on the center of the bottom electrode. RF heating was applied to each prepared sample and heated to about 90℃ to maximize the efficiency of pasteurization while maintaining product quality.

2.2.5. Temperature measurement

A fiber optic temperature sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a temperature signal conditioner was used to measure real-time temperatures in samples during RF heating. The sensor was inserted directly into the center of the non-inoculated rice located in middle, and the temperature was recorded at 1 s intervals.

2.2.6. Bacterial enumeration

For enumeration of pathogens, 25 g of treated rice was transferred immediately into the sterile stomacher bag containing 100 ml of 0.2% PW pre-cooled in a 4℃ refrigerator (detection limit, 0.7 log CFU/g), and homogenized for 2 min with a 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 a selective medium, xylose lysine deoxycholate agar (XLD; Difco) for enumeration of *S.* Typhimurium and Baird-Parker agar (Baird-Parker Agar Base; Difco) for enumeration of *S. aureus*. All agar plates were incubated at 37℃ for 24 h. After that, black colonies were counted on XLD, and grey-black colonies with clear zones formed on Baird-Parker agar.

2.2.7. Color measurement

Colors of RF-treated and untreated uninoculated rice were measured using a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan). The values of L^*, a^*, and b^* were used to quantify color attributes and indicate lightness, redness, and yellowness of the sample, respectively.

2.3. Experiments of roasted grain powder treated by RF heating and UV radiation

2.3.1. Bacterial strains

E. coli (ATCC 10536, ATCC 25922, and B/4), *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), and *B. cereus* (ATCC 10876, ATCC 13061, ATCC 14579) were obtained from the Bacterial Culture Collection of Seoul National University (Seoul, South Korea) and were used in the experiments. Stock cultures were kept frozen at -80℃ in 0.7 ml of tryptic soy broth (TSB; Difco) and 0.3 ml of 50% (vol/vol) glycerol. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37℃ for 24 h, and stored at 4℃.

2.3.2. Preparation of pathogen inoculum

All strains of *E. coli*, *S.* Typhimurium, and *B. cereus* were inoculated individually in 5 ml of TSB and incubated at 37℃ for 24 h. Overnight culture (1 ml) of each strain of *E. coli* and *S.* Typhimurium was spread onto three TSA plates to make a bacterial lawn. 9 ml of 0.2% peptone water (PW; Difco) was

added to each plate, and cell suspensions were made by rubbing the agar surface with a sterile swab (3M pipette swab, 3M Korea Ltd.) to remove cells. Those cell suspensions (ca. 10^{11}-10^{12} CFU/ml) were mixed to make a mixed culture cocktail. Also, overnight culture (1 ml) of each strain of *B. cereus* was inoculated into two 250 ml conical flasks which contain 50 ml TSB to increase the cell number. They were incubated at 37°C for 24 h, collected by centrifugation at 4,000 × g for 20 min at 4°C, and washed three times with 0.2% PW. The final pellets were resuspended in 0.2 % PW, corresponding to approximately 10^{11} to 10^{12} CFU/ml.

2.3.3. Sample preparation and inoculation

Roasted grain powder (ChungO, Kyung-gi, South Korea) called “Misugaru” in Korean was purchased from a local grocery store (Seoul, South Korea). It is a combination of many different grains. For inoculation, 5 ml of culture cocktail was added in drops to 250 g of samples inside sterile low-density polyethylene (LDPE) 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 roasted grain powder. The inoculated

roasted grain powder samples were then immediately used in each experimental trial.

2.3.4. Combined treatment of RF heating and UV radiation

Firstly, for the RF heating treatment, 25 g of inoculated roasted grain powder was put in a polypropylene jar, 6.3 cm in diameter and 6.1 cm deep (NALGENE 2118-0004; Thermo Scientific, Hudson, NH), which was placed on the center of the bottom electrode. RF heating was applied to each prepared sample and heated to about 120℃ to maximize the efficiency of pasteurization while maintaining product quality. Secondly, UV experiments were carried out in a UV radiation apparatus consisted of two banks of germicidal emitting lamp (254 nm, G6T5, Sankyo Denki, Japan). The UV lamps were located at the ceiling and bottom of the radiation chamber and were turned on for at least 10 min to stabilize. Sample was spread out on a thickness of 0.07 mm and 25 × 25 cm polypropylene (PP) film located at the middle of two lamps. The reaction time for UV treatment was 3 min, and the corresponding UV doses were 1.90 kJ/m^2 calculating by multiplying the irradiation time for the intensity of UV lamp. The UV intensity was measured by using a spectrometer

at 253.7 nm wavelength (AvaSpec-ULS2048-USB2-UA-50, Avantes, Netherlands).

2.3.5. Temperature measurement

A fiber optic temperature sensor (FOT-L) connected to a temperature signal conditioner was used to measure real-time temperatures in samples during RF heating. The sensor was inserted directly into the center of the non-inoculated roasted grain powder located in middle, and the temperature was recorded at 2 s intervals.

2.3.6. Bacterial enumeration

For enumeration of pathogens, 25 g of treated roasted grain powder was transferred immediately into the sterile stomacher bag containing 225 ml of 0.2% PW pre-cooled in a 4°C refrigerator (detection limit, 1 log CFU/g), and homogenized for 2 min with a stomacher. 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 a selective medium, xylose lysine deoxycholate agar (XLD; Difco) for enumeration of *S.* Typhimurium and

Mannitol-Egg Yolk-Polymyxin agar (MYP; Difco) for enumeration of *B. cereus*. Also, 1 ml of sample or diluent was dispensed onto Petrifilm (3M Petrifilm Coliform Count Plates; 3M Korea Ltd.) for enumeration of *E. coli*. All agar plates and Petrifilms were incubated at 37℃ for 24 h. After that, black colonies were counted on XLD, pink-red colonies with the zone of white precipitate forms on MYP, and blue colonies with bubbles on Petrifilm.

2.3.7. Color measurement

Colors of RF-treated and untreated uninoculated roasted grain powder were measured using a Minolta colorimeter (CR400). The values of L^*, a^*, and b^* were used to quantify color attributes and indicate lightness, redness, and yellowness of the sample, respectively.

