

# **Synergistic Effects of Mild Heating and Dielectric Barrier Discharge Plasma on Reduction of *Bacillus cereus* in Red Pepper Powder**

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**Abstract:** The synergistic efficacy of combined treatment mild heat (MH) and dielectric barrier discharge (DBD) plasma in *Bacillus cereus*-contaminated red pepper powder was tested. A cocktail of three strains of *B. cereus* (NCCP 10623, NCCP 14579, ATCC 11778) was inoculated onto red pepper powder and then treated with MH (60 °C for 5-20 min) and DBD plasma (5-20 min). Treatment with MH and DBD plasma alone for 5~20 min resulted in reductions of 0.23~1.43 and 0.12~0.96 log CFU/g, respectively. Combined treatment with MH and DBD plasma was the most effective at reducing *B. cereus* counts on red pepper powder and resulted in log-reductions of  $\geq 6.0$  log CFU/g. The largest synergistic values (4.24-4.42 log) against *B. cereus* in red pepper powder were obtained by the combination of 20 min MH and 5~15 min DBD plasma. Hunter color “L”, “a”, and “b” values of the combination-treated samples were not significantly different from those of non-treated samples. Also, no significant ( $p > 0.05$ ) differences in pH values between samples were observed. Therefore, these results suggest that the combination of MH treatment and DBD plasma can be potentially utilized in the food industry to effectively inactivate *B. cereus* without incurring quality deterioration of red pepper powder.

**Keywords:** *Bacillus cereus*; mild heating; dielectric barrier discharge plasma; red pepper powder; quality

## 1. Introduction

Red pepper powder is an essential dietary spice in Korea and the principle ingredient and determinant of the color, taste and quality of traditional fermented *kochujang* (fermented red pepper paste). This spice is also commonly used as a cooking sauce for processed foods (e.g., Korean ramen noodles, traditional Korean soups and stews) [1]. Because the cultivation period of red peppers occurs during hot and humid seasons, they are likely to be contaminated by pests or microorganisms. Moreover, there is a high likelihood of microbial cross contamination during red pepper powder processing, the extent of which depends on the sanitary conditions of the operator, manufacturing facility and surrounding environment. Harvesting red peppers and drying them in a conventional manner (i.e., with a dryer, using sunlight) includes no sterilization process, leaving them exposed to potential contamination from many microorganisms [2]. The use of contaminated red pepper powder in a food can lead to rapid spoilage. In a study by Chun et al. (2009) [3], the contamination level in red pepper powder was 6.72 and 6.57 log CFU/g for aerobic bacteria and *Bacillus cereus*, respectively.

Plasma is a quasi-neutral ionized gas state composed of ions, free electrons, atoms and molecules in their fundamental or excited states with a net neutral charge [4]. Plasma technology is commonly used in biomedical, semiconductor manufacturing, and displays (e.g. fluorescent, televisions, monitors, lighting and equipment) [5]. Plasma can be artificially generated by subjecting a neutral gas to a wide range of temperatures and pressures, therefore it is classified into thermal and non-thermal plasma (NTP). NPT, also named cold plasma is a promising new food sterilization technology and is proven to be effective against pathogenic microorganisms with relatively little impact on the nutritional value of a treated food. Dielectric barrier discharge (DBD) plasma is used as an NTP and can be generated between two electrodes that are covered [6]. DBD plasma is a type of discharge suitable for food processing because it can be applied to a large area [7]. Kim et al. (2014) [8] reported that microwave-powered cold plasma at 900W and 667 Pa for 20 min inhibited naturally occurring total aerobic bacteria in red pepper powder by approximately 1 log CFU/g.

Pasteurization, a relatively mild heat (MH) treatment in which food is heated to <100 °C, is commonly used in the food industry. It is also frequently applied as a critical control point in the hazard analysis critical control point (HACCP) system. Hurdle technology (eg., combination of non-thermal and thermal processing techniques) can control the growth of spoilage and pathogenic microorganisms in foods, thus extending the shelf-life of foods [9]. UV-C irradiation/hot air heating or cold plasma treatment/hot water immersion have been shown to specifically inactivated foodborne pathogens in red pepper powders [8, 10]

To date, a few studies have researched combinations of non-thermal and thermal treatments on reducing foodborne pathogens in pepper powders. Cheon et al. (2015) [10] reported that the combination of ultraviolet radiation with MH treatment was more effective than UV-C irradiation alone for inactivation of *Escherichia coli* and *Salmonella*. Moreover, this combined approach does not cause quality deterioration of powdered red pepper. Choi et al. (2018) [11] reported that microbiological analysis of powders inoculated with *E. coli* O157:H7 and *Staphylococcus aureus* following radio frequency thermal treatment and plasma treatment combination resulted in 2 log CFU/g reductions in the microbial counts proportional with increasing treatment cycles.

