A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Inactivation of *Salmonella, Escherichia coli* and *Bacillus cereus* spores on foods using superheated steam

과열수증기를 이용한 식품의 ***Salmonella,*** ***Escherichia coli*** 및 ***Bacillus cereus*** 포자의 살균

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석사학위논문

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지도교수 최 영 진

이 논문을 석사학위논문으로 제출함
2017 년 8 월

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배 혜 련

배혜련의 석사학위논문을 인준함
2017 년 8 월

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Abstract

The consumption of spices and nuts has been increased due to the public favoring healthy and fresh foods. Since nuts and spices are prone to deteriorating quality during cooking and sterilization procedure, they are usually consumed as raw material or treated with minimal subsequent sterilization processes. However, these foods are often exposed to foodborne pathogens such as *Salmonella* during the harvesting, which is a serious hazard for the food safety or due to the low moisture contents during the drying and pulverizing processes promoting the forming of strains of *Bacillus* spores, some foods are easy to get damages in quality from thermal treatment. Thus, the red peppers were sterilized to prevent the formation of spores from the vegetative *Bacillus* cells. Based on the previous study that the lab-scale sterilization using superheated steam (SHS) was conducted on black peppercorns, pecans, and almonds, the SHS equipment was improved for the industrial applications as a scale-up study in consideration of increasing the treatment amount of samples and changing the direction of SHS flow in order to reduce heat loss. In this study, the applicability of the modified equipment was verified as a sterilization apparatus on food. *Salmonella* inoculated on black peppercorns, pecans, and almonds was decontaminated below to detection limit (1 log CFU/g) by 100℃ saturated steam, 140 and 180℃

superheated steam. And *E. coli*, and *Salmonella* inoculated on red pepper were achieved below to detection limit (0.6 log CFU/g) within 10 s by 120, 150, and 180℃ SHS, and total aerobic bacteria was reduced within 20, 30, and 50 s by 120, 150, and 180℃ SHS, respectively. Due to SHS treatment at a high temperature for a short time, capsaicin content did not change.

Recently, instant foods are more demanded as the number of singles and dual income households increase. Garlic, one of the main ingredients in instant food, is easily contaminated with *Bacillus*, which allows heat-resistant spores to form and be present in garlics at the level of 2-3 log CFU/g even after washing step. For retort foods containing garlic, sterilization is done at 121.1℃. However, the time for an additional processing is necessary owing to deal with heat-resistant bacteria and *Bacillus* spores including *Bacillus cereus* strain in the garlic, causing the quality loss of the foods. For this reason, *B.* cereus spores were pretreated with SHS to reduce the total time for subsequent retort processing and minimize the quality deterioration of foods. The study for inactivating the B. cereus spores by SHS was conducted with garlics cloves and sliced garlics, and germinants were used to induce the spore germination in order to increase the efficiency of the SHS sterilization process. As a result, the treatment of SHS at 180℃ on garlic cloves inactivated the contamination of the spores by 3 log CFU/g within 90 s. Inactivation of the spores on garlic cloves was more efficient than the sterilization on garlic slices,

because garlic slices were dried up faster during the SHS treatment. Effects of the germination inducers were investigated using a germinant buffer with L-alanine, inosine (as famously known as germination inducers), and disodium 5'-inosinate (IMP). The Ala+IMP buffer was applied to real food sample with spores inoculated. The sample treated with the germinant buffer was about 0.5 log CFU/g more effective to reduce the spores than the sample without germinant buffer treatment. As a result, a synergistic effect of Ala+IMP was confirmed. The inactivation pattern of *B. cereus* spores by SHS on garlic was fitted by the Weibull model and t_R-value calculated from the Weibull model was tended to decrease as temperature increased. Through pre-treatment sterilization of spores in garlic, total retort processing time could be reduced which minimizes food quality loss.

***Key words*:** superheated steam, sterilization, black peppercorns, pecan halves, almond kernels, red peppers, garlics, *Salmonella*, *Escherichia coli*, *Bacillus cereus* spore.

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Part 1.

Applicability verification of superheated steam (SHS) sterilization equipment on foods

I. INTRODUCTION

As the recent demands for healthy and convenient foods have grown, the importance of nuts and spices has also increased every year. Due to the growing consumption of nuts and spices, a safe supply of food production is necessary [1].

Since the quality of taste, texture, and color for nuts and peppers change greatly when cooked, they are usually consumed after minimal sterilization and cooking process. It is known that *Salmonella* serovars are widespread in nature and are frequently found on the surface of tree nuts and spices, therefore *Salmonella* contamination is easy to occur when harvesting by hand, or when products are dried in the sun. Owing to these reasons, outbreaks of *Salmonella* food poisoning derived from nuts and spices are being reported [2].

The previous study of sterilization process using superheated steam resulted in that did not damage the quality of nuts and spices, but that required a modified superheated steam equipment that reflected a size-up research for the application in the food industry [3]. Two points were considered before the modification. (i) Increasing the amount of samples to be treated, (ii) Minimizing heat loss. The size of reacting cell was increased responsible for the sterilization treatment. When samples were placed in the reacting cells on

a flat surface, the previous reacting cell was capable of holding 3 g of black peppercorns, 9 g of pecans, and 9 g of almonds, whereas the remodeled reacting cell was able to treat 50 g of black peppercorns, 150 g of pecans, and 150 g of almonds at once [4]. The reacting chamber was also recreated with insulation materials to minimize equipment related heat-loss. Also, in the original apparatus, the direction of the fluid flowed from bottom to up, reducing heat transfer efficiency by interfering with fluid flow and causing heat loss. To compensate this flaw, the direction of the fluid flow was modified from top to bottom so that the heat energy from the superheated steam could be utilized for the sterilization process of the food, and the steam that lost heat energy were then directed further downward into the condenser for discharge. To further decrease the heat-loss during the steaming process, nozel-type manifold pipes were used to connect the superheater to the reacting chamber rather than the conventional enlargement pipe [5].

Thus, after the superheated steam equipment was modified, the new equipment was used to inactivate food-borne pathogens in black peppercorns, pecan halves, almond kernels and red pepper to verify applicability for the food sterilization, and compared them with results with the previous equipment.

Superheated steam (SHS) is a steam heated above the boiling point. When the food is treated with SHS, condensate water was occurred in the early stage because of the low temperature of the food. At this time, high latent heat of condensation is instantaneously transmitted to the microorganisms on the surface of food so that moist heat sterilization was processed. When SHS is continuously supplied and the condensate water on the surface of the food is completely evaporated, the sterilization process by dry heat in which the contaminated microorganisms are inactivated by the sensible heat of SHS occurs [6]. In other words, SHS sterilization has both the effect of moist heat sterilization and dry heat sterilization, so the speed of microbial sterilization is fast, which has the advantage of instant surface sterilization [7].

Previous studies have been conducted to decrease *E. coli*, *Salmonella* and total aerobic bacteria in red pepper powder [8] [9]. However, the vegetative cells in red pepper formed spores and biofilms during the drying/grindling processes as the growth environment became poor due to the decrease of moisture content, and the rise of temperature [10]. There was a limitation in inactivating heat resistant bacterial spores without changing the quality of red pepper powder. Therefore, in this study, changing the process order from washing → drying → grinding → sterlization → packaging to washing → sterilization → drying → grinding → packaging was

proposed. By changing the process order, it was expected to sterilize the raw food material without damaging its quality, thereby preventing the bacterial spore and biofilm formation, decreasing the number of contaminated bacteria, reducing the risk of the contamination of machinaries in the food industry, and the cross-contamination during the manufacturing process at the same time.

II. MATERIALS AND METHODS

2.1. Superheated steam (SHS) equipment

The SHS equipment in this research consisted of a water reservoir, a steam boiler (model DWE-15, Dae-Woo Steam Boiler, Daegu, Korea), 5 kW superheater, pipelines, reacting chamber and power controller. A steam boiler generated 100℃ saturated steam with 15 kWh^{-1} heater capacity, 20.19 kWh^{-1} steam evaporative capacity and 5 $kg_f(cm^2)^{-1}$ of maximum usage pressure. A superheater played a role of turning 100℃ saturated steam into superheated steam (5 kW, 101-220℃, 1 atm). To increase the amount of samples and compensate the thermal losses due to condensate water, steam direction, pipelines and reacting chamber were modified from the machinery of previous research (Figure 1 and 2). Steam temperature was controlled by the power of superheater, and monitored consistently at the exit of superheater and processing chamber.

Figure 1. Schematic diagram of the previous superheated-steam sterilization equipment: (A) water reservoir, (B) boiler (15 kW), (C) superheater (5 kW), (D) reacting chamber with band heater, (E) power controller, (F) SHS temperature monitoring system.

Figure 2. Schematic diagram of the modified superheated-steam sterilization equipment: (A) water reservoir, (B) boiler (15 kW), (C) superheater (5 kW), (D) reacting chamber, (E) power controller, (F) SHS temperature monitoring system (G) thermocouple #1, (H) thermocouple #2, (I) condenser.

2.1.1. SHS pipelines and temperature monitoring

As the sample throughput increased, the superheated steam outlet was expanded. Using 5 nozzles from the outlet of the superheater, steam could be sprayed evenly. In addition, to minimize heat loss, the manifold pipes were insulated with heat insulating material and the length of the manifold pipes was made as short as it could be. In order to solve the condensate water problem, the flow direction of steam was changed from up to down.

Temperature of superheated steam was automatically measured through two thermal couples of PT 100Ω which is a line connection type of temperature sensors. Two thermal sensors were installed at the exit of the superheater and the inlet of the reacting chamber, respectively (Figure 3).

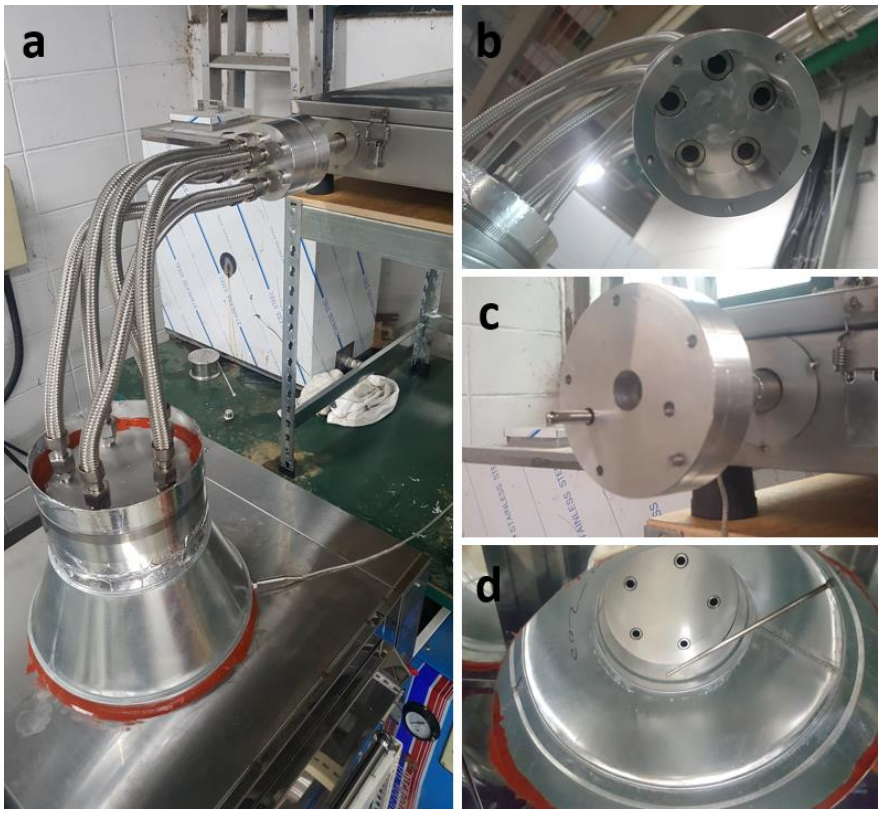


Figure 3. Superheated-steam sterilization equipment. (a) manifold pipes, (b) 5 nozzles connected to the outlet of superheater, (c) thermocouple #1 at the superheater outlet, (d) thermocouple #2 at the reacting chamber inlet.

2.1.2. SHS reacting chamber and reacting cell

In order to increase the amount of treated sample, the diameter of reacting cell was increased from 6 cm to 30 cm (Figure 4). Based on almond kernels and pecan halves, using reacting cells (300 mm D) up to 200 g of samples were treated as monolayer.

In the previous superheated steam system, the reacting chamber was composed of an outer reacting chamber shell, a band heater, an air jacket, a silicon gasket, a standard clamp, and an internal reacting cell. In case of the modified equipment, the reacting chamber (480 mm × 380 mm × 500 mm) was manufactured that can be insulated, so that it did not require the use of external components.

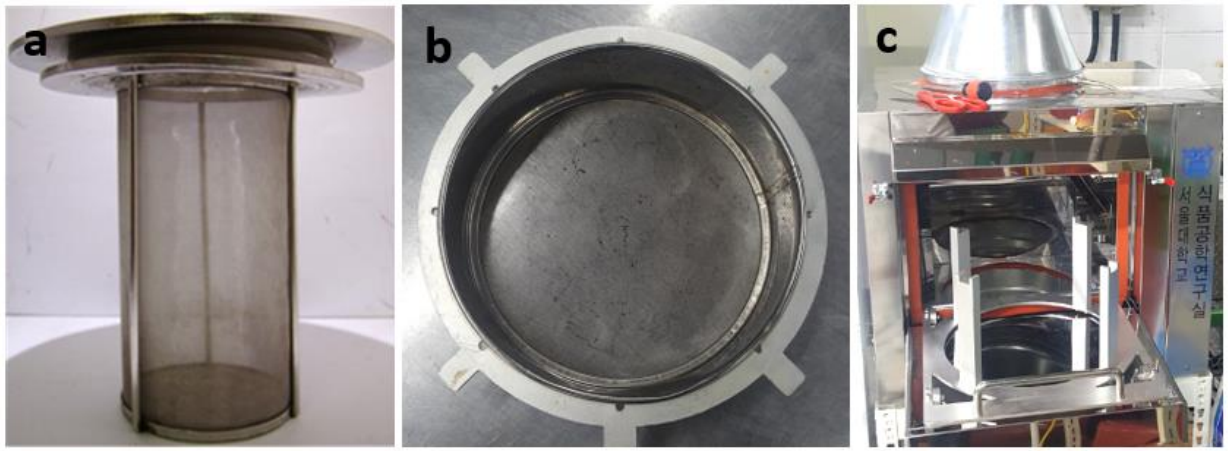


Figure 4. Reacting cell. (a) Internal reacting cell of previous reacting chamber (60 mm D), (b) reacting cell of modified reacting chamber (300 mm diameter), (c) reacting chamber and reacting cell frame.

2.2. Bacterial strains and inoculum preparation

2.2.1. Bacterial strains

In this study, three serotypes of *Salmonella* (*S.* Typhimurium ATCC 19585, *S.* Enteritidis PT-30, and *S.* Enteritidis NCCP 12236) and *E. coli* K-12 MG 1655 were used from the bacterial culture collection of Seoul National University (Seoul, South Korea). All bacteria stored at -80℃ in 0.7 mL of tryptic soy broth (TSB, Difco, Franklin Lakes, NJ, USA) and 0.3 mL of 50% glycerol (v/v). Bacterial strains were streaked onto tryptic soy agar (TSA, MB Cell, Los Angeles, CA, USA), and incubated at 37℃ for 24 h and stored at 4℃.

2.2.2. Inoculum preparation

Three strains of *Salmonella* were cultured in 5 mL of TSB at 37℃ for 24 h. After incubation, 0.2 mL of each culture was spread onto TSA and incubated at 37℃ for 24 h. Bacterial lawns were scraped using sterile spreader with 10 mL of 0.2% (w/v) buffered peptone water (BPW, Difco) and harvested together to obtain the cocktailed *Salmonella* inoculum (total 30 mL, mixing ratios 1:1:1), corresponding to 10.39 ± 0.11 log CFU/g.

Each strain of *E. coli* K-12 and *S.* Typhimurium was cultured in 5 mL of TSA at 37℃ for 24 h, harvested by centrifugation at 4000×g for 20 min at

4℃ and washed three times with 5 mL of BPW, corresponding to approximately 10^9-10^{10} CFU/mL. Subsequently, suspended pellets of the two pathogen species were combined to produce the *Salmonella* and *E. coli* cocktailed inoculum (10 mL).

2.3. Sample preparation and inoculation

Whole raw almond kernels, pecan halves and black peppercorns were purchased at a local market (Seoul, South Korea). 500 g of each samples was used to inoculate. Samples were placed in a sterilized polyethylene bag (12 x 18 cm) and mixed with the cocktailed *Salmonella* inoculum (sample 500 g : inoculum 150 mL). Hand shaking was carried out for 2 min and dried for 2 h in a biosafety hood.

