# A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

# Inactivation of pathogens in particulate foods using superheated steam

과열수증기를 이용한 분체 식품의 위해균 저감화

**February, 2016**

**Department of Agricultural Biotechnology**

**Seoul National University**

**Seong, Haejin**

석사학위논문

# Inactivation of pathogens in particulate foods using superheated steam

## 과열수증기를 이용한 분체 식품의 위해균 저감화

지도교수 최영진

이 논문을 석사학위 논문으로 제출함

2016년 2월

서울대학교 대학원 농생명공학부

성 해 진

성해진의 석사 학위논문을 인준함

2016년 2월

위원장 유상렬 (인)

부위원장 최영진 (인)

위원 하남출 (인)

# ABSTRACT

Particulate foods could be corrupted easily because of improper handling during storage and distribution. To sterilize particulate foods, non-thermal sterilization such as ethylene oxide and gamma irradiation was used. However, these processes have limitation because of consumers' perception and potential hazard of residue. Superheated steam (SHS) has the advantage of high rate of heat transfer. The latent heat transferred to food when SHS condenses on food surfaces. In this study, the effect of SHS on decontamination of bacteria on the surface of particulate foods including oats, flaxseeds, and red pepper powder was investigated. Each sample contaminated with *Salmonella* Typhimurium and *Escherichia coli* K-12 was treated by SHS with various temperatures and times. Within less than 10 s of SHS treatment, both pathogens were reduced below the detection limit in all samples without quality loss. In case of red pepper powder, after treated by 160℃ SHS for 15 s, the total aerobic bacteria were reduced by 1.5 log CFU/g without quality change. However, after treated by 180℃ SHS for 75 s, the sample was tanned because of the high temperature and long treatment time although the total aerobic bacteria were reduced by 3 log CFU/g. The contamination level of *Bacillus* spp. is found to be high in red pepper powder. *Bacillus* spp. are capable of forming spores which are the potential risk for

food safety. Therefore, the effect of SHS on *Bacillus cereus* spores was investigated. After the exposure to 180℃ SHS for 300 s, the reduction of viable spores was 2.7 log CFU/g. In order to reduce the spore more effectively, the spores in red pepper powder were geminated by known germination methods before SHS treatment. As a result, the reduction of viable spores was 1.9 and 2.4 log CFU/g after germinated by mild heating and germinant buffer, respectively. On the other hand, the reduction of viable spores of non-geminated sample was only 1 log CFU/g. These results suggest that SHS can be effectively applied for the reduction of bacteria on the surface which have the possibility of cross contamination. In addition, SHS could reduce *Bacillus cereus* spore about 3 log CFU/g, and pretreatment with SHS could reduce total sterilization time by lowering the initial contamination of a raw material. Moreover, further study about combination SHS with spore germination is required.

*Keywords*: particulate food, superheated steam, oat, flaxseed, red pepper powder, *Bacillus cereus* spore

Student number: 2014-20692

# Contents

# LIST OF TABLES

# LIST OF FIGURES

# I. INTRODUCTION

As changing in dietary patterns with an emphasis on health and convenience, a variety of cereals, fruits and vegetables are consumed in a powder or particulate form. In particular, the demand for diet foods and health supplement foods is increased (Won et al., 2012). However, consumers recognize that microorganisms do not grow well in the powder food so they store it wrong way or manufacturer distribute the foods carelessly. As well as contamination of the raw materials, the possibility of microbial contamination may be increased in drying process after harvest or grinding process. Especially, cereal ingredients may become contaminated by *Salmonella* on their farms of origin because *Salmonella* is widespread in nature (Davies et al., 2013).

Oat grain (*Avena sativa* L.) is a good source of quality protein, unsaturated fatty acids, minerals and vitamins, phenolic compounds as well as dietary fiber, especially its soluble fraction (Head et al., 2010). Although oats were contaminated with a variety of bacteria, including mycotoxin (Perkowski et al., 2002), there is no proper sterilization technique except for the steam drying (up to 100 min at 88–98℃) (Cenkowski et al., 2006). However, because of the long time treatment with relatively high temperature, a degradation of oat lipids via enzymatic hydrolysis was

facilitated and oxidation was followed, which leads to development of rancidity and off-flavors (Lehtinen et al., 2003).

Flaxseeds are selected as super-foods for their impressive nutrient profile and powerful health benefits. The majority of flax seed oil is an essential fatty acid, alpha linolenic acid (ALA), which is a vegan source of omega-3 and is the precursor to eicosapentaenoic acid (EPA) (Cunnane et al., 1995). Because of its high oil content, it oxidizes easily and must be stored in a tightly sealed container in the refrigerator or freezer (Accorsini et al., 2011). Since it became known as a diet food recently, domestic consumption is increasing. However, in USA, flaxseeds were recalled several times because it might be contaminated with *Salmonella*.

The red pepper (*Capsicum annuum* L.) is a natural spice with a pungent flavor and widely used around the world including Korea. Color and spicy flavor that determine the commercial quality of spicy is mainly capsaicinoid, in particular dependent on the content of capsaicin and dihydrocapsaicin (Jung et al., 2015). Because the red pepper cultivated between April and August, with high temperature and frequent rain, it is susceptible to microorganism. In addition, the cross-contamination could be occurred depending on the hygiene of workers and the manufacturing facility. (Woo et al., 2012). Especially, contamination of *Bacillus cereus* in red

pepper suggests that there is a potential health risk associated with *Bacillus cereus* (Oh et al., 2012).

For sterilization of these foods, ethylene oxide and irradiation are used currently. However, ethylene oxide is limited in many countries because of the potential hazards. Moreover, consumers have unwelcome perception about irradiation (Lee et al., 2004). Since most of the powdered foods are sensitive to heat, the quality and functionality is likely to be damaged. Therefore, a new sterilization technology was needed.

Superheated steam (SHS) is a steam that heated over the saturation temperature or more, and it has attracted attention as an excellent heating medium. Although widely used mainly in the drying process (Sagar et al., 2010), it has been used in the sterilization process due to the advantages such as the high heat transfer efficiency and anaerobic conditions (Cenkowski et al., 2007). The biggest advantage of superheated steam is having the effect of moist heat sterilization and dry heat sterilization (James et al., 2000). When the superheated steam heated the food, because the temperature of food is low, the steam condenses on the surface of the food. Then, high latent heat of condensation is instantly delivered to the microorganisms attached to the surface of food so that moist heat sterilization process takes place and that could sterilize the bacteria. After all the condensed water of food surface evaporated, dry heat sterilization process could kill the bacteria due to the

sensible heat of superheated steam. Therefore, using superheated steam, instant disinfection of powdered food could be possible.

The objectives of this study were to investigate the effects of superheated steam on *Salmonella* Typhimurium and *Escherichia coli* K-12 on the surface of oats, flaxseeds and red pepper powder and to reduce the total aerobic bacteria in red pepper powder using superheated steam.

# II. MATERIALS AND METHODS

## 2.1. Sample preparation and inoculation

### 2.1.1. Bacteria inoculation

*E. coli* K-12 (MG1655) and *S.* Typhimurium (ATCC 19585) were used. Each strain of *E. coli* K-12 and *S.* Typhimurium was cultured in 5 mL of Tryptic Soy Broth (TSB, Difco, Franklin Lakes, NJ, USA) at 37℃ for 24 h, harvested by centrifugation at 4000×g for 20 min. at 4℃ and washed with 0.2% (w/v) buffered peptone water (BPW, Difco). The final pellets were resuspended in BPW, corresponding to approximately $10^9$–$10^{10}$ CFU/mL. Subsequently, suspended pellets of each strain of the two pathogen species were combined to produce culture cocktails.

Red pepper powder, oat and flaxseed used for this study were purchased at a local grocery store (Seoul, Korea). For red pepper powder, three different sizes of samples were used (300, 500 and 1000 μm). 0.5 mL of culture cocktail was applied to 25 g sample, for oat and flaxseed, 0.3 mL of culture cocktail was applied to 25 g sample inside a sterile polyethylene bag. The inoculated samples were mixed by hand massage for 1 min and dried for 1 h in a biosafety hood. Final inoculum was confirmed as $10^7$ CFU/g.

### 2.1.2. Spore production and inoculation

One colony of *Bacillus cereus* ATCC 14579 (Seoul National University, Seoul, Korea) vegetative cell was cultivated in 30% diluted Luria Bertani broth (LB broth, MB cell, Seoul, Korea) at 37℃ for 11 days. Through the microscopic observation, it was confirmed that the 80% of the vegetative cells changed to spores. The spores were centrifuged (3,600×*g*, 20 min, 4℃) and washed with BPW. The spore suspension was heat-treated for 10 min at 80℃ with a water bath to inactivate vegetative cells. One mL of the suspension was mixed with 10 g of sterilized sand in a sterilized polyethylene bag by hand massage and dried for 2 h in a biosafety hood. The final spore concentration of the spore-sand mixture was $5\times10^5$ CFU/g.