There is a need to examine the potential synergistic effects of combined treatment with MH and DBD plasma for the decontamination of red pepper powder. The present study was therefore undertaken to determine the synergistic effects of combined treatment with MH (60 °C for 5-20 min) and DBD plasma (5-20 min) on the reduction of *B. cereus* in red pepper powder.

## 2. Materials and Methods

### 2.1. Bacterial strain

Three strains of *B. cereus* (NCCP 10623, NCCP 14579, ATCC 11778) were tested. A stock culture ( $10^8$  CFU/mL) was maintained at  $-80^{\circ}\text{C}$  in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI) containing 30% glycerol. Bacteria were cultured on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI) plates at  $37^{\circ}\text{C}$  for 24 h. Afterwards, a single colony was inoculated into TSB and grown at  $37^{\circ}\text{C}$  for 12 h with shaking at  $150 \times g$ .

### 2.2. Culture preparation

Each strain was cultured in 5 mL of TSB at  $37^{\circ}\text{C}$  for 24 h, harvested by centrifugation at  $5400 \times g$  for 10 min at  $4^{\circ}\text{C}$  and washed three times with 0.85% sterile saline water. The final pellets were resuspended in 10 mL of 0.85% sterile saline water, corresponding to approximately  $10^7$ – $10^8$  CFU/mL. Suspended pellets from each of the three *B. cereus* strains were combined into a mixed culture cocktail at a final concentration of approximately  $10^7$  CFU/mL for use in this study.

### 2.3. Inoculation and sample preparation

Commercially available dried red pepper powders were purchased at a traditional market (Tongyeong, Korea). Red pepper powder samples were autoclaved for 15 min at  $121^{\circ}\text{C}$  before inoculation to remove preexisting microorganisms. For inoculation, 1 mL of culture cocktail was added to 25 g of samples in 500 mL glass beakers, and then mixed by sterilized stainless steel spoon for 5 min to ensure uniform distribution. After mixing, samples were dried for 1 h inside a clean bench ( $25 \pm 1^{\circ}\text{C}$ ) with the fan running. The concentration of final samples was  $10^6$ – $10^7$  CFU/g.

### 2.4. MH treatment and DBD plasma treatment

To pasteurize using MH treatment, the sterilized petri dish containing 25 g of red pepper powder was sealed with parafilm (Heathrow Scientific, Vernon Hills, IL, USA) to prevent water vapors from going into the sample. Samples were treated at  $60^{\circ}\text{C}$  in a water bath for 5, 10, 15, and 20 min, and then immediately cooled in an ice water bath for 5 min to stop further thermal inactivation.

The DBD plasma device ( $\mu$ -DBD Surface Plasma Generator, Model; Micro DBD plasma) was supplied by

Plasma Biomedicine Institute (Plasma Bioscience Research Center, Seoul, Korea) and was described by Ryu et al. (2013) [7]. The silver electrode which served as a high voltage electrode was screen printed (thickness of 10  $\mu\text{m}$ ) on glass (thickness of 1.8 mm); the dielectric material (consisting of  $\text{SiO}_2$ ) was also screen printed to 100  $\mu\text{m}$  in polylactic acid. A metal mesh grid was attached on the rear side of the glass and used as a grounded electrode. Gas flow can be guided to the mesh surface by polylactic acid cover via gas injection hole. DBD plasma was generated on the rear glass surface between the glass and metal mesh grid using a nitrogen flow rate of 1.5 liters per minute. The DBD plasma under a driving frequency 43 kHz shows voltage and current characteristics with low discharge voltage of approximately 1 kV and discharge peak current 40 mA, respectively. The minimum discharge voltage for plasma production by DBD plasma devices used in this experiment was 1.1 kV. The optical emission profile was measured 3 mm from the mesh surface with a 10 s integration time using a spectrometer (HR4000, Ocean optics). The device was turned on at least 10 min before the start of the experiment, and the surface of the red pepper powder previously inoculated with *B. cereus* was treated with DBD plasma for 5, 10, 15, and 20 min in a sterile petri dish (35 x 15 mm). A distance of 3 mm was maintained between the plasma-emitting electrode and the sample during treatment.