2.4. Conditions for germination of Bacillus cereus spore in roasted grain powder

2.4.1. Bacterial strains

Bacillus cereus (ATCC 10876, ATCC 13061, ATCC 14579) were obtained from the Bacterial Culture Collection of Seoul National University (Seoul, South Korea) and were used in the experiments. Stock cultures were kept frozen at -80℃ in 0.7 ml of tryptic soy broth (TSB; Difco Becton, Dickinson, Sparks, MD, USA) and 0.3 ml of 50% (vol/vol) glycerol. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37℃ for 24 h, and stored at 4℃.

2.4.2. Preparation of pathogen inoculum

All strains of *B. cereus* were inoculated individually in 5 ml of TSB and incubated at 37℃ for 24 h. Overnight culture (1 ml) of each strain of *B. cereus* was spread onto three TSA plates to make a bacterial lawn. Each plate was incubated at 30℃ for 5 days to make spores, and 9 ml of 0.2% peptone water (PW; Difco) was added to each plate. Then, cell suspensions were made by

rubbing the agar surface with a sterile swab to remove cells. Those cell suspensions were mixed, collected by centrifugation at 4,000 × g for 20 min at 4°C, and washed three times with 0.2% PW to eliminate impurities. The final pellets were resuspended in 0.2% PW, corresponding to approximately 10^9 to 10^{10} CFU/ml.

2.4.3. Sample preparation and inoculation

Roasted grain powder was purchased from a local grocery store (Seoul, South Korea). It is a combination of many different grains. For inoculation, 5 ml of spore suspension was added in drops to 250 g of samples inside sterile low-density polyethylene (LDPE) 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 roasted grain powder. The inoculated roasted grain powder samples were then immediately used in each experimental trial.

2.4.4. Conditions for germination of B. cereus spore

Firstly, for the first heating treatment, 25 g of inoculated roasted grain powder were place in a polypropylene jar, 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) of various conditions. The conditions were composed of temperature, relative humidity, and duration time. The conditions of temperature were 80 and 90℃, those of the relative humidity were 30, 50, and 70%, and lastly those of the duration time were 1, 2, 3, and 4 hours. Secondly, samples were placed in the low temperature incubator (IL-11; JEIO TECH, Daejeon, Korea) of 30℃ for 1 hour. Lastly, RF heating was applied to each prepared sample and heated to about 120℃ to inactivate germinated cells in roasted grain powder.

2.4.5. Mild heat treatment

Before treatment, the water bath was set to 60, 70, 80℃. 25 g of roasted grain powder inoculated with vegetative cells or spore of *B. cereus* for each sample was placed in a PP jar. Samples inoculated with spore were treated in

the constant temperature and humidity chamber of 90℃ and 70% for 4 hours followed by cooling down at 30℃ in the low temperature incubator for 1 hour. Then, 25 g of samples inoculated with vegetative cells or spore with treatment were transferred into the sterile stomacher bag containing 225 ml of 0.2% PW and homogenized for 2 min with a stomacher. After homogenization, 1-ml aliquots of the samples inoculated with vegetative cells or spore were put in 9-ml blanks of 0.2% PW in the water bath preheated to 60, 70, 80℃, respectively. Then 1-ml aliquots of the samples were enumerated immediately by serial dilution.

2.4.6. Bacterial enumeration

For enumeration of pathogens, 25 g of treated roasted grain powder was transferred immediately into the sterile stomacher bag containing 225 ml of 0.2% PW pre-cooled in a 4℃ refrigerator (detection limit, 1 log CFU/g), and homogenized for 2 min with a stomacher. After homogenization, 1-ml aliquots of the sample inoculated with *B. cereus* spore were put in 9-ml blanks of 0.2% PW in an 80℃-water bath (BW-05G; JEIO TECH, Daejeon, Korea) for 20 min to eradicate all vegetative cells of *B. cereus*. Then, 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 a selective medium, Mannitol-Egg Yolk-Polymyxin agar (MYP; Difco) for enumeration of *B. cereus*. All agar plates were incubated at 37℃ for 24 h. After that, pink-red colonies with the zone of white precipitate forms on MYP.

2.5. Statistical analysis

All experiments were performed in triplicate. Data were analyzed by the analysis of variance procedure and Duncan's multiple-range test of the Statistical Analysis System (SAS Institute, Cary, NC). Significant differences in the processing treatments were determined at a significance level of p = 0.05.

III. RESULTS

3.1. Effect of milling degree of rice on the performance of RF heating for inactivation of foodborne pathogens

3.1.1. Temperature curves of rice with different milling degrees

Average temperatures of rice with milling degrees varying from 0 to 10% during RF heating at a constant frequency of 27.12 MHz are shown in Fig. 2. At the same milling degree, the temperature increased with increasing treatment time. The heating rate of rice was dependent on milling degree, but there is limit when milling degree went under the 2% level. Rice with 2% milling degree which had the highest heating rate increased from 21.3℃ to 91.1℃ when exposed to RF energy for 56 s. For the same treatment time, the temperature of rice with 10% milling degree was 65.7℃.