## 2.2. Superheated steam equipment and treatment

The equipment used in this research consisted of steam generator (boiler), superheater, water reservoir, processing chamber, and sample tray (Figure 1). Whole pipe was made of stainless steel, and covered with heat insulating material in order to prevent heat loss. Processing chamber was insulated additionally with band heater. The velocity of steam passing through the processing chamber was 3.0±0.2 m/s. Temperature of steam could be

controlled by power of superheater, and monitored consistently at the exit of superheater and processing chamber.

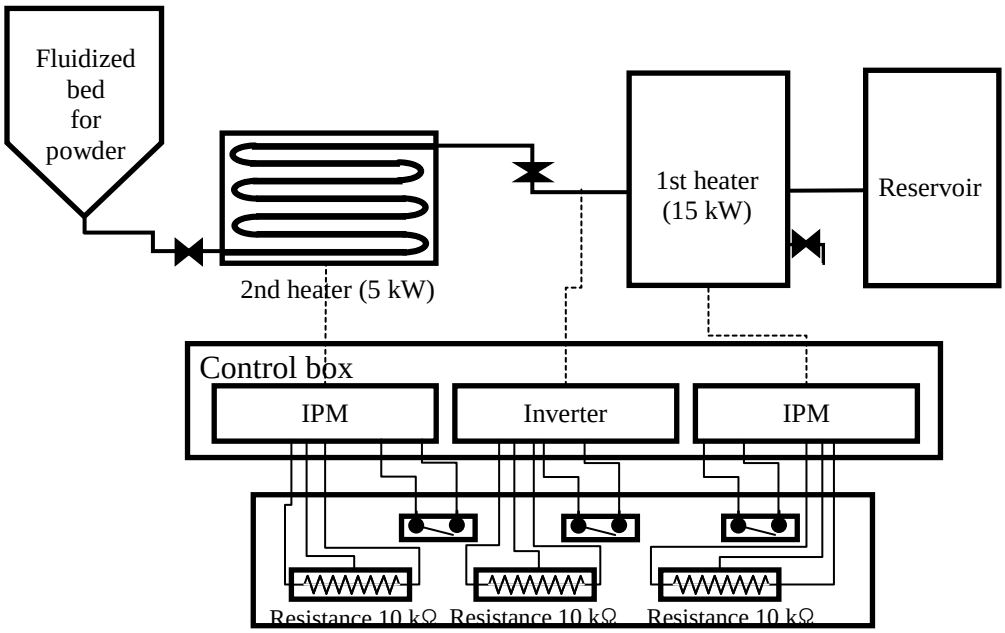


Figure 1. Schematic diagram of superheated steam sterilization equipment.

All inoculated samples (1 g) were transferred into reacting cell and treated with 120-180℃ of SHS for various times. SHS treated samples were immediately transferred to sterile stomacher filtra-bag (Labplas Inc., Sainte-Julie, QC, Canada).

For *Bacillus cereus* spore inactivation, sample tray was developed. It consisted of two stainless steel meshes (30 μm mesh opening; 55 mm diameter; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA). A layer of spore-sand mixture (2 g) was placed between the two meshes, and the top and bottom of the mesh was fixed with stainless steel ring which can fit tightly. Sample was treated with 100℃ of saturated steam and 120, 140, 160, and 180℃ of superheated steam for 15, 30, 45, 60, 180, and 300 s. SHS treated samples were immediately transferred to sterile stomacher bag.

## 2.3. Bacteria enumeration

Treated red pepper powder, oat and flaxseed were diluted in 9 mL of BPW and homogenized for 2 min in a stomacher (Hansol Tech Co., Seoul, Korea). Treated spore-sand mixture was diluted in 18 mL of BPW. After homogenization, samples were serially diluted in BPW, and 0.1 mL of diluent was spread onto *Salmonella* selective agar (xylose lysine desonycholate agar, XLD, Oxoid, Basingstroke, UK), *E. coli* selective agar

(Sorbitol-MacConkey agar, HiMedia, Mumbai, India) and Tryptic Soy Agar (TSA, MB cell, Seoul, Korea) for *Bacillus cereus* spore enumeration. All plates were incubated at 37℃ for 24 h and colonies were counted.

## 2.4. Superheated steam treatment after germination of *Bacillus* spores

### 2.4.1. Heat-induced germination of *Bacillus* spores

*Bacillus* spores were germinated according to the method of Joan F. Powell (1955). One gram of red pepper powder was heated in 80℃ water bath for 15 min. Spore germination was observed by spreading on the MYP agar (Mannitol-egg yolk-polymyxin agar, Oxoid).

### 2.4.2. L-alanine-induced germination of *Bacillus* spores

*Bacillus* spores were germinated by germinant buffer (Joan F. Powell, 1950). Germinant buffer was made with 5 mM L-alanine, 50 mM glucose and 33 mM phosphate buffer (pH 7.0). One gram of red pepper powder was mixed with 1 mL of germinant buffer. Then, after that mixture was cultivated at 37℃ for 2 h, dried for 1 h in a biosafety hood. All germinated red pepper powders were treated with 140℃ SHS for 30 s.

## 2.5. Moisture content

After treated with SS and SHS, spore-sand mixture were transferred in aluminum dish which was reached constant weight in advance. By conducting all processes in several times, the amount of the sample was to 8 g. The samples were allowed to cool for 30 min using a desiccator and measured the moisture content with infrared moisture analyzer (A & D Company, Tokyo, Japan) at 120℃.

## 2.6. Color measurement

Hunter's color values (L*, a*, b*) of all treated samples were measured using a Chroma Meter CR-400 (KONICA MINOLTA, Sensing, Inc., Osaka, Japan) after SHS treatment until *S.* Typhimurium and *E. coli* K-12 reduced by detection limit at each temperature.

## 2.7. Oil content measurement

After SHS treatment until *S.* Typhimurium and *E. coli* K-12 reduced by detection limit at each temperature, flaxseed oil was extracted by the Soxhlet method with *n*-hexane as a solvent followed by AOAC (1990).

## 2.8. Statistical analysis

All data were analyzed with SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Tukey's multiple comparison test was used for investigating if there was significant difference ($p < 0.05$) in mean values of color values.

# III. RESULTS AND DISCUSSION

## 3.1. Inactivation of *E. coli* K-12 and *S.* Typhimurium in oats and flaxseeds

Inoculation level of *E. coli* K-12 and *S.* Typhimurium in oats was approximately $10^7$ CFU/g. After the exposure to 110, 120 and 130℃ SHS for 5 s, both bacteria were decreased to below the detection limit (Figure 2). Reduction of pathogens increased as the temperature increased. After 110℃ SHS treatment for 5 s, lightness was decreased (Table 1). However, there was no significant difference observable with the naked eye between before and after treatments.

Inoculation level of *E. coli* K-12 and *S.* Typhimurium in flaxseeds was also approximately $10^7$ CFU/g. After the exposure to 110, 130 and 150℃ SHS for 15 s, both bacteria were decreased to below the detection limit (Figure 3). In case of color change, there was no significant difference between before and after treatment (Table 2). Oil content of flaxseeds was measured 41.16%, even after 110, 130 and 150℃ SHS treatment, 40.72, 40.37 and 41.24%, respectively.

