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# Reduction of *Bacillus cereus* in Cooked Rice Treated with Sanitizers and Disinfectants

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**Abstract** This study aimed to identify effective washing and sanitation programs to minimize the contamination of cooked rice by *B. cereus*. As such, the effectiveness of five sanitizers, including QAC, alcohol, chlorine, CaO, and $H_2O_2$, was evaluated in relation to the survivability of *B. cereus* spores in cooked rice and resulting sensory properties of the rice. The water-treated cooked rice showed remaining *B. cereus* spores at 1.09 $\log_{10}$CFU/g. In contrast, treatment with the minimum inhibitory concentrations of the sanitizers, such as 200 ppm of QAC, 50% of alcohol, 100 ppm of chlorine, 650 ppm of CaO, and 500 ppm of $H_2O_2$, destroyed all the spores in the cooked rice below a non-detection limit (ND< 0.15 CFU/g). The sensory properties of the sanitizer-treated (1,000 ppm of $H_2O_2$, 100 ppm of chlorine, and 800 ppm of CaO) cooked rice did not differ significantly from those of the water-treated cooked rice. As a result, 500 ppm of $H_2O_2$, 650 ppm of CaO, and 100 ppm of chlorine were found to effectively eliminate *B. cereus* spores in rice while cooking.

**Key words:** *Bacillus cereus*, rice, quaternary ammonium compound, alcohol, calcium oxide, hydrogen peroxide, chlorine

*Bacillus cereus* is Gram-positive and spore-forming, and causes diarrheal and emetic-type food poisoning. Widely distributed in nature, including soil, air, dust, and water, *B. cereus* can also be isolated from raw and processed foods, including, rice, dairy products, meat, and spices. In particular, since *B. cereus* spores are heat resistant, they can survive the cooking process. Thus, when cooked rice is stored at room temperature, the spores can germinate, proliferate, produce emetic toxins, and cause food poisoning [1, 11, 30]. Emetic food poisoning is associated with rice-based products and starch foods [6]. The total infective dose of *B. cereus* causing emetic food poisoning seems to vary from $10^5$ to $10^8$ colony forming units/g (CFU/g). In addition, since the bacteria produce the toxins in the food, emetic food poisoning has a short incubation time, between 30 min and 5 h [7]. It has been reported that *B. cereus* caused 1–22% of all foodborne outbreaks in Europe, Japan, and North America from 1960 to 1992 [27], plus 2 cases of Bacillosis were identified among the 135 pathogenic outbreaks in 2003 [15] and 2 more cases among the 165 pathogenic outbreaks in 2004 [14]. Berghofer *et al.* [3] reported the detection of *B. cereus* in 81% of wheat and 93% of flour with a high frequency ranging from 0.1 to 1 CFU/g, whereas Sarrías *et al.* [30] detected 1.2–3.5×$10^3$ CFU/g of *B. cereus* in husked rice, and Lee and Chang [19] reported *B. cereus* contamination levels of 5.9×$10^2$, 2.6×$10^3$, 2.6×$10^6$, and 2.3×$10^6$ CFU/g in rice, barley, cooked rice, and *Kimbab*, respectively, with raw rice more contaminated by *B. cereus* than cooked rice and *Kimbab*. However, preventing contamination is difficult, as *B. cereus* and its spores are widely dispersed in nature. Nonetheless, various methods of disinfection or *B. cereus* inactivation have been developed, including controlling the pH, electrolyzed water, sodium chlorite, ozone, hydrogen peroxide, organic acids, ethanol, sodium chloride, grapefruit extracts, nisin, storage at a low temperature, and electron beam irradiation [2, 4, 8, 10, 13, 16, 19, 21–25, 30, 32, 33]. Accordingly, the purpose of this study was to identify effective methods of reducing *B. cereus* spores in rice and cooked rice using sanitizers and disinfectants.

The *B. cereus* used in this study was ATCC 14893, and the spore suspension prepared as described by prEN 14347

