# ANTIMICROBIAL EFFECT OF SUPERCRITICAL CARBON DIOXIDE TREATMENT ON *BACILLUS CEREUS* IN MARINADES AND MARINATED PORK

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***Abstract*—This study was conducted to evaluate the effectiveness of supercritical carbon dioxide (SC-$CO_2$) treatment of a soy sauce marinade, hot-pepper paste marinade, and marinated pork products for the inhibition of *Bacillus cereus*. Marinades and marinated pork were treated with SC-$CO_2$ at 10 to 14 MPa and temperatures of 40 or 45 °C for 20 to 40 min. SC-$CO_2$ was more effective at destroying *B. cereus* when applied to the soy sauce marinade or soy sauce–marinated pork than when applied to the hot-pepper paste marinade or hot-pepper paste–marinated pork. When SC-$CO_2$ was applied at 14 MPa and 45°C for 40 min, the reduction levels were 1.82 and 1.20 log CFU/cm$^2$ in soy sauce and hot-pepper paste marinades, and were 2.02 and 1.31 log CFU/cm$^2$ in soy sauce– and hot-pepper paste–marinated pork, respectively. These results may be useful in the processing of marinades and marinated meat products to help improve microbial safety.**

***Index Terms*— *Bacillus cereus*, marinade, marinated pork, meat safety, supercritical carbon dioxide**

## I. INTRODUCTION

The consumption of commercial marinades and ready-to-cook marinated meat products has increased in Korea in response to increased consumer demand. However, marinades and marinated products have limited food safety due to possible pathogenic contamination. Thus, the improvement of microbial safety is a major concern during the processing of marinated meat products. Supercritical carbon dioxide (SC-$CO_2$) treatment is a nonthermal sterilization technique that can inactivate microorganisms without or minimally impacting the sensory and nutritional qualities of solid and liquid foods (Jung, Choi & Rhee, 2009; Choi, Bae, Kim, Kim & Rhee, 2009; Garcia-Gonzalez et al., 2007). *Bacillus cereus* is one of most common pathogenic spore-forming bacteria (Bryne, Dunne and Bolton, 2006). It is found in raw meat, meat products, and food ingredients, and can produce an enterotoxin that causes food poisoning. Although most *B. cereus* spores are moderately heat-resistant, some are markedly heat-resistant (Bryne et al., 2006), making the organism difficult to control in a food processing environment. This study was conducted to evaluate the effects of SC-$CO_2$ treatment on the reduction of *B. cereus* levels in marinades and marinated pork.

## II. MATERIALS AND METHODS

### *A. Bacterial strains*

*B. cereus* (ATCC 10876, ATCC 13061 and ATCC 11778) was obtained from the Food Microbiology Culture Collection at Korea University (Seoul, Korea). The cultures were maintained on tryptic soy agar (TSB; Difco Laboratories, Detroit, Mich.) slants at 4°C and subcultured monthly. Cultures used in all experiments were freshly prepared by the same procedure.

### *B. Culture and cell suspension*

Each strain was cultured overnight in TSB at 37 °C. The cultures were then combined in plastic centrifuge tubes (Corning Inc., NY), and the cells were harvested by centrifugation (Centri-CL2; IEC, Needham Heights, MA) at 2,500 × *g* for 30 min. The supernatant was discarded and the pellet was washed twice with 0.2% sterile peptone water. The final pellet was resuspended in 0.2% sterile peptone to a final concentration of ~ $10^9$ CFU/ml of sample.

### *C. Treatments of marinades and marinated pork*

Two marinades were used (Table 1) that contained either soy sauce or hot-pepper paste. The marinades were inoculated with cell suspensions of *B. cereus* (~ $10^7$ CFU/ml). Six boneless pork loins were obtained from carcasses at a local slaughterhouse 24 h post-slaughter. Muscle samples were trimmed using a stainless steel knife that had been sanitized with alcohol and flame. Each pork loin was cut into nineteen rectangular-shaped pieces (10 x 10 x 100 mm, each weighing 20 g) that were then randomly selected for use to minimize bias. Chops were added to each inoculated

sauce in a 1:1 ratio, and were placed in culture tubes and incubated overnight at 4 °C. Inoculated samples, including marinades and marinated pork, were subjected to SC-$CO_2$ treatments using an SC-$CO_2$ system (ISA-LSFECO, Ilshin Co., Korea) at 10, 12, or 14 MPa and temperatures of 40 or 45 °C, for 20, 30 or 40 min. The whole experiment was replicated three times.

*D. Microbial analysis*

Treated samples were placed in stomacher bags containing 180 ml of buffered peptone water, and homogenized for 2 min (Seware, Stomacher 400, U.K.). After homogenization, the samples underwent serial 10-fold dilutions with 9 ml of sterile buffered peptone water. Mannitol-egg yolk-polymyxin agar (Difco) was used as selective media for the enumeration. Samples were incubated at 37 °C for 24 h, after which enumerations were performed on samples collected before and after each treatment.