### 2.5. Determining synergistic reduction effects

To estimate any synergistic effect on bacterial inactivation, the inactivation values of combined MH and DBD plasma were compared with those of MH or DBD plasma alone. The disinfection efficacy after different treatments was determined by measuring microbial reduction. The synergistic reduction effect values of combined MH and DBD plasma were calculated using the following equation:

$$\text{Value of synergistic reduction} = A - (B + C)$$

Where A was the reduction from combined mild heating and DBD plasma disinfection, B was the reduction from MH treatment disinfection alone, and C was the reduction from DBD plasma disinfection alone. On the basis of this equation, synergistic and antagonistic reduction effects are indicated as plus values and minus values, respectively.

### 2.6. Microbial enumeration

For enumeration of *B. cereus*, a treated red pepper powder sample was transferred into a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 mL of 0.85% sterile saline water and then homogenized in a stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min. After homogenization,

sample aliquots were serially tenfold diluted in 9 mL blanks of 0.85% sterile saline water, and 0.1 mL of diluted samples were duplicate spread-plated onto selective media. TSA was used as a media for the enumeration of *B. cereus*. All plates were incubated at 37 °C for 48 h before counting.

## 2.7. Quality measurement

In order to characterize any potential changes in the quality of red pepper powder after combined treatment with MH and DBD, color and pH assessments were made. All treated samples were stored in a clean bench for 1 h to allow for equilibration to room temperature. Subsamples of powdered red peppers were selected from three random locations.

### 2.7.1. Hunter color

After treatment with MH and DBD plasma the colors of red pepper powders were measured using a color difference meter (UltraScan PRO, Hunterlab, USA) which was calibrated with the original value from a standard plate ('L'=98.48, 'a'=0.14, and 'b'=0.41). 'L' (brightness+, darkness–), 'a' (redness+, greenness–), and 'b' (yellowness+, blueness–) values were measured and the mean of three measurements was recorded for each sample.

### 2.7.2. pH values

To measure pH, 100 mL of distilled water was added to 1 g of red pepper powder, stirred at room temperature for 5 min, and filtered using Whatman paper (Whatman inc., Piscataway, New jersey, USA). The supernatant was measured three times using a pH meter (A211, Thermo Orion, Benchtop, MI, USA).

## 2.8. Statistical analysis

Data are presented as the mean of three determinations  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was performed using the SPSS software system. Bacteria enumeration presented as log CFU/g reductions (treated vs pretreated), color, and pH values were analyzed with the Duncan's multiple range test to identify any potential differences among mean values. Statistical significance was tested at 5% probability level ( $p < 0.05$ ).

### 3. Results

#### 3.1. Synergistic reductions of *Bacillus cereus* in red pepper powder were achieved by combined treatment with MH treatment and DBD plasma

To characterize the combined treatment effect of MH and DBD plasma on *B. cereus*-contaminated red pepper powder, reductions in *B. cereus* counts was determined (Table 1). After 5, 10, 15, and 20 min of treatment with MH alone, *B. cereus* counts were significantly ( $p < 0.05$ ) reduced—0.23, 0.93, 1.16 and 1.43 log CFU/g, respectively. *B. cereus* counts were also significantly ( $p < 0.05$ ) reduced by 0.12, 0.28, 0.61, and 0.96 log CFU/g after treatment with DBD plasma alone for 5, 10, 15, and 20 min, respectively. Treatment with DBD plasma resulted in a decrease of 0.96 log CFU/g after the 20 min maximum treatment, while MH treatment for 10 min resulted in a decrease of 0.93 log CFU/g. These results indicate that *B. cereus* reduction was primarily dependent on MH treatment rather than DBD plasma.

