**Sorbitol Minimizes Calcium Carbonate Scale Generation While Maintaining the Disinfection Effect of Heated Scallop-Shell Powder for Fresh Produce**

YURI NOMOTO, SHUN SAWADA, SHINYA ABE, JIN WAKAZAWA, MIKIO KIKUCHI, AND JUN SAWAI

Faculty of Applied Bioscience, Kanagawa Institute of Technology, 1030 Shimo-Ogino, Atsugi, Kanagawa 243-0292, Japan

Received 24 June, 2017/Accepted 26 October, 2017

Scallop shells subjected to heat treatment exhibit antimicrobial activity, and heated scallop-shell powder (HSSP) has recently been reported to be effective for disinfecting food. However, because the main component of these shells is calcium oxide, there is a problem that scales of calcium carbonate ($\text{CaCO}_3$) become established on the surface of equipment used for food processing. In this study, we thus investigated whether the addition of sugar to HSSP slurry suppressed CaCO$_3$ scale generation and whether the sugar-supplemented HSSP could be applied to the disinfection and preservation of fresh lettuce. The results showed that glucose, sucrose, and sorbitol could suppress the scale generation in HSSP slurry. However, glucose and sucrose decreased the antibacterial activity of HSSP. Since the addition of sorbitol did not affect the antibacterial activity of HSSP slurry, it was used for subsequent experiments because of its low bioavailability. Sorbitol effectively suppressed scale formation by dissolving it before the addition of HSSP. The disinfection and preservative effects of sorbitol-supplemented HSSP (S-HSSP) treatment on lettuce did not decrease compared with those upon HSSP treatment and were almost equal to or higher than those of sodium hypochlorite treatment at 200 mg/l. The addition of sorbitol solved the major problem of scale generation by HSSP containing CaO, which contributes to expansion of usage of heated shell powder, such as HSSP, in food processing.

Key words: Heated scallop-shell powder / Calcium oxide / Calcium carbonate scale generation / Lettuce / Sorbitol.

**INTRODUCTION**

In Japan, the scallop catch in 2016 was approximately $4.3 \times 10^5$ tons, according to statistics from the Ministry of Agriculture, Forestry, and Fisheries. The edible portion of the scallop constitutes only about 20% of its total weight, leaving nearly $4 \times 10^5$ tons of shell waste. Although some parts of scallop shells are recycled as food additives and in plastering and paving materials, most of the shell is considered commercial waste. In scallop-harvesting regions, large numbers of shells are heaped on shore, creating serious problems such as offensive odors and soil pollution from heavy metals contained in the viscera (Ghimire et al., 2008; Marcus et al., 1988). However, previous studies reported that scallop-shell powder heated at temperatures of at least 700°C exhibited antibacterial activity against vegetative cells of bacteria. The main component of scallop shells is CaCO$_3$, and its antibacterial activity is due to the generation of CaO through heat treatment (Sawai et al., 1995; 2001a; 2001b). The antibacterial activity of the powder heated at 1000°C was comparable to that of pure CaO. When all of the Ca (70.8 wt%) existed as CaO, shells heated at 1000°C contained 99% CaO by weight (Sawai et al., 2001a). Therefore, the use of heated scallop-shell powder (HSSP) in food processing can provide a source of minerals and prolong the shelf life of foodstuffs. Besides scallop shells, the shells of oyster (Bari et al., 1999; Fransisca et al. 2011), surf
calm (Okawa et al., 2000), and mussel (Li et al., 2014) were also reported to exert antimicrobial activity by heat treatment.

HSSP was reported to exhibit antimicrobial activity against bacteria (Bae et al., 2006; Ro et al., 2015, 39), fungi (Sawai and Shiga, 2006; Xing et al., 2013), heat-resistant bacterial spores (Sawai et al., 2003, 2011), and viruses (Thammakarn et al., 2014; 2015). In addition, HSPP treatment killed and eliminated bacterial biofilms as well as planktonic cells (Bodur and Cagri-Mehmetoglu, 2012; Kubo et al., 2013; Sawai et al., 2013; Shimamura et al., 2015). Recently, nanoparticles of HSSP were also shown to have higher bactericidal, sporicidal, and virucidal effects than microparticles of HSSP (Jeong et al., 2007; Watanabe et al., 2014). HSSP was also applied to actual food, and was shown to disinfect fresh vegetables and sprouts (Kim et al., 2011; Mamun et al., 2012; Sawai et al., 2001c), frankfurters (Bodur et al., 2010), chicken (Cagri-Mehmetoglu, 2011), frozen meat (Ro et al., 2015), and fresh whole fish (Ahmed et al., 2015).