*E. Statistical analysis*

The average of duplicate plate counts for three replications was converted to units of log CFU/$cm^2$. All experiments were conducted in triplicate, with duplicate samples analyzed at each treatment. All data were analyzed by the general linear model procedure contained in the SAS statistical package (SAS Institute, 2004). Significance was reported at the $P < 0.05$ level.

**Table 1**
Components of the soy sauce and hot-pepper paste marinades

| **Soy sauce marinade (%)** | | **Hot-pepper paste marinade (%)** | |
|---|---|---|---|
| Soy sauce | 14.48 | Hot-pepper paste | 20.00 |
| Water | 43.45 | Water | 37.96 |
| Garlic powder | 3.64 | Garlic powder | 3.64 |
| Chopped spring onion | 3.64 | Chopped spring onion | 3.64 |
| Chopped onion | 3.64 | Chopped onion | 3.64 |
| Pear puree | 7.24 | Pear puree | 7.24 |
| Ginger powder | 0.72 | Ginger powder | 0.72 |
| Sugar | 7.24 | Sugar | 7.24 |
| Starch syrup | 7.24 | Starch syrup | 7.24 |
| Refined rice wine | 7.27 | Refined rice wine | 7.24 |
| Ground black pepper | 0.28 | Ground black pepper | 0.28 |
| Sesame seed | 0.44 | Sesame seed | 0.44 |
| Sesame oil | 0.72 | Sesame oil | 0.72 |
| Total | 100.00 | Total | 100.00 |

## III. RESULTS AND DISCUSSION

Figure 1 shows the inhibitory effects of SC-$CO_2$ treatments under various conditions on *B. cereus* in the soy sauce marinade. All samples treated with SC-$CO_2$ at 14 MPa displayed reduced *B. cereus* levels. A significantly higher number of *B. cereus* were observed in the control (6.96 log CFU/$cm^2$) than in samples treated with SC-$CO_2$ at 12 MPa and 40 °C (12M-40T) for 40 min (5.95 log CFU/$cm^2$, $P < 0.01$). In the case of hot-pepper paste (Fig. 1B), the level of microbial reduction was not affected by treatment for 20 or 30 min in the 10M-40T or 10M-45T group. The *B. cereus* level was reduced from 6.80 to 5.60 log CFU/$cm^2$ ($P < 0.01$) after 40 min in the 14M-45T treatment group.

Figure 2 shows the effects of SC-$CO_2$ treatments on the inhibition of *B. cereus* in marinated pork. When SC-$CO_2$ was applied at 10 MPa at 40 °C, the *B. cereus* levels were unaffected by treatment. In the case of soy sauce–marinated pork, treatment at 14T-45M for 20 min did not reduce the *B. cereus* level. After 40 min of treatment, a significantly lower *B. cereus* level was observed in the samples treated at 14M-45T than in the control (5.23 vs. 7.25 log CFU/$cm^2$, $P < 0.001$). In the case of hot-pepper paste–marinated pork, the initial count of *B. cereus* was reduced from 6.93 to 5.62 log CFU/$cm^2$ ($P < 0.01$) after 40 min in the 14T-45M treatment group. However, after 40 min, the 12T-40M and 14T-45T groups had similar levels of *B. cereus* reduction.

Generally, the SC-$CO_2$ mediated decontamination of solid foods is more difficult than that of liquid foods due to the more limited $CO_2$ solubility (Garcia-Gonzalez et al., 2007). Moreover, the SC-$CO_2$ treatment conditions, including

pressure, temperature, and exposure time, can deteriorate meat quality, especially surface colour and texture (Choi et al., 2008). In previous studies, the treatment consisted of pressures or temperatures that were too high or exposure times that were too long (Sirisee, Hsieh & Huff, 1998; Wei, Balaban, Fernando & Peplow, 1991). Therefore, from a food quality point of view, the treatment conditions of SC-$CO_2$ in this study were also minimized to the comparison of previous studies. The reduction of *B. cereus* levels in the marinades ranged from 1.20 to 1.82 log CFU/cm$^2$, and those of the marinated pork ranged from 1.31 to 2.02 log CFU/cm$^2$.