After combined treatment with MH and DBD plasma, *B. cereus* counts in contaminated red pepper powder were reduced by 0.33–6.43 log CFU/g. This combined treatment led to decreases of more than 1 log CFU/g in most combinations except for 5-min MH + 5-min DBD plasma (0.33 log CFU/g), and 5-min MH + 10-min DBD plasma (0.42 log CFU/g). As the MH treatment time and DBD plasma treatment time increased, the reduction effect was greater. Among these combination treatments, 5-min MH + 15-min DBD plasma, 5-min MH + 20-min DBD plasma, 10-min MH + 5-min DBD plasma, 10-min MH + 10-min DBD plasma, 15-min MH + 5-min DBD plasma, and 15-min MH + 10-min DBD plasma resulted in a > 1–2-log reduction of *B. cereus* in contaminated red pepper powder. Also, 10-min MH + 15-min DBD plasma, 10-min MH + 20-min DBD plasma, 15-min MH + 15-min DBD plasma, 15-min MH + 20-min DBD plasma, and 20-min MH + 5-min DBD plasma resulted in a > 3-log reduction. 20-min MH + 10-min DBD plasma, 20-min MH + 15-min DBD plasma, and 20-min MH + 20-min DBD plasma produced > 6-log reductions. The maximum reduction of *B. cereus* was 6.43 log CFU/g after treatment with a combination of 20-min MH and 20-min DBD plasma. The results of these experiments demonstrate that combined treatment with MH and DBD plasma led to a greater reduction in *B. cereus* compared with each individual treatment.

Table 2 reveals the synergistic effects of MH treatment combined with DBD plasma on the reduction of *B. cereus* in contaminated red pepper powder. Among them, synergistic effects were observed for most combination treatments except for 5-min MH + 5-min, 5-min MH + 10-min DBD plasma, 10-min MH + 5-min DBD plasma,

and 10-min MH + 10-min DBD plasma. Unfortunately, antagonistic effects did appear in certain combinations, including 5-min MH + 5-min DBD plasma (−0.22 reduction) and 5-min MH + 10-DBD plasma (−0.28 reduction). All synergistic reduction values were above 1 log CFU/g. Additionally maximum synergistic reduction values of greater than 4 log CFU/g (99.99%) were achieved when samples were treated with MH for 20-min and DBD plasma for 5-min, 10-min or 15-min. These results suggest that the synergistic effects against *B. cereus* were dependent on both MH and DBD plasma treatment times.

### 3.2. Hunter color and pH value on red pepper powder treated by combined MH treatment and DBD plasma

To identify any potential mechanical color differences between combined treatments, Hunter color “L” (lightness), “a” (redness), and “b” (yellowness) values were measured on the red pepper powder (Table 3). Hunter color “L”, “a”, and “b” values of the combination treated samples were not significantly ( $p > 0.05$ ) different from those of non-treated samples. Also, there were no significant ( $p > 0.05$ ) differences in pH values among the treated samples (Table 4).

## 4. Discussion

*B. cereus* is a soil bacterium [12] and commonly found in raw plants including rice, potatoes, peas, beans, herbs and spices [13]. Additionally, *B. cereus* is regarded as a food poisoning bacterium that can occasionally be an opportunistic human pathogen [14]. *B. cereus* can survive harsh environments including normal cooking temperatures. Herbs and spices are the main source of spore-forming bacteria such as *Bacillus* spp. and *Clostridium* spp. in food products (e.g., soups, cooked or stewed dishes, sauces) which can provide good conditions for the growth of these microorganisms; *B. cereus*, has also been shown to cause food poisoning in consumers [15]. It was also reported that *B. cereus* was considered as the main pathogenic microorganism detected in red pepper powder [3, 16, 17]. Red pepper powder is of agricultural origin and is therefore generally contaminated by this bacterium during the cultivation, drying, grinding, and storage processes.

Various methods such as UV irradiation, chlorine dioxide treatment, infrared treatment, radiation, and electron beam irradiation have been used to reduce microorganism contamination of red pepper powder [18-19]. Although these methods have proven effective at removing pathogenic bacteria, they may also lead to undesirable chemical (lipid oxidation) and sensory (color, odor and texture) changes in seafoods and vegetables [20]. Based

on these studies, the current study used a combination of MH and DBD plasma to sterilize red pepper powder in the hopes that it would not affect quality or leave any chemical residues.