As mentioned above, although the effectiveness of HSSP in the disinfection of food has been reported, in actual use, heated shell powder including HSSP is associated with the problem of the generation of scales of CaCO₃ on the surface of the equipment used for processing because CaO is the main component of the heated shell powder. The scales of CaCO₃ generated by the reaction of CaO with CO₂ in the air, have no antimicrobial activity, cause an unpleasant appearance, result in resisting heat transfer and fluid, and can also be a hotbed for biofilm formation via the adherence of microorganisms. Therefore, there is a need to suppress scale generation.

CaO accounts for approximately 60% of the volume of cement (Jun et al., 2015). It has long been reported that sugars can be utilized to control the coagulation (hardening) of cement in cement makers (Tomas and Brichall, 1983). Saccharide reactions and interactions at hydrating inorganic oxide surfaces significantly influence diverse surface chemistry processes, including carbonate formation. Saccharides are often used to slow hydration processes or alter the rheological properties of cement-water mixtures, commonly referred to as cement “ slurries” (Bishop and Barron, 2006; Ghio et al., 1994; Peschard et al., 2006; Smith et al., 2011, 2012). Based on these findings, the objective of this study was to investigate whether the addition of sugars as food additives could suppress the generation of CaCO₃ scales in HSSP slurry and whether the sugar-supplemented HSSP slurry could be applied to disinfect and preserve fresh food.

**MATERIALS AND METHODS**

**Preparation of HSSP**

Powder from scallop shells (*Patinopecten yessoensis*) was obtained from Soycom Co. Ltd. (Atsugi, Japan). The powder was heated with an electric oven (F-120-SP; TGK, Tokyo, Japan) in air (1000°C, 1 h) and ground in a ball mill. The mean particle size of the HSSP was approximately 5 µm and it was stored in a desiccator before use.

**Observation of CaCO₃ scale generation**

HSSP and a sugar were added simultaneously to a 100-ml beaker containing 40 ml of pure water. The sugars were used as shown in Table 1. The concentration of HSSP was constant at 5 mg/ml (0.5%), while the sugar concentration was varied. The uncovered beakers were left at 25°C±2°C. After 12-72 h, the slurry was discarded. The inside wall of the beaker was rinsed three times with pure water, and the generation of scales was observed. Erythritol was donated by Mitsubishi-Kagaku Foods Co. (Tokyo, Japan) and the other sugars were purchased from Wako Pure Chemicals Co. Ltd.

**TABLE 1. Effect of sugar addition on scale formation in HSSP slurry.***

| Sugar | Molar mass (g/mol) | Concentration (mol/l) |
|-------|-------------------|-----------------------|
|       |                   | 0.01 0.1 0.2 0.3 0.4 0.5 0.7 1.0 |
| Glucose | 180.2         | ±  -  -   -  ±  +  +  +  +  +  + |
| Sucrose | 342.3         | +  +  +  +  +  -  -  -  -  -  - |
| Xylitol | 152.2         | ++ ++ ++ ++ ++ ++ ++ +  +  + |
| Erythritol | 122.1    | +  +  +  +  +  +  +  +  +  +  +  |
| Sorbitol | 182.2        | +  +  +  -  -  -  -  -  -  -  -  |

*HSSP (5 mg/ml) and sugar were simultaneously added to 40 ml of pure water. HSPP slurry containing sugar was left at 25°C for 72 h. Abbreviations: HSSP, heated scallop-shell powder.

- : no scale formation
+ : scale formation
++ : scale formation (strong)
Sorbitol inhibits scale in CaO slurry

To investigate the influence of the timing of addition of sorbitol, the sorbitol was added and dissolved completely in pure water in 6 min, followed by the addition of HSSP after 6 min. Alternatively, HSSP was added to pure water, followed by the addition of sorbitol 1, 2, or 8 h later. Then, the generation of scales was observed as described above.