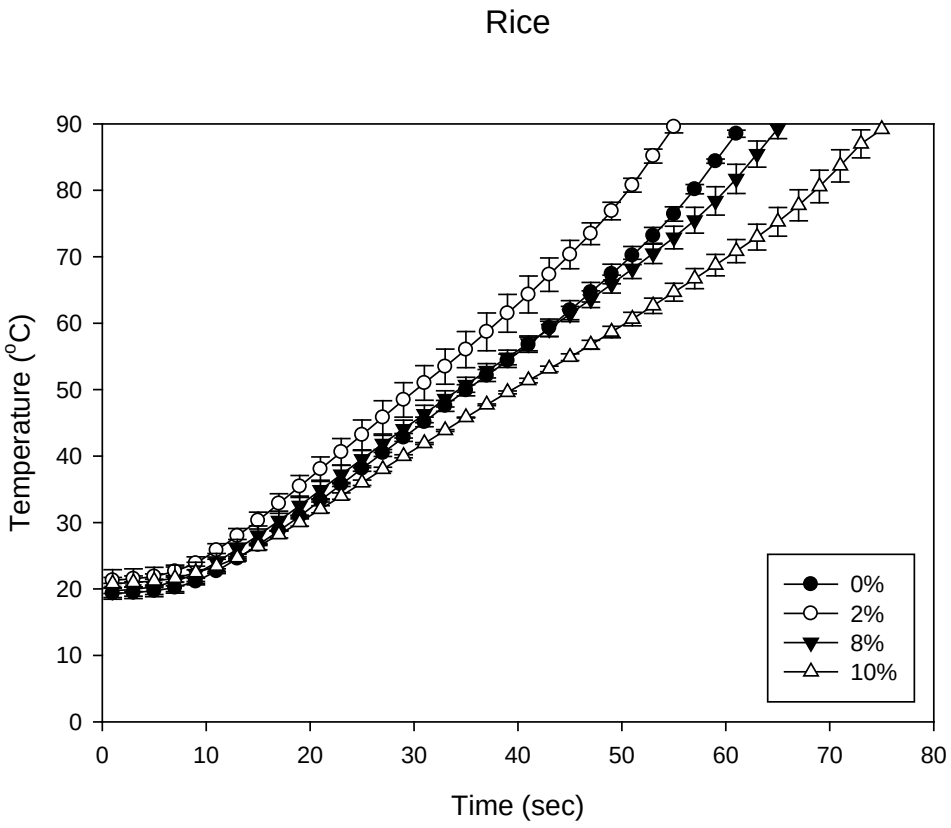


Fig. 2. Temperature curves of center of four kinds of rice during RF heating at the milling degree of 0% (●), 2% (○), 8% (▼), and 10% (△). The results are means from three experiments, and error bars indicate standard deviations.

3.1.2. Effect of milling degree on the inactivation of foodborne pathogen in rice

The survival of pathogens subjected to RF heating at various milling degrees is shown in Table 1 and 2. Populations of *S.* Typhimurium and *S. aureus* decreased with increasing treatment time. Populations of *S.* Typhimurium and *S. aureus* in rice with high milling degrees were inactivated more effectively compared to those in rice with low milling degrees. After 55 s for both pathogens, the reductions by various milling degrees were significantly different. Both for two pathogens, the reductions at milling degree of 2% were 5.19 and 5.08, respectively.

Table 1. Effect of RF heating on populations of *S*. Typhimurium on rice

Degree of milling (%)	**Time (sec)**							
	20	40	50	55	60	65	70	75
	Log reduction (log CFU/g)							
0	0.17 ± 0.06 A	1.25 ± 0.14 A	2.30 ± 0.64 AB	3.64 ± 0.38 A	> 5.97	> 5.97	> 5.97	> 5.97
2	0.20 ± 0.11 A	1.45 ± 0.35 A	2.56 ± 0.23 A	5.19 ± 0.50 B	5.89 ± 0.62 A	> 6.09	> 6.09	> 6.09
8	0.07 ± 0.30 A	1.06 ± 0.36 A	1.91 ± 0.07 AB	2.18 ± 0.16 C	3.19 ± 0.67 B	4.50 ± 0.40 A	6.01 ± 0.62 A	> 6.31
10	0.13 ± 0.40 A	0.97 ± 0.54 A	1.70 ± 0.14 B	1.80 ± 0.16 C	2.18 ± 0.03 C	3.10 ± 0.75 B	4.70 ± 0.56 B	5.76 ± 0.53

Mean values ± standard deviation.

Values in the same column followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Effect of RF heating on populations of *S. aureus* on rice

Degree of milling (%)	**Time (sec)**							
	20	40	50	55	60	65	70	75
	Log reduction (log CFU/g)							
0	-0.01 ± 0.04 A	0.56 ± 0.20 A	1.72 ± 0.30 A	2.77 ± 0.14 A	7.59 ± 0.17 A	> 7.84	> 7.84	> 7.84
2	0.13 ± 0.05 B	0.71 ± 0.20 A	1.77 ± 0.40 A	5.08 ± 0.79 B	7.79 ± 0.11 A	> 7.90	> 7.90	> 7.90
8	0.04 ± 0.02 AB	0.23 ± 0.05 B	0.72 ± 0.18 B	1.15 ± 0.23 C	1.53 ± 0.31 B	2.64 ± 0.53 A	5.09 ± 0.45 A	> 7.80
10	0.01 ± 0.05 A	0.19 ± 0.11 B	0.71 ± 0.12 B	0.90 ± 0.17 C	1.28 ± 0.15 B	2.15 ± 0.46 A	3.53 ± 0.55 B	5.03 ± 0.32

Mean values ± standard deviation.

Values in the same column followed by the same letter are not significantly different ($P > 0.05$).

3.1.3. Effect of RF heating on product quality

The color values of rice of various milling degrees after RF heating are shown in Table 3. The color The L*, a*, and b* values of RF-treated rice by various milling degrees were not significantly ($P > 0.05$) different from those of nontreated samples. Therefore, RF heating did not affect the quality of rice for all milling degrees.

Table 3. Surface color values of RF-treated and untreated rice[a]

Parameter	Degree of milling (%)			
	0	2	8	10
L* [b]				
Control	59.43 ± 0.81 a	61.98 ± 0.87 a	66.35 ± 1.53 a	65.57 ± 0.75 a
RF treated	59.71 ± 0.74 a	64.16 ± 2.37 a	69.07 ± 1.34 a	67.72 ± 1.70 a
a*				
Control	3.35 ± 0.18 a	3.55 ± 0.12 a	-0.19 ± 0.08 a	-0.43 ± 0.06 a
RF treated	3.15 ± 0.10 a	3.37 ± 0.17 a	-0.30 ± 0.14 a	-0.36 ± 0.06 a
b*				
Control	20.91 ± 0.67 a	22 ± 0.35 a	11.34 ± 0.39 a	10.69 ± 0.52 a
RF treated	21.24 ± 0.43 a	24.70 ± 0.61 a	12.83 ± 1.05 a	10.72 ± 0.57 a

[a] Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).

