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# Establishment of Microbiological Safety on the Raw Materials or Products of *Saengshik*

**Hyo-Jung Chu, Min-Hee Oh and Deog-Hwan Oh***

School of Bioengineering and Biotechnology, Kangwon National Univesity
E-mail: deoghwa@kangwon.ac.kr

## INTRODUCTION

There has been a noticeable increase in the consumption of fresh fruits and vegetables as well as fresh cereals, and a marked increase in the global distribution of produce. An abundance of research over last decade has shown that diets which are low in fat and high in fiber, and that include liberal consumption of fruits and vegetables, are protective against many cancers and lessen the risk of coronary heart disease (Kim & Bang, 1998).

Recently, the consumption of *Saengshik* has been dramatically increased due to the consumer's recognition as healthy food. In general, *Saengshik* consists of 40～50 kinds of mixed cereals, soybeans, fruits and vegetables, mushrooms and marine algae with uncooked and grounded. *Saengshik* contains live nutrients such as fiber, enzymes, vitamine, mineral because of uncooked conditions (Lee, 2002)

The *Saengshik* market in Korea has been dramatically grown since 1997 and might be expected approximately 300,000,000,000 won in 2005 (Park, 2002). However, relative standard on the definition and regulation to establish safe consumption of *Saengshik* has not been made even though the rapid market growth in the food industry was happened Thus, great concern on the microbiological safety has been occurred (Chang et al., 2004).

*Saengshik* products may be simply produced by trimmed, peeled, washed and disinfected cereals or vegetables, freezed dried, grounded, mixed and packged (Hwang, 2002). Such *Saengshik* products may be contaminated with pathogens when they enter the processing chain. The product itself and its source will often determine the numbers and types of microorganisms initially present. Pathogens may form part of this microflora, posing a potential safety problem.

Outbreaks of foodborne disease have increased around the world. In the United States, each year foodborne illness affect 6 to 80 million persons, cause 9,000 deaths, and cost an estimated 5 billion U.S. dollors (Altekruse et al., 1997). The epidemiology of foodborne disease is rapidly changing as newly recognized pathogens emerge and well recognized pathogens increase in prevalence or become associated with new food vehicles. Among the known foodborne pathogens, *Bacillus cereus* and *Clostridium perfringence* were widely distributed on plant vegetation and likely contaminates the latter via many routes, including soil, water, animal manure, decaying vegetation, including rice, cereals, bean sprouts, cabbage, cucumber, potatoes, and radishes have been found to be contaminated with *B. cereus* (Girardin et al., 2002)

Strict hygiene is critical during washing and disinfection in order to avoid contamination of these pathogens. Sanitizers such as hypochlorite solutions and quaternary ammonium compounds have been used in food processing facilities to control contaminant microorganisms, particularly those causing foodborne disease. Use of some sanitizers has been limited or banned because of the potential health hazard. On the other hand, the need for potent antimicrobial agents has increased in recent years due to increasing disease outbreaks and emergence of new foodborne pathogens. Illnesses arising from the presence of foodborne pathogens have renewed interest in effective control measures. Therefore, the food industry is in search of disinfectants that are effective against common and emerging pathogens and safe to use in many specific applications of food processing.

One of widely interesting potential sanitizers in the *Saengshik* industry is electrolyzed water that has been utilized as a sanitizer in water treatment plants in Europe and Japan. Electrolyzed water has been used with mixed success to inactivate contaminant microflora on meat, poultry, eggs, fish, fruit, vegetables and dry foods (Venkitanarayanan et al., 1999b; Park, 2002; Russell, 2003). The purpose of this study was to determine the antimicrobial effects of electrolyzed water alone or combined with organic acids on the reduction of microbial flora, such as total counts, yeast and mold on the vegetables and cereals as well as *B. cereus* inoculated into raw materials of *Saengshik*.

## MATERIALS AND METHODS

### Sampling

Five kinds of cereals (barley, browny rice, sticky rice, black bean, adlay), four kinds of vegetables (carrot, spinach, kayle, burdock) were collected at random from five supermarkets located at Chunchon city in Korea. The tested samples were already trimmed, peeled, washed, and kept at refrigeration temperature for vegetables or room temperature for cereals in the supermarkets. All samples were transferred to the laboratory for analyses on the same day in an ice cooler.

### Bacterial cultures

*B. cereus* ATCC 14579, ATCC 53522 and ATCC 25621 strains were used for this experiments. Each culture was grown in tryptic soy broth (Difco Laboratories, Detroit, MI) and maintained in tryptic soy broth (TSB) - glycerol (50 : 50, vol/vol) at -20°C. To prepare inocula for the test media, the bacteria were grown in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE; Difco) at 37°C for 24 h. Each working culture was combined in equal volumes to serve as test organisms.

### Preparation of antimicrobial agents

Electrolyzed water was obtained from electrolyzer (Enfirst A2, Chungju, Korea). Also, ten% stock solutions of known concentrations of reagent-grade acetic, citric, hydrochloric, and lactic acid (Sigma Chemical Co., St. Louis, MO, v/v) were prepared in sterile distilled water. The antimicrobial solutions were filter-sterilized using a 0.22 membrane filter (Milipore Products Division, Bedford, MA). These were prepared fresh before each experiment. The kinds of natural antimicrobials used in this study are shown in Table 1.

**Table 1.** Kinds of natural antimicrobials used in this study

| | Kinds of antimicrobials |
|---|---|
| 1 | Control |
| 2 | 100 ppm |
| 3 | 1% citric acid |
| 4 | 1% acetic aicd |
| 5 | 1% lactic acid |
| 6 | electrolyzed acid water |
| 7 | electrolyzed acid water + 1% citric acid |
| 8 | electrolyzed acid water + 1% acetic aicd |
| 9 | electrolyzed acid water + 1% lactic acid |

**Experimental procedure**

From the $10^9$ CFU/mL working culture of the organism, 10 mL aliquots were aseptically transferred into each beaker containing 100 mL of sterile distilled water and each cereal and vegetable was dipped to give a final inoculum of $10^7$ CFU/mL of *B. cereus* and dried with sterile paper tower. Twenty five gram of cereals (barley, browny rice, sticky rice, black bean, adlay) were dipped in the electrolyzed acid water and 1% each organic acid either alone or in combination for 3 h and 25 grams of four kinds of vegetables (carrot, spinach, kayle, burdock) for 3 min, respectively. Then, each treatment was dehydrated with centrifugation for 3 min at 3,000 rpm. Following the treatments, uninoculated and inoculated cereals and vegetables (25 g) subjected to various treatments and storage conditions were analyzed for population of *B. cereus.* Each sample was combined with 225 mL of sterile 0.1% pepton water in a sterile polyethylene abg and pummeled at medium speed with a stomacher (Seward stomacher 400, London, UK) for 2 min. Homogenized samples were serially diluted and pour plated, in duplicate, using TSA for total counts and MYP (Mannitol-Egg York-Polymyxin, Difco) and plates were incubated at 37°C for 24 h. Each treatment was analyzed separately and average counts (log CFU/g) were calculated.

## RESULTS AND DISCUSSION

The effect of electronized acid water or organic acid either alone or in combination against inactivation of *B. cereus* inoculated into barley are shown in Fig. 1.

The treatment of 100 ppm sodium chlorite was not significantly effective compared to control, but strong inactivation was observed in 1% acetic and lactic acid. Also, electronized acid water alone showed approximately 1 log CFU/g reduction on *B. cereus* inoculated into barley. However, the strongest reduction (3.1 log CFU/g) was observed in the combined electronized acid water with 1% acetic acid, and followed by lactic acid and citric acid. Fig. 2 shows the effect of electronized acid water or organic acid either alone or in combination against inactivation of *B. cereus* inoculated into sticky rice.

The treatment of 100 ppm sodium chlorite did not affect the inhibition of *B. cereus* inoculated into barley. Three log CFU/g reduction was observed in 1% acetic and followed by lactic acid and citric acid. However, the strong synergistic effect (3.5 log CFU/g) was observed in the combined electronized acid water with 1% citric acid, and followed by lactic acid and acetic acid. In the meantime, the effect of electronized acid water or organic acid either alone or in combination against inactivation of *B. cereus* inoculated into Job's tears are shown in Fig. 3.

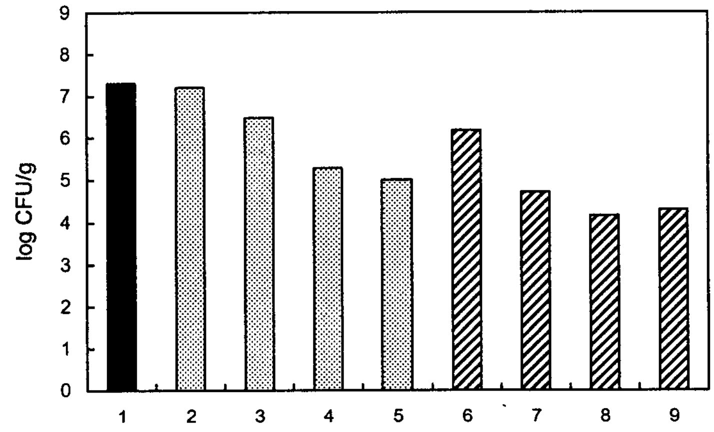

**Fig. 1.** Effect of electrolyzed acid water and organic acid, either alone or in combined on inactivation of *B. cereus* on barley.

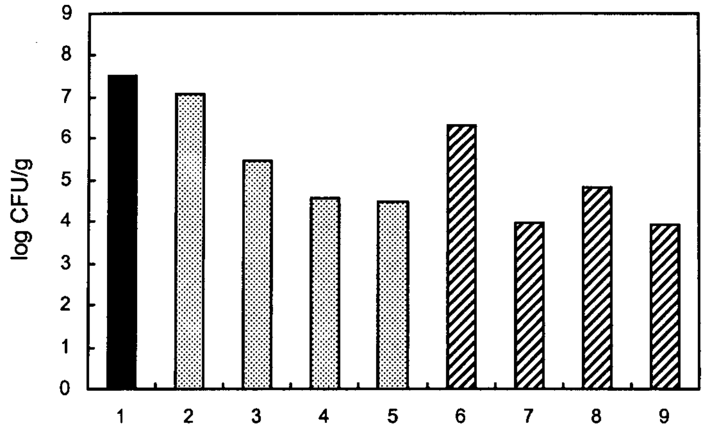

**Fig. 2.** Effect of electrolyzed acid water and organic acid, either alone or in combined on inactivation of *B. cereus* on sticky rice.

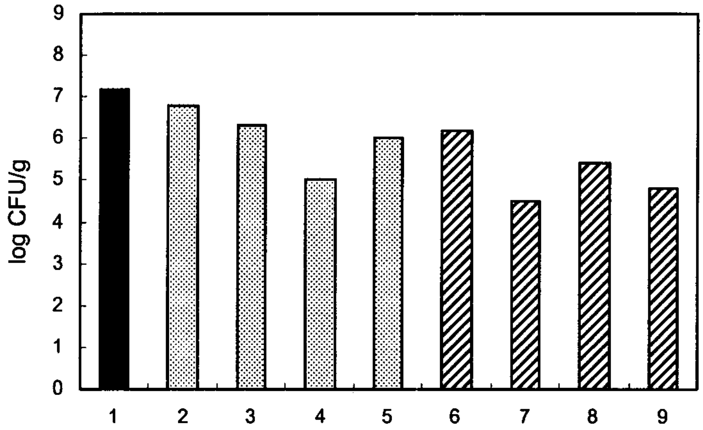

**Fig. 3.** Effect of electrolyzed acid water and organic acid, either alone or in combined on inactivation of *B. cereus* on Job's tears.

Similar results was observed with Fig. 2. The treatment of 100 ppm sodium chlorite was not significantly effective compared to control, but 2 log reduction was observed in 1% acetic acid. Also, electronized acid water alone showed approximately 1 log CFU/g reduction on *B. cereus* inoculated into barley. However, the strongest reduction (2.7 log CFU/g) was observed in the combined electronized acid water with 1% citric acid, and followed by lactic acid and acetic acid.

Fig. 4 shows the effect of electronized acid water or organic acid either alone or in combination against inactivation of *B. cereus* inoculated into kale. The treatment of 100 ppm sodium chlorite did not affect the inhibition of *B. cereus* inoculated into kale. On the contray with other raw materials, acetic acid and citric acid did no reduce the *B. cereus* inoculated into kale, but lactic acid gave a 1 log reduction. However, the strongest inactivation effect (2.9 log CFU/g) was observed in the combined electronized acid water with 1% citric acid, and followed by lactic acid and acetic acid. These results indicate that the combined electronized acid water with organic acids (especially citric acid) might play a important role in the reduction of *B. cereus* contaminated in the RTE vegetables and cereals.

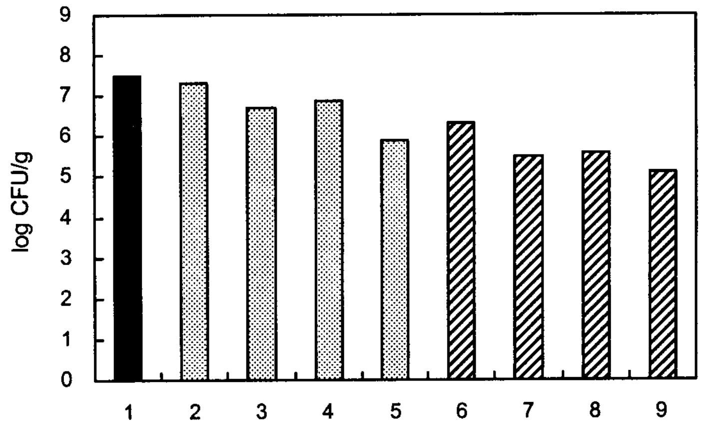

**Fig. 4.** Effect of electrolyzed acid water and organic acid, either alone or in combined on inactivation of *B. cereus* on kale.

## CONCLUSIONS

This study was to determine the antimicrobial effects of electrolyzed water alone or combined with organic acids on the reduction of *B. cereus* inoculated into raw materials of *Saengshik.* The treatment of 100 ppm sodium chlorite was not significantly effective compared to control, but strong inactivation was observed in 1% acetic and lactic acid. Also, electronized acid water alone showed approximately 1 log CFU/g reduction on *B. cereus* inoculated into barley. However, the strongest reduction (3.1 log CFU/g) was observed in the combined electronized acid water with 1% acetic acid, and followed by lactic acid and citric acid. However, similar inactivation in the other raw materials such as sticky rice Job's tears and kale was observed for the organic acids alone or electrolyzed acid water, but the strongest inactivation was observed in the combined electrolyzed acid water with citric acid, opposed to that of barley. These results indicate that the combined electronized acid water with organic acids (especially citric acid) might play a important role in the reduction of *B. cereus* contaminated in the RTE vegetables and cereals.

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