Red pepper was purchased at a local grocery store (Seoul, South Korea), removed the seeds and head of the red pepper and washed in running water. 10 mL of culture cocktail was applied to 100 g of red pepper in a sterile polyethylene bag and mixed by hand massage for 1 min and dried for 2 h in a biosafety hood.

2.4. SHS treatment

25 g of black peppercorns, pecan halves and raw almond kernels for *Salmonella* were individually treated using 100℃ saturated steam, 140 and 180℃ superheated steam. Black peppercorns were treated at an interval of one second for nine seconds and pecan halves and almonds were treated at intervals of three seconds for 30 s. The trimmed peppers were placed on a reacting cell and heat treated by 120, 150, and 180℃ of SHS at intervals of ten seconds for 50 min. Treated samples were immediately transferred to sterile stomacher filtra-bag (Labplas Inc., Sainte-Julie, QC, Canada).

2.5. Bacterial enumeration

Treated samples were 10-fold diluted by adding BPW, and homogenized for 2 min in a stomacher (Hansol Tech Co., Seoul, South Korea). After homogenization, the resulting suspension was serially diluted in 9 mL of BPW, and 0.1 mL of diluent was plated onto *Salmonella* selective agar (xylose lysine desonycholate agar, XLD, Oxoid, Basingstroke, UK), *E. coli* selective agar (Sorbitol MacConkey agar, Oxoid, Basingstoke, UK) and TSA for total aerobic bacteria enumeration. At the level of detection limit, 1 mL of the resulting suspension was directly plated. All plates were incubated at 37℃ for 24 h, and colonies were counted.

Injured cells of *Salmonella* were enumerated using the overlay (OV) method after samples were heat treated. 0.1 mL of the resulting sample solutions was spread plated onto a non-selective media, TSA. For enumerating the recovered number of *Salmonella* at the detection limit level, 1 mL of the resulting sample solution was plated onto TSA. The plates were incubated at 37℃ for 2 h to allow injured cells to recovery, and then over-poured with XLD, *Salmonella* selective agar (OV-XLD). The plates were incubated at 37℃ for 22 h and colonies were counted [11].

2.6. Quality evaluation of samples

2.6.1. Color measurement and moisture content

Chromaticity, and moisture content were measured to evaluate of quality. Color Hunter's color values (L*, a*, b*) of samples were measured by a Chroma Meter CR-400 (KONICA MINOLTA, Sensing, Inc., Osaka, Japan).

Moisture content was measured as follows. The weighing glass bottle was placed in the drying oven and dried to constant weight at 105℃. Afterward, the weighing glass bottle was brought to the desiccators and cooled before being weighed (W_1). SS/SHS treated, samples were placed inside the weighing glass and weighed (W_2). After samples were dried at 105℃, samples

were weighed with the weighing glass bottle (W_3) in a second time. The sample's moisture content was then calculated by the formula below in terms of % dry basis (1).

$$\text{Moisture content (\% dry basis)} = \left(\frac{W_2 - W_3}{W_2 - W_1}\right) \times 100 \qquad (1)$$

2.6.2. Capsaicin content

The capsaicin content was estimated by high performance liquid chromatography (HPLC; 2695 Separations module, WATERS, Milford, MA, USA) following the Hoffman's method. The capsaicin (Sigma, St. Louis, MO, USA) standard curve was obtained for quantification. The capsaicin was extracted from red pepper powder which was SHS treated/non-treated, dried and pulverized. The quantification of capsaicin was done on a C18 Novapak column with detection at 280 nm. The mobile phase of 50% acetonitrile, injection volume of 20 μL, and a flow rate of 0.8 mL/min was used [12].

1 g of the sample was precisely weighed, and 13 mL of acetone was added. The sample was extracted in a water bath at 30℃ for 5 h and filtered. The acetone was completely removed by evaporator (30℃), and 5 mL of methanol was again added. The resulting solution was filtered through a 0.45 μm filter, passed through a Sap-Pak cartrige.

2.7. Heat resistance parameter

To determine the *D*-values, the data corresponding to each temperature were used and plotted a thermal death curve (TDC). TDC was fitted with first order kinetics model (2)

$$\log\left(\frac{N}{N_0}\right) = \quad -kt \tag{2}$$

where N_0 is the initial number of microorganisms CFU/g, N is the number of microorganism at time t (CFU/g), t is treatment time (sec or min) and k is a rate constant (s^{-1} or min^{-1}).

The *D*-values were determined using the average slope ($D = -1/\text{slope}$) for each treatment.

The *z*-values were calculated by plotting the log *D* against temperature and calculating the inverse negative of the slope of the thermal death time curve $z = -1/\text{slope}$)

2.8. Statistical analysis

All experiments were conducted in triplicate and the data were analyzed using IBM SPSS 21.1 (SPSS Inc., Chicago, IL, USA). Duncan's multiple comparison test was used for investigating in order to determine significant difference ($p < 0.05$) of the results.

III. RESULTS AND DISCUSSION

3.1. Operation of the equipment for SHS treatment

Temperature profiling was performed to verify that the temperature of the new installed reacting chamber was well controlled (Figure 5). The temperature difference between the superheater outlet (#1) and the inlet of the reacting chamber (#2) was about 20℃ in case of 140℃ setting up. At 180℃ setting, the temperature difference was approximately 30℃. This was due to the fact that heat loss was occurred during the superheated steam generation. The enthalpy of 120, 140, 150, and 180℃ superheated steam were 2716, 2756, 2776, and 2835 kJ/kg, respectively. The heat loss, thus, was about 40 and 60 kJ/kg at 140, and 180℃ each. The higher temperature of superheated steam, the faster rates of heat transfer leading to increase heat loss. After 1 h from the operation of the device, it was confirmed that the temperature was well controlled.

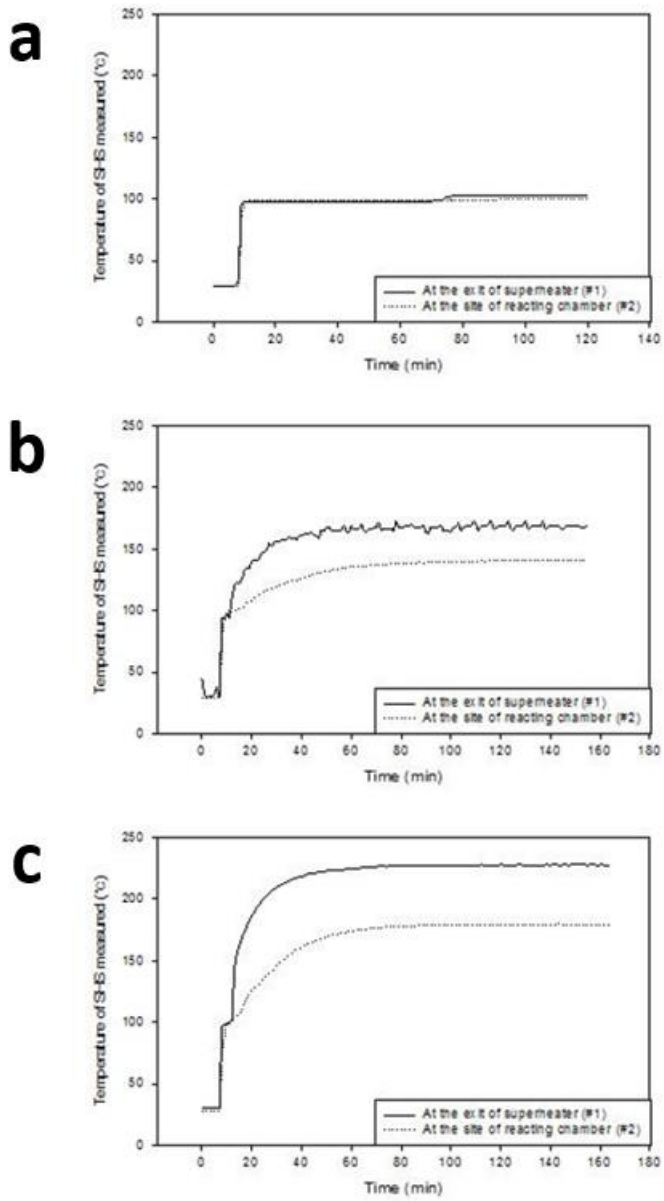


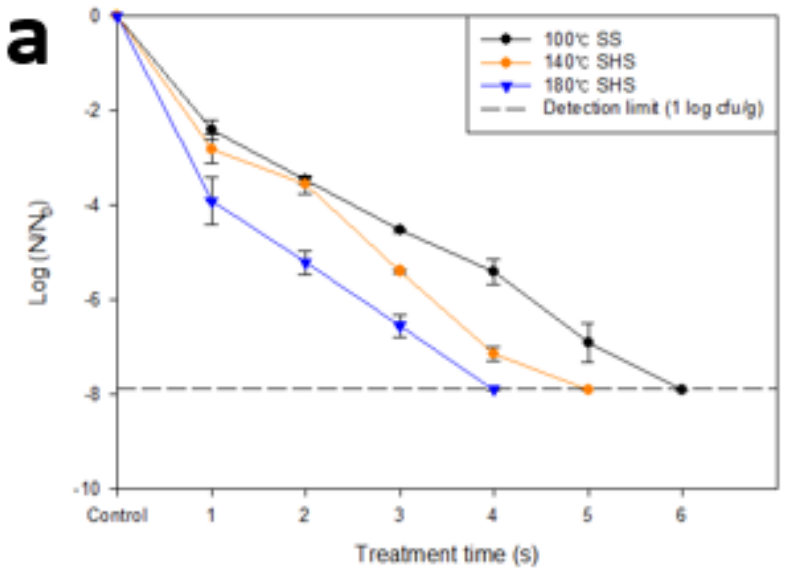
Figure 5. Temperature profiling at thermal couples #1 and #2 according to the initial operation of the superheated steam system (Boiler rating output: 13.5 kW, superheater rating output: 1.68 kW). Temperatures were set up to (a) 100℃, (b) 140℃, (c) 180℃.

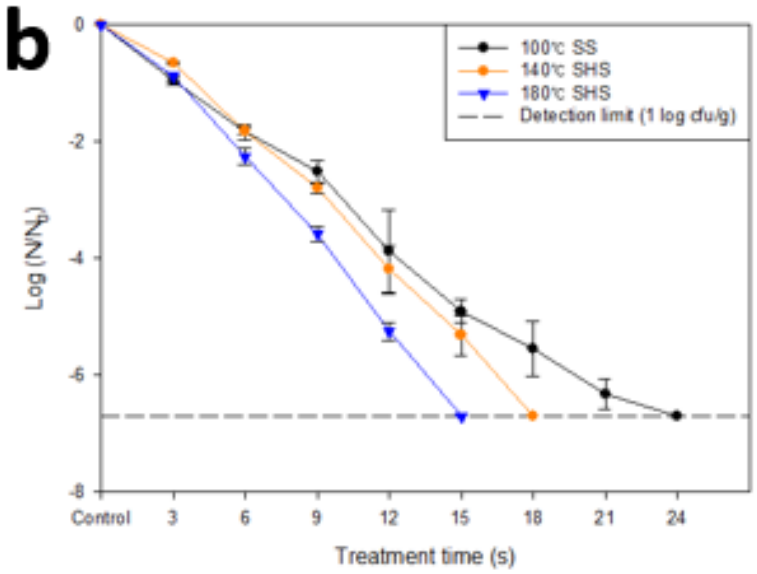
3.2. Inactivation of the cocktailed of *Salmonella* Typhimurium and *Salmonella* Enteritidis on black peppercorns, pecan halves, and raw almond kernels

The initial levels of inoculation were 8.89 ± 0.06 log CFU/g for black peppercorns, 7.69 ± 0.08 log CFU/g for pecan halves, 7.43 ± 0.10 log CFU/g for almond kernels. After SS/SHS treatment, the survival curves were obtained as Figure 6. Regardless of the samples, inoculated *Salmonella* was decreased to below the detection limit, but the rates of decrease were different depending on the type of samples. *Salmonella* was inactivated most effectively in black peppercorns with a decline in 6, 5 and 4 s at 100, 140, and 180℃, respectively. It is presumed that the heat transfer was efficiently carried out to the large surface area of the black peppercorns. *Salmonella* was effectively reduced killed within 24 s in pecans and almonds by 100℃ SHS and in case of the SHS treatment at 140 and 180℃, *Salmonella* was killed more rapidly than when treated at 100℃.

Injured cells of *Salmonella* were enumerated using OV-XLD. After 180℃ SHS treated for 6 s on black peppercorns, no recovered cells were detected. In case of pecan halves, *Salmonella* was decreased below the detection limit when treated at 180℃ for 15 s, but recovered cells were observed on OV-XLD. Recovered cells were not enumerated when SHS was

treated for 18 s. When heat treated with 180℃ SHS for 12 s, *Salmonella* population were reduced to below the detection limit, and recovered cells were also not detected.


a
100℃ SS
140℃ SHS
180℃ SHS
Detection limit (1 log cfu/g)
Log (N/N0)
0
-2
-4
-6
-8
-10
Control
1
2
3
4
5
6
Treatment time (s)


b
100℃ SS
140℃ SHS
180℃ SHS
Detection limit (1 log cfu/g)
Log (N/N0)
0
-2
-4
-6
-8
Control
3
6
9
12
15
18
21
24
Treatment time (s)

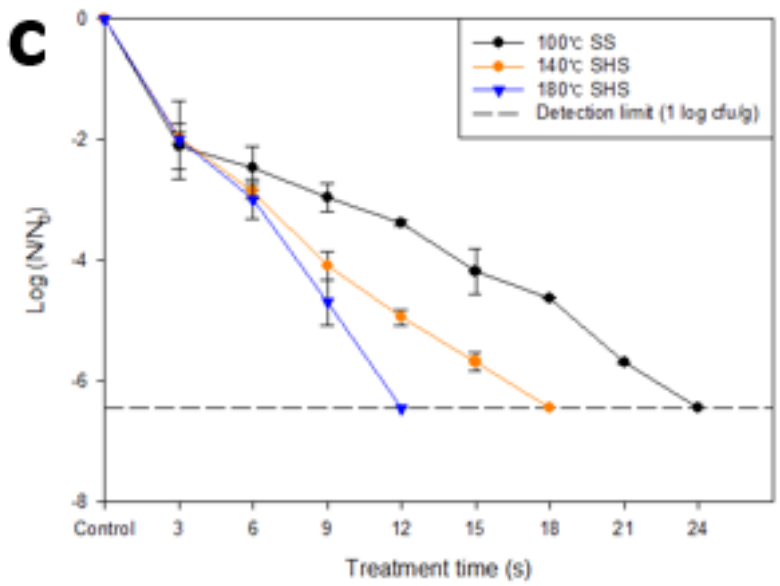


Figure 6. Survival curves of homogeneously cocktailed *S.* Typhimurium, *S.* Enteritidis PT-30 and NCCP 12236 by SHS treatment on (a) black peppercorns, (b) pecan halves, and (c) raw almond kernels.

Compared to the decontamination performance of the previous equipment and modified equipment, the sterilization rate at 100℃ was faster, 140℃ was slightly faster, and in the case of 180℃, approximately 1.5 times slower tendency was shown (Table 1). First of all, it would have affected the rate of inactivation since there was a difference in the speed of the fluid in the device after retrofitting. The volumetric flow rate could be determined according to volumetric flow rate (3).

$$\mathrm{Q} = \dot{V} = \lim_{\Delta t \to 0} \frac{\Delta V}{\Delta t} = \frac{dV}{dt} \tag{3}$$

where, Q ($\dot{V}$): the volumetric flow rate ($m^3 \ / \ s$), V: the flow rate (m/s), and t: time (s). And volumetric low rate is also defined by (4)

$$U = \frac{Q}{A} \tag{4}$$

where, *U*: flow velocity (m/s), *Q*: the flow of volume ($m^3 \ / \ s$), and *A*: cross-sectional vector area (m^2).

The above equation is applicable only for the flat or plane cross-section.

The volumetric flow rates were constant before and after the customized SHS equipment was modified because heater capacity (15 kW/h) and steam evaporative capacity (20.19 kg) of the boiler were the same and the specification (5 kW single-phase) of the 2nd superheater was the same. And the area of the reacting chamber inlet of the modified equipment was wider than that of the previous equipment. Therefore, the flow velocity (*U*, m/s) of modified equipment was slower than that of previous equipment.

In the 1st boiler, 18.171 kg/h (13.5 kW) of 100℃ saturated steam was generated. Specific volume of 100℃ saturated steam is 1.67 m^3/kg. It was thus 30.35 m^3/h of the flow of volume. Since the diameter was 6 cm, the flow velocity could be derived by substituting into equation (4), as about 2.98, 3.37 and 3.71 m/s at 100, 140 and 180℃, respectively.