[b] Color parameters are L* (lightness), a* (redness), b* (yellowness).

3.2. Effect of RF heating for inactivation of foodborne pathogens in roasted grain powder

3.2.1. Temperature curves of roasted grain powder

Average temperature of roasted grain powder during RF heating at a constant frequency of 27.12 MHz are shown in Fig. 3. During 120 s, roasted grain powder center temperature reached 120℃. Especially, the heating speed slowed down about during last 30 s.

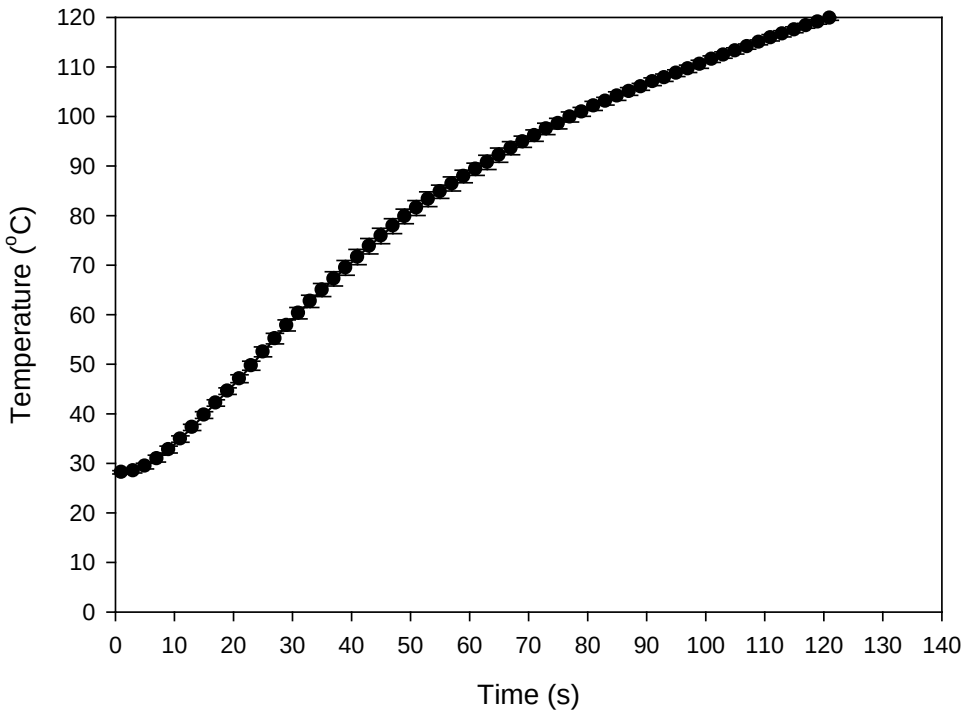


Fig. 3. Average temperature-time history of the center of roasted grain powder during RF heating. The results are means from three experiments, and error bars indicate standard deviations.

3.2.2. Survival of foodborne pathogens in roasted grain powder subjected with RF heating

Populations (log CFU/g) of *E. coli*, *S.* Typhimurium, and *B. cereus* in roasted grain powder during RF heating are shown in Table 4. When RF heating was treated for the maximum time, 120 s, it achieved 4.68, 3.89, and 4.54-log reductions in *E. coli*, *S.* Typhimurium, and *B. cereus*, respectively. Although reductions of three pathogens decreased slightly while treatment time increased. The rate of reductions of *E. coli*, *S.* Typhimurium decreased last few seconds, but the longer tail was made in reduction curve of *B. cereus* vegetative cells. For the first 60 s, RF heating achieved 4.18-log reduction in *B. cereus*, but for the last 60s, just 0.36-log reduction was achieved.

Moreover, survival of vegetative cells and spore for *B. cereus* in roasted grain powder during RF heating are shown in Fig. 4. Significant differences in survival of two forms of vegetative cells and spore were shown for all treatment time. At 120 s, there was big difference of 3.9-log reduction for two forms of *B. cereus*.

Table 4. Effect of radio-frequency heating on populations of *Escherichia coli*, *Salmonella* Typhimurium, and vegetative cells of *Bacillus cereus* in roasted grain powder[a]

Pathogen	**Time (sec)**					
	0	60	80	100	110	120
	Population (log CFU/g)					
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*_vegetative cells**	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

[a]Means ± standard deviations from three replications.

