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# Bactericidal Effects of CaO (Scallop-Shell Powder) on Foodborne Pathogenic Bacteria

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This study was investigated the bactericidal effects of calcium oxide (CaO) on three common foodborne pathogenic bacteria: *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhimurium*. Each bacteria level was determined in a CaO solution (0.01, 0.03, 0.05, 0.10, 0.15, and 0.20% [w/v]) exposed for either15 sec, 30 sec, 1 min, 2 min, 3 min, 5 min, 10 min, or 30 min. All three bacteria were not greatly affected by CaO solutions at concentrations of 0.01 and 0.03%, however, the decline of *E. coli* (99%; 2.78 $\log_{10}$CFU/mL), *L. monocytogens* (45%; 1.44 $\log_{10}$CFU/mL), and *S. typhimurium* (70%; 2.08 $\log_{10}$CFU/mL) was greatest when they were exposed to 0.05% CaO solution for 10 min. Moreover, the bactericidal action of CaO was maintained for at least 24 h of storage. The results of this study provide evidence that CaO, as a substitute for synthetic chemical substances has potential for use in the disinfection and sanitization of foods and food processing equipment.

**Key words:** CaO, Bactericidal effects, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurun*

## INTRODUCTION

Approximately 10,000 Korean people suffered from food borne illnesses in 2004. Therefore, attention to the existing physical, chemical, and biological methods used to control food borne pathogens has become increasingly important, especially in food processing plants (Cho *et al.*, 2004, 2005; Lim *et al.*, 2005). The physical methods available include high voltage pulsed electric fields (Kim *et al.*, 2003; Qin *et al.*, 1995), oscillating magnetic fields, high hydrostatic pressures (Kalchayanand *et al.*, 1994), sonication, and microwave treatments (Park *et al.*, 2004; Shin and Pyun, 1997). Chemical methods in use include disinfectants and sanitizers such as alcoholic compounds (Shin *et al.*, 2001), quaternary ammonium compounds, iodopores, acid/alkali solutions (Kim *et al*, 2001a, 2001b; Shin *et al.*, 2001) and surfactants. Most food processing plants tend to avoid using synthetic chemical substances over concerns about the toxicity and safety (Jin *et al.*, 1995) although the chemical methods are popular because of their convenience and cost. Therefore, increasing demands for alternative and preferably natural biological disinfectants and sanitizers to be used by food processing plants are on the rise.

Scallop shells are regarded as waste products because in the scallop harvesting districts of Korea, a great quantity of shells, when accumulated at the seaside, produces critical social and environmental problems such as the discharge of offensive odors and soil pollution from the heavy metals left in the residual mollusk viscera. Heat treatment at higher than 700, however, converts calcium carbonate ($CaCO_3$), the main component of scallop shells, to calcium oxide (CaO) which is known to possess antibacterial properties (Sawai *et al.*, 1996a, 1995, 1996b, 1997, 1999, 2003., 2001a, 2001b). Therefore, we have examined the possible bactericidal effects of CaO on pathogenic bacteria for the potential use of CaO as a practical disinfectant and sanitizer of foods and food processing equipments.

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## MATERIALS AND METHODS

### Test bacteria

Microflora consisting of *Escherichia coli* ATCC 10536, a cocktail of *Listeria monocytogenes* (ATCC 19112, 19113, and 19115), and *Salmonella typhimurium* resistant to novobiocin (NO) and nalidixic acid (NA) were used to test the bactericidal effects of CaO (Sufcera Chemicals, Tokyo, Japan). Each strain of bacteria was maintained at -70°C in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, U.S.A.) containing 50% glycerol until use; each strain was thawed at room temperature and evenly spread on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 12 to 24 h.

### Determination of CaO effects

CaO was added to sterile distilled water in differing volumes to obtain six different concentrations (0.01, 0.03, 0.05, 0.10, 0.15, and 0.20% [w/v]) mixed with a magnetic stirrer. The following amounts of microflora were inoculated in the CaO solution: 2.88 $log_{10}$CFU/mL of *E. coli*, 3.18 $log_{10}$CFU/mL of *L. monocytogens*, and 3.7 $log_{10}$CFU/mL of *S. typhimurium* NO/NA. Each strain was exposed in the CaO solution for 15 sec, 30 sec, 1 min, 2 min, 3 min, 5 min, 10 min, or 30 min. After each exposure, 50 mL of the CaO solution was plated as pouring on TSA (tryptic soy agar) plate. The amounts of each surviving strain were determined by colony counting and the results expressed as CFU/mL; the data were converted to values of $log_{10}$CFU/mL.