The Reynolds number (*Re*) helps to predict the pattern of the fluid, and widely used in fluid flow situation such as in a pipe. The Reynolds number is a dimensionless number that can be used to determine the flow conditions of laminar or turbulent flows in a pipe and to know the effect of fluid flow on heat transfer, and can be given as follows (5).

$$Re\ \# = \frac{\mathcal{D} \cdot v \cdot \rho}{\mu} \quad (5) \quad \begin{cases} Re < 2100 : laminar\ \ flow \\ 2100 < Re < 4000 : transition\ \ flow \\ Re > 4000 : turbulent\ \ flow \end{cases}$$

where, $\mathcal{D}$: the diameter of the pipe (m), v: the mean velocity of the fluid (m^2/s), ρ: the density of the fluid (kg/m^3), μ: the dynamic viscosity of the fluid ($Pa \cdot s$or $N \cdot s/m^2$ or $kg/(m \cdot s)$).

Each value was substituted to obtain the Reynolds number of SS/SHS in case of the previous system, and the results were 13199, 15999, and 14258 at 100, 140, and 180℃, respectively, which means that all flows were turbulent.

Due to the flow direction from bottom to top, SS/SHS steam was disturbed by the net at the bottom of the reacting cell in the previous system. There were two possibilities to be hindered. First, the important reason was that the cell net acted as an obstacle, causing flow volume reduction and heat energy loss. In practice, the flow rate just above the sample was measured at 0.5 m/s, leading to decrease in the flow of volume to 14.14 m^3/s. This value was less than half of the flow volume that generated in the 1st boiler. When the Reynolds numbers were calculated, each value was 2214 (100℃), 2373 (140℃), and 1921 (180℃) which was transition flow or laminar flow which has lower the thermal energy transfer capability than that of turbulent flow [6]. Second, the steam consumes the heat energy to evaporate the condensed water on the cell net, so that all heat energy could not be used to inactivate bacteria in the sample, which would have slowed the death rate. The steam that lost

heat energy would slow down and form a stagnation zone right above the net of cell bottom where the sample was placed, which in turn interferes with the progress of the new steam with thermal energy. Consequently, the heat energy could not be efficiently transmitted.

The points dropped below the detection limit for each sample were fitted based on temperature and time in the previous and modified equipment system as seen in the figure 7-9. The fitting model was applied differently for each food. In the graphs, there were crossing points, suggesting that other factors such as geometrical factors, the thermal conductivity of the fluid (k), the convective heat transfer coefficient (h), the characteristic length, the thermal loss of variance, and amount of samples, besides temperature and the velocity also affected. But, temperature-dependent sterilization curves were indicated that temperature was an important factor in decontamination of microorganism on foods. Thus, it is considered that the modified equipment could be applicable to inactivation of pathogens.

Table 1. Comparison of the treated sample amounts and treatment time with before/after modified SHS equipment

Samples	Temperature (℃)	Previous SHS equipment		Modified SHS equipment	
		Sample amount	Treatment time	Sample amount	Treatment time
Black peppercorns	100	3 g	25 s	25 g	6 s
	140	3 g	13 s	25 g	5 s
	180	3 g	3 s	25 g	4 s
Pecan halves	100	9 g	35 s	25 g	24 s
	140	9 g	19 s	25 g	18 s
	180	9 g	13 s	25 g	15 s
Almond kernels	100	9 g	45 s	25 g	24 s
	140	9 g	30 s	25 g	18 s
	180	9 g	8 s	25 g	12 s

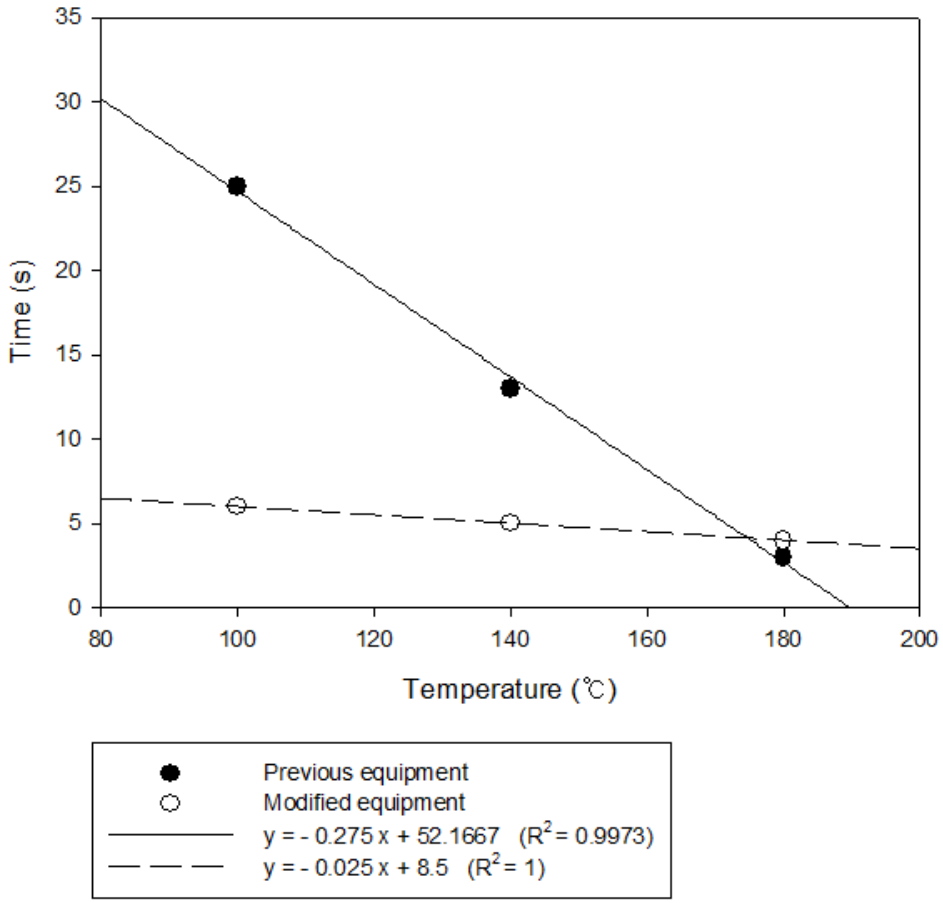


Figure 7. Temperature vs. time correlation comparison between the previous and modified equipment fitted with a linear model in the case of black peppercorns.

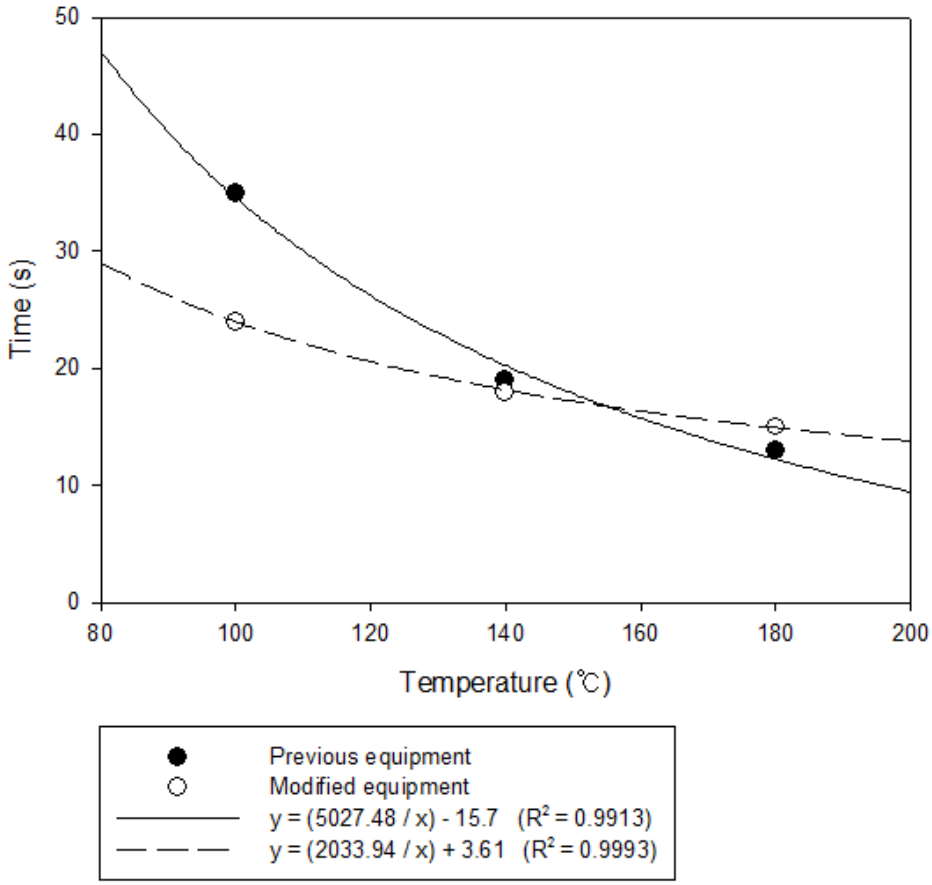


Figure 8. Temperature vs. time correlation comparison between the previous and modified equipment fitted with an inverse first order model in the case of pecan halves.

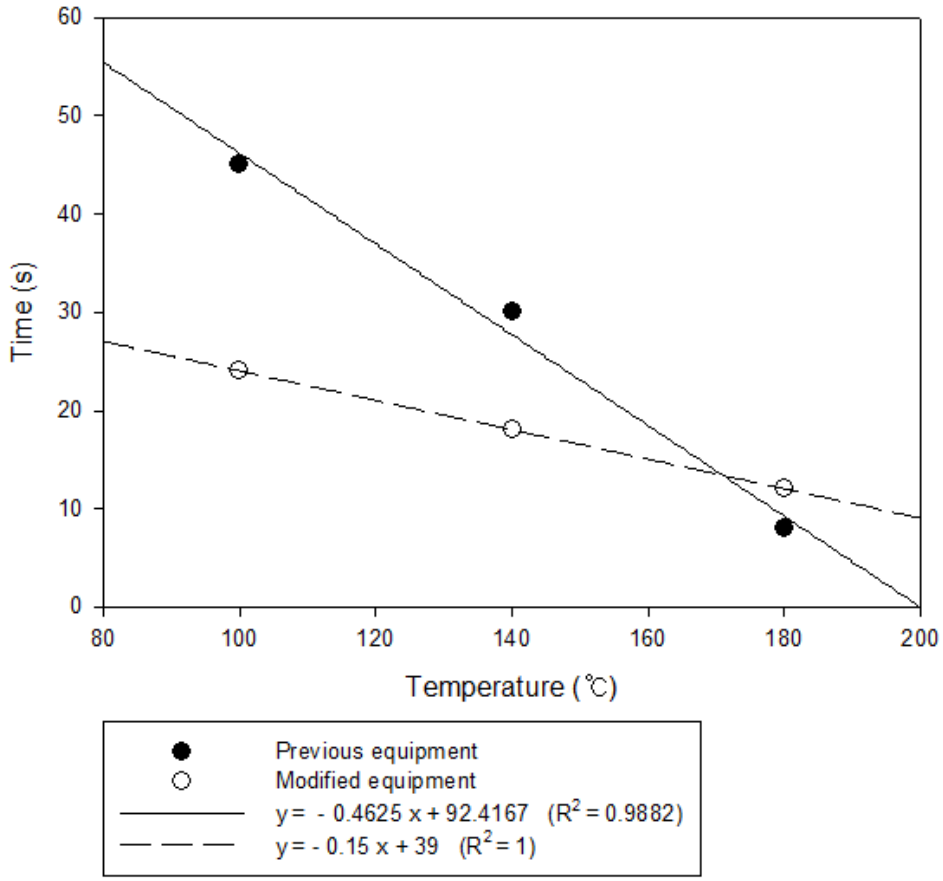


Figure 9. Temperature vs. time correlation comparison between the previous and modified equipment fitted with a linear model in the case of raw almond kernels.

3.3. Inactivation of the cocktailed *Escherichia coli* K-12 and *Salmonella* Typhimurium, and total aerobic bacteria on red peppers

The level of inoculation on red pepper was confirmed as 8.42 ± 0.08 log CFU/g for *S.* Typhimurium and 8.36 ± 0.18 log CFU/g for *E. coli* by plate counts. After 10 s exposure of 120, 150, and 180℃ SHS treatment, both bacteria were reduced to below the detection limit, leading to 7 log CFU/g reduction (Figure 10). To inactivate the total aerobic bacteria, more SHS treatment time was required so that 50, 30, and 20 s were taken when red peppers were treated with 120, 150, and 180℃ SHS. From the results, microbial safety can be more effectively achieved when the sterilization process is performed in the raw material than when it is performed in the final product. Not only that, the secondary contamination can be reduced thanks to supply of clean raw materials.

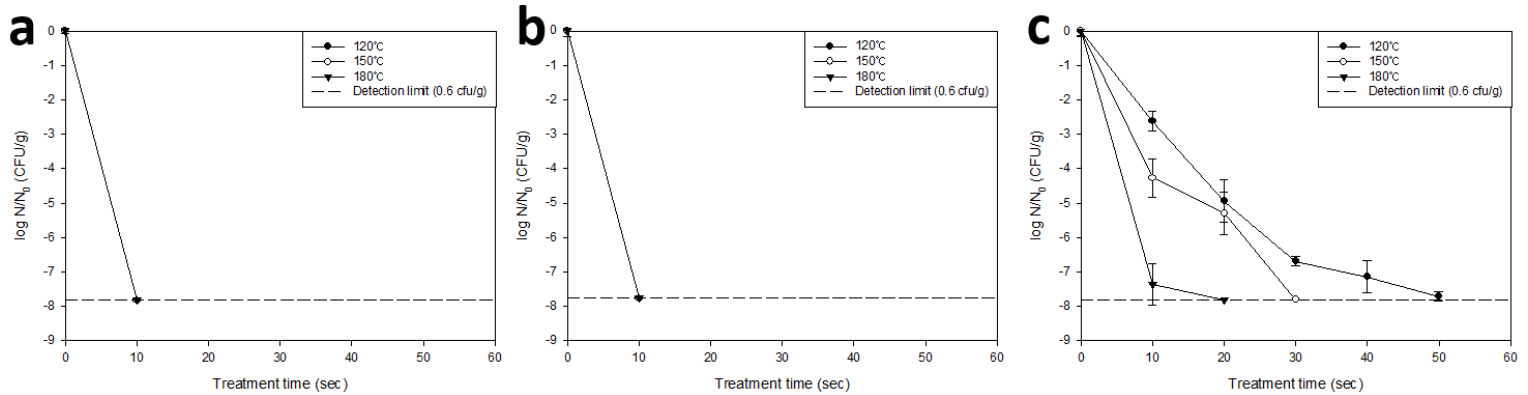


Figure 10. Survival curves of (a) *Salmonella* Typhimurium, (b) *E. coli* and (c) total aerobic bacteria inoculated in red pepper after treated with SHS.

3.4. Effect of SHS treatment on foods quality

Photographs were taken to observe changes in the appearance of samples, black peppercorns, pecan halves, almond kernels and red pepper which were non-treated and treated by SHS.

3.4.1. Color and moisture content

The chromaticity and moisture content were measured and the results were listed (Table 2-9). In case of black peppercorns, there was no difference in treated and non-treated black peppercorns under superheated steam. Whereas, after SS treatment, the condensed water remained enough to be visually distinguishable, and black peppercorns were clump up by the unremoved condensate water. Considering microbial reduction, color change, and moisture content, treatment with 180℃ SHS in a short time would appropriate for sterilization of black peppercorns. For pecan halves, there was no visible difference from the pecan halves between before and after SHS treated while condensate water was observed when 100℃ SS was treated. And there was also no significant difference in chromaticity in all samples. After treated by 140 and 180℃ SHS, water content was not much change. When the pecan was treated with 100℃, on the other hand, condensate water was observed with the naked eye, and the moisture content also showed an increase in water content about 3 times as compared with a control sample.

Nevertheless, the pecan inherent odor was generated during the heat treatment regardless of steam temperature, suggesting that quality loss occurred. As for almond kernels, condensate water was observed under SS treatment and skin loosening was caused compared with the control group. While there was no significant difference in lightness after SHS exposure, there was a statistically significant difference with a* and b* values. Notwithstanding, no visible difference was observed and there was no change in moisture content. Regarding red pepper, the values of L, a, and b were slightly decreased in all samples after SHS treatment. The color change was the smallest when treated with 150℃ SHS for 30 s, compared with 120℃ for 50 s and 180℃ for 20 s. Although there was a statistically significant difference in chromaticity when treated with SHS, as shown in the figure 11, no visible change in appearance was observed.