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[5]. The *B. cereus* was inoculated into sterile tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, U.S.A.) for 24 h at 37°C. Thereafter, 2.0 ml of the liquid culture was dispersed on a manganese sulfate agar (MSA) [5] surface that had been dried for 7 days, then 1.0 ml of the MSA was removed and incubated for 1 week at 30°C. To harvest the spores, 2.0 ml of sterile distilled water and glass beads (Glastechnique MFG, Germany) were placed in the incubated bottles. The same procedure was then repeated one more time with another 2.0 ml of sterile distilled water. Next, the spore suspension was diluted with 500 ml of sterile distilled water to achieve a concentration of 5.5–6.0 $\log_{10}$CFU/ml, and rice immersed in the diluted spore suspension for 30 min for inoculation with *B. cereus*. After being immersed, the rice was dried for 2 days and stored at room temperature for 1 week to allow the spores to adhere to the rice. The inoculated concentration of *B. cereus* spores in the rice was 3.51±0.21 $\log_{10}$CFU/g. To analyze the *B. cereus*, 225 ml of a sterile mannitol-egg yolk-polymyxin agar (MYP, Difco Laboratories, Detroit, MI, U.S.A.) containing 12.5 ml of egg yolk (Difco Laboratories, Detroit, MI, U.S.A.) and 4.1 ml of antimicrobic vial P (Difco Laboratories, Detroit, MI, U.S.A.) that had been dissolved in 5.0 ml of sterile distilled water was prepared [19]. To enumerate the surviving *B. cereus*, the sanitizer-treated, untreated, and cooked rice were serially diluted and plated on 15–20 ml of MYP using a pour plate technique. The tested sanitizers were alcohol (fermented ethanol 95%, Korea Ethanol Supplies Co., Korea), chlorine (Kirbychlor, Schering-Plough Ltd, England), quaternary ammonium compounds (QAC, Akzo Qrquad MCB-80, Namkang, Korea), hydrogen peroxide (Huwa-san TR-50, Roam Chemical NV, Belgium), calcium oxide (Yaizu Suisan Kagaku Industry Co. Ltd., Japan), and glutaric dialdehyde (GD, Acrose Organics, U.S.A.). The sanitizers were all diluted with distilled water to their respective normal target concentrations, including 200 ppm of QAC, 50% of ethanol, 100 ppm of chlorine, 0.01–2.0% of hydrogen peroxide, and 500–2,000 ppm of calcium oxide. The rice was washed with sterile distilled water for 2 min, treated with the sanitizers for 5 min, and rinsed with sterile distilled water for 2 min. The *B. cereus* in the treated, untreated, and cooked rice was then enumerated. The sensory characteristics, such as the color, odor, taste, and overall acceptability, of the cooked rice were evaluated by a trained panel of 15 using a 5-scale method and compared with the sensory characteristics after water treatment (treated with only water for 2, 5, and 2 min). The panelists washed out their mouth after evaluating each sample. The data from the sensory evaluation were analyzed using a statistical analysis system (SAS, SAS Institute, version 8.1), and the average and significance analyzed using Duncan's multiple range test.

Table 1 shows the *B. cereus* counts for the raw and cooked rice before and after sanitizer treatment, such as 200 ppm of QAC, 50% of ethanol with and without 0.3% of GD, 100 ppm of chlorine, 0.01–2.0% of hydrogen peroxide,

**Table 1.** Inhibitory effect of sanitizers on *B. cereus* spores in raw and cooked rice.

| Sanitizers | | Raw rice ($\log_{10}$ CFU/g rice) | Cooked rice ($\log_{10}$ CFU/g rice) |
|---|---|---|---|
| Treatment | Concentration (ppm) | | |
| No treatment | | 3.51±0.21$^{ab}$ | |
| Water treatment | | 3.45±0.20$^{b}$ | 1.09 |
| QAC* | 200 | 2.35±0.05$^{de}$ | ND** |
| Alcohol | 500,000 | 2.32±0.02$^{e}$ | ND |
| Chlorine | 100 | 2.29±0.02$^{e}$ | ND |
| Calcium oxide | 500 | 3.37±0.12$^{b}$ | 0.18 |
| | 600 | 3.35±0.06$^{b}$ | 0.30 |
| | 650 | 3.32±0.02$^{bc}$ | ND |
| | 700 | 3.24±0.02$^{c}$ | ND |
| | 800 | 3.25±0.04$^{c}$ | ND |
| | 1,000 | 3.25±0.05$^{c}$ | ND |
| | 2,000 | 2.29±0.05$^{e}$ | ND |
| Hydrogen peroxide | 100 | 3.70±0.05$^{a}$ | 0.45 |
| | 300 | 3.68±0.05$^{a}$ | 0.15 |
| | 500 | 3.65±0.04$^{a}$ | ND |
| | 700 | 3.56±0.03$^{a}$ | ND |
| | 1,000 | 3.44±0.20$^{b}$ | ND |
| | 10,000 | 3.10±0.12$^{c}$ | ND |
| | 20,000 | 2.57±0.06$^{d}$ | ND |

*QAC: Quaternary ammonium compound.