Many studies on the inactivation of pathogenic bacteria in vegetable by MH treatment have been conducted. Son et al. (2016) [21] reported that spinach inoculated with *E. coli* O157: H7 decreased from 5.98 log CFU/g to 4.16 log CFU/g after treatment at 60 °C for 5 min (1.82 log CFU/g decrease). Koseki et al. (2005) [22] reported that lettuce treated at 50 °C reduced the surviving numbers of each *E. coli* O157:H7 and *Salmonella* by 2.73 and 2.81 log CFU/g, respectively. MH treatments reduced vegetative cells of pathogenic species present in foods. Such MH treatments are also capable of inactivating microbial spores and many enzymes and toxins present in foods. Generally, MH treatment can kill microbes by altering their cell membranes and denaturing proteins [23].

In the food processing industry, cold plasma treatment represents an innovative technology, especially since it was proven to be effective against foodborne pathogens with relatively little effect on food nutritional value [24]. Yong et al. (2015) [25] reported that the populations of *E. coli*, *S. Typhimurium*, and *L. monocytogenes* on cheese slices treated with plasma for 5 min (approximately 5 log CFU/g) were decreased by 1.75, 1.97, and 1.65 log CFU/g, respectively. Deng et al. (2007) [26] observed that DBD treatment for 30 seconds at 16 kV, 20 kV and 25 kV could yield 1-, 2.43-, and 4.12-log reduction of *E. coli* counts on almond samples. This decrease is high compared with the present results, however, it is considered to be caused by differences in the voltage and frequency of the plasma. Similar to the results presented here, Won et al. (2016) [27] recently determined that the growth of *E. coli* on onion powders inoculated with *E. coli* O157: H7 treated with atmospheric plasma for 5, 10, 15, and 20 min were inhibited by 0.4, 0.8, 0.9 and 1.4 log CFU/g, respectively.

There are various active species in the activated plasma (e.g., electrons, cations, anions, free radicals, ultraviolet photons). Cold plasma for the inactivation of microorganisms is mainly associated with the reactive species generated, particularly reactive nitrogen species (RNS) and reactive oxygen species (ROS) [28]. Rupturing bacterial cell walls using a build-up of charged particles and/or bombardment of free radicals have been proposed as possible modes of action for bacteria inactivation [28]. Although greater microbial inhibition is achieved by increasing the non-thermal treatment power and time, in practice a possible negative effect on quality must be taken into account [26, 8]. For these reasons, there has been increasing interest throughout the world in employing hurdle approaches to enhance the shelf life of red pepper powder and to ensure the microbiological safety of the products in food production and processing. Gayán et al. (2013) [29] studied the synergistic inactivation of *E. coli* and *Salmonella enterica* when treated with UV-C light at mild temperature. Simultaneously treatment with sanitizers, UV-irradiation and MH was shown to be more effective than either treatment alone or when applied

sequentially. Ha and Ha (2011) [30] reported that combined sanitizers (ethanol, hydrogen peroxide, and sodium hypochlorite)/UV treatments resulted in greater reductions in bacterial counts compared with either treatment alone. Xiang et al. (2019) [31] reported that *E. coli* O157:H7 were reduced from about 8.28 log CFU/mL to undetectable levels following exposure to the combined treatment of plasma-activated water and 60 °C (approximately 8 log CFU/mL reduction). Kim et al. (2017) [32] reported that red pepper flakes inoculated with *B. cereus* spores decreased from 6.0 log CFU/g to 3.4 log CFU/g after microwave-combined cold plasma for 20 min (2.60 log CFU/g decrease). Seong et al. (2017) [33] reported that *E. coli* O157: H7, *S. Typhimurium*, and *L. monocytogenes* on fresh lettuce were reduced by 1.55, 2.39, and 2.45 log CFU/g, respectively, following combined treatment with cold plasma and UV-C. The combinations of MH treatment and DBD plasma with other food preservation technologies not only allow the optimization of microbial inactivation, but also effectively maintain the nutritional and sensory attributes of food products during storage [34].

To evaluate the suitability of this combined decontamination approach, quality properties of red pepper powder were measured. The results from the Hunter color and pH value assessments demonstrated the potential for combined treatment with MH and DBD plasma as bacterial decontamination approach did not noticeably alter the color properties of red pepper powder. According to Li et al. (2014) [35] here was no significant difference between the treatments as a result of examining the surface color, texture, and overall acceptability of potato with ascorbic acid and mild heat treatment.

Combine treatment with MH and DBD plasma is more effective at inactivating *B. cereus* compared with MH treatment alone. Moreover, this combined approach does not lead to a deterioration in the quality of red pepper powder. Thus, this combined treatment can be utilized as an alternative to conventional decontaminating interventions (e.g., super-heated steam).