Table 2. Color change of black peppercorns after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Samples	L*	a*	b*
Control	35.70 ± 0.56[a]	1.55 ± 0.13[a]	4.19 ± 0.45[a]
100℃, 6 s	33.06 ± 0.29[b]	1.58 ± 0.38[a]	3.33 ± 0.49[ab]
140℃, 5 s	33.12 ± 0.23[b]	1.61 ± 0.01[a]	2.93 ± 0.20[b]
180℃, 4 s	32.29 ± 0.63[b]	1.27 ± 0.30[a]	2.51 ± 0.47[b]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 3. Moisture content change of black peppercorns after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Quality parameters	Control	100℃, 6 s	140℃, 5 s	180℃, 4 s
Moisture content (% dry basis)	5.83 ± 0.60^{a}	30.10 ± 0.84^{c}	12.30 ± 0.72^{b}	6.78 ± 0.94^{a}

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 4. Color change of pecan halves after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Samples	L*	a*	b*
Control	37.97 ± 0.73[a]	8.56 ± 0.76[a]	9.45 ± 0.33[a]
100°C, 24 s	38.11 ± 0.33[a]	8.32 ± 0.62[a]	10.48 ± 2.26[a]
140°C, 18 s	36.11 ± 1.57[a]	9.12 ± 1.05[a]	9.24 ± 1.47[a]
180°C, 15 s	38.13 ± 1.72[a]	8.92 ± 1.23[a]	8.86 ± 1.81[a]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 5. Moisture content change of pecan halves after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Quality parameters	Control	100℃, 24 s	140℃, 18 s	180℃, 15 s
Moisture content (% dry basis)	3.32 ± 0.27[a]	9.77 ± 0.28[c]	4.23 ± 0.68[ab]	4.81 ± 0.53[b]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 6. Color change of almond kernels after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Samples	L*	a*	b*
Control	47.39 ± 1.65[a]	9.97 ± 0.33[a]	19.01 ± 1.66[a]
100℃, 24 s	49.25 ± 1.93[a]	11.23 ± 0.43[ab]	20.63 ± 1.73[ab]
140℃, 18 s	47.54 ± 0.31[a]	11.77 ± 0.78[b]	17.60 ± 1.48[ab]
180℃, 12 s	50.58 ± 1.79[a]	11.97 ± 1.10[b]	23.17 ± 1.44[b]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 7. Moisture content change of almond kernels after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Quality parameters	Control	100 ℃, 24 s	140 ℃, 18 s	180 ℃, 12 s
Moisture content (% dry basis)	4.12 ± 0.34^{a}	6.63 ± 0.82^{b}	4.84 ± 0.44^{a}	4.51 ± 0.52^{a}

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 8. Color change of red pepper after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Samples	L*	a*	b*
Control	39.10 ± 0.18[a]	38.31 ± 3.48[a]	20.80±1.59[a]
120℃, 50 s	35.83 ± 0.47[b]	30.77 ± 1.96[b]	14.17±0.86[ab]
150℃, 30 s	37.34 ± 1.62[ab]	33.37 ± 2.36[ab]	20.08±5.39[ab]
180℃, 20 s	35.28 ± 1.21[b]	32.38 ± 0.97[ab]	16.59±1.93[b]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 9. Moisture content change of red pepper after SS/SHS treatment until *Salmonella* reduced blow the detection limit[1]

Quality parameters	Control	100 ℃, 24 s	140 ℃, 18 s	180 ℃, 12 s
Moisture content (% dry basis)	70.30 ± 1.55[a]	70.95 ± 1.20[a]	73.35 ± 2.74[b]	68.49 ± 1.27[a]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Figure 11. Appearances of non-treated and treated samples for the time required to reduce below the detection limit.

3.3.2. Capsaicin content

Measuring the change in capsaicin was necessary to investigate changes in physicochemical properties of pepper after SHS treatment in terms of the most important component of pepper flavor, a lipid-soluble ingredient with a spicy taste. The residual rate of capsaicin, when heated at 100℃ for 10 h, was 84.7% in the atmosphere [13]. Although capsaicin is known to be highly thermo-stable, it was necessary to confirm the thermal stability of capsaicin when treated with 180℃ SHS.

The HPLC system was conducted to determine the capsaicin standard curve with 10, 20, 40, 60, 80 and 100 mg% of capsaicin standard solution and retention time was approximately 11 min. The capsaicin content of control was 17.55 ± 0.51 mg% and the capsaicin content of the SHS heat treated sample was 19.04 ± 0.38. mg% (Figure 12-b).

Statistical analysis was carried out and it was concluded that there was no significant difference between before and after SHS treatment. Even though SHS is at a high temperature, it is treated for a short time, it has little effect on the loss of capsaicin, which is the most important ingredient in the quality of pepper.

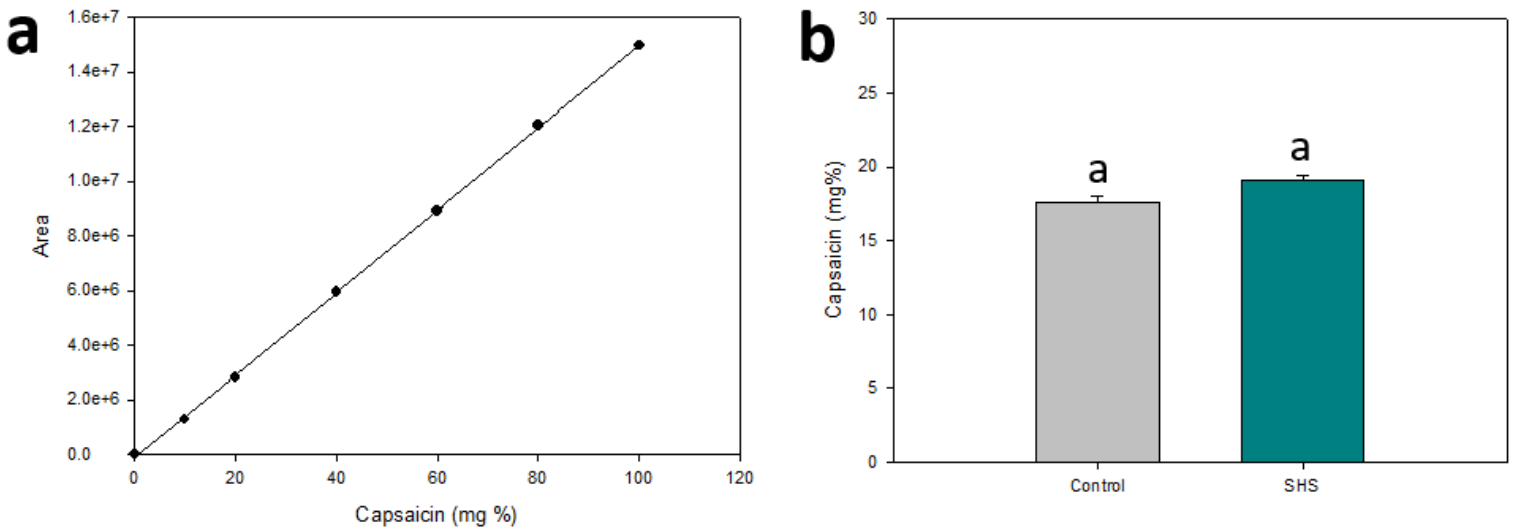


Figure 12. Capsaicin content (a) standard curve of capsaicin (b) capsaicin content before/after SHS treatment. The equation of capsaicin standard curve was $y = 151259x - 136072$.

IV. CONCLUSIONS

More than 5 log CFU/g of food-borne pathogens such as the cocktailed *Salmonella* on black peppercorns, pecan halves, and almond kernels were inactivated in a short time using SHS of the modified equipment. The time to reduce the bacteria according to the temperature of the previous and the modified equipment was different depending on the difference in factors such as the flow rate, sample amount, size of the reacting chamber, obstacles in the flow direction of the steam, etc.

In case of the treatment microorganisms in red peppers which are raw materials of red pepper powder, the populations of *Salmonella* and *E. coli* were reduced within 10 s regardless of the temperature of SHS, and more than 7 log CFU/g reduction of total aerobic bacteria were achieved after treated with 120℃ for 50 s, 150℃ for 30 s and 180℃ for 20 s without the capsaicin content loss. As the result, superheated steam (SHS) equipment was verified the applicability of sterilization microorganism in foods.

Part 2.

Inactivation of *Bacillus cereus* spores on garlic using superheated steam

I. INTRODUCTION

The consumption and demand of instant foods and retort foods are increasing as the number of single and working households increase. Accordingly, food companies produce more and more different types of retort foods, so the types of retort foods have been more diversified. Retort products contain lots of spices in general, and garlic (*Allium sativum* L.) is one of the representative spices since garlic has a unique flavor and aroma that are from diallyl thiosulfinate (allicin) discovered by Cavallito and Bailey, and has been reported to be good for health [14].

However, garlic is easily contaminated by *Bacillus cereus* which is a soil originated spore-forming microorganism having a remarkable thermal resistance [15] [16] [17]. Controlling *Bacillus cereus* spores is important in food industry because they can provoke infection and toxin types of foodborne illnesses when they are germinated [18] .

Retort food is a ready-to-eat food that can be stored and distributed with a long shelf-life at room temperature. So, complete sterilization is recommended in food industry. Thermal processing is a widely used cooking and sterilization method, but it is not always sufficient to decontaminate all bacterial spores due to the presence of cold point [19]. Despite the fact that both microbiological safety and quality for taste and nutrition are important,

excessive heat treatment, which causes damage of food quality, is inevitable to ensure microbiological safety.

Indeed, many studies have been conducted to control spores [20] [21] [22] but there is still no effective technique, so it is clear that more research is needed in terms of application to food.

In addition, germinant was used to sterilize spores more efficiently [23]. Germinant could be initiators to induce germination of the spores, which makes it easier to decontaminate the spores due to the fact that germinated spores and vegetative cells do not have as high heat resistance as spores [24]. According to advanced research of the genome, there are 7 putative germination (*ger*) operons in *Bacillus cereus* ATCC 14579, and in particular, reacted to amino acids or purine ribosides [25] [26]. In this study, experiments were conducted with L-alanine, and inosine, one of the well-known components for a germinant. Unfortunately, because inosine is not permitted to be used as a food additive by CAC (Codex Alimentarius Commission; the International Food Standards Committee), it was necessary to find a substitute for inosine. Disodium 5'-inosinate (IMP, Disodium inosine 5'-monophosphate) is the disodium salt of inosinic acid and not only allowed to be used as a food additive but also now widely used on food industry as a flavor enhancer [27]. Consequently, L-alanine, inosine, and disodium 5'-inosinate were studied as single germinants or in combination.

To the best of my knowledge, there are no reported decimal reduction time (*D*-value) for *B. cereus* spores in garlic to superheated steam treatment. Therefore, the aim of this study was to evaluate the effect of superheated steam on garlic and to obtain the thermal resistance data of *B. cereus* spores in garlic. Survival curves were fitted by Weibull model to find out the kinetics of *B. cereus* spores, and t_R-value, and *z*-value were calculated based on these data.

II. MATERIALS AND METHODS

2.1. Bacterial strain and preparation of the inoculum of *Bacillus cereus* spore

2.1.1. Bacterial strain

B. cereus KCTC 3624 (ATCC 14579) used in this research was obtained from Korean collection for type cultures (KCTC). Stock culture was kept frozen at -80℃ in 0.7 mL of Tryptic Soy Broth (TSB, Difco, Franklin Lakes, New Jersey, USA) and 0.3 mL of 50% glycerol (v/v). Bacterial strain was streaked onto tryptic soy agar (TSA, MB Cell, Los Angeles, CA, USA), and incubated at 30℃ for 24 h and stored at 4℃.

2.1.2. Production and purification of *B.* cereus spore and preparation of the inoculum of *B. cereus* spore

B. cereus was pre-cultured in 5 mL of Tryptic Soy Broth (TSB, Difco, Franklin Lakes, New Jersey, USA) at 30℃ for 24 h. After incubation, 0.2 mL of culture was spread onto Difco Sporulation Medium (DSM) containing per liter 8 g bacto nutrient broth (Difco), 10 mL of 10% (w/v) KCl, 10 mL of 1.2% (w/v) $MgSO_4 \cdot 7H_2O$, ~1.5 mL (pH to 7.6) of 1 M NaOH, with 1.5% micro agar

and after autoclave, 1 mL of 1 M $Ca(NO_3)_2 \cdot 4H_2O$, 1 mL of 0.01 M $MnCl_2 \cdot 4H_2O$, and 1 mL of 1 mM $FeSO_4 \cdot 7H_2O$, and incubated at 30℃ for 5 days to produce a bacterial lawn.

Spores were gradually formatted for 5 days and it was observed through optical microscope (×1000). Spores were collected by scraping the surface of the DSM agar with 10 mL of sterile distilled water and washed three times by centrifugation (10,000×*g*, 15 min, 4℃) for *B. cereus* spore purification. The pellet was resuspended in 5 mL distilled water and 5 mL ethanol and the suspension was kept at 4℃ for 12 h in order to reduce the number of vegetative cells [28, 29] [30]. It was confirmed through preliminary experiments that 99.99% of *B. cereus* vegetative cells were eliminated by the method of spore purification with 50% ethanol. The obtained suspension was washed three times by centrifugation under the same conditions, and the finial pellet was resuspended in 10 mL of 0.2% (w/v) buffered peptone water (BPW, Difco, Franklin Lakes, New Jersey, USA) as the spore inoculum corresponding to approximately 10^9 CFU/mL.

2.1.3. Sample preparation and inoculation

Garlics (Hapcheon-gun, Gyeongsangnam-do, Korea) for this study were purchased at a local market (Seoul, Korea). The garlic cloves were washed with running water and water was removed from garlic cloves. Sliced

garlics (about 2 mm thickness) were used.

Samples were inoculated with the *B. cereus* spore inoculum using a spot-inoculation method. Inoculated garlics were dried by low temperature drying at 4℃ to prevent the spore from germinating. Samples were placed in a desiccator which had been pre-cooled for 12 h.

2.2. SHS treatment

The whole garlics inoculated with *B. cereus* spores were treated with 120, 150, and 180℃ of superheated steam for 0.5, 1, 1.5, 2, 2.5, and 3 min. For SHS treatment to garlic cloves with 3 log CFU/mL inoculation level, SHS was exposed to the same temperature for 10, 20 and 30 s. SHS treated samples were then immediately transferred to a sterile stomacher bag.

2.3. Germinants-induced germination of *B. cereus* spore

L-alanine, inosine, and disodium 5'-inosinate (IMP) as germinants were used due to the fact that bacterial spores are once germinated, the heat resistance becomes weak. 50 mM of L-alanine, 5 mM of inosine, and 5 mM of IMP were used. The combination of L-alanine (50 mM) + inosine (5 mM) and L-alanine (50 mM) + IMP (5 mM) were done to investigate the synergistic effect [31] [32] [16] [25] [24].

B. cereus spores were germinated preferentially in the germination buffer. The germination buffer was made by adding germinants to distilled water. 1 mL of the purified spore inoculum was put into micro-tube, centrifuged and discarded the supernatant. 1 mL of the germination buffer was filled in the micro-tube, and mixed well with a pipette. Each micro-tube was allowed to leave at a room temperature for a set period of time and then the germination buffer was removed by centrifugation. After repeating washing three times with distilled water, 0.5 mL of distilled water and 0.5 mL of ethanol were added and left at 4℃ for 12 h. Germinated spores were eliminated by 50% ethanol and the remaining spores were counted.

2.4. SHS treatment after the germination of *B. cereus* spore

2 mL of germinant buffer, the mixture of 50 mM L-alanine and 5 mM disodium 5'-inosinate, was sprayed on about 100 g of the garlic cloves and 50 g of sliced garlic and left for 15-20 min. And then samples were treated with 120, 150, and 180℃ of SHS. SHS treated garlics were immediately transferred to a sterile stomacher bag.

2.5. Bacterial enumeration

The number of *B. cereus* was enumerated by spreading on the MYP agar (Mannitol-egg yolk-polymyxin agar, Oxoid, Basingstoke, UK). The population of germinated spores was calculated by subtracting the number of survivors after purification with 50% ethanol from the initial population of spores (6) [31] [33].

$$\text{Germination} = \text{Initial number of spore} - \text{Spore survivors} \quad (6)$$

Garlics treated by SHS were placed into a sterile stomacher bag with the 0.2% BPW as much as 9 times the weight of samples and homogenized for 2 min with a stomacher (Hansol Tech Co., Seoul, Korea). Samples were serially diluted in BPW, and 0.1 mL of diluent was spread onto MYP (*B. cereus* selective agar) for *B. cereus* enumeration. All plates were incubated at 30℃ for 24 h and colonies were counted.