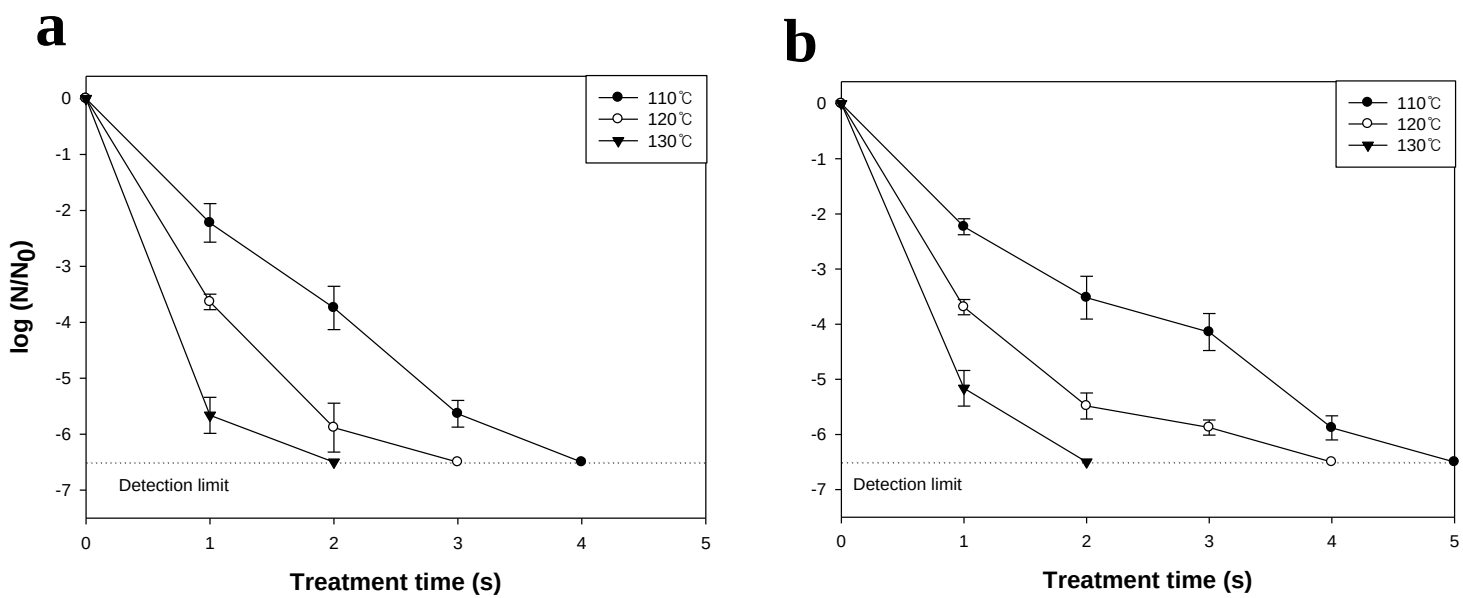


Figure 2. Survival curves of *S.* Typhimurium (a) and *E. coli* K-12 (b) inoculated on oats treated with 110 (●), 120 (○) and 130℃ (▼) SHS for 5 s.

Table 1. Color change of oats after SHS treatment until *S.* Typhimurium and *E. coli* K-12 reduced below the detection limit [1)]

| | **L*** | **a*** | **b*** |
|---|---|---|---|
| **Control** | 62.03 ± 0.49 [a] | 2.64 ± 0.13 [a] | 18.63 ± 0.68 [a] |
| **110°C, 5 s** | 58.39 ± 0.43 [b] | 2.86 ± 0.32 [ab] | 18.20 ± 0.49 [a] |
| **120°C, 4 s** | 59.32 ± 0.71 [a] | 3.28 ± 0.19 [b] | 18.92 ± 0.33 [a] |
| **130°C, 2 s** | 61.28 ± 0.58 [a] | 2.62 ± 0.37 [a] | 19.39 ± 0.26 [a] |

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

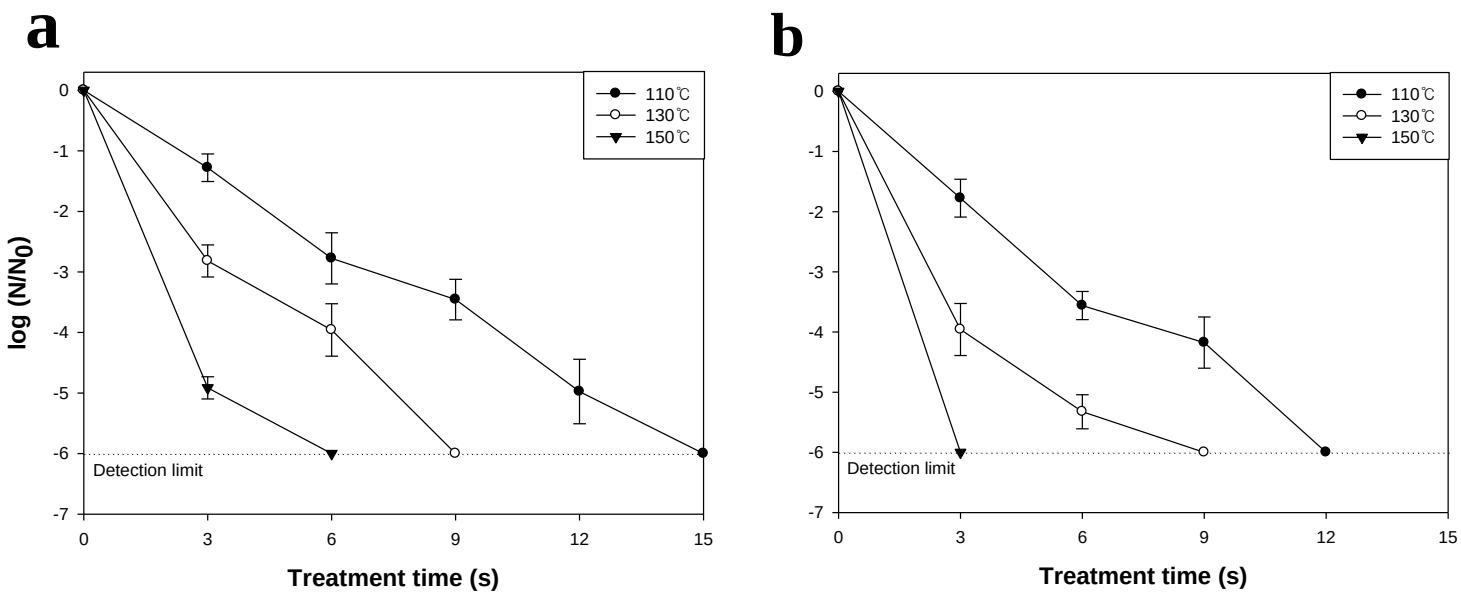


Figure 3. Survival curves of *S.* Typhimurium (a) and *E. coli* K-12 (b) inoculated on flaxseeds treated with 110 (●), 130 (○) and 150℃ (▼) SHS for 15 s.

Table 2. Color change of flaxseeds after SHS treatment until *S.* Typhimurium and *E. coli* K-12 reduced below the detection limit [1)]

| | **L*** | **a*** | **b*** |
|---|---|---|---|
| **Control** | 49.05±0.21 [a] | 6.95±0.42 [a] | 18.75±0.26 [a] |
| **110 ℃, 15 s** | 50.11±0.33 [a] | 6.69±0..46 [a] | 19.44±0.63 [a] |
| **130 ℃, 9 s** | 50.03±0.76 [a] | 7.39±0.22 [a] | 19.32±0.21 [a] |
| **150 ℃, 6 s** | 50.25±0.70 [a] | 7.28±0.57 [a] | 19.23±0.43 [a] |

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

*S.* Typhimurium and *E. coli* K-12 were inactivated more effectively in oats than flaxseed. It can be explained by shape of samples. Oats are in the shape of an oval, so SHS could reach more easily to the sample than flaxseeds which are in shape of sphere. These results suggest that SHS is effective to reduce the pathogens on the surface of cereal foods.

Flaxseed is a rich source of alpha linolenic acid (ALA). It is generally recognized that ALA in an isolated form or as a component in an oil exposed to air or high temperature is susceptible to autoxidation. According to previous study, after treated with 178℃ oven for 2 hrs, ALA content did not change (Chen et al., 1994). The composition of volatile compounds of raw oats is highly dependent on the parameters of heat treatment, especially when Maillard reactions take place and the flavor may be affected by pyrazines, pyrroles and furans (Sides et al., 2001). In this study, it is expected that ALA contents of flaxseeds and volatile compounds of oats have no significant changes due to short time treatment.

## 3.2. Inactivation of *E. coli* K-12 and *S.* Typhimurium in red pepper powder

Inoculation level of *E. coli* K-12 and *S.* Typhimurium in three different size of red pepper powder was $10^7$ CFU/g. For 300 μm red pepper

powder, after 12 s exposure of all temperature, both bacteria were decreased to below the detection limit. In all temperature, treated for 5 s, both bacteria were decreased to below the detection limit at 500 and 1000 μm red pepper powder (Figure 4). In the larger size of red pepper powder, pathogens reduced in shorter time. It is thought that because the condensed steam might evaporate easily in the larger size of sample.

After treated with SHS, color of red pepper powder was slightly changed (Table 3). However, there was no significant difference observable with the naked eye between before and after treatment. Preference for red pepper powder is mainly influenced by the color and flavor components. The most important ingredient of the flavor component of red pepper powder is capsaicin (Jung et al., 2007). Because the thermal stability of capsaicin is high, the residual ratio of capsaicin after treated with 100℃ hot air for 10 hours were 84.7% (Bae et al., 1991). It is expected that the capsaicin contents have no significant changes due to short time treatment in this study.