## 5. Conclusions

Here, it was noted that combined treatment of red pepper powder with MH and DBD plasma were more effective compared with treatment with MH or DBD alone. *B. cereus* counts in contaminated red pepper powder were significantly ( $p < 0.05$ ) reduced as treatment times with MH and DBD plasma increased; log-reductions of  $\geq 6.0$  log CFU/g were achieved. Synergistic reduction values for combined treatment with MH and DBD plasma against *B. cereus* in red pepper powder were  $-0.22$  to  $4.42$  log CFU/mL, respectively and the largest synergistic

reduction values (4.24-4.42 log CFU/mL) of *B. cereus* in red pepper powder were obtained by a combination of 20 min MH and 5~15 min DBD plasma. Hunter color “L”, “a”, and “b” values of the combination-treated samples were not significantly ( $p > 0.05$ ) different from those of non-treated samples. Also, there were no significant ( $p > 0.05$ ) differences in pH value. This combined treatment approach did not result in any deterioration in quality of red pepper powder. These results suggest that combined treatment with MH and DBD plasma is a potentially a novel method to improve the microbial safety of dried spices including red pepper powder.

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**Table 1.** Reduction of *Bacillus cereus* in red pepper powder after the combined treatment of MH and DBD plasma.

		Mean ( $\pm$ SD) reduction value (log CFU/g)				
		DBD plasma (min)				
Target organism	MH treatment (min)	0	5	10	15	20
<i>B. cereus</i>	0	–	0.12 $\pm$ 0.21	0.28 $\pm$ 0.32	0.61 $\pm$ 0.30	0.96 $\pm$ 0.17
	5	0.23 $\pm$ 0.24	0.33 $\pm$ 0.2	0.42 $\pm$ 0.1	1.97 $\pm$ 0.26 <sup>gh</sup>	2.57 $\pm$ 0.33 <sup>f</sup>
	10	0.93 $\pm$ 0.18	1.45 $\pm$ 0.23 <sup>ij</sup>	1.70 $\pm$ 0.13 <sup>hi</sup>	3.08 $\pm$ 0.32 <sup>e</sup>	3.64 $\pm$ 0.24 <sup>d</sup>
	15	1.16 $\pm$ 0.18 <sup>j</sup>	2.34 $\pm$ 0.19 <sup>fg</sup>	2.60 $\pm$ 0.24 <sup>f</sup>	3.87 $\pm$ 0.22 <sup>d</sup>	5.53 $\pm$ 0.16 <sup>c</sup>
	20	1.43 $\pm$ 0.23 <sup>ij</sup>	5.77 $\pm$ 0.26 <sup>bc</sup>	6.05 $\pm$ 0.27 <sup>ab</sup>	6.20 $\pm$ 0.31 <sup>ab</sup>	6.43 $\pm$ 0.21 <sup>a</sup>

The data indicates means with standard deviations (three samples/treatment).

MH: mild heat

DBD plasma: dielectric barrier discharge plasma

Gray box: Among all combination treatments having > 1 log reductions, mean with different letters differ significantly ( $p < 0.05$ ) by Duncan multiple range test.

**Table 2.** Synergistic and antagonistic effects of *B. cereus* in red pepper powder after the combined treatment MH and DBD plasma.

		Mean ( $\pm$ SD) synergistic and antagonistic value of reduction (log CFU/g)			
		DBD plasma (min)			
Target organism	MH treatment (min)	5	10	15	20
<i>B. cereus</i>	5	$-0.22 \pm 0.6$	$-0.28 \pm 0.7$	$1.24 \pm 0.3^d$	$1.20 \pm 0.6^d$
	10	$0.47 \pm 0.3$	$0.57 \pm 0.9$	$1.65 \pm 0.6^{cd}$	$1.58 \pm 0.5^{cd}$
	15	$1.13 \pm 0.4^d$	$1.24 \pm 0.1^d$	$2.18 \pm 0.3^c$	$3.20 \pm 0.6^b$
	20	$4.32 \pm 0.5^a$	$4.42 \pm 0.3^a$	$4.24 \pm 0.7^a$	$3.83 \pm 0.4^{ab}$

The data indicates means with standard deviations (three samples/treatment).