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

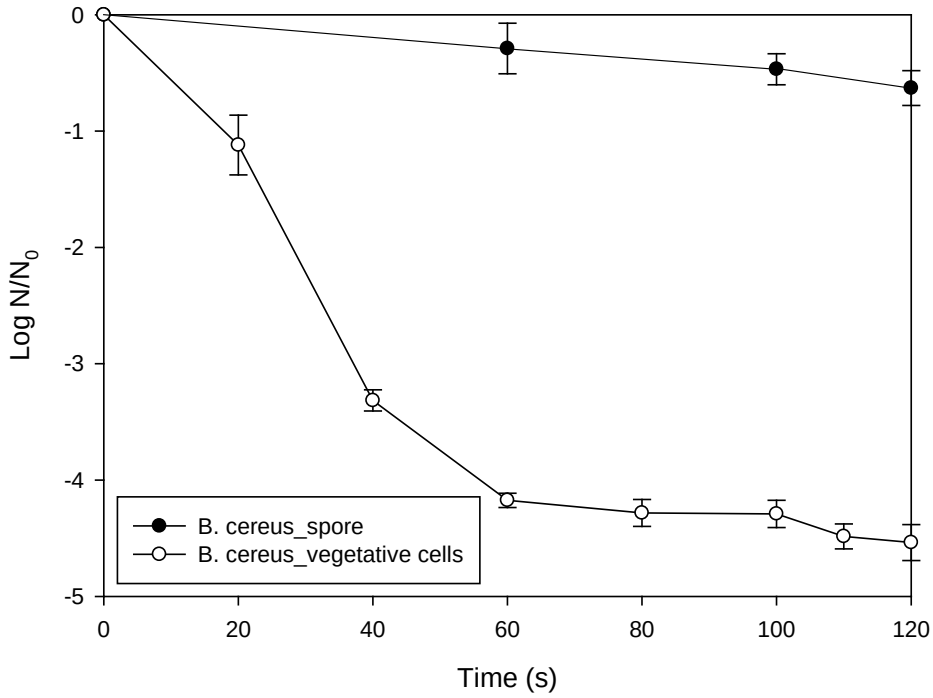
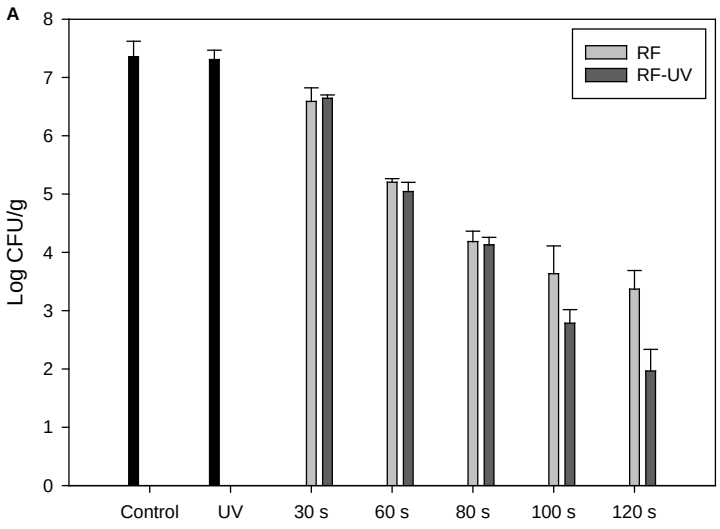
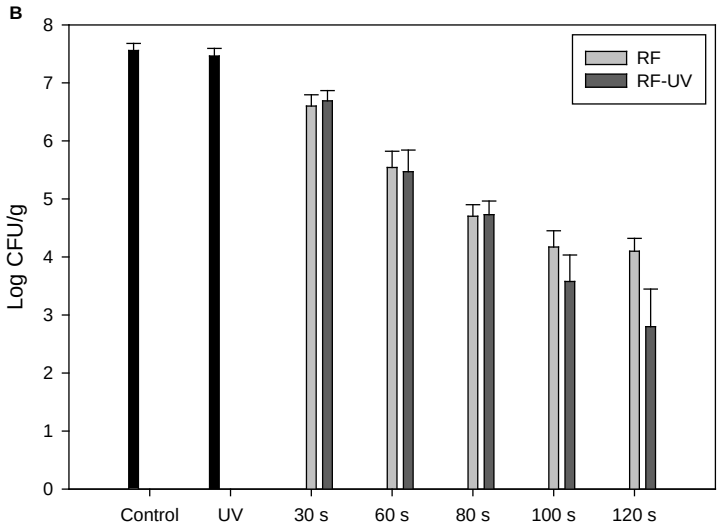


Fig. 4. Survival curves for spore and vegetative cells of *Bacillus cereus* in roasted grain powder treated with RF heating. The results are means from three experiments, and error bars indicate standard deviations.

3.2.3. Synergistic bactericidal effect of combined treatment

Fig. 5 represented the effect of RF heating, UV radiation, and sequential treatment of both technologies on the survival numbers of *E. coli*, *S.* Typhimurium, and *B. cereus* cells in roasted grain powder. Reductions of 4.48 and 5.39 log units were observed in *E. coli* after sequential RF-UV combined treatment for 100 and 120 s, respectively. Also, reductions of 3.98 and 4.76 log units were observed in *S.* Typhimurium after the same treatment for the same time before. The sums of results for RF and UV inactivation were lower than values obtained by the sequential treatment of both technologies. That is, synergistic effects were observed for *E. coli* and *S.* Typhimurium. However, statistically significant ($P < 0.05$) differences between the sums of RF and UV inactivation and values for inactivation achieved with combination treatment were observed only after treatment times of 120 s. Log reductions from the synergistic effects after 120 s, calculated by subtracting the sums of RF and UV reductions from the values obtained during sequential RF-UV treatment, were 1.4 and 1.31 log units at 120 s for each pathogen, respectively.


A
Log CFU/g
RF
RF-UV
Control
UV
30 s
60 s
80 s
100 s
120 s


B
Log CFU/g
RF
RF-UV
Control
UV
30 s
60 s
80 s
100 s
120 s

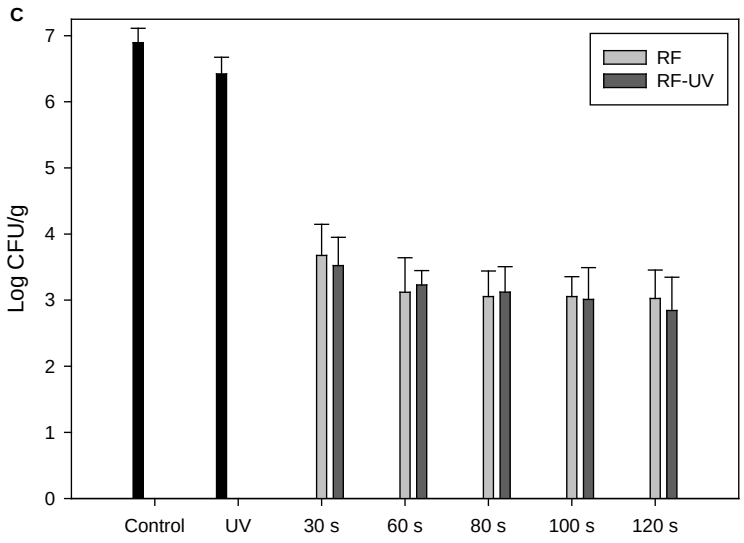


Fig. 5. Survival (log CFU/g) of (a) *Escherichia coli*, (b) *Salmonella* Typhimurium, and (c) *Bacillus cereus* in roasted grain powder during RF heating, UV radiation, and combined application of both technologies. The results are means from three experiments, and error bars indicate standard deviations.