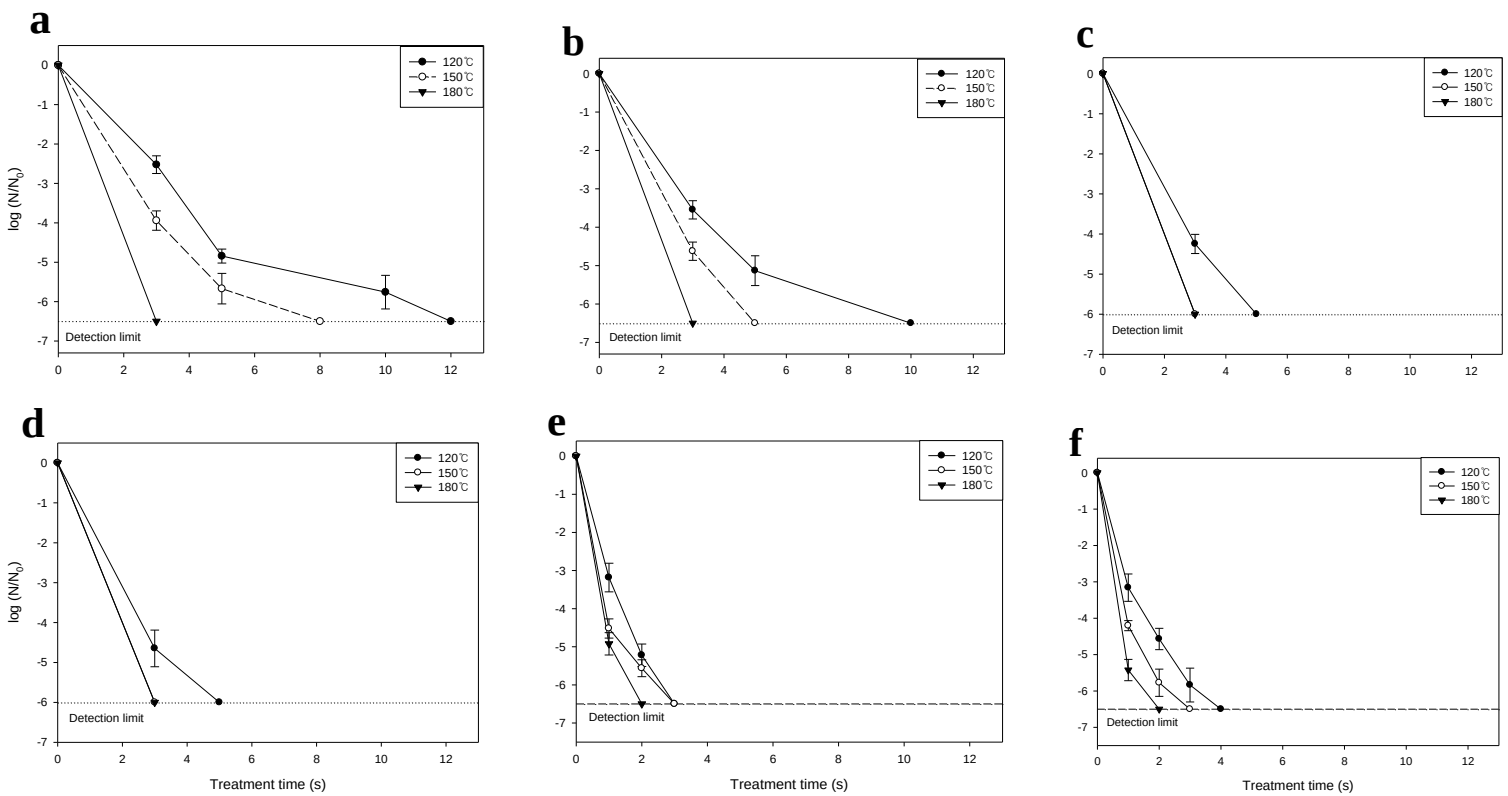


Figure 4. Survival curves of *S.* Typhimurium (a, c, e) and *E. coli* K-12 (b, d, f) in red pepper powder of 300 μm (a, b), 500 μm (c, d) and 1000 μm (e, f) after treated with 120, 150 and 180℃ SHS for 12 s.

Table 3. Color change of red pepper powders after SHS treatment until *S.* Typhimurium and *E. coli* K-12 reduced below the detection limit [1)]

| | | L* | a* | b* |
|---|---|---|---|---|
| **300 μm** | **Control** | 39.04 ± 0.56 [a] | 17.53 ± 0.57 [a] | 13.42 ± 0.54 [a] |
| | **120 ℃, 12 s** | 38.00 ± 0.21 [a] | 15.92 ± 0.65 [b] | 11.65 ± 0.39 [a] |
| | **150 ℃, 8 s** | 38.22 ± 0.35 [a] | 16.72 ± 0.45 [ab] | 12.78 ± 0.54 [a] |
| | **180 ℃, 3 s** | 38.21 ± 0.24 [a] | 16.32 ± 0.38 [ab] | 12.37 ± 0.53 [a] |
| **500 μm** | **Control** | 39.41 ± 0.47 [a] | 14.88 ± 0.42 [a] | 11.18 ± 0.42 [a] |
| | **120 ℃, 5 s** | 37.74 ± 0.51 [b] | 14.23 ± 0.68 [a] | 11.55 ± 0.38 [ab] |
| | **150 ℃, 3 s** | 38.26 ± 0.31 [ab] | 14.80 ± 0.54 [a] | 11.75 ± 0.34 [ab] |
| | **180 ℃, 3 s** | 38.76 ± 0.74 [ab] | 14.56 ± 0.67 [a] | 12.67 ± 0.48 [b] |
| **1000 μm** | **Control** | 44.15 ± 0.87 [a] | 13.29 ± 0.81 [a] | 17.11 ± 0.54 [a] |
| | **120 ℃, 3 s** | 43.21 ± 0.57 [a] | 11.23 ± 0.85 [a] | 14.98 ± 0.33 [b] |
| | **150 ℃, 3 s** | 44.53 ± 0.64 [a] | 12.45 ± 0.59 [a] | 15.43 ± 0.64 [ab] |
| | **180 ℃, 2 s** | 44.41 ± 0.51 [a] | 13.11 ± 0.77 [a] | 15.34 ± 0.97 [ab] |

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

## 3.3. Inactivation of total aerobic bacteria in red pepper powder

Contamination level of the sample before sterilization was 7.42 log CFU/g. After the exposure to superheated steam, total aerobic bacteria reduced about 4 log CFU/g. As the superheated steam temperature increased, total aerobic bacteria reduced faster (Figure 5). After the exposure to 120, 140, 160 and 180℃ SHS for 75 s, the reduction of viable bacteria was 0.75, 1.4, 2.6 and 4.06 log CFU/g, respectively. However, sample was tanned because of the long treatment time (Figure 6). To minimize the change in quality, experiments were repeated with new conditions.

When treated with 180℃ SHS, 1.1 log reduction was achieved in 3 s, after 15 s, total aerobic bacteria were reduced 2.33 log CFU/g. For 200℃, 1.86 log reduction was achieved in 3 s, after 15 s, 3.04 log reduction was achieved. However, 6 s later, sample was still tanned. Therefore, the optimum microbial reduction condition that could maintain the quality was 200℃ SHS treatment for 6 s. When treated with SHS during short time, condensed water did not dry, so the sample was agglomerated. Therefore, long time treatment with SHS was required for drying condensed water, or a grinding process was needed after short time treatment with SHS to disassembly the sample agglomeration.