Injured cells of *B. cereus* were enumerated using the overlay (OV) method after samples were heat treated. 0.1 mL of the resulting sample solutions was spread plated onto a non-selective media, TSA. For enumerating the recovered number of *B. cereus* at the detection limit level, 1 mL of the resulting sample solution was plated onto TSA. The plates were incubated at 37℃ for 2 h to allow injured cells to recovery, and then over-poured with

Brilliance Bacillus Cereus agar (BBC, Oxoid, Basingstoke, Hampshire, England). The plates were incubated at 37℃ for 22 h and colonies were counted after the overlaid medium was solidified.

2.6. Scanning electron microscopy (SEM)

The skins of garlic cloves and sliced garlics which were inoculated by *B. cereus* spores were fixed inside the petri dish lid using a tape. Three droplets of 2% osmium tetroxide were dropped on a petri dish and it was covered with the lid and sealed using parafilm. The petri dish was left in the safety hood for more than 24 h so that the moisture in garlics was completely eliminated. Dehydrated samples were mounted on the stubs and samples were sputter coated with platinum (Pt) using a vacuum coater (EM ACE200, Leica, Germany). The SEM images were obtained using a Filed-Emission Scanning Electron Microscope (SUPRA 55VP, Carl Zeiss, Germany).

2.7. Measurement of water activity (A*w*)

Water activity measurement was practiced using water activity meter 4TE (Aqua Lab, WA, USA) and repeated three times. The temperature range during the measurement was 25.0 ± 1.0℃ during the measurement.

2.8. Statistical analysis

All experiments were conducted in triplicate and one way analysis of variance (ANOVA) was performed using IBM SPSS 21.1 (SPSS Inc., Chicago, IL, USA). Duncan's multiple comparison test was used for investigating in order to determine significant difference ($p < 0.05$) of the results.

III. RESULTS AND DISCUSSION

3.1. Inactivation of *B. cereus* spore on garlics

In order to investigate the reduction pattern of *B. cereus* spore depending on shapes of garlic, garlic cloves and sliced garlics inoculated by *B. cereus* spores were used to SHS treatment. The initial inoculation levels of spore were 6.22 ± 0.26 log CFU/g on garlic cloves, and 6.44 ± 0.15 log CFU/g on sliced garlics, and garlics were treated by 120, 150, and 180℃ of SHS for 0.5, 1, 1.5, 2, 2.5, and 3 min. The results were shown in the following (Figure 13).

3.1.1. Inactivation of *B. cereus* spore on garlic cloves and sliced garlics

The results showed that the inactivation of spores on sliced garlic was slightly slower than that on garlic cloves. Thus, three factors were assumed to be affected. (i) Influence on the internal cracks or structure, (ii) influence on the outer coating film, and (iii) the water activity. First of all, scanning electron microscopy (SEM) images were taken to confirm the effect of the internal structure or cracks. Secondly, the same experiment was carried out with the garlic cloves removed the outer coating film to examine the influence of the

coating film. Lastly, to investigate the water activity (Aw) which is well-known to affect the thermal resistance of spores, were measured by a water activity meter after garlic cloves, sliced garlics and peeled garlics were treated by SHS [34].

SEM images of the garlic cloves and the sliced garlics surface were obtained (Figure 14). As a result of SEM images, spores were well attached to the surface of garlic cloves and sliced garlics, and it is hard to conclude that inner cracks and structures by the exposed surface affected.

In order to confirm the effect of outer coating film, *B. cereus* spore was inoculated on garlic cloves without coating film and 120, 150, and 180℃ of SHS treatment was performed and the initial inoculation level was 6.26 ± 0.14 log CFU/g on peeled garlic. Regardless of outer coating film, the reduction pattern was similar when SHS was treated on garlic cloves and peeled garlic cloves (Figure 15).

Water activities of garlic cloves, sliced garlics and peeled garlics were then measured and the following results were obtained (Figure 16). It was initially considered that water activities had an effect on the heat resistance of spores. However, as the water content gradually decreased, the tendency that the resistance of spores became stronger did not coincide. The thermal energy applied to the spore on garlics was not mainly transmitted by the conduction of moisture in the food, and rather, the heat energy of the SHS was directly

transmitted to the spore on the food surface by the convection [35]. It was therefore concluded that thermal energy was used to dry the food moisture leading to a slower spore inactivation in the sliced garlic than a reduction in the garlic clove [36] [37] [38].

In addition, as a result of the measurement of water activity, it was confirmed that the latent heat was instantaneously transferred, resulting in rapid sterilization rate due to the generation of condensate water on the surface of the garlic in the early 0-0.5 min of SHS treatment.

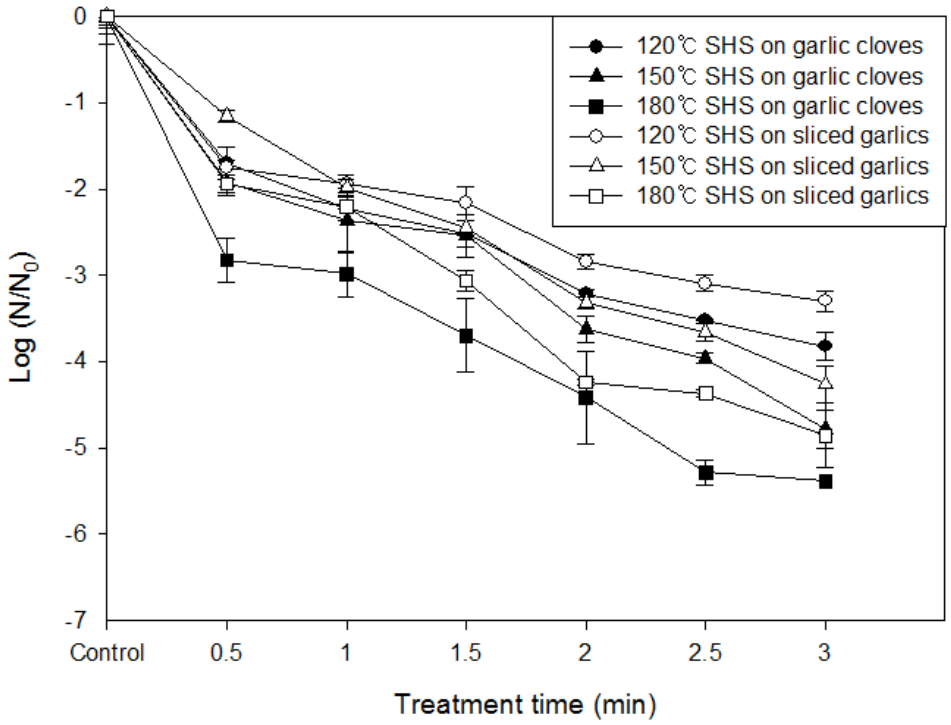


Figure 13. Survival curves for *B. cereus* spores inoculated on garlic cloves and sliced garlics treated with 120℃ (●, ○), 150℃ (▲, △), and 180℃ (■, □) SHS for 3 min.

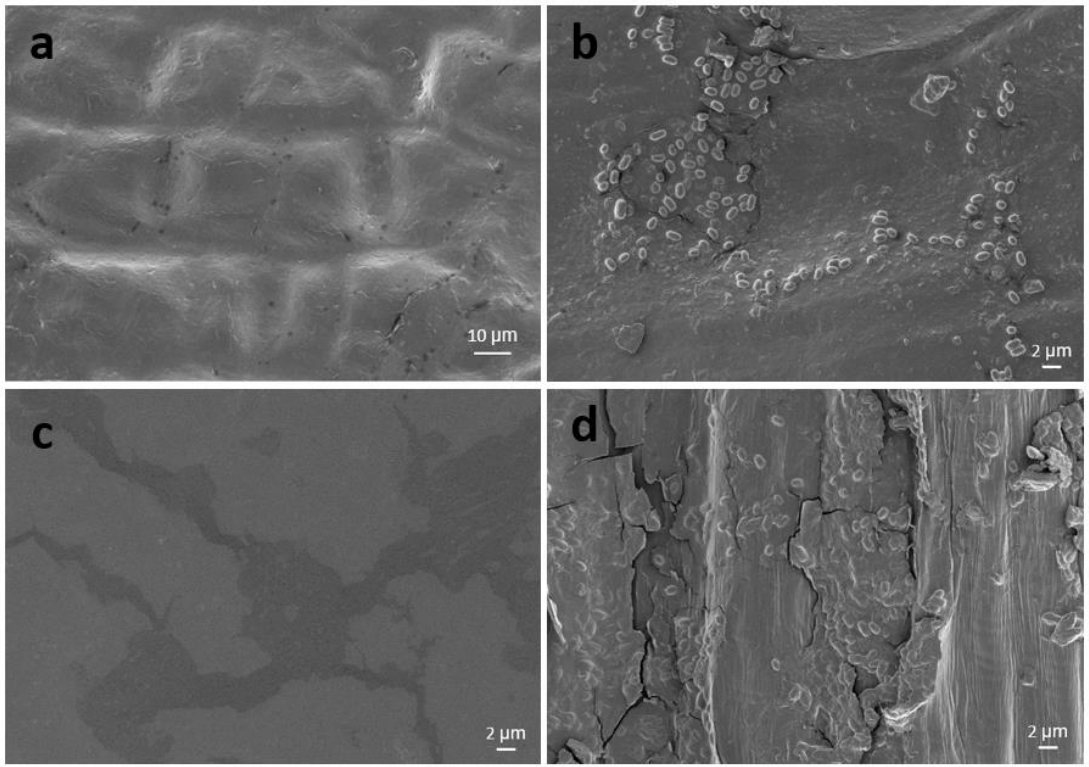


Figure 14. Scanning electron microscopy photomicrographs. Surface of whole garlic (a) non-inoculated, (b) *B. cereus* spore inoculated, and surface of sliced garlic (c) non-inoculated, (d) *B. cereus* spore inoculated.

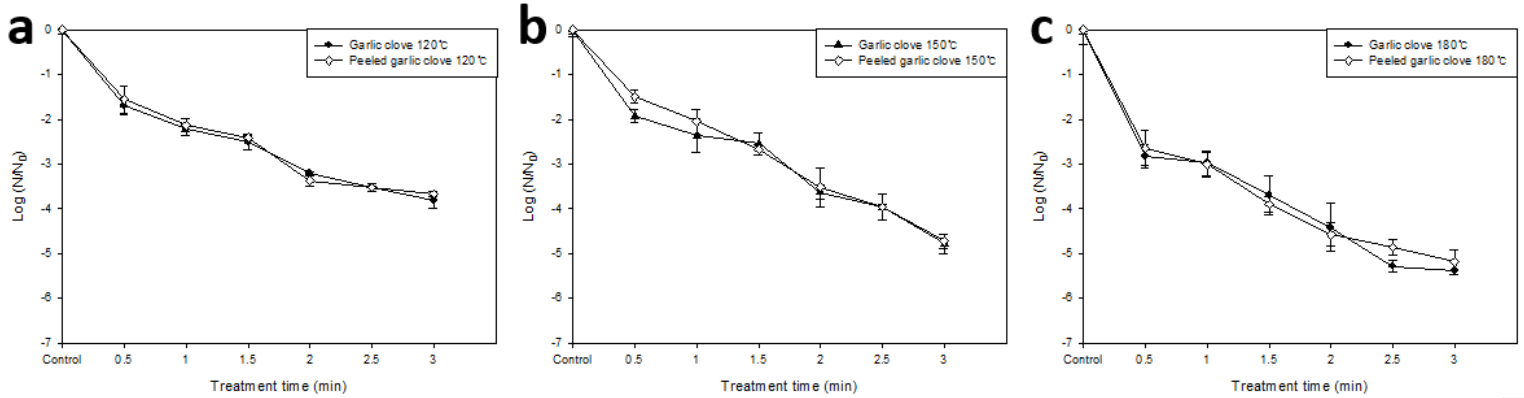


Figure 15. Survival curves for *B. cereus* spores inoculated on garlic cloves and garlic cloves removed outer coating film treated with 120℃ (●, ○), 150℃ (▲, △), and 180℃ (■, □) SHS for 3 min.

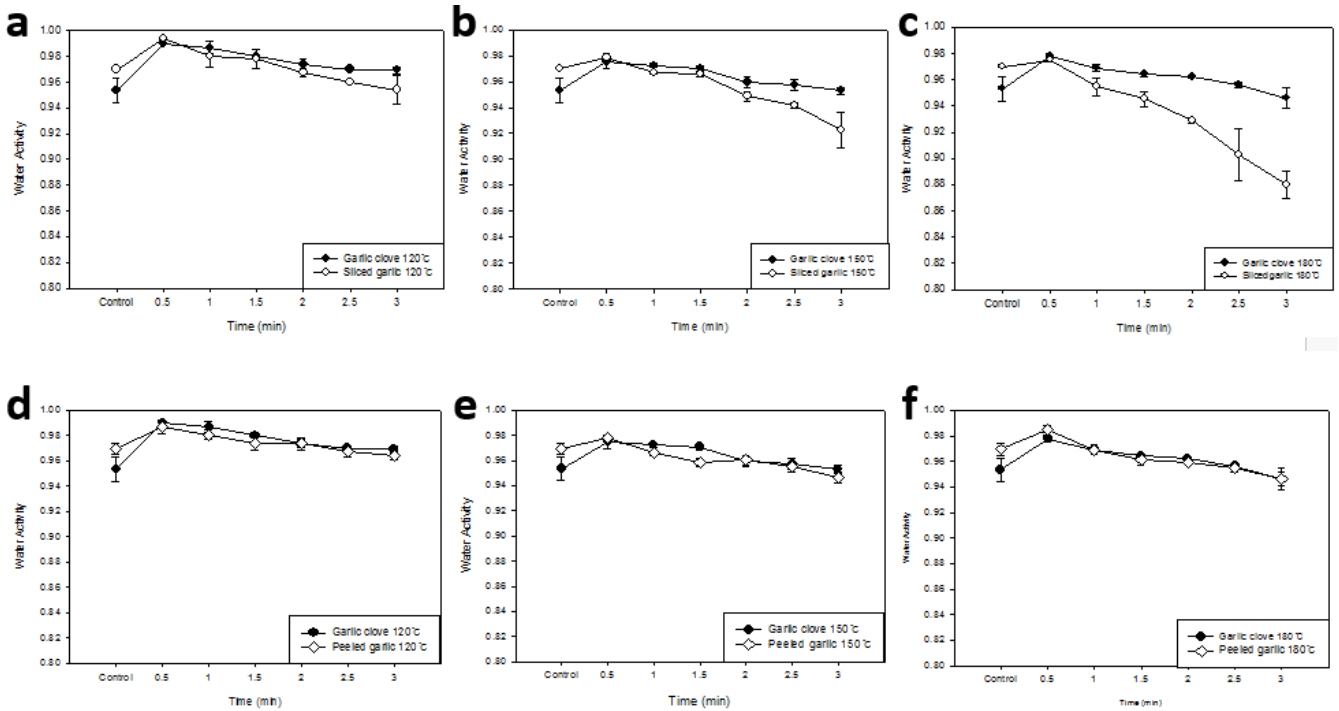


Figure 16. Water activity measurement. A*w* of the garlic cloves (●) and the sliced garlics (○), treated by (a) 120℃, (b) 150℃, and (c) 180℃ SHS and A*w* of the garlic cloves (●) and the peeled garlics (◇), treated by (d) 120℃, (e) 150℃, and (f) 180℃ SHS.

3.2. Inactivation of *B. cereus* spore using germinants

To investigate the effects of germinants on germination of *B. cereus* spore, experiments were carried out in the buffer. Non-treated (control), Sterile water (water), 50 mM L-alanine (Ala), 5 mM inosine (Ino), 5 mM disodium 5'-inosinate, 50 mM L-alanine + 5 mM inosine (IMP), and 50 mM L-alanine + 5 mM disodium 5'-inosinate were used as germinants [39]. Initial number of spores on buffer was 9.01 ± 0.08 log CFU/mL. Each germinants was treated to spores and leaving for 60 min at a room temperature. As a result, 0.10 log CFU/mL in water, 0.25 log CFU/mL in the L-alanine buffer, 0.83 log CFU/mL in the inosine buffer, 1.41 log CFU/mL in the IMP buffer, 1.21 log CFU/mL in the Ala+Ino combination buffer, and 2.10 log CFU/mL in the Ala + IMP combination buffer were germinated, respectively (Figure 17). When L-alanine and IMP were treated, the effect of the germination rate was the highest.