### Determination of CaO stability for potential bactericidal effects

CaO was added to sterile distilled water to obtain three different concentrations (0.05, 0.10, and 0.20% (w/v)) and mixed with a magnetic stirrer. The CaO solutions were stored at room temperature (25°C) for two days. Five point thirty three $log_{10}$CFU/mL of *E. coli* ATCC 10536 were exposed to the CaO solution for five min immediately and thereafter every 3, 6, and 12 h of storage. Fifty μL of the CaO solution were pour-plated on a TSA plate after every 3, 6, and 12 h. The number of surviving *E. coli* colonies were counted and the results expressed as CFU/mL; the data were converted to values of $log_{10}$CFU/mL.

## RESULTS AND DISCUSSION

### Bactericidal effects of CaO

The bactericidal action of the CaO solutions against *E. coli* at room temperature are shown in Fig. 1. The reduction rate of *E. coli* colonies appeared to increase as the concentrations of CaO solutions increased of and as exposure time increased. *E. coli* was not reduced by CaO solutions at the lower concentrations of 0.01 and 0.03%. The overall decrease of *E. coli* when immersed in the 0.05% of CaO solution for 10 min, 0.10% of CaO solution for 5 min, and over 0.15% of CaO solution for 2 to 3 min, were 99% (2.78 $log_{10}$CFU/mL), 80% (2.18 $log_{10}$CFU/mL), and more than 80-90% (2.18-2.48 $log_{10}$CFU/mL), respectively.

The bactericidal action of the CaO solutions against *L. monocytogenes* at room temperature is shown in Fig. 2. The reduction of *L. monocytogenes* also appeared to increase as the concentrations of CaO solution increased and exposure times increased. *L. monocytogenes* was not reduced by CaO solutions at the lower concentrations of 0.01 and 0.03%. The overall decrease of *L. monocytogenes* immersed in the 0.05% of CaO solution for 10 min, 0.10% of CaO solution for 5 min, and over 0.15% of CaO solution for 2 to 3 min were 45% (1.44 $log_{10}$CFU/mL), 40% (1.36 $log_{10}$CFU/mL), and 30-50% (0.99-1.57 $log_{10}$CFU /mL), respectively. The decreases of *L. monocytogenes* colonies were less than those of *E. coli* and *S. typhimurium* when the CaO effects were evaluated for bacterial survival. *L. monocytogenes* appeared to be more resistant to CaO solutions than *E. coli* and *S. typhimurium*.

The bactericidal action of the CaO solution against *S. typhimurium* at room temperature is shown in Fig. 3. The same responses as identified for *E. coli* and *L. monocytogenes* to concentrations of CaO solutions and exposure times also appeared to be the case for the decreases of *S. typhimurium* i.e. increased as the concentrations of CaO solution and exposure times increased. *S. typhimurium* colonies were not reduced by CaO solution at concentrations of 0.01 and 0.03%. The overall decreases of *L. monocytogenes* immersed in the 0.05% of CaO solution for 10 min, 0.10% of CaO solution for 5 min, and over 0.15% of CaO solution for 2 to 3 min were 70% (2.08

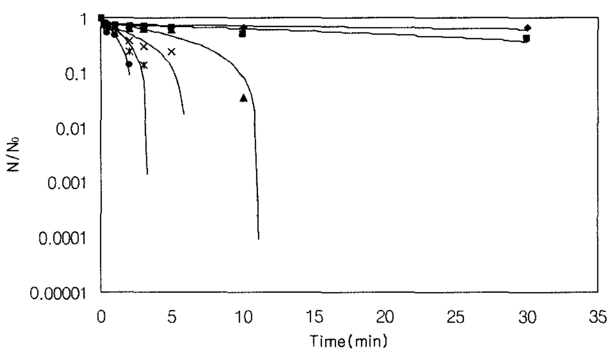


**Fig. 1.** Effects of CaO concentrations on the survivability of *E. coli*. Effects of the CaO concentrations (0.01% (◆), 0.03% (■), 0.05% (▲), 0.10% (×), 0.15% (∗), 0.20% (◆)) on survival of *E. coli* were compared. The ordinate is the ratio of *E. coli* cfu post-treatment (N) divided by non-treated cfu (No) to represent the survival ratio; the abscissa is the time of exposure.