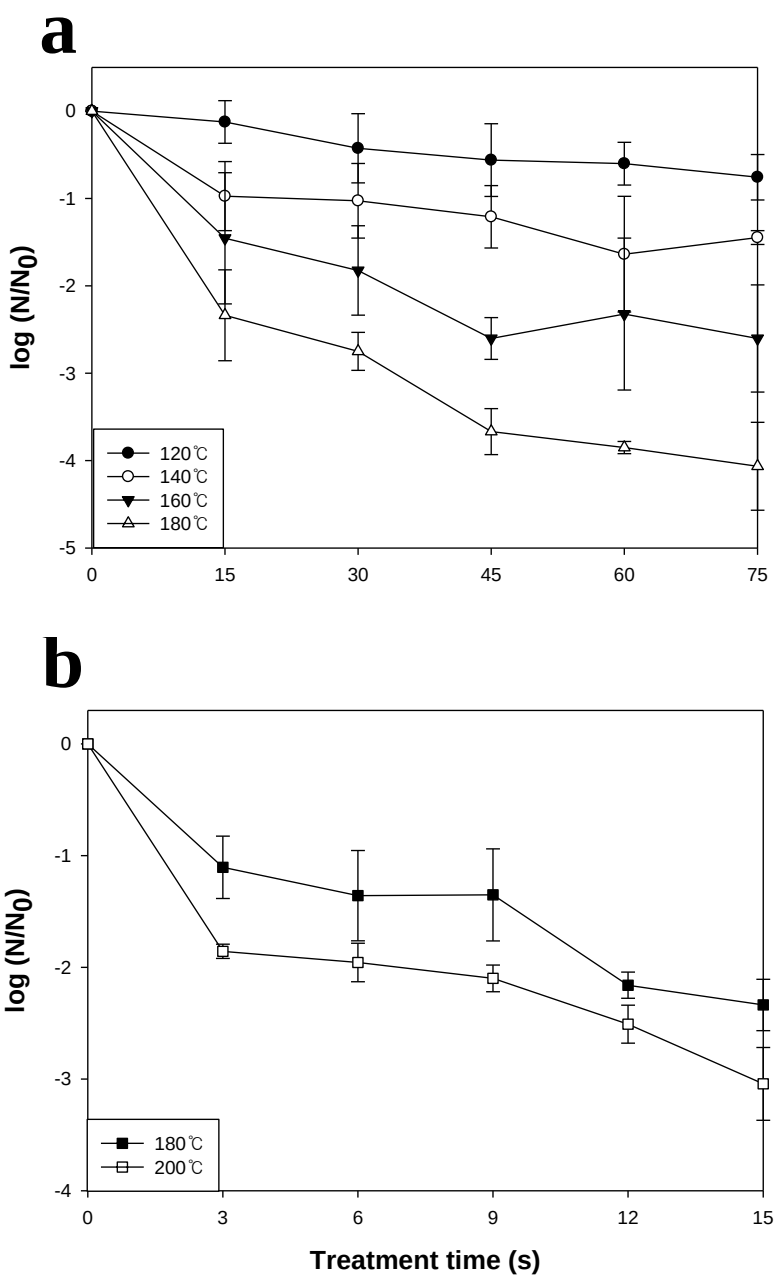


Figure 5. Survival curves of total aerobic bacteria in red pepper powder: (a) 120 (●), 140 (○), 160 (▼) and 180℃ (△) SHS treatment for 75 s, (b) 180 (■) and 200℃ (□) SHS treatment for 15 s.

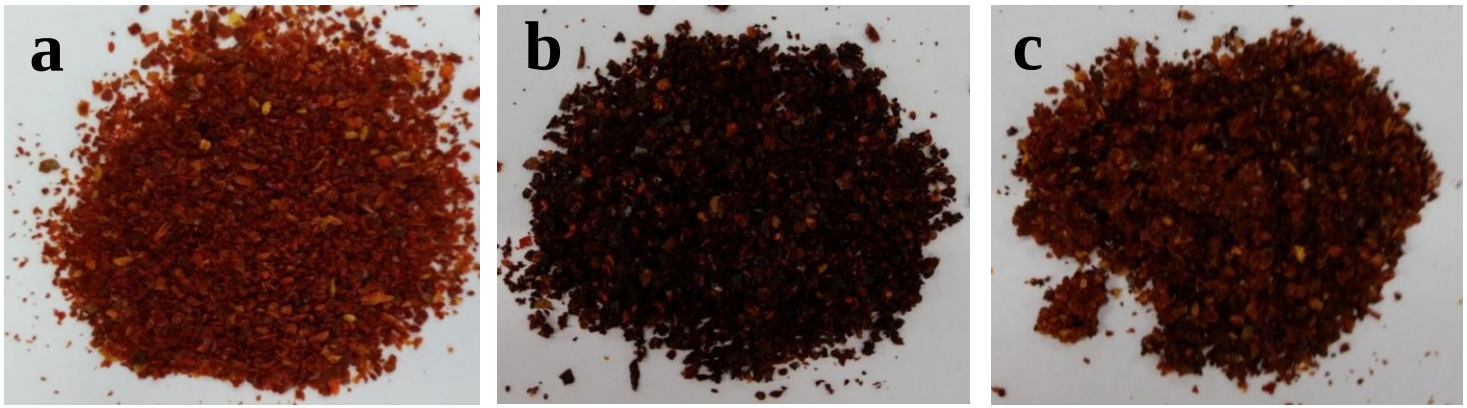


Figure 6. Appearance change of red pepper powder after SHS treatment: (a) non-treated, (b) 180℃ SHS treatment for 75 s, (c) 200℃ SHS treatment for 15 s.

SHS couldn’t reduce the total aerobic bacteria in red pepper powder effectively. It could be due to various causes. The microorganisms in a variety of crops formed a biofilm that has thermal resistance. The microorganisms in red pepper powder could be also generated a biofilm during the manufacturing process and storage so that sterilizing effect was inhibited (Han et al., 2000).

The spore is also an important factor. According to Choo (2007), *Bacillus cereus* was found in 84.3% of the dried red pepper samples, with an average concentration of $1.9\times10^4$ CFU/g. *Bacillus cereus* is a foodborne spore-forming bacterial pathogen that is ubiquitous in the natural environment. Infections with this pathogen manifest as diarrheal or emetic types of food poisoning (Valero et al., 2002). As a result of identification of bacteria contaminated in red pepper powder using VITEK 2 (bioMérieux, Marcy l'Etoile, France), contamination of spore-forming bacteria such as *Bacillus* spp. was confirmed (Table 4). Therefore, it was thought that total aerobic bacteria could be reduced through the reduction of *Bacillus* spores contaminated with red pepper.

Table 4. Identification of bacteria contaminated in red pepper

| **Type of Samples** | **Bacteria** |
|---|---|
| **Red pepper powder** | *Staphylococcus lentus, Bacillus vallismortis, Bacillus megaterium* |
| **Whole red pepper** | *Staphylococcus lentus, Bacillus vallismortis* |

## 3.4. Inactivation of *Bacillus cereus* spores

*Bacillus cereus* spores which have higher risk of safety than other *Bacillus* spp. were inoculated in the sterilized sand and treated with SHS to find out the reduction pattern of spores for the SHS. After the exposure to saturated steam, and 120, 140, 160 and 180˚C SHS for 5 min, the reduction of viable spores was 1.3, 2.3, 2.5, 2.5 and 2.7 log CFU/g, respectively (Figure 7). According to previous study (Van Opstal et al., 2004), when *Bacillus cereus* spore inoculated in 0.15% NaCl buffer solution was treated with 25kV/cm intensity of pulsed electric field, the reduction of spore was 0.4 log CFU/mL. Compared with this result, SHS could reduce *Bacillus* spores effectively.

As the temperature of SHS increased, and the treatment time get longer, spores were reduced more. In addition, the spore reduction of all samples became slow after certain times. It can be explained by the drying effect of the superheated steam. When the water activity is from 0.3 to 0.4, heat-resistance of *Bacillus* spores becomes high (Murrell et al., 1966). If the water activity is high or low, thermal resistance of spores becomes lower. Superheated steam treatment could bring the change of moisture content because of the condensation, evaporation and drying. Therefore, the moisture content was measured and compared with the spore reduction (Figure 8).