3.2.4. Effect of RF-UV combined treatment on product quality

The color values of roasted grain powder after RF- and combined RF-UV treatment are shown in Table 2. The L*, a*, and b* values of RF-UV-treated roasted grain powder were not significantly ($P > 0.05$) different from those of nontreated samples. Therefore, both RF heating and combined treatment of RF and UV treatment did not affect the quality of roasted grain powder.

Table 5. Surface color values of RF-treated and untreated roasted grain powder[a]

Treatment	Color		
	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

[a] Means ± standard deviations from three replications. Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).

[b] Color parameters are L* (lightness), a* (redness), b* (yellowness).

3.2.5. Effect of temperature and humidity on germination rate and survival of Bacillus cereus spore

The germination rate (%) of *B. cereus* spore is shown in Fig. 6. *B. cereus* spore germination rate was identified in conditions of fixed temperature, 90℃ in the constant temperature humidity chamber and three different relative humidity, such as 30, 50, and 70%. For 1 h in that situation, there was no significant change in the form of spore, but 99.41% of germination rate was achieved in the 90℃ and 70% chamber. Also, a comparison at fixed relative humidity, 70% was confirmed at 80 and 90℃. Even for 4 h, only 63.9% of germination rate achieved in the 80℃ and 70% chamber.

After the first heat treatment in the constant temperature and humidity chamber went by and the cooling time in 30℃ for 1 h passed as mentioned above, RF heating until 120℃ was treated for roasted grain powder. However, there was no significant difference in treated and untreated samples in *B. cereus* populations. Under 1-log reductions were shown for both pretreatment time, 3 or 4 h in Fig. 7.

From above results, it is possible to think that there was difference in heat resistance for just vegetative cells and germinated cells in roasted grain powder. For all treatment temperature, such as 60, 70, and 80℃, there were

significant differences for all the temperature and time. Especially, at 70℃, there was a difference in 3.73-log reduction even for 30 s. From all temperature in the water bath treatment, heat resistance of germinated cells was remarkably lower than that of vegetative cells.

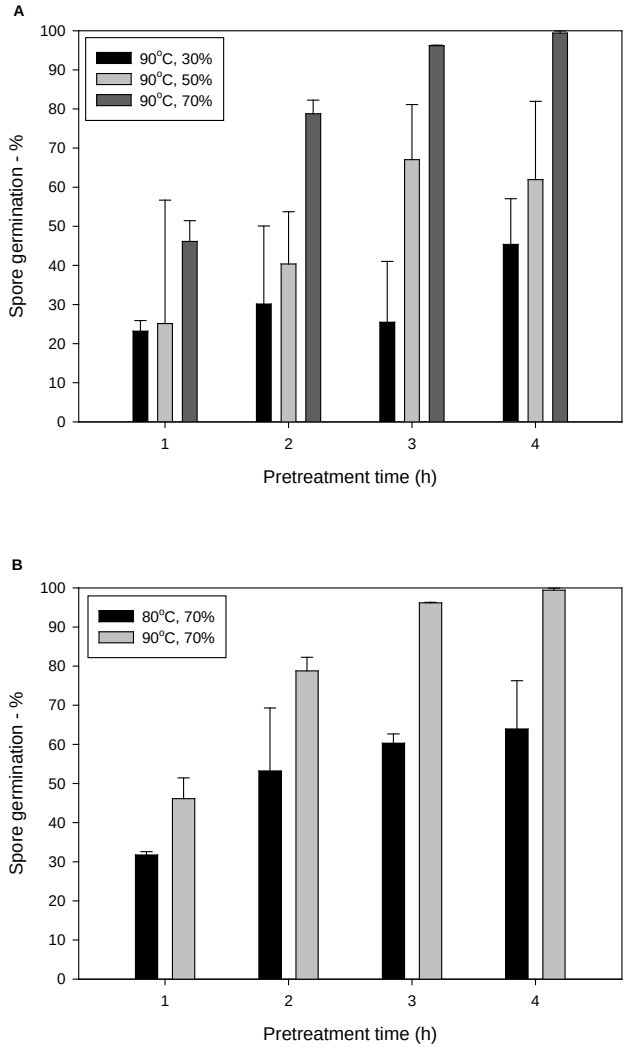


Fig. 6. *B cereus* spore germination rate (%) after being stored in constant temperature and humidity chamber. The results are means from three experiments, and error bars indicate standard deviations.