So, the survival number of spores after the combination buffer with 50 mM L-alanine and IMP 5 mM was conducted in order to find out the change patter of germination depending on over time (Figure 18). The germination was reached a maximum at 15 min, and no further germination occurred after the lapse of time. Thus, the germination treatment time was set at 15 min, and the germination in the buffer was performed and the result was as follows (Figure 19). The initial level of spore was 9.02 ± 0.05 log CFU/mL. According

to the result, the germinated spore population was decreased when inosine (0.69 log CFU/mL) or IMP (1.34 log CFU/mL) was treated for 15 min, and increased when sterile water (0.29 log CFU/mL), L-alanine (0.52 log CFU/mL), Ala+Ino (1.58 log CFU/mL), or Ala+IMP (2.41 log CFU/mL) was treated for 15 min. Moreover, synergistic effects of germinants combination were observed, both the 50 mM Ala + 5 mM Ino buffer and the 50 mM Ala + 5 mM IMP.

The germination buffer of 50 mM Ala + 5 mM IMP, which had the best germination effect, was applied to the real food and the spore inactivation experiment was conducted. Sterile water was sprayed and compared the results for exclusion the effect of moisture. The initial contamination level of *B. cereus* spore was 6.42 ± 0.30 log CFU/g. From the figure 20, Ala+IMP combined germinant buffer could induce the spore germination in garlic leading to more effective inactivation of spore than that the samples without germinant buffer. When water was sprayed instead of the germinant buffer, water played a role of a protective layer serving as conserve bacteria, and thermal energy by SHS was used to evaporate water. Consequentially, the reduction rate of spores was slower with water than samples that were untreated.

Expletively, no apparent change in the quality was observed when treated with germinant buffer and water.

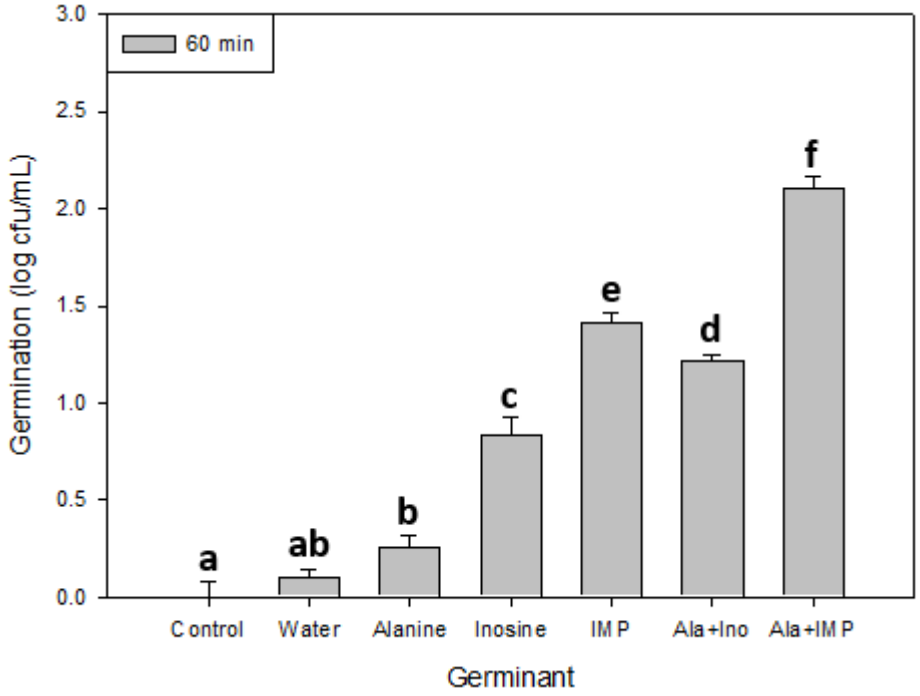


Figure 17. Germination of *B. cereus* spores after germinants were treated for 60 min in the buffer.

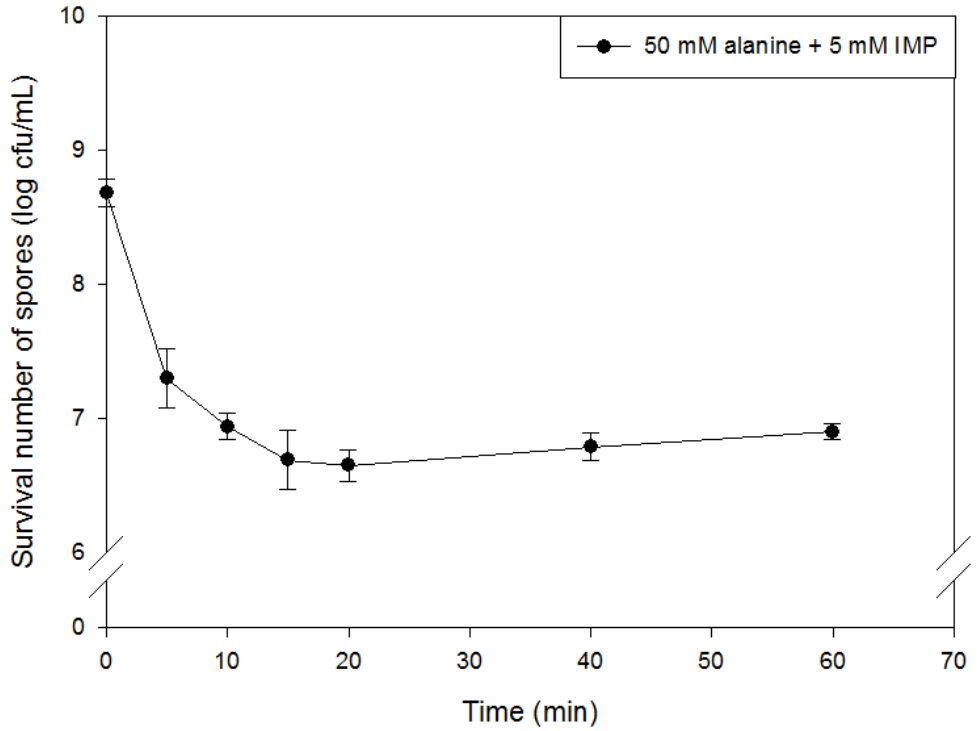


Figure 18. Survival number of *B. cereus* spores over time after disodium 5'-inosinate (IMP) was treated for 60 min in the buffer.

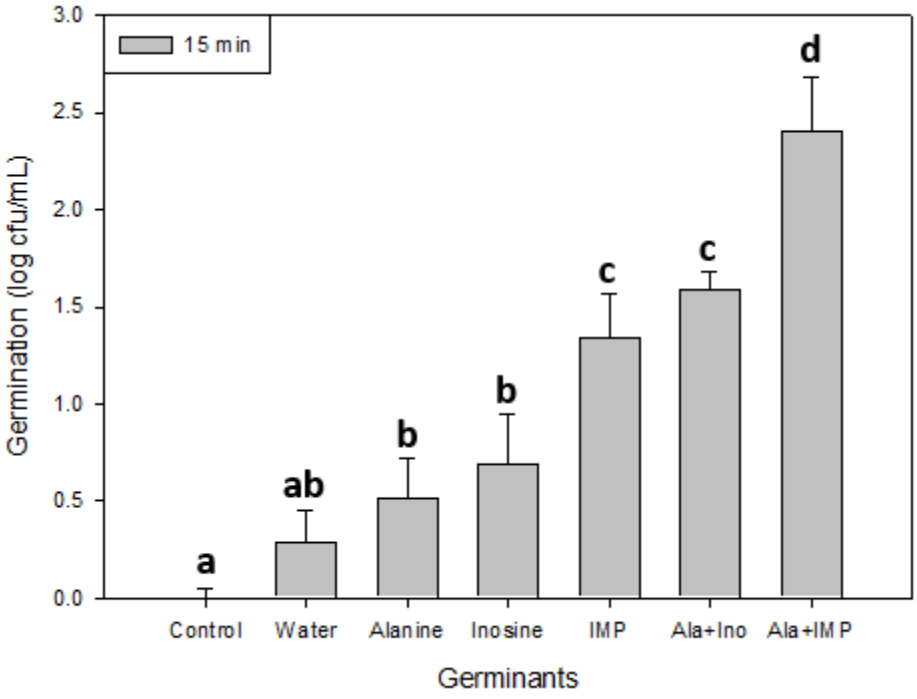


Figure 19. Germination of *B. cereus* spores after germinants were treated for 15 min in the buffer.

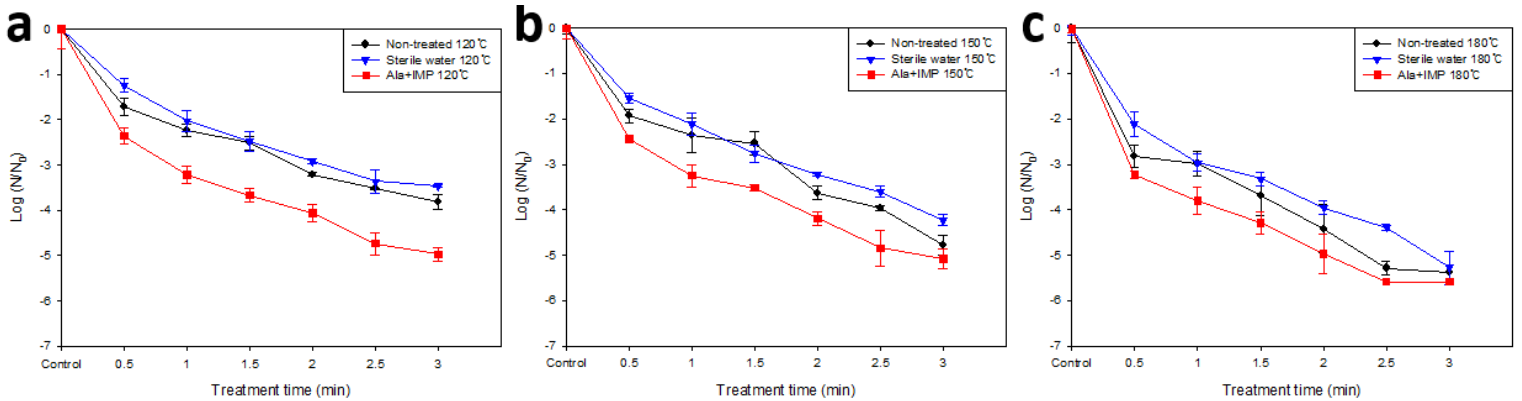


Figure 20. Reduction of *B. cereus* spore inactivation curves after germination buffer (●: non-treated, ▼: sterile water treated, and ■: Ala+IMP buffer treated) was used for 15 min and then treated with (a) 120℃, (b) 150℃, and (c) 180℃ SHS.

3.3. Recovery of *B. cereus* after the SHS treatment

Table 10 and 11 show levels of sublethally injured *B. cereus* on garlic cloves and sliced garlic after SHS treatment. And table 12 and 13 were for recovered *B. cereus* with treated Ala+IMP germinant buffer and with spraying water. Injured cells showed a tendency to recover slightly, but there was no statistically significant difference in overall treatment results.

Table 10. Survival (log CFU/g) of uninjured and recovered *B. cereus* on garlic cloves after SHS treatment

Treatment time (min)	120℃		150℃		180℃	
	MYP	OV-BBC	MYP	OV-BBC	MYP	OV-BBC
0	6.25 ± 0.26[a]	6.25 ± 0.26[a]	6.25 ± 0.26[a]	6.25 ± 0.26[a]	6.25 ± 0.26[a]	6.25 ± 0.26[a]
0.5	4.31 ± 0.19[a]	4.41 ± 0.19[a]	4.43 ± 0.15[a]	4.76 ± 0.10[a]	3.56 ± 0.25[a]	3.75 ± 0.32[a]
1	3.78 ± 0.13[a]	3.90 ± 0.15[a]	4.00 ± 0.37[a]	4.11 ± 0.32[a]	3.40 ± 0.27[a]	3.49 ± 0.25[a]
1.5	3.50 ± 0.15[a]	3.53 ± 0.18[a]	3.82 ± 0.25[a]	3.93 ± 0.28[a]	2.69 ± 0.43[a]	3.04 ± 0.28[a]
2	2.80 ± 0.04[a]	2.87 ± 0.02[a]	2.73 ± 0.15[a]	2.83 ± 0.13[a]	1.97 ± 0.53[a]	1.56 ± 0.40[a]
2.5	2.50 ± 0.01[a]	2.67 ± 0.03[a]	2.40 ± 0.06[a]	2.50 ± 0.13[a]	1.10 ± 0.14[a]	1.49 ± 0.16[a]
3	2.19 ± 0.16[a]	2.46 ± 0.01[a]	1.58 ± 0.22[a]	1.77 ± 0.10[a]	<1.00[a]	0.53 ± 0.76[a]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 11. Survival (log CFU/g) of uninjured and recovered *B. cereus* on sliced garlics after SHS treatment

Treatment time (min)	120°C		150°C		180°C	
	MYP	OV-BBC	MYP	OV-BBC	MYP	OV-BBC
0	6.44 ± 0.15[a]	6.44 ± 0.15[a]	6.44 ± 0.15[a]	6.44 ± 0.15[a]	6.44 ± 0.15[a]	6.44 ± 0.15[a]
0.5	4.59 ± 0.09[a]	4.67 ± 0.13[a]	5.32 ± 0.07[a]	5.61 ± 0.16[a]	4.55 ± 0.10[a]	4.98 ± 0.04[a]
1	4.41 ± 0.11[a]	4.43 ± 0.08[a]	4.50 ± 0.09[a]	4.75 ± 0.18[a]	4.29 ± 0.03[a]	4.40 ± 0.07[a]
1.5	4.18 ± 0.19[a]	4.31 ± 0.30[a]	4.03 ± 0.08[a]	4.59 ± 0.26[a]	3.43 ± 0.13[a]	3.54 ± 0.11[a]
2	3.50 ± 0.08[a]	3.75 ± 0.04[a]	3.16 ± 0.07[a]	3.67 ± 0.30[a]	2.25 ± 0.04[a]	2.32 ± 0.04[a]
2.5	3.25 ± 0.09[a]	3.40 ± 0.08[a]	2.82 ± 0.10[a]	3.04 ± 0.10[a]	2.12 ± 0.04[a]	2.18 ± 0.06[a]
3	3.05 ± 0.12[a]	3.06 ± 0.08[a]	2.22 ± 0.21[a]	2.55 ± 0.28[a]	1.40 ± 0.38[a]	1.53 ± 0.33[a]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 12. Survival (log CFU/g) of uninjured and recovered *B. cereus* on garlic cloves with sterile water spraying after SHS treatment.

Treatment time (min)	120℃		150℃		180℃	
	MYP	OV-BBC	MYP	OV-BBC	MYP	OV-BBC
0	6.43 ± 0.19[a]	6.43 ± 0.19[a]	6.43 ± 0.19[a]	6.43 ± 0.19[a]	6.43 ± 0.19[a]	6.43 ± 0.19[a]
0.5	5.14 ± 0.15[a]	5.23 ± 0.12[a]	4.83 ± 0.10[a]	5.04 ± 0.32[a]	4.42 ± 0.26[a]	4.65 ± 0.27[a]
1	4.36 ± 0.24[a]	4.71 ± 0.24[a]	4.26 ± 0.25[a]	4.38 ± 0.25[a]	3.57 ± 0.19[a]	3.96 ± 0.03[a]
1.5	3.90 ± 0.23[a]	4.12 ± 0.16[a]	3.62 ± 0.20[a]	3.82 ± 0.24[a]	3.21 ± 0.15[a]	3.40 ± 0.14[a]
2	3.46 ± 0.06[a]	3.50 ± 0.04[a]	3.16 ± 0.05[a]	3.21 ± 0.05[a]	2.58 ± 0.14[a]	2.90 ± 0.04[a]
2.5	3.02 ± 0.25[a]	3.29 ± 0.03[a]	2.78 ± 0.13[a]	3.04 ± 0.03[a]	2.14 ± 0.06[a]	2.53 ± 0.10[a]
3	2.92 ± 0.02[a]	3.15 ± 0.04[a]	2.15 ± 0.12[a]	2.97 ± 0.02[b]	1.26 ± 0.37[a]	2.03 ± 0.04[a]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

Table 13. Survival (log CFU/g) of uninjured and recovered *B. cereus* on garlic cloves with L-alanine + disodium 5'-inosinate spraying after SHS treatment

Treatment time (min)	120℃		150℃		180℃	
	MYP	OV-BBC	MYP	OV-BBC	MYP	OV-BBC
0	6.58 ± 0.33[a]	6.58 ± 0.33[a]	6.58 ± 0.33[a]	6.58 ± 0.33[a]	6.58 ± 0.33[a]	6.58 ± 0.33[a]
0.5	4.41 ± 0.18[a]	5.54 ± 0.81[a]	3.96 ± 0.08[a]	4.74 ± 0.13[a]	3.36 ± 0.08[a]	3.67 ± 0.11[a]
1	3.56 ± 0.19[a]	3.74 ± 0.43[a]	3.15 ± 0.24[a]	3.96 ± 0.08[a]	2.78 ± 0.29[a]	3.27 ± 0.06[a]
1.5	3.10 ± 0.14[a]	3.38 ± 0.35[a]	2.88 ± 0.07[a]	3.19 ± 0.21[a]	2.30 ± 0.25[a]	2.99 ± 0.17[a]
2	2.71 ± 0.19[a]	3.17 ± 0.49[a]	2.20 ± 0.14[a]	2.82 ± 0.16[a]	1.62 ± 0.44[a]	2.71 ± 0.19[a]
2.5	2.03 ± 0.24[a]	2.71 ± 0.10[a]	1.55 ± 0.40[a]	2.40 ± 0.38[a]	<1.00[a]	1.93 ± 0.18[b]
3	1.80 ± 0.15[a]	2.43 ± 0.15[a]	0.98 ± 0.70[a]	1.90 ± 0.27[a]	<1.00[a]	1.52 ± 0.06[b]

[1] Data represent average value ± standard deviation. The values with different superscripts in a column are significantly different (p <0.05).