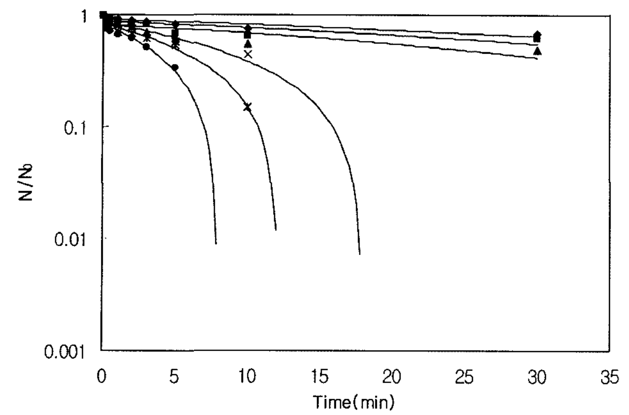


**Fig. 2.** Effects of CaO concentrations on the survivability of *L. monocytogenes*. Effects of CaO concentrations (0.01% (◆), 0.03% (■), 0.05% (▲), 0.10% (×), 0.15% (∗), 0.20% (◆)) were compared. The ordinate was the ratio of *L. monocytogenes* cfu post-treatment (N) divided by non-treated cfu (No) and represented the survival ratio.

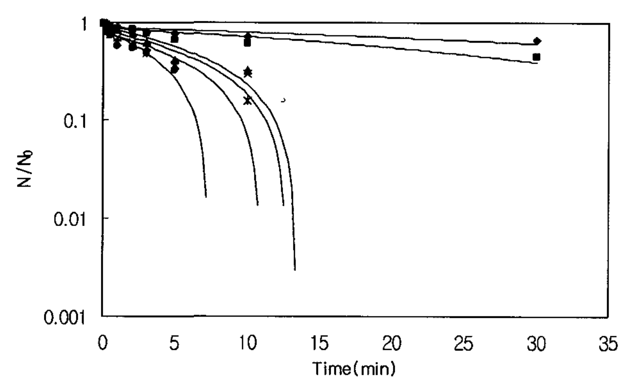


**Fig. 3.** Effects of CaO concentrations on the survivability of *S. typhimuriuim*. Effects of CaO concentrations ((0.01% (◆), 0.03% (■), 0.05% (▲), 0.10% (×), 0.15% (∗), 0.20% (◆)) were compared. The ordinate was the ratio of *S. typhimurium* cfu post-treatment (N) divided by non-treated cfu (No) and represented the survival ratio.

$log_{10}$CFU/mL), 60% (1.93 $log_{10}$CFU/mL), and 40-50% (1.31-1.59 $log_{10}$CFU/mL), respectively.

The pH values of CaO solutions at each of the six concentrations approximated 11-12. Increases in the CaO solution concentrations were accompanied by increases in the pH of the CaO solution (data not shown), suggesting that the bactericidal action of CaO is a pH effect (Catalano *et al.*, 1994; Kinner and Moats, 1981; Laird *et al.*, 1991; Pearson *et al.*, 1987; Sawai *et al.*, 2001a; Southam *et al.*, 1987). Moreover, the bactericidal actions of CaO may just as likely reflect the action of activated oxygen generated from CaO (Sawai *et al.*, 1996a; Sawai *et al.*, 2001a).

### CaO stability for bactericidal effects

The stability of the CaO solutions against *E. coli* at room temperature during two days of storage are shown in Fig.