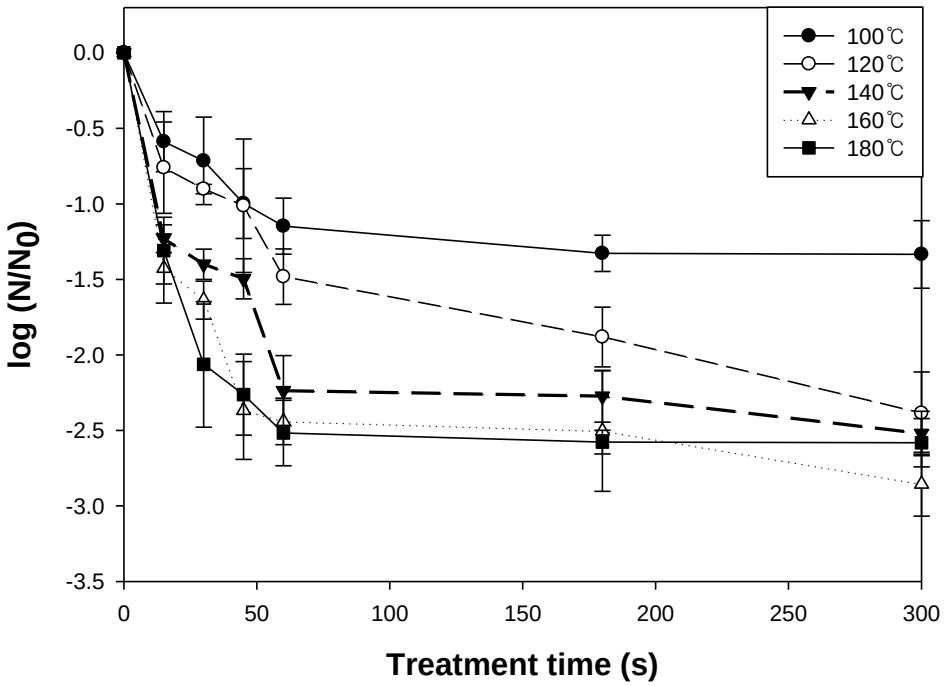


Figure 7. Survival curves of *Bacillus cereus* spores inoculated in sterilized sand after treated with 100 (●), 120 (○), 140 (▼), 160 (△), 180℃ (■) SHS for 5 min.

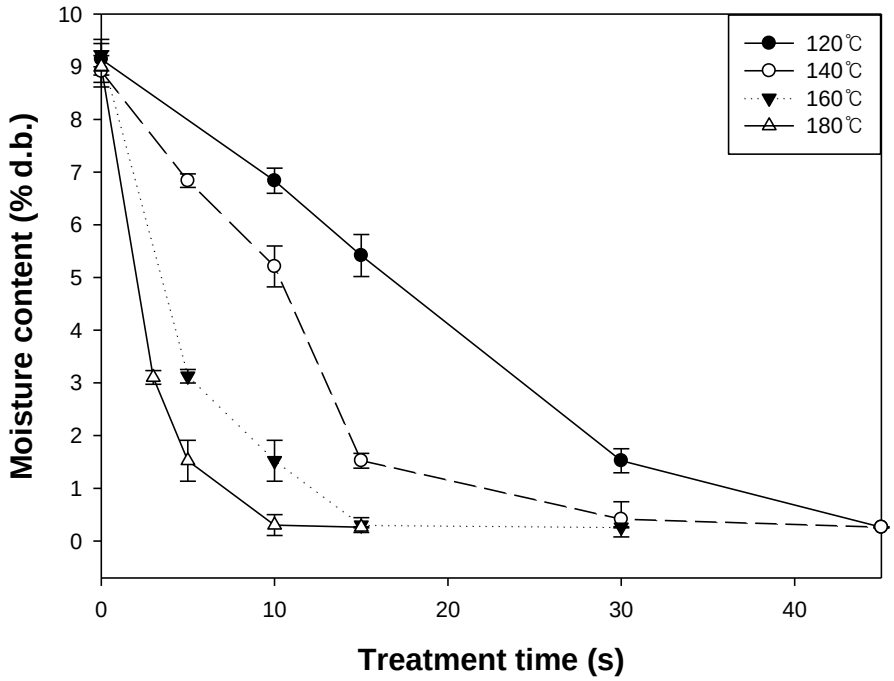


Figure 8. Moisture content change of spore-sand mixture after treated with 120 (●), 140 (○), 160 (▼) and 180℃ (△) SHS for 45 s.

The moisture content of sand was 9.14 ± 0.31% before the SHS treated, the higher temperature of SHS can desiccate easily the moisture. After the exposure to 120, 140, 160 and 180˚C SHS, moisture was dried in 45, 30, 15 and 15 s, respectively. It was a similar pattern with time that the slope of survival curve changes. In order to analyze the survival kinetics of *Bacillus cereus* spore after SHS treatment, a biphase model was used (Figure 9). Fitting equation was as follows:

$$\log\frac{\mathrm{N}}{\mathrm{N}_0}(y) = \begin{cases} -\frac{t}{D_1} & 0 < t < t_c \\ -\frac{t}{D_2} + \frac{t_1 y_1 - t_c y_2}{t_1 - t_c} & t_c < t < 300 \end{cases} \quad (1)$$

$D_1$ is the $D$-value of initial phase, $D_2$ is the $D$-value of second phase, $t_c$ is the time that slope changes, $t_1$ is the final treatment time (300 s), $y_1$ is the reduction level at $t_1$, and $y_2$ is the reduction level at $t_c$. By fitting the curve, $D_1$, $D_2$ and $t_c$ of *Bacillus cereus* spores of each temperature was calculated (Table 5).

The inhibitory mechanism of *Bacillus subtilis* spore by the moist heat and the dry heat is known to be different (Setlow, 2006). Spore killing by wet heat is often accompanied by inactivation of core enzymes and rupture of the spore's inner membrane permeability barrier (Warth, 1980). In contrast to spore killing by wet heat, killing of wildtype spores by dry heat is

accompanied by accumulation of DNA damage. The precise mechanism of this damage has not been determined but this damage is mutagenic and gives a mutation spectrum slightly different than that of wet heat treatment of spores (del Huesca-Espita et al. 2002).

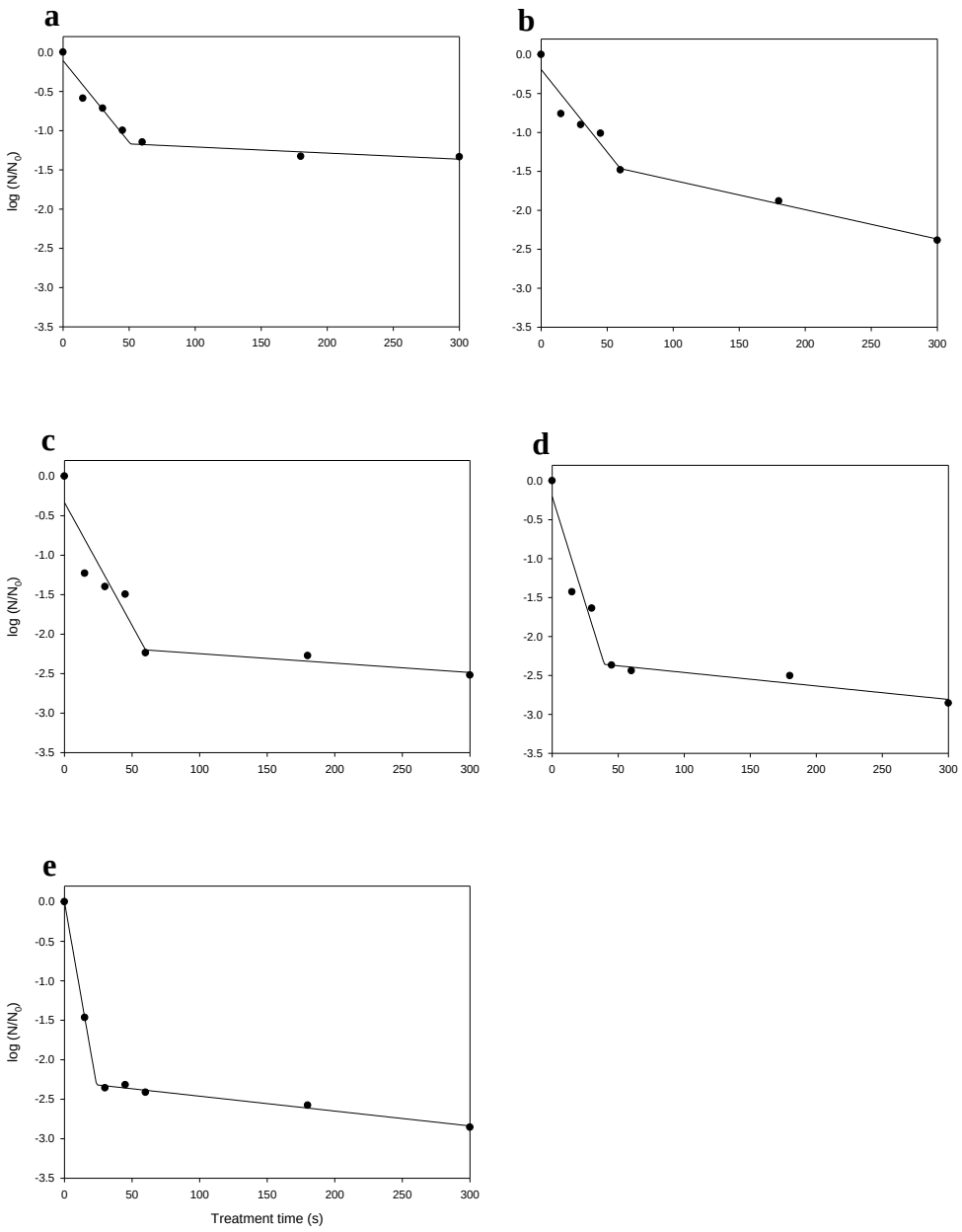


Figure 9. Survival curves of *Bacillus cereus* spores fitted with biphase model at (a) 100℃, (b) 120℃, (c) 140℃, (d) 160℃ and (e) 180℃.