A pH probe (S220 Seven Compact; Mettler-Toledo International Inc., Greifensee, Switzerland) was inserted into the slurry and used for continuous monitoring of the pH until it was confirmed to have reached a steady state; then, the pH of the slurry was recorded.

Antibacterial test of sugar-supplemented HSSP

Escherichia coli NBRC 3306 was used as the test strain. The bacteria were stored in 10% glycerol solution at −85°C. Prior to the experiment, they were thawed and pre-incubated in nutrient broth (Eiken Chemicals Co. Ltd., Tokyo, Japan) at 37°C for 24 h. The pre-incubated E. coli cells were washed twice (4,000 × g, 5 min) and resuspended in sterile 0.85% saline to a concentration of 10⁶ CFU/ml.

HSSP and a sugar shown in Table 1 were added at the same time to a 100-ml beaker containing 40 ml of sterile pure water. A portion of the bacterial suspension (0.1 ml) was inoculated into a test tube containing 10 ml of the as-prepared sorbitol-HSSP slurry. The experiment was conducted at 35°C. At intervals, a sample (0.1 ml) was withdrawn, diluted with saline and spread onto nutrition agar (Eiken Chemicals) in duplicate, and viable colonies were counted after incubation at 37°C for 24 h.

Sorbitol-added HSSP (S-HSSP) treatment for fresh vegetables

Lettuce was purchased from a city supermarket, and several of its outer leaves were removed. The lettuce leaves were cut into slices, approximately 2 mm wide, with a slicer disinfected with ethanol. Two stainless vessels containing 3 liters of sterile pure water were set around the beaker discontinuously or continuously even after rinsing. The case where the scale formation was observed as no scale formation was defined as no scale formation (-). On the other hand, the scale formation (+) or scale formation (strong: +++) was used when the scale was formed around the beaker discontinuously or continuously even after rinsing, respectively. Here HSSP and sugar were simultaneously added to pure water. The addition of glucose, sucrose, or sorbitol clearly suppressed scale generation, but xylitol did not. The effect of erythritol was very weak.

Table 1 shows the presence or absence of scale generation of HSSP slurry when each type of sugar was added. The case where the scale on the beaker wall was completely removed by rinsing with pure water (3 times) was defined as no scale formation (-). On the other hand, the scale formation (+) or scale formation (strong: +++) was used when the scale was formed around the beaker discontinuously or continuously even after rinsing, respectively. Here HSSP and sugar were simultaneously added to pure water. The addition of glucose, sucrose, or sorbitol clearly suppressed scale generation, but xylitol did not. The effect of erythritol was very weak.

Table 2 shows the effects of glucose and sucrose, for which the suppression of scale generation was observed as shown in Table 1, on the antibacterial activity of HSSP against E. coli. HSSP alone decreased the survival ratio to below the detection limit within 20 s. However, the addition of glucose or sucrose caused a significant reduction of the antibacterial activity of HSSP (p < 0.05).
Y. NOMOTO ET AL.

TABLE 2. Effect of addition of glucose, sucrose and sorbitol on antibacterial activity of HSSP against E. coli at 37°C.*

| Sugar       | Concentration (%, w/v) | Survival ratio (–) | 0 s | 20 s | 40 s | 80 s | 120 s | 240 s | 480 s |
|-------------|------------------------|--------------------|-----|------|------|------|-------|-------|-------|
| (HSSP: control) | 0                      | 1.00               | N.D.| N.D. | N.D. | N.D. | N.D.  | N.D.  | N.D.  |
| Glucose     | 1.8                    | 0.1                | 1.00| 0.20±0.06 | 0.046±0.013 | N.D. | N.D.  | N.D.  | N.D.  |
|             | 5.4                    | 0.3                | 1.00| –    | –    | –    | 0.14±0.09 | 0.09±0.03 | N.D.  |
|             | 18.0                   | 1.0                | 1.00| –    | –    | –    | 0.88±0.07 | 0.50±0.10 | 0.16±0.14