MH: mild heat

DBD plasma: dielectric barrier discharge plasma

Synergistic effects indicated as + = [reduction achieved by the combination of MH + DBD plasma] – [(reduction achieved by MH) + (reduction achieved by DBD plasma)]

Antagonistic effects indicated as – = [reduction achieved by the combination of MH + DBD plasma] – [(reduction achieved by MH) + (reduction achieved by DBD plasma)]

Gray box: Among all combination treatments having > 1 log synergistic reductions, mean with different letters differ significantly ( $p < 0.05$ ) by Duncan multiple range test.

**Table 3.** Effects of combined treatment of MH and DBD plasma on the Hunter color of red pepper powder.

Treatment	Hunter color		
	'L'-value	'a'-value	'b'-value
Control	26.88 ± 0.17	22.01 ± 0.23	14.12 ± 0.23
5 min MH + 5 min DBD	26.76 ± 0.21	22.08 ± 0.13	14.09 ± 0.25
5 min MH + 10 min DBD	26.65 ± 0.18	22.15 ± 0.24	14.07 ± 0.20
5 min MH + 15 min DBD	26.60 ± 0.10	22.07 ± 0.11	14.13 ± 0.14
5 min MH + 20 min DBD	26.66 ± 0.14	22.09 ± 0.16	14.17 ± 0.18
10 min MH + 5 min DBD	26.72 ± 0.18	22.12 ± 0.21	14.21 ± 0.11
10 min MH + 10 min DBD	26.61 ± 0.12	22.16 ± 0.26	14.16 ± 0.18
10 min MH + 15 min DBD	26.63 ± 0.14	22.12 ± 0.18	14.12 ± 0.08
10 min MH + 20 min DBD	26.64 ± 0.07	22.06 ± 0.14	14.21 ± 0.15
15 min MH + 5 min DBD	26.65 ± 0.28	22.08 ± 0.21	14.24 ± 0.14
15 min MH + 10 min DBD	26.76 ± 0.24	22.06 ± 0.24	14.13 ± 0.16
15 min MH + 15 min DBD	26.64 ± 0.15	22.12 ± 0.14	14.21 ± 0.19
15 min MH + 20 min DBD	26.77 ± 0.16	22.16 ± 0.16	14.09 ± 0.21
20 min MH + 5 min DBD	26.75 ± 0.22	22.01 ± 0.19	14.18 ± 0.18
20 min MH + 10 min DBD	26.76 ± 0.18	22.06 ± 0.12	14.11 ± 0.17
20 min MH + 15 min DBD	26.64 ± 0.13	22.12 ± 0.16	14.22 ± 0.17
20 min MH + 20 min DBD	26.77 ± 0.23	22.16 ± 0.14	14.09 ± 0.14

The data indicates means with standard deviations (three samples/treatment).

Control: non-treated sample

Values with the same letter in the same column are not significantly different ( $p < 0.05$ ) by Duncan's multiple range test.

Hunter 'L' values= lightness

Hunter 'a' values= redness+, greenness–  
 Hunter 'b' values= yellowness+, blueness–

**Table 4.** Effects of combined treatment of MH and DBD plasma in the pH value of red pepper powder.

		Mean ( $\pm$ SD) reduction value (log CFU/g)				
		DBD plasma (min)				
	MH treatment (min)	0	5	10	15	20
pH	0	4.86 $\pm$ 0.04	4.89 $\pm$ 0.02	4.87 $\pm$ 0.03	4.88 $\pm$ 0.03	4.84 $\pm$ 0.02
	5	4.87 $\pm$ 0.04	4.90 $\pm$ 0.02	4.88 $\pm$ 0.03	4.86 $\pm$ 0.04	4.84 $\pm$ 0.03
	10	4.85 $\pm$ 0.03	4.79 $\pm$ 0.02	4.87 $\pm$ 0.03	4.86 $\pm$ 0.03	4.75 $\pm$ 0.03
	15	4.84 $\pm$ 0.05	4.80 $\pm$ 0.03	4.81 $\pm$ 0.04	4.77 $\pm$ 0.04	4.76 $\pm$ 0.05
	20	4.83 $\pm$ 0.05	4.80 $\pm$ 0.04	4.78 $\pm$ 0.05	4.74 $\pm$ 0.05	4.73 $\pm$ 0.04

The data indicates means with standard deviations (three samples/treatment).