Table 5. *D*-values of *Bacillus cereus* spores for saturated steam and superheated steam

| **Temp (°C)** | $D_1$ **(s)** [1)] | $D_2$ **(s)** [1)] | $t_c$ **(s)** [2)] | $R^2$ |
|---|---|---|---|---|
| **100** | 48.08 | 1281.24 | 51.06 | 0.983 |
| **120** | 47.17 | 265.40 | 60.00 | 0.983 |
| **140** | 32.12 | 839.16 | 60.00 | 0.957 |
| **160** | 18.32 | 1733.54 | 40.84 | 0.976 |
| **180** | 10.23 | 530.92 | 23.71 | 0.999 |

[1)] $D_1$: *D*-value for initial stage, $D_2$: *D*-value for second stage

[2)] $t_c$: the time that inactivation rate was changed

Superheated steam is initially formed condensed water on the surface of food and the latent heat is transferred to the surface so that the microorganisms could be killed. After all water dried, the dry heat sterilization occurs because microorganisms are killed by the sensible heat of superheated steam. Since superheated steam has both effect of moist heat sterilization and dry heat sterilization, it can inhibit *Bacillus cereus* spores with two mechanisms so two slopes were shown in survival curve. Compared with *D*-values of initial phase at different temperature, temperature of SHS get higher, $D_1$ and $t_c$ tended to be shorter. However, to achieve 2.5 log CFU/g reduction, high temperature and a long time more than 5 minutes were required. However, it is expected to adversely affect food quality if this condition is actually applied to foods. Therefore, these results suggest that pretreatment with SHS could reduce total sterilization time by lowering the initial contamination of a raw material.

## 3.5. Effect of germination of *Bacillus* spores in red pepper powder

Although spores have heat-resistance, once they germinate to vegetative cells, it could be inactivated easily by thermal sterilization. *Bacillus* spores can germinate by mild heat and germinants (Yi et al., 2010). Spores in nature germinate in response to nutrients, termed germinants.

These germinants are generally single amino acids or sugars (Setlow, 2003). Mild heating and L-alanine as a germinant were used for germination of spores. Therefore, in this study, *Bacillus* spores contaminated in red pepper powder were germinated and then treated with SHS.

The contamination level of *Bacillus* spp. in red pepper powder was about 5 log CFU/g. When germinated by mild heating, the population of *Bacillus* spp. was increased about 0.7 log CFU/g and 1.3 log CFU/g by alanine germinant buffer (Figure 10a).

When treated with 140℃ SHS for 30 s, 1 log CFU/g of *Bacillus* spp. in the original sample were reduced. However, after heat germination and alanine germination, *Bacillus* spp. reduced 1.8 log CFU/g and 2.4 log CFU/g, respectively. This difference was consistent with the amount of germinated *Bacillus* spores, and it shown that a similar degree of reduction in the total bacteria. Germinant buffer could induce the germination of spore more effectively than mild heating (Figure 10b, c).

However, the amount of germinant buffer used in this study induced aggregation of red pepper powder. Therefore, using the drying process, which is characteristic of superheated steam, further study about combination SHS with spore germination will be required.

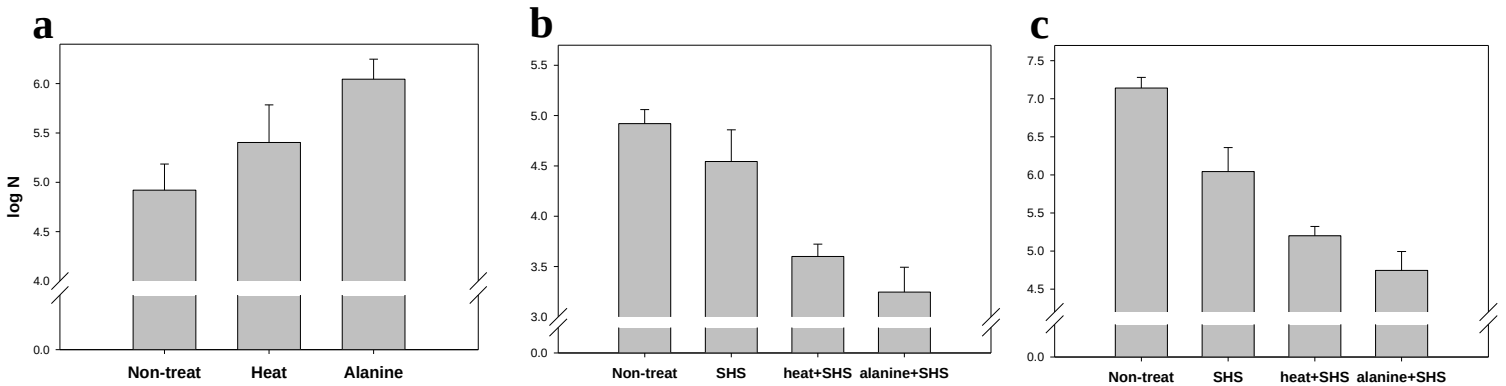


Figure 10. Germination of *Bacillus* spores in red pepper powder by mild heat treatment and alanine germinant buffer (a), and the reduction of *Bacillus* spores in red pepper powder germinated by different methods were cultivated on MYP agar (b) and TSA agar (c) after treated with 140℃ SHS for 30 s.

# IV. CONCLUSIONS

Superheated steam could successfully inactivate *Salmonella* Typhimurium and *E. coli* K-12 on the surface of oats, flaxseeds and red pepper powder without quality deterioration mainly due to the short time treatment. For all samples used in this study, the reduction time was faster with higher SHS temperature. 150℃ SHS could reduce 6 log CFU/g of both pathogens within 10 s.

For total aerobic bacteria population in red pepper powder, however, 3 log CFU/g reduction was achieved after treated with 180℃ SHS for 75 s. After that treatment, sample was burnt seriously. Therefore, the cause of difficulty to inactivate the total aerobic bacteria was investigated and it was thought that *Bacillus cereus* spore might be the obstacle of sterilization. After treated with SHS, *Bacillus cereus* spore reduced 2.7 log CFU/g and survival curve was fitted with biphase model. *D*-values for 100, 120, 140, 160 and 180℃ SHS in initial stage were calculated as 48.1, 47.2, 21.4, 10.5 and 10.2 s, respectively.

As the result of SHS treatment after germination of spores by mild heating and germinant buffer, total aerobic bacteria reduced 1.8 log CFU/g and 2.4 log CFU/g, respectively more than non-treated sample. Further study about combination SHS with spore germination will be required.

# V. REFERENCES


1. Won, H. S., Lee, H. J., Kwak, J. S., Kim, J., Kim, M. K., & Kwon, O. (2012). Study on purchase and intake patterns of individuals consuming dietary formula for weight control or health/functional foods. *Korean Journal of Nutrition*, 45, 541-551.
2. Davies, R. H., & Wales, A. D. (2013). *Salmonella* contamination of cereal ingredients for animal feeds. *Veterinary Microbiology*, 166, 543-549.
3. Head, D. S., Cenkowski, S., Arntfield, S., & Henderson, K. (2010). Superheated steam processing of oat groats. *LWT-Food Science and Technology*, 43, 690-694.
4. Perkowski, J., & Basiński, T. (2002). Natural contamination of oat with group A trichothecene mycotoxins in Poland. *Food Additives & Contaminants*, 19, 478-482..
5. Cenkowski, S., Ames, N., & Muir, W. E. (2006). Infrared processing of oat groats in a laboratory-scale electric micronizer. *Canadian Biosystems Engineering*, 48, 3.
6. Lehtinen, P., Kiiliäinen, K., Lehtomäki, I., & Laakso, S. (2003). Effect of heat treatment on lipid stability in processed oats. *Journal of Cereal Science*, 37, 215-221.