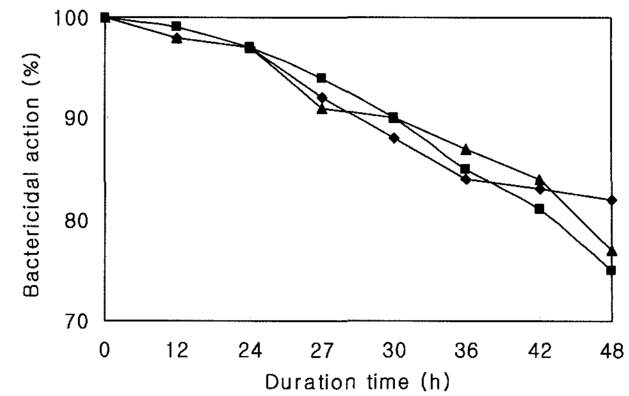


**Fig. 4.** The stability of diluted CaO solutions against *E. coli* during two days of storage. The stability of CaO solutions (0.05% (◆), 0.10% (■), 0.20% (▲)) against *E. coli* was compared according to their storage times. The bactericidal action of CaO was expressed as 100% at the initiation of storage.

4. Bactericidal actions of the CaO solution steadily decreased over the time of storage and most profoundly decreased after 24 h of storage. This observation suggests that CaO solutions for sanitization should be used within 24 h of preparation. Based on the results of this study, CaO would be expected to be used as a practical disinfectant and sanitizer for foods and for food processing equipment. The optimum concentration of CaO and the exposure time for sanitation were essentially 0.05% and 10 min, respectively. The treatment protocol of 0.05% of CaO for 10 min had some merits such as no residual effects, low cost, and reasonable decreases of bacteria. The CaO solutions should be used within 24 h for the greatest bactericidal effects. Therefore, we suggest that CaO can be used as a practical disinfectant and sanitizer for foods and food processing equipments.

## REFERENCES

Catalano, V. R. and Knabel, S. J., Incidence of *Salmonella* in Pennsylvania egg processing plants and destruction by high pH. *J. Food Prot.*, 57, 587-591 (1994).

Cho, S. A., Lee, I. S., Park, J. H., Seok, S. H., Lee, H. Y., Kim, D. J., Back, M. W., Lee, S. H., Hur, S. J., Ban, S. J., Lee, Y. K., and Park, J. H., Safety and immunogenicity of *Salmonella enterica serovar typhimurium* LlaB in mice. *J. Microbiol. Biotechnol.*, 15, 609-615 (2005).

Cho, S. Y., Park, B. K., Moon, K. D., and Oh, D. H., Prevalence of *Listeria monocytogenes* and related species in minimally processed vegetables. *J. Microbiol. Biotechnol.*, 14, 515-519 (2004).

Jin, S. K., Song, D. J., Lee, H. G., Kim, Y. G., Park, T. S., and Park, G. B., Effects of sodium lactate addition and lactic acid dipping on the cooking loss, salt, nitrite content, pH, WHC, water activity of sausage. *Kor. J. Animal. Sci.*, 37, 379-386

(1995).

Kalchayanand, N., Sikers, T., Dunne, C. P., and Ray, B., Hydrostatic pressure and electroporation have increase bactericidal efficiency in combination with bacteriocins. *Appl. Environ. Microbiol.,* 60, 4174-4177 (1994).

Kim, K. T., Kim, S. S., Hong, H. D., Ha, S. D., and Lee, Y. C., Quality changes and pasteurization effects of citrus fruit juice by high voltage pulsed electric fields (PEF) treatment. *Kor. J. Food Sci. Technol.,* 35, 635-641 (2003).

Kim, W. J., Shin, S. Y., and Hwang, H. J., Inhibitory Effects of acetic acid and temperature on growth of *campylobacter jejuni* ATCC 33291. *J. Microbiol. Biotechnol.,* 11, 934-939 (2001).

Kim, S. Y., Lee, Y. M., Lee, S. Y., Lee, Y. S., Kim, J. H., Ahn, C., Kang, B. C., and Ji, G. E., Synergistie effect of citric acid and pediocin K1, a bacteriocin produced by *pediococcus* sp. K1, on inhibition of *Listeria monocytogenes*. *J. Microbiol. Biotechnol.,* 11, 831-837 (2001).