3.4. Kinetic study for the SHS inactivation of *B. cereus* spores on garlics

Because spores in garlics were contaminated about 2-3 log CFU/g, *B. cereus* spores were inoculated at 3 log CFU/g level in garlic cloves and sliced garlics in order to investigate the reduction patterns for the SHS and the results are shown in the figure 21. 120, 150, and 180℃ of SHS were treated to samples for 30 s. To find out the effect of germination buffer on inactivation of *B. cereus* spores, Ala+IMP germinant buffer was sprayed on garlics. Spores were slightly more reduced in the germination buffer sprayed samples. After the exposure to 120, 150 and 180℃ SHS on garlic cloves for 30 s, 1.59, 1.84, and 2.38 log CFU/g of spores were decreased without germinant buffer, and 2.12, 2.16 and 3.09 log CFU/g of spores were reduced with germinant buffer. At 120, 150 and 180℃ SHS in sliced garlic, 1.12, 1.44, and 2.01 log CFU/g of spores were decreased without germinant buffer, and 1.59, 2.19, 2.56 log CFU/g of spores were reduced with germinant buffer. In this study, spores corresponding to 0.52 ± 0.16 log CFU/g on garlic cloves and 0.59 ± 0.12 log CFU/g on sliced garlics were more affected by the germinant buffer on average. *B. cereus* spores in garlics were more effectively decreased with higher SHS temperature and longer treatment time.

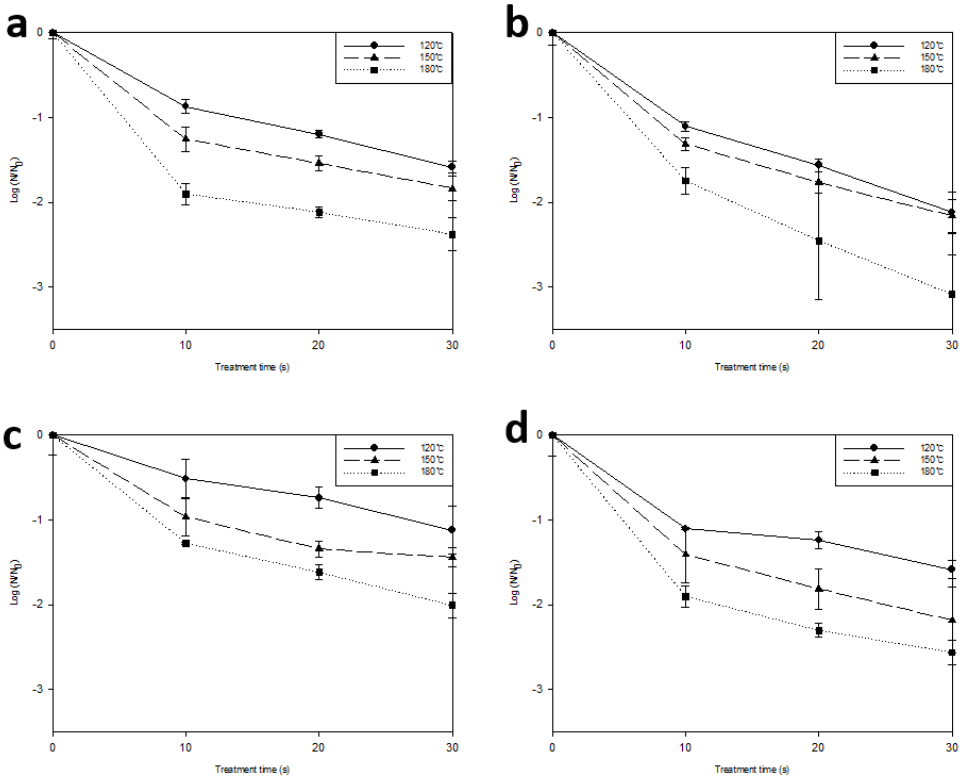


Figure 21. Survival curves of *B. cereus* spores inoculated approximately 3 log CFU/g after treated with SHS for 30 s in garlic cloves (a) without germinant buffer, (b) with germinant buffer, and in sliced garlics (c) without germinant buffer, (d) with germinant buffer.

The inactivation pattern of spores was analyzed to figure out the survival kinetics of *B. cereus* spores with Weibull model [40]. Fitting equation of the Weibull model was as follows (7):

$$\log\left(\frac{\mathrm{N}}{N_0}\right)(y) = ax^b \tag{7}$$

The survivor curves fitted by Weibull model for *B. cereus* spores on garlic cloves and sliced garlic with 120, 150, and 180℃ SHS were shown in the figure 22 and 23, and table 14 and 15. α and β values were used to calculate t_R-value which indicates stochastic 1 log reduction time [41], and *z*-value. The t_R-values of all samples, garlic cloves and sliced garlics with/without germinant buffer, were calculated and tended to decrease as the SHS temperature were risen.

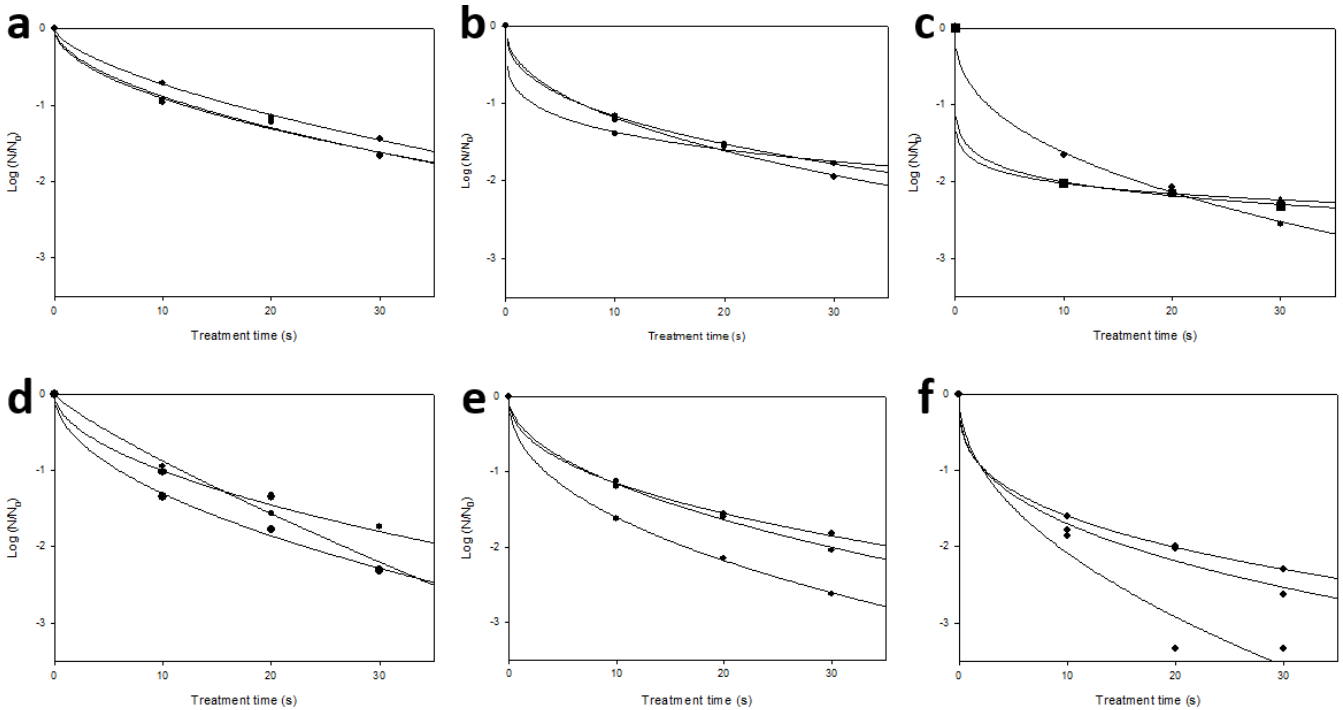


Figure 22. Survival curves of *B. cereus* spores fitted with Weibull model to SHS treatment in garlic cloves without germinant buffer at (a) 120℃, (b) 150℃, and (c) 180℃, and with germinant buffer at (d) 120℃, (e) 150℃, and (f) 180℃.

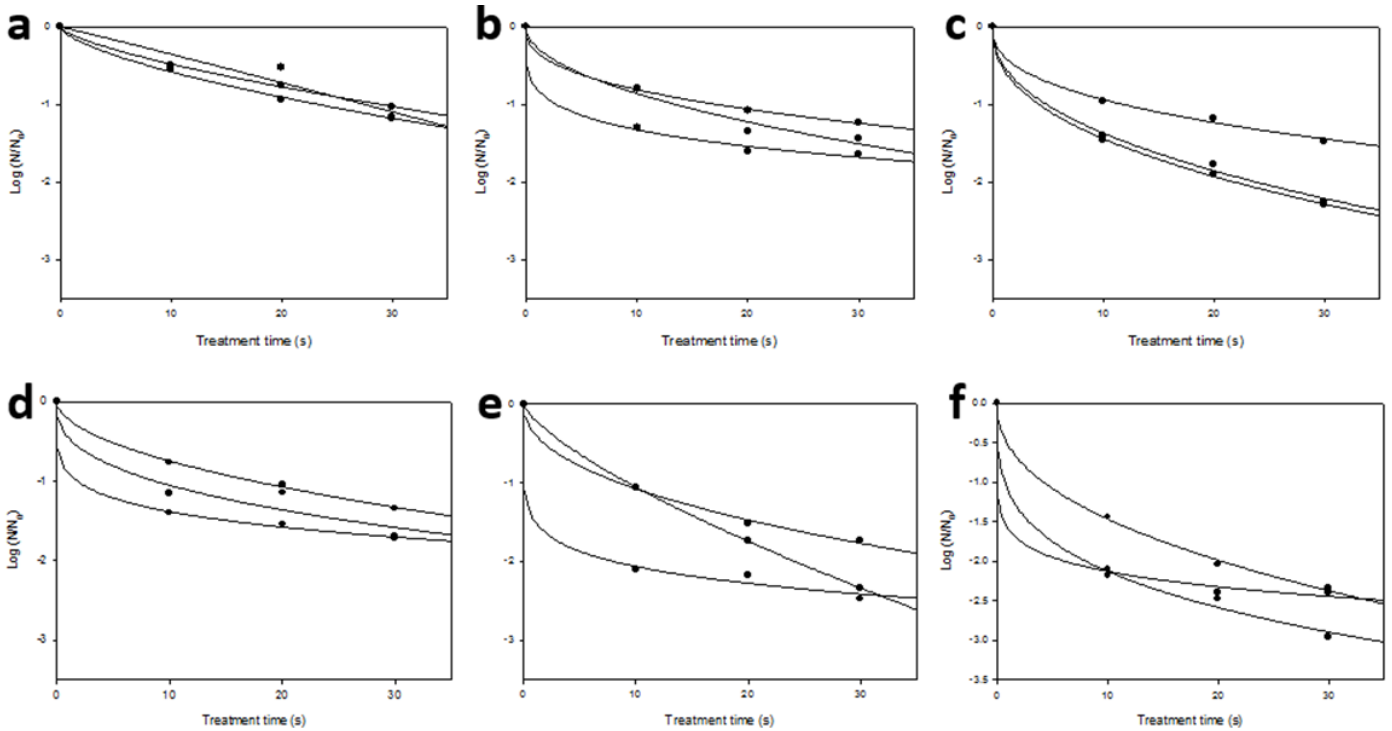


Figure 23. Survival curves of *B. cereus* spores fitted with Weibull model to SHS treatment in sliced garlics without germinant buffer at (a) 120℃, (b) 150℃, and (c) 180℃, and with germinant buffer at (d) 120℃, (e) 150℃, and (f) 180℃.

Table 14. The Weibull model fitting values for the *B. cereus* spores on garlics

Samples	Temp (℃)	α-value	β-value	t_R (s) [1)]	z-value (℃)
Garlic cloves without germinant buffer	120	2.37 ± 0.49[a]	1.78 ± 0.15[a]	3.83 ± 0.96[a]	23.26
	150	1.27 ± 0.17[ab]	3.13 ± 0.99[b]	1.73 ± 0.35[b]	
	180	1.03 ± 0.11[b]	7.12 ± 3.46[b]	1.25 ± 0.30[b]	
Garlic cloves with germinant buffer	120	3.04 ± 1.84[a]	1.68 ± 0.35[a]	5.58 ± 4.08[a]	15.06
	150	1.46 ± 0.16[ab]	2.20 ± 0.14[a]	2.14 ± 0.28[a]	
	180	1.14 ± 0.05[b]	2.61 ± 0.41[a]	1.59 ± 0.16[a]	
Sliced garlics without germinant buffer	120	3.66 ± 1.19[a]	1.77 ± 0.39[a]	6.19 ± 2.44[a]	14.12
	150	1.61 ± 0.52[a]	4.08 ± 2.78[b]	2.32 ± 1.06[b]	
	180	1.37 ± 0.08[a]	2.40 ± 0.10[b]	1.94 ± 0.09[b]	
Sliced garlics with germinant buffer	120	1.54 ± 0.59[a]	3.12 ± 1.03[a]	2.18 ± 1.08[a]	64.94
	150	1.48 ± 0.10[a]	2.19 ± 0.10[a]	2.17 ± 0.19[a]	
	180	1.00 ± 0.03[a]	4.31 ± 1.70[a]	1.25 ± 0.11[a]	

[1)] t_R: the time that 1 log reduction in the number of surviving microorganism.

Table 15. Analysis of variance (ANOVA) through the Weibull model fitting for *B. cereus* spore on garlics

Samples	Temp (℃)	MS[1)]	*F*-value	*p*-value	R^2
Garlic cloves	120	0.461	743.800	0.0029	0.9943
	150	0.658	34631.353	0.0011	0.9986
	180	1.191	3550.028	0.0004	0.9991
Garlic cloves with germinant buffer	120	0.834	293.218	0.0073	0.9855
	150	0.907	2049.694	0.0010	0.9980
	180	1.613	18374.779	0.0119	0.9843
Sliced garlics	120	0.186	1097.755	0.0072	0.9857
	150	0.462	5543.827	0.0076	0.9900
	180	0.787	2268.645	0.0013	0.9975
Sliced garlics with germinant buffer	120	0.497	1024.525	0.0129	0.9746
	150	0.809	270526.639	0.0075	0.9950
	180	1.378	1444.995	0.0013	0.9975

[1)] MS: Mean of square.

In the initial stage, the spores were effectively reduced by the latent heat effect from the condensation water, and after the condensate water was removed, the spore reduction was explained by the sensible heat energy. In other words, moist heat sterilization and dry heat sterilization occurred. The two different sterilization mechanism in survival curves of *B. cereus* spores could explain as known that the core enzymes and the inner membranes were damaged by the wet heat sterilization, and DNA of spores were injured by the dry heat sterilization process [20].

When the SHS was treated on garlic cloves and sliced garlics for 30 s, tanned samples were not observed. Therefore, 2-3 log CFU/g of *B. cereus* spores in garlics could be controlled using the SHS pretreatment. In addition, the spores could be more efficiently decreased if the germinant buffer was used properly. It is expected that the pre-treatment with SHS could be effective to reduce the total sterilization time of retort products such as cream base sauce and soup, in which garlic is the raw material containing spores with the highest heat resistance. Therefore, the SHS pretreatment for controlling spores can be applied as an important process in the food industries in order to minimize the quality loss of retort products.