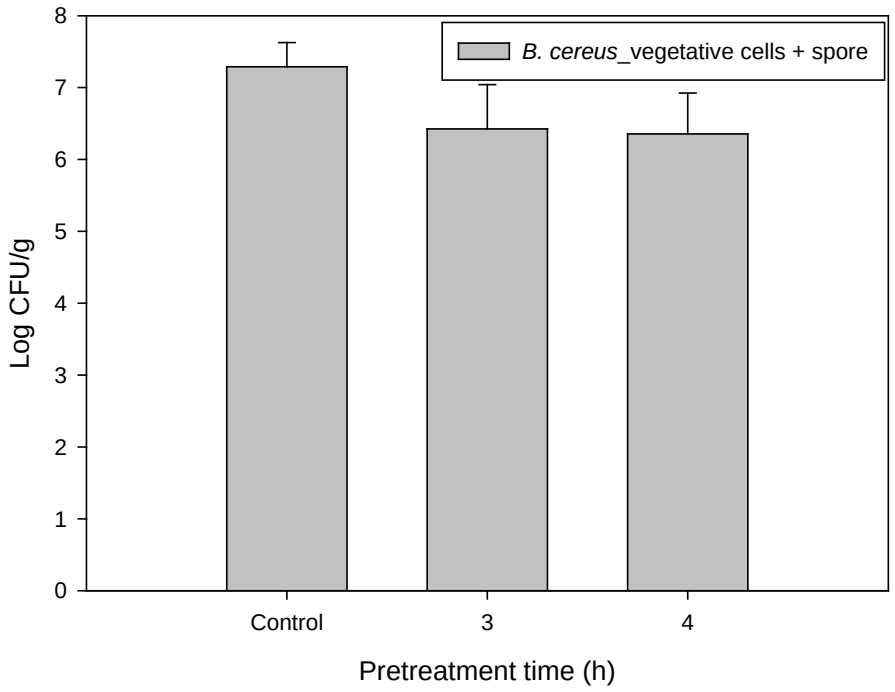
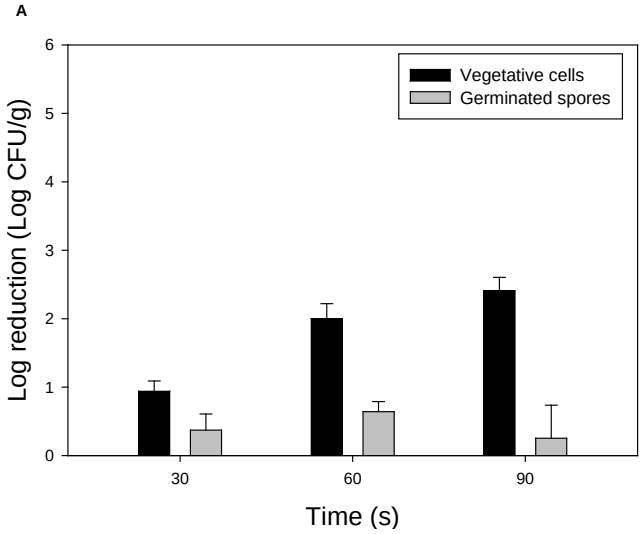
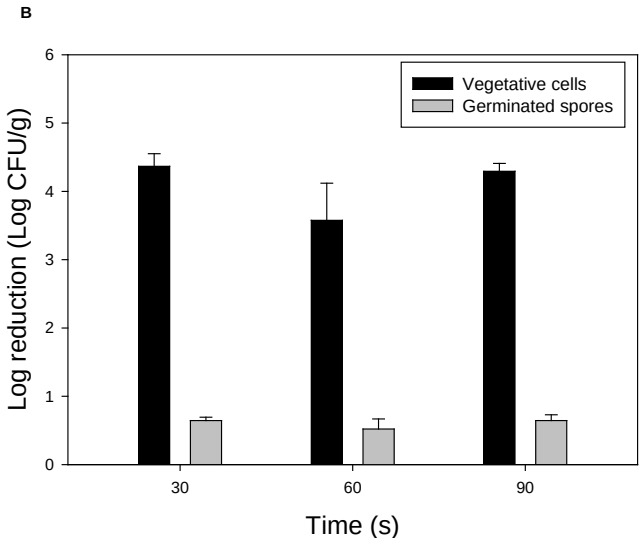


Fig. 7. Populations of vegetative cells and spores of *B. cereus* in roasted grain powder treated with RF heating after pretreatments. The results are means from three experiments, and error bars indicate standard deviations.

60 ^{o}C


A
Vegetative cells
Germinated spores
Log reduction (Log CFU/g)
0
1
2
3
4
5
6
30
60
90
Time (s)

70 ^{o}C


B
Vegetative cells
Germinated spores
Log reduction (Log CFU/g)
0
1
2
3
4
5
6
30
60
90
Time (s)

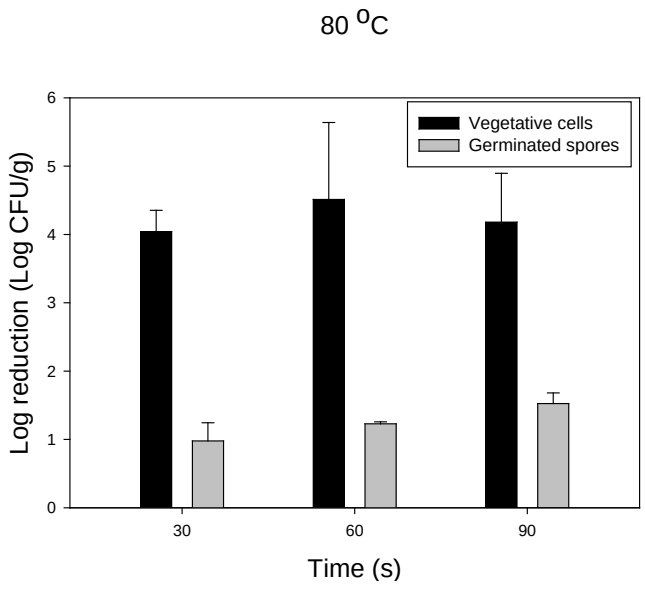


Fig. 8. Log reductions for vegetative cells and germinated spore of *Bacillus cereus* in roasted grain powder treated with (a) 60℃, (b) 70℃, (c) 80℃ mild heat in the water bath. The results are means from three experiments, and error bars indicate standard deviations.

IV. DISCUSSION

RF heating showed much faster heating rate than conventional convective heating did. During conventional heating, heat transfer can be achieved via conduction and convection (Zhu et al., 2014). RF heating could be applied to solid and semi-solid foods which have low thermal conductivities. RF heating is a promising food processing technology due to internal heating resulting from the direct interaction between electromagnetic waves and the material (Gao et al., 2011; Ha et al., 2013; Kim, Sagong, et al., 2012). Therefore, the temperature rising rate of RF heating was 7.5 times as fast as that of conventional convective heating. There are no published data showing the inactivation of foodborne pathogens in rice of different milling degrees using RF heating. The more milling degrees fell down, the more the temperature, heating rate, and inactivation of *S.* Typhimurium and *S. aureus* went up and became faster, but there was a low limit, the milling degree of 2% at which decreasing milling degrees led to a leveling of RF treatment time. This result showed milling degree-dependent heating rate in rice, since RF energy has no non-thermal effect on microbial inactivation (Geveke et al., 2002; Ponne et al., 1996). Thus, it is reasonable expectation that pathogens are inactivated more rapidly at the higher temperature.