7. Cunnane, S. C., Hamadeh, M. J., Liede, A. C., Thompson, L. U., Wolever, T. M., & Jenkins, D. J. (1995). Nutritional attributes of traditional flaxseed in healthy young adults. *The American Journal of Clinical Nutrition*, 61, 62-68.
8. Accorsini, D. (2011). Ancient Grains, Ancient Seeds.
9. Chen, Z. Y., Ratnayake, W. M. N., & Cunnane, S. C. (1994). Oxidative stability of flaxseed lipids during baking. *Journal of the American Oil Chemists' Society*, 71, 629-632.
10. Sides, A., Robards, K., Helliwell, S., & An, M. (2001). Changes in the volatile profile of oats induced by processing. *Journal of Agricultural and Food Chemistry*, 49, 2125-2130.
11. Jung, K., Song, B. S., Kim, M. J., Moon, B. G., Go, S. M., Kim, J. K., & Park, J. H. (2015). Effect of X-ray, gamma ray, and electron beam irradiation on the hygienic and physicochemical qualities of red pepper powder. *LWT-Food Science and Technology*.
12. Woo, H. I., Kim, J. B., Choi, J. H., Kim, E. H., Kim, D. S., Park, K. S., & Om, A. S. (2012). Evaluation of the level of microbial contamination in the manufacturing and processing company of red pepper powder. *Journal of Food Hygiene and Safety*, 27, 427-431.
13. Oh, S. W., Koo, M., & Kim, H. J. (2012). Contamination patterns and molecular typing of Bacillus cereus in red pepper powder

processing. *Journal of the Korean Society for Applied Biological Chemistry*, 55, 127-131.

14. Lee, J. H., Sung, T. H., Lee, K. T., & Kim, M. R. (2004). Effect of gamma-irradiation on color, pungency, and volatiles of Korean red pepper powder. *Journal of Food Science*, 69, C585-C592.
15. Sagar, V. R., & Kumar, P. S. (2010). Recent advances in drying and dehydration of fruits and vegetables: a review. *Journal of Food Science and Technology*, 47, 15-26.
16. Cenkowski, S., Pronyk, C., Zmidzinska, D., & Muir, W. E. (2007). Decontamination of food products with superheated steam. *Journal of Food Engineering*, *83*(1), 68-75.
17. James, C., Göksoy, E. O., Corry, J. E. L., & James, S. J. (2000). Surface pasteurisation of poultry meat using steam at atmospheric pressure. *Journal of Food Engineering*, 45, 111-117.
18. Powell, J. F., & Hunter, J. R. (1955). Spore germination in the genus Bacillus: the modification of germination requirements as a result of preheating. *Journal of General Microbiology*, 13, 59-67.
19. Powell, J. F. (1950). Factors affecting the germination of thick suspensions of *Bacillus subtilis* spores in L-alanine solution. *Journal of General Microbiology*, 4, 330-338.
20. Jung, J. K., Lee, S. J., Park, S. W., Kim, W. G., & Moon, S. I. (2007).

Characteristics of taste on red pepper powder products in Daegu and Gyeongbuk area. *Government Public Institute of Health & Environment*.

21. Bae, T. J., Choi, O. S., Bahk, J. R., Kim, M. N., & Han, B. H. (1991). Studies on oleoresin product from spices - 2. Quality stability of red pepper oleoresin. *Journal of the Korean society of Food Science and Nutrition*, 20, 609-614.
22. Han, Y., Linton, R. H., Nielsen, S. S., & Nelson, P. E. (2000). Inactivation of *Escherichia coli* O157: H7 on surface-uninjured and-injured green pepper (*Capsicum annuum* L.) by chlorine dioxide gas as demonstrated by confocal laser scanning microscopy. *Food Microbiology*, 17, 643-655.
23. Choo, E., Jang, S. S., Kim, K., Lee, K. G., Heu, S., & Ryu, S. (2007). Prevalence and genetic diversity of *Bacillus cereus* in dried red pepper in Korea. *Journal of Food Protection*, 70, 917-922.
24. Valero, M., Hernández-Herrero, L. A., Fernández, P. S., & Salmerón, M. C. (2002). Characterization of *Bacillus cereus* isolates from fresh vegetables and refrigerated minimally processed foods by biochemical and physiological tests. *Food Microbiology*, 19, 491-499.
25. Van Opstal, I., Bagamboula, C. F., Vanmuysen, S. C., Wuytack, E. Y., & Michiels, C. W. (2004). Inactivation of *Bacillus cereus* spores in milk by mild pressure and heat treatments. *International Journal of Food*

*Microbiology*, 92, 227-234.

26. Murrell, W. G., & Scott, W. J. (1966). The heat resistance of bacterial spores at various water activities. *Journal of General Microbiology*, 43, 411-425.
27. Setlow, P. (2006). Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *Journal of Applied Microbiology*, 101, 514-525.
28. Warth, A. D. (1980). Heat stability of *Bacillus cereus* enzymes within spores and in extracts. *Journal of Bacteriology*, 143, 27-34.
29. Espitia, L. D. C. H., Caley, C., Bagyan, I., & Setlow, P. (2002). Base-change mutations induced by various treatments of *Bacillus subtilis* spores with and without DNA protective small, acid-soluble spore proteins. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 503, 77-84.
30. Yi, X., & Setlow, P. (2010). Studies of the commitment step in the germination of spores of *Bacillus* species. *Journal of Bacteriology*, *192*(13), 3424-3433.
31. Setlow, P. (2003). Spore germination. *Current Opinion in Microbiology*, *6*(6), 550-556.

# VI. 국문초록

최근 건강 지향성 및 편의성을 중시한 식생활 패턴의 변화로, 분체 형태로 가공된 곡류 및 과채류의 수요가 늘고 있다. 하지만 분체 식품은 보관이나 유통 과정을 소홀히 하여 부패를 초래하는 경우가 많다. 이러한 분체 식품을 살균하기 위해 에틸렌옥사이드나 감마선 조사 등의 비가열 살균이 이용되지만, 소비자의 인식이나 잠재적 위해성으로 인하여 사용이 제한되었다. 과열 수증기는 열전도율이 높고, 초기 응축 시 전달되는 응축 잠열로 인하여 표면의 순간 살균에 이용되고 있다. 본 연구에서는 최근 *Salmonella* 오염으로 회수 조치를 받은 귀리, 아마씨, 고춧가루를 시료로 선정하고, 과열 수증기를 이용하여 시료에 오염된 위해균을 저감해보고자 하였다. 각 시료에 *Salmonella* Typhimurium 과 *E. coli* K-12 를 $10^7$ CFU/g 접종하여 과열 수증기를 처리하였다. 그 결과, 모든 시료에서 150℃ 의 과열수증기를 처리하였을 때, 10 초 이내에 품질 변화 없이 두 위해균이 검출 한계 이하로 저감되었다. 고춧가루의 총호기성균을 저감화하기 위해 180℃ 의 과열 수증기를 처리한 결과, 75 초 처리 시 3 log CFU/g 저감화되었다. 하지만 이러한 조건에서는 높은 온도로 인하여 시료가 탄화되는

현상이 나타났다. 고춧가루에 포자를 형성하는 *Bacillus* 의 오염도가 높기 때문에, *Bacillus* 속의 포자의 저감을 통해 고춧가루의 총호기성균을 저감해보고자 하였다. 멸균된 모래에 *Bacillus cereus* 의 포자를 $10^5$ CFU/g 접종하여 과열 수증기를 처리하였다. 그 결과, 180℃ 의 과열 수증기를 5 분 처리 시 약 3 log CFU/g 저감화되었다. 모든 조건에서 포자의 저감 속도는 특정 시간 후에 느려졌는데, 이는 과열 수증기의 건조 효과에 기인한 것으로 판단된다. 더 효과적으로 포자를 제어하기 위해, 이미 알려져 있는 발아 방법으로 고춧가루에 오염되어 있는 포자를 영양세포로 전환시킨 후 과열 수증기를 처리해보았다. 그 결과, 발아를 유도하기 전 보다 열로 발아시킬 경우 약 1.9 log CFU/g, 발아유도 용액으로 발아시킬 경우 약 2.4 log CFU/g 더 저감화되는 것을 알 수 있었다. 이러한 결과들을 통해 교차 오염의 가능성이 있는 표면 위해균을 저감화하는 데에 과열 수증기가 효과적으로 적용될 수 있음을 확인하였다. 또한 식품 제조 시 과열 수증기를 전처리 공정으로 이용하여 위해균의 포자 등 원료의 초기 오염을 감소시킴으로써 총 살균 시간을 줄일 수 있음을 시사한다. 포자를

발아시킨 후 과열 수증기를 처리하여 저감하는 방법은 추가 연구가 더 필요할 것으로 사료된다.

주요어: 분립체 식품, 과열 수증기(superheated steam), 귀리, 아마씨, 고춧가루, *Bacillus cereus* spore.