**ND: not detected (<0.15 CFU/g for *B. cereus*).

**Table 2.** Sensory evaluation of cooked rice treated by various sanitizers.

| Attributes \ Sanitizers | Control | Hydrogen peroxide (1,000 ppm) | Chlorine (100 ppm) | Calcium oxide (800 ppm) |
|---|---|---|---|---|
| Color | 3.00 | 3.07±1.03 | 2.07±0.96 | 2.93±1.22 |
| Taste | 3.00 | 2.67±1.29 | 2.40±1.12 | 2.53±1.19 |
| Odor | 3.00 | 2.93±0.88 | 2.60±1.06 | 2.67±1.11 |
| Overall acceptability | 3.00 | 2.73±1.10 | 2.60±0.99 | 2.73±1.03 |

Data not significantly different at P<0.05.

and 500–2,000 ppm of calcium. The water treatment reduced the microbial load to 0.06 $\log_{10}$CFU/g compared with the initial microbial load of 3.51 $\log_{10}$CFU/g. Meanwhile, the treatment with 200 ppm of QAC, 50% of ethanol, 100 ppm of chlorine, and 2,000 ppm of calcium oxide produced a similar reduction to approximately 1.2 $\log_{10}$CFU/g of *B. cereus* spore in the raw rice. The addition of 0.3% GD to 50% ethanol did not enhance the sporicidal effect of the 50% ethanol, indicating a poor spore-controlling ability for GD. Jang *et al.* [8] noted that the numbers of *B. cereus*, *Escherichia coli*, and *Salmonella typhimurium* were reduced to 5–6 $\log_{10}$CFU/g after treatment with 30% ethanol for 5 min, plus they noted that 20% ethanol with 10% sodium chloride reduced each bacterium to 3–5 $\log_{10}$CFU/g. According to Peng *et al.* [27], treatment with 100 and 200 ppm of QAC reduced the number of *B. cereus* in a single cell to 4.8 and 4.5–4.8 $\log_{10}$CFU/g, respectively. In the case of *B. cereus* in biofilm, 25 and 50 ppm of sodium hypochlorite reduced the number of *B. cereus* to 1.9–2.1 and 2.1–2.3 $\log_{10}$CFU/chip, respectively [26]. Jang *et al.* [9] also noted that *B. cereus* was inhibited by treatment with hydrogen peroxide at a concentration higher than 75 ppm at pH 6, and heat-resistant *Staphylococcus aureus* stopped growing in the presence of 50 ppm hydrogen peroxide at pH 6. Plus, Sagripanti *et al.* [29] reported that 5 $\log_{10}$CFU/ml of *B. cereus* was reduced by treatment with 0.05% sodium hypochlorite and 10% hydrogen peroxide. According to Jang *et al.* [10], electrolyzed and ozonated water inactivated 0.1–1.8 $\log_{10}$CFU/g of *B. cereus* in job's tears, sorgum, millet, and Italian millet. Although the treatment with sanitizers did not significantly reduce the *B. cereus* spores compared with the water treatment, after cooking, no active spores were found in the sanitizer-treated rice, whereas 1.09 $\log_{10}$CFU/g of spores still remained in the water-treated rice. This implies that, although the sanitizers did not inactivate the spores immediately, they may have had a synergistic effect in reducing the heat-resistant ability of the *B. cereus* spores. Melly *et al.* [21] previously noted that hydrogen peroxide releases pyridine-2,6-dicarboxylic acid (dipicolinic acid) that affects the heat-resistant ability of spores and inactivates the enzymes necessary for germination. According to Jun *et al.* [12], 12,500 ppm of calcium oxide disinfected vegetative cells and spores of *Bacillus subtilis*. The effects of the treatments on the sensory properties of the cooked rice are described in Table 2. The cooked rice treated with ethanol and QAC was excluded from the sensory evaluations, owing to the bad taste caused by the residual ethanol and QAC. The calcium oxide-treated rice exhibited a yellowish color, although this was hardly detected by the panelists. The results of the sensory analysis revealed slightly lower scores for the sanitizer-treated rice, yet not significantly different ($p$<0.05) from the control. Kim *et al.* [17] reported on the relationship between the time taken to wash rice and the texture of the cooked rice, where the hardness of the cooked rice decreased when increasing the washing time ($p$<0.01), whereas the springiness and cohesiveness did not change significantly. In addition, Kim *et al.* [18] also reported that the difference in color and odor of cooked rice treated by three surfactants was not significant at a 5% level.

Consequently, in the present study, 500 ppm of hydrogen peroxide, 650 ppm of calcium oxide, and 100 ppm of chlorine were found to effectively eliminate *B. cereus* spores in rice during cooking.

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