Meanwhile, RF heating treatment alone made a tail during inactivating *E. coli*, *S.* Typhimurium, and *B. cereus* in roasted grain powder. Hurdle combinations for controlling microorganisms in powdered foods have been reported for many times. Microwave heating followed by refrigerated storage at 5℃ resulted in the progressive death of *C. sakazakii* in the reconstituted form of powder infant formula (Pina-Pérez et al., 2014). Cheon et al. (2015) obtained the synergistic effect with combined UV-C irradiation and mild heating of *Escherichia coli* O157:H7 and *S.* Typhimurium in powdered red pepper. Hamanaka et al. (2011) treated fresh fig fruit with sequential UV and infrared treatment and achieved 3 log reductions in the counts of fungi after 30 s infrared heating followed by 30 s UV radiation. Therefore, application of hurdle technology is becoming more attractive maintaining the product quality, and single treatment such as heating can be utilized at lower level. In this study, combined RF and UV treatment resulted in more reductions in populations of *E. coli* and *S.* Typhimurium than did either treatment alone, so it showed the synergistic effect. However, population of *B. cereus* showed no significant difference in RF- and RF-UV treatment and it is explainable with UV resistance in *B. cereus* species (Setlow, 2001). Moreover, further studies about damages of relationship between cell wall and membrane with intracellular

substance such as nucleic acids are needed to understand the mechanism of the synergistic effect of combined RF-UV treatment.

In the other hand, it is important to control spore of *B. cereus* in powdered foods. The conditions of *B. cereus* germination were established, but heat resistance problem remained. It is reasonable that the condition of 90℃ and 70% storage for 4 h was harsh and lethal enough. Dormant spores return to their vegetative growth cycle in a process including germination (Ghosh and Setlow, 2010; Moir, 2006). Germinated spores have a lower heat resistance than dormant spores (Setlow, 2003). Thus, induced germination and subsequent inactivation of germinated spores have been evaluated as a spore inactivation method (Brown et al., 1979; Gould et al., 1968). However, no research for germination of *B. cereus* spore in powdered foods was investigated. Because of high heat resistance after modified tyndallization in this study, other methods are need to devise. If I modify little bit from this method, additional treatment combined with modified tyndallization can be injection with carbon dioxide or addition of germinants such as L-alanine and inosine.

In order to verify commercial application of this new germicidal technology, it is necessary to investigate quality changes of the treated food. After the maximum treatment of rice of various milling degrees applied to inactivation

of foodborne pathogens, color values (L*, a*, b*) of samples were not significantly ($P > 0.05$) different from those of the untreated controls. Similarly, following maximum treatment of roasted grain powder with RF heating alone or RF heating combined with UV radiation, color values of samples were not significantly ($P > 0.05$) different from those of the control. Other studies also reported that RF heating treatment did not affect qualities of agricultural products. Gao et al. (2010) reported that the RF treatment did not affect the almond quality such as peroxide values, fatty acid, and kernel color. Zheng et al. (2017) reported that RF treated corn maintained the levels of moisture content, water activity, protein, starch, ash, fat, fatty acid, and color.

As demonstrated in this study, RF heating led to effective inactivation of *S.* Typhimurium and *S. aureus* in rice of different milling degrees, without causing any quality loss. Also, about a 5-log reduction of *E. coli* and *S.* Typhimurium could be achieved in roasted grain powder by performing RF heating with UV radiation without affecting product quality, but not in *B. cereus*. Although the pilot apparatus used in this study was batch type and its capacity was comparatively small, RF and UV combined processing for powdered foods could be expanded to industrial scale by using it in the form of continuous line processing. Also, 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* spore was possible, but there are many factors to be considered due to increased resistance of germinated spores.

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VI. 국문초록

본 연구의 목적은 (1) 쌀의 도정도에 따른 고주파 가열의 *Salmonella* Typhimurium 과 *Staphylococcus aureus* 살균 효율 평가, (2) 선식에서 고주파 가열 및 고주파 가열과 자외선의 조합 처리를 통한 *Escherichia coli* 와 *S.* Typhimurium 의 제어 효과 규명, 그리고 (3) 선식에서 *Bacillus cereus* 포자의 발아 조건 평가이다. 고주파 가열은 두 전극 사이에서 교류 전기장이 형성되어 식품 내부로부터 열이 발생하기 때문에 효과적인 가열 처리 기술이라고 할 수 있다. 그래서 고주파 가열은 식품에서 빠르고 고른 온도 상승을 가능하게 한다. 고주파 가열은 품질 파괴 없이 곡물 소재의 식품에 사용할 수 있다. 그 중에서도 쌀에 적용했을 때, 쌀의 도정도가 낮아질수록 고주파 가열 속도가 빨라지다가 2% 이하에서는 더 이상 증가하지 않고 감소하였다. 또한, *E. coli* 와 *S.* Typhimurium 이 접종된 선식에도 고주파 가열 처리를 하여 식중독균을 제어하였다. 선식에서 고주파 가열 처리 시, 마지막 10 초 동안 이전 처리 시간에 비해 유의적인 저감화 차이가 나지 않았기 때문에 살균 효율 증진을 위해 자외선

조사와의 병행 처리 연구를 수행한 결과, 시너지 효과를 보여줄 수 있었다. 따라서 고주파 가열 처리와 다른 기술과의 조합 처리는 기존 가열 방식의 대안이 될 수 있을 것이다. 또한, 곡물을 소재로 한 분말 식품에서 주로 발생하는 *B. cereus* 포자 문제를 변형된 간헐 멸균법으로 발아 조건을 확인하고 저감화 실험을 진행하였으나 세포의 열 저항성에 대한 추가적인 연구가 더 필요하다. 실제 식품 산업에서 곡물 소재의 식품 처리 시 여러가지 사항을 고려해야하기 때문에 본 연구를 통해 도출된 결과는 실제 식품 산업에서 고주파 가열 기술의 살균 효과를 최적화 하는 데 유용하게 사용될 수 있을 것이다.

주요어: 고주파 가열, 식품매개 병원균, 도정도, 자외선 조사,
바실러스 포자
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