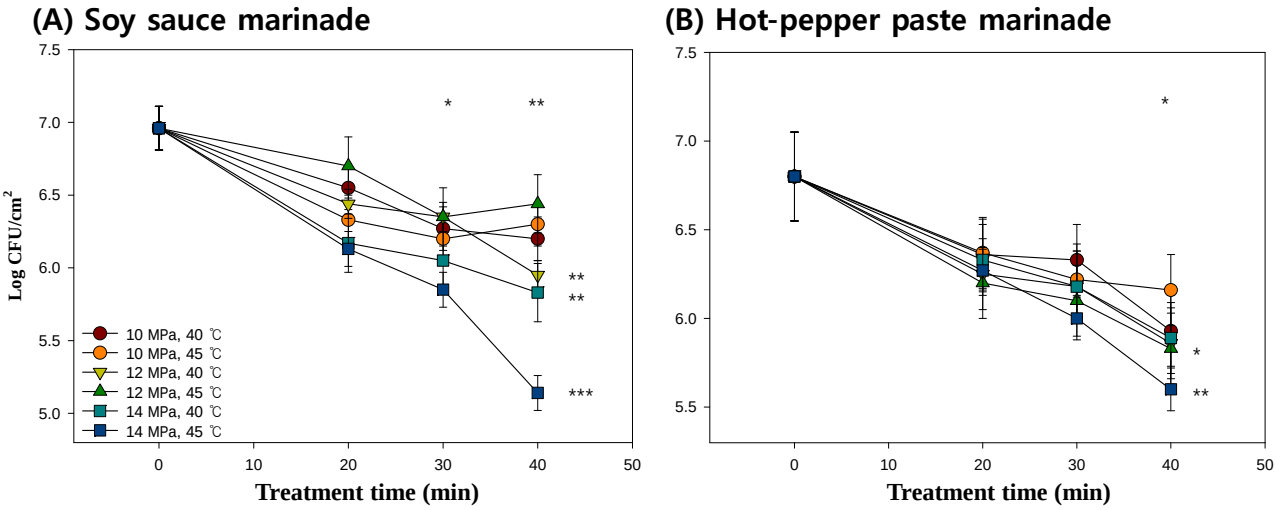


**Fig. 1.** Effects of supercritical carbon dioxide treatment of soy sauce (A) and hot-pepper paste (B) marinades on the inhibition of *Bacillus cereus*. Bar indicates standard errors. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

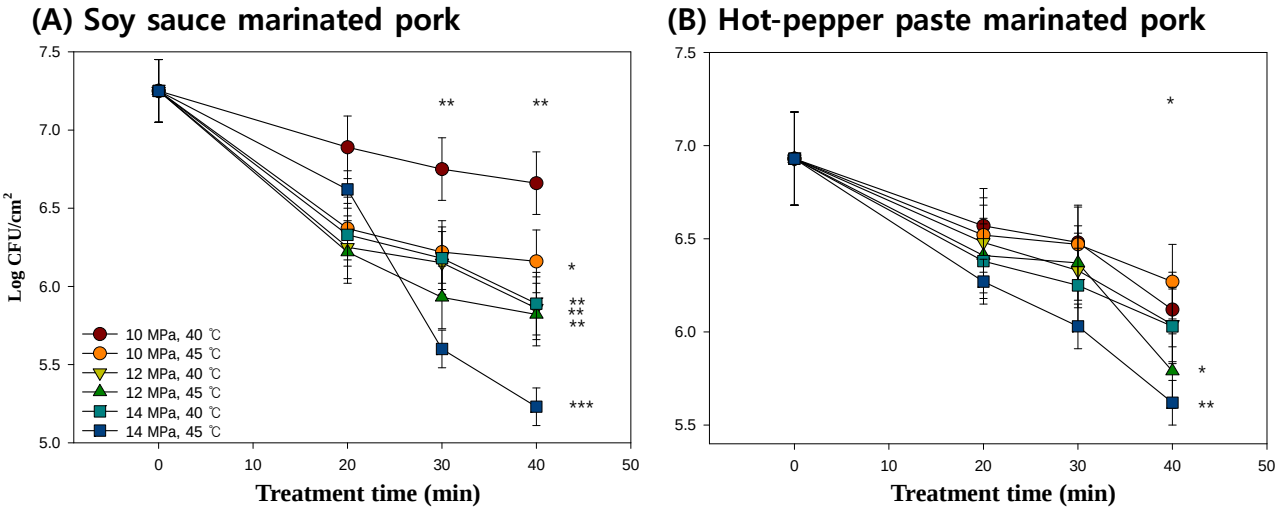


**Fig. 2.** Effects of supercritical carbon dioxide treatment of soy sauce– (A) and hot-pepper paste– (B) marinated pork on the inhibition of *Bacillus cereus*. Bar indicates standard errors. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

## IV. CONCLUSION

These results may be useful in the processing of marinades and marinated meat products to help improve microbial safety.


## ACKNOWLEDGEMENTS

This work was supported by the Agricultural R & D Promotion Centre (Korea). The authors thank the Korea University Food Safety Centre for the use of their equipment and facilities.

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