IV. CONCLUSIONS

In order to investigate the reduction pattern of *Bacillus cereus* spores on the superheated steam depending on the shape of garlics, the effect of inactivation was better in the garlic cloves than in sliced garlics. The reason for this result was assumed to be surface effects and water activity. Through the experiment carried out by removing the outer coating film and the SEM images, it was not thought to have been influenced by the surface effect. The spore reduction effect was better than that of sliced garlic, and it was thought to be due to water activity. The rapid decrease of water activity in sliced garlic could be interpreted that the heat energy of the superheated steam partially lost to evaporate the water. In other words, more heat energy was used to kill spores due to the low evaporation of water, which resulted in better bactericidal effects. For more effectively sterilize the spores, the germination experiment was conducted. It was done in germinant buffers with L-alanine and inosine which were well-known substance of germination. Disodium 5'-inosinate (IMP) was used as a substitute for inosine, which is not approved as a food additive. The germinant buffer combined 50 mM of L-alanine and 5 mM of IMP was confirmed to have a synergistic effect on the buffer. Approximately 15 min after the reaction, the germination rate was reached its maximum level. When the germinant buffer was applied to garlic cloves, the

decontamination effect of spores was slightly better than the samples without the germination inducer. In addition, when the sterile water was sprayed as a control for the water content of the germination inducing agent, the sterilization effect was rather reduced because the moisture acted as a protective layer and had to eliminate. In experiments with 3 log CFU/g level which was the actual contamination levels, spores corresponding to about 0.5 log CFU/g were more effectively decreased with Ala+IMP germinant buffer. Especially, when the garlic cloves were sprayed by Ala+IMP germinant buffer, and treated with 180℃ of SHS for 30 s, approximately 3 log CFU/g of the spore population could be reached. The inactivation of *B. cereus* spores in garlic with SHS treatment was fitted with the Weibull model. It is significant that there was little research on the synergistic effect of L-alanine and disodium 5'-inosinate as germinants. When IMP, which is allowed to be used as a food additive, was applied in combination with L-alanine, the synergistic effect on spore germination was confirmed. The potential of IMP in the food industry was presented not only as a sweetener but also as a spore germinant substance.

V. REFERENCES

1. **Little, C., Rawal, N., et al.**, Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. *Food Microbiology*, 2010. **27**(1): p. 171-174.

2. **Atungulu, G., Pan, Z., et al.**, Microbial decontamination of nuts and spices. 2012: Woodhead Publishing, Philadelphia, USA.

3. **Berghel, J. and Renström, R.**, Basic design criteria and corresponding results performance of a pilot-scale fluidized superheated atmospheric condition steam dryer. *Biomass and Bioenergy*, 2002. **23**(2): p. 103-112.

4. 이대한, Inactivation of *Salmonella* on particulate foods using superheated steam (SHS). 2015, 서울대학교 대학원: 서울.

5. **Dincer, I. and Rosen, M.**, Thermal energy storage: systems and applications. 2002: John Wiley & Sons.

6. **Cenkowski, S., Pronyk, C., et al.**, Decontamination of food products with superheated steam. *Journal of Food Engineering*, 2007. **83**(1): p. 68-75.

7. **James, C., Göksoy, E., et al.**, Surface pasteurisation of poultry meat using steam at atmospheric pressure. *Journal of Food Engineering*, 2000. **45**(2): p. 111-117.

8. **성해진**, Inactivation of pathogens in particulate foods using superheated steam. 2016, 서울대학교 대학원: 서울.

9. **Kim, J.E., Lee, D.U., and Min, S.C.**, Microbial decontamination of red pepper powder by cold plasma. *Food Microbiology*, 2014. **38**: p. 128-136.

10. **Lindsay, D., Brözel, V., and Von Holy, A.**, Biofilm-spore response in *Bacillus cereus* and *Bacillus subtilis* during nutrient limitation. *Journal of Food Protection*, 2006. **69**(5): p. 1168-1172.

11. **Byrne, B., Dunne, G., and Bolton, D.**, Thermal inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores in pork luncheon roll. *Food Microbiology*, 2006. **23**(8): p. 803-808.

12. **식품의약품안전청 and 경상북도보건환경연구원**, 대구·경북 지역에서 생산·유통되는 고춧가루제품의 품질 모니터링 및 관리방안 연구. 2007, 식품의약품안전청: 서울.

13. **배태진**, **최옥수**, **et al.**, 향신재료를 이용한 Oleoresin 제조에 관한 연구 – 2. 고추 Oleoresin 의 품질안정성. *한국식품영양과학회지*, 1991. **20**(6): p. 609-614.

14. **Bocchini, P., Andalo, C., et al.**, Determination of diallyl thiosulfinate (allicin) in garlic (*Allium sativum* L.) by high-performance liquid chromatography with a post-column photochemical reactor. *Analytica Chimica Acta*, 2001. **441**(1): p. 37-43.

15. **Voort, M. and Abee, T.**, Sporulation environment of emetic toxin-producing *Bacillus cereus* strains determines spore size, heat resistance and germination capacity. *Journal of Applied Microbiology*, 2013. **114**(4): p. 1201-1210.

16. **de Vries, Y.P.**, *Bacillus cereus* spore formation, structure, and germination. 2006, Wageningen Universiteit: Netherlands.

17. **Soni, A., Oey, I., et al.**, *Bacillus* spores in the food industry: a review on resistance and response to novel inactivation technologies. *Comprehensive Reviews in Food Science and Food Safety*, 2016. **15**(6): p. 1139-1148.

18. **Fernández, A., Collado, J., et al.**, Empirical model building based on Weibull distribution to describe the joint effect of pH and temperature on the thermal resistance of *Bacillus cereus* in vegetable substrate. *International Journal of Food Microbiology*, 2002. **77**(1): p. 147-153.

19. **Cattani, F., Dolan, K., et al.**, One-step global parameter estimation of kinetic inactivation parameters for *Bacillus sporothermodurans* spores under static and dynamic thermal processes. *Food Research International*, 2016. **89**: p. 614-619.

20. **Setlow, P.**, Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *Journal of Applied Microbiology*, 2006. **101**(3): p. 514-525.

21. **Cléry-Barraud, C., Gaubert, A., et al.**, Combined effects of high hydrostatic pressure and temperature for inactivation of *Bacillus anthracis* spores. *Applied and Environmental Microbiology*, 2004. **70**(1): p. 635-637.

22. **Akbas, M.Y. and Ozdemir, M.**, Application of gaseous ozone to control populations of *Escherichia coli*, *Bacillus cereus* and *Bacillus cereus* spores in dried figs. *Food Microbiology*, 2008. **25**(2): p. 386-391.

23. **Kumari, S. and Sarkar, P.K.**, *Bacillus cereus* hazard and control in industrial dairy processing environment. *Food Control*, 2016. **69**: p. 20-29.

24. **Løvdal, I.S., Hovda, M.B., et al.**, Promoting *Bacillus cereus* spore germination for subsequent inactivation by mild heat treatment. *Journal of Food Protection*, 2011. **74**(12): p. 2079-2089.

25. **Hornstra, L.M., de Vries, Y.P., et al.**, Characterization of germination receptors of *Bacillus cereus* ATCC 14579. *Applied and Environmental Microbiology*, 2006. **72**(1): p. 44-53.

26. **Hornstra, L.M., de Vries, Y.P., et al.**, *GerR*, a novel ger operon involved in L-alanine-and inosine-initiated germination of *Bacillus cereus* ATCC 14579. *Applied and Environmental Microbiology*, 2005. **71**(2): p. 774-781.

27. **Yamaguchi, S.**, The synergistic taste effect of monosodium glutamate and disodium 5′-inosinate. *Journal of Food Science*, 1967. **32**(4): p. 473-478.

28. **Leguérinel, I. and Mafart, P.**, Modelling the influence of pH and organic acid types on thermal inactivation of *Bacillus cereus* spores. *International journal of Food Microbiology*, 2001. **63**(1): p. 29-34.

29. **López-Pedemonte, T.J., Roig-Sagués, A.X., et al.**, Inactivation of spores of *Bacillus cereus* in cheese by high hydrostatic pressure with the addition of nisin or lysozyme. *Journal of Dairy Science*, 2003. **86**(10): p. 3075-3081.

30. **Crane, J., Frodyma, M., and Bergstrom, G.**, Nutrient-induced spore germination of a *Bacillus amyloliquefaciens* biocontrol agent on wheat spikes. *Journal of Applied Microbiology*, 2014. **116**(6): p. 1572-1583.

31. **Hornstra, L., De Leeuw, P., et al.**, Germination of *Bacillus cereus* spores adhered to stainless steel. *International Journal of Food Microbiology*, 2007. **116**(3): p. 367-371.

32. **Barlass, P.J., Houston, C.W., et al.**, Germination of *Bacillus cereus* spores in response to L-alanine and to inosine: the roles of *gerL* and *gerQ* operonsc. *Microbiology*, 2002. **148**(7): p. 2089-2095.

33. **Shin, H.W., Lim, Y.-H., et al.**, Effect of commercial antimicrobials in combination with heat treatment on inactivation of *Bacillus cereus* spore. *Food Science and Biotechnology*, 2008. **17**(3): p. 603-607.

34. **Hätnulv, B., Johansson, M., and Snygg, B.G.**, Heat resistance of *Bacillus stearothermophilus* spores at different water activities. *Journal of Food Science*, 1977. **42**(1): p. 91-93.

35. **El-Nour, S.A. and Hammad, A.**, Inactivation of *Baccillus cereus* spores in liquid food by combination treatments of heat and irradiation. *Food Science and Quality Management*, 2013. **19**: p. 1-40.

36. **Iyota, H., Nishimura, N., et al.**, Drying of sliced raw potatoes in superheated steam and hot air. *Drying Technology*, 2001. **19**(7): p. 1411-1424.

37. **Tang, Z., Cenkowski, S., and Izydorczyk, M.**, Thin-layer drying of spent grains in superheated steam. *Journal of Food Engineering*, 2005. **67**(4): p. 457-465.

38. **XIAO, H.W., YAO, X.D., et al.**, Effect of SSB (superheated steam blanching) time and drying temperature on hot air impingement drying kinetics and quality attributes of yam slices. *Journal of Food Process Engineering*, 2012. **35**(3): p. 370-390.

39. **Gounina-Allouane, R., Broussolle, V., and Carlin, F.**, Influence of the sporulation temperature on the impact of the nutrients inosine and l-alanine on *Bacillus cereus* spore germination. *Food Microbiology*, 2008. **25**(1): p. 202-206.

40. **Tola, Y.B. and Ramaswamy, H.S.**, Combined effects of high pressure, moderate heat and pH on the inactivation kinetics of *Bacillus licheniformis* spores in carrot juice. *Food Research International*, 2014. **62**: p. 50-58.

41. **Buzrul, S.**, A suitable model of microbial survival curves for beer pasteurization. *LWT-Food Science and Technology*, 2007. **40**(8): p. 1330-1336.

VI. 국문초록

건강식품 및 신선식품을 선호하는 식생활의 변화에 따라 향신료 및 견과류에 대한 소비가 증가하고 있다. 견과류와 향신료는 수확 시 살모넬라균의 오염에 노출되어 식품 안전에 위험요소로 남아있음에도 불구하고 조리, 살균 등의 식품 가공 공정 중 품질손상이 일어나기 쉽기 때문에 수확된 이후 날 것 그대로 혹은 최소한의 살균 처리만을 하여 섭취되고 있다. 또, 분체 식품은 내열성균 또는 포자가 오염되었을 때, 낮은 수분함량으로 인해 열 처리시 식품의 품질이 크게 손상을 받고, 방사선 살균은 소비자의 좋지 않은 인식 때문에 분체 식품에 적합한 살균방법이 정립되지 못한 상태이다. 통후추, 피칸, 아몬드의 살모넬라균에 대한 과열수증기살균의 lab-scale 연구가 선행 되었으며, 본 연구에서는 산업에 적용시키기 위한 전단계로 scale-up 연구를 진행하였다. 과열수증기 장치의 반응기 부분의 재제작을 통해 샘플의 증량을 할 수 있었고, 열 손실을 줄이기 위해 유체 흐름의 방향을 위에서 아래로 흐르도록 과열수증기 장치를 개선하였다. 또한 살균이 되지 않은 고추에 있던 영양세포 상태의 균들이 건조, 분쇄 공정을 거치면서 바실러스 계통의

균들이 포자를 형성하여 내열성이 강해져 과열수증기 처리로 고춧가루의 품질 변화가 없는 시간 내에 고춧가루에 오염되어 있는 내열성균과 포자를 사멸시키는 것의 한계가 있었다. 이를 극복하기 위해 고춧가루가 건조, 분쇄 되기 전의 원물 상태로의 홍고추를 살균하여 영양세포들이 내열성을 가지는 형태로의 전환을 막을 수 있도록 공정 순서의 변화를 제시해보았다. 100℃의 포화수증기, 140 그리고 180℃의 과열수증기로 살모넬라균이 오염된 통후추는 6 초 이내, 피칸, 아몬드는 24 초 이내로 검출한계 미만 (1 log CFU/g)으로 저감화 시킬 수 있었다. 그리고 홍고추 원물에 오염된 대장균, 살모넬라균은 120, 150, 그리고 180℃의 과열수증기로 10 초 이내로 저감화되었으며, 총호기성균은 온도별로 각각 20 초, 30 초, 50 초에 검출한계 미만 (0.6 log CFU/g)으로 저감화 되었다. 고온 단시간으로 처리되는 과열수증기 살균으로 인한 캡사이신 함량의 변화는 없었음을 확인하였다. Part1 에서 확인된 재제작된 장치의 적용성을 통후추, 피칸, 아몬드, 홍고추의 미생물 저감화를 통해 살균장치로써 적용가능성을 검증하였다.

최근 1 인가구, 맞벌이부부가 많아짐에 따라 즉석편의식품의 수요가 늘어 나고 있다. 즉석편의식품에 포함되는 향신료 중 마늘에는 토양유래의 바실러스가 오염이 되어 강한 열저항성을 가지는 포자를 형성하게 되어 세척 후에도 2~3 log CFU/g 의 수준으로 오염이 되어있다. 마늘에 있는 내열성균이나 바실러스 포자를 사멸시키기 위해 121.1℃에서 레토르트 살균이 이루어지지만 과도한 열처리로 인한 품질 저하가 큰 문제가 되고 있다. 따라서 마늘에 오염된 바실러스 세레우스 포자를 과열수증기로 전처리 살균을 통해 레토르트 총 처리 시간을 줄여서 식품의 품질 저하를 최소한으로 하고자 하였다. 마늘의 형태에 따른 포자의 저감화 효과를 보기 위해 통마늘과 슬라이스 마늘에 포자를 접종하여 실험을 진행하였으며, 과열수증기 살균을 좀 더 효과적으로 하기 위해 포자의 발아를 유도하기 위해 germinant 를 사용하였다. 기존에 알려져 있는 알라닌과 이노신으로 실험을 하였으며, 식품첨가물로 허용이 되지 않는 이노신의 대체 물질로 식품첨가물로 사용이 가능하며 생체 내에서 이노신과 무기인산으로 분해가 되는 disodium 5'-inosinate (IMP)로 실험을 하였다. 그 결과, 통마늘에서 180℃의 과열수증기

처리시 1 분 30 초에 3 log CFU/g 의 저감화를 할 수 있었고, 슬라이스 마늘을 살균할 때보다 통마늘을 살균했을 시 더 효과적으로 살균을 할 수 있었다. 슬라이스 마늘의 경우 수분활성도 측정결과 건조가 더 빨리 일어났으며, 수분 증발에 열에너지를 일부 뺏겨 통마늘을 살균할 때보다 살균효과가 저하되었다고 판단되었다. Germinants 의 실험 결과, Ala+IMP 의 시너지 효과가 있음을 확인하였다. 이를 실제 마늘에 적용해본 결과, germinant 를 사용하지 않았을 때 보다 동일 시간에 평균적으로 0.5 log CFU/g 정도 살균효과가 더 있음을 확인하였다. 마늘에 있는 바실러스 세레우스 포자의 저감화 양상은 비선형으로 나타났으며, 이를 Weibull model 에 적용하여 얻은 t_R 값을 통해 온도가 증가할수록 t_R 값이 감소하는 경향을 알 수 있었다.

본 연구에서는 scale-up 연구를 위해 개선된 과열수증기 장치가 식품 살균에 적합하다는 것을 견과류 및 향신료 살균 실험을 통해 확인하였고, 고춧가루 살균의 한계를 원물 고추를 살균 함으로서 극복하고자 하였고 식품 산업에서 공정 순서 변경 제안을 위한 학문적인 기초 자료를 얻을 수 있었다. 식품 원료에

있는 포자의 전처리 살균을 통해 레토르트 총 처리시간을 감소시켜 식품품질 변화를 줄일 수 있는 방안을 모색해보았다.

주요어: 과열 수증기(superheated steam), 살균, 통후추, 피칸, 아몬드, 고추, 마늘, *Salmonella*, *Escherichia coli*, *Bacillus cereus* spore.

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