Kinner, J. A. and Moats, W. A., Effect of temperature, pH and detergent on the survival of bacteria associated with shell eggs. *Poultry Sci.,* 60, 761-767 (1981).

Laird, J. M., Bartlett, F. M., and Mckellar, R. C., Survival of *Listeria monocytogenes* in egg washwater. *Int. J. Food Microbiol.,* 12, 115-122 (1991).

Lim, M. S., Lee, M. H., Lee, J. H., Ju, H. M., Park, N. Y., Jeong, H. S., Rhee, J. E., and Choi, S. H., Identification and characterization of the *Vibrio vulnificus* malPQ operon. *J. Microbiol. Biotechnol.,* 15, 616-625 (2005).

Park, J. C., Park, B. J., Han, D. W., Lee, D. H., Lee, I. S., Hyun, S. O., Chun, M. S., Chung, K. H., Aihara, M., and Takatori, K., Fungal sterilization using microwave-induced argon plasma at atmospheric pressure. *J. Microbiol. Biotechnol.,* 14, 188-192 (2004).

Pearson, J, Southam, G. G., and Holley, R. A., Survival and transport of bacteria in egg washwater. *Appl. Environ. Microbiol.,* 53, 2060-2065 (1987).

Qin, B. L., Pothakamurry, U. R., Vega, H., Martin, O., Babosa-Canovas, G. V., and Sanson, B. G., Food pasteurization using high- intensity pulsed electric fields. *Food Technol.,* 49, 55-60 (1995).

Sawai, J., Kawada, E., Kanou, F., Igarashi, H., Hashimoto, A., Kokugan, T., and Shimizu, M., Detection of active oxygen generated from ceramic powders having antibacterial activity. *J. Chem. Eng. Japan,* 29, 627-633 (1996).

Sawai, J., Igarashi, H., Hashimoto, A., Kokugan, T., and Shimizu, M., Evaluation of growth inhibitory effect of ceramics powder slurry on bacteria by conductance method. *J. Chem. Eng. Japan,* 28, 288-293 (1995).

Sawai, J., Igarashi, H., Hashimoto, A., Kokugan, T., and Shimizu, M., Effect of particle size and heating temperature of ceramic powders on antibacterial activity of their slurries. *J. Chem. Eng. Japan,* 29, 251-256 (1996).

Sawai, J., Kojima, H., Igarashi, H., Hashimoto, A., Shoji, S., Takehara, A., Sawaki, T., Kokugan, T., and Shimizu, M., *Escherichia coli* damage by ceramic powder slurries. *J. Chem. Eng. Japan,* 30, 1034-1039 (1997).

Sawai, J., Kojima, H., Igarashi, H., Hashimoto, A., Shoji, S., and Shimizu, M., Bactericidal action of calcium oxide powder. *Trans. Mater. Res. Soc. Japan,* 24, 667-670 (1999).

Sawai, J., Miyoshi, H., and Kojima, H., Sporicidal kinetic of *Bacillus subtilis* spore by heated scallop shell powder. *J. Food Prot.,* 66, 1482-1485 (2003).

Sawai, J., Shiga, H., and Kojima, H., Kinetic analysis of the bactericidal action of heated scallop-shell powder. *Int. J. Food Microbiol.,* 71, 211-218 (2001).

Sawai, J., Satoh, M., Horikawa, M., Shiga, H., and Kojima, H., Heated scallop-shell powder slurry treatment of shredded cabbage. *J. Food Prot.,* 64, 1579-1583 (2001).

Shin, J. K. and Pyun, Y. R., Inactivation of Lactobacillus plantrum by pulsed-microwave irradiation. *J. Food Sci.,* 62, 163-166 (1997).

Shin, S. Y., Hwang, H. J., and Kim, W. J., Inhibition of *Campylobacter jejuni* in chicken by ethanol, hydrogen peroxide, and organic acids. *J. Microbiol. Biotechnol.,* 11, 418-422 (2001).

Southam, G., Pearson, J., and Holley, R. A., Survival and growth of *Yersinia enterocolirrca* in egg washwater. *J. Food Prot.,* 50, 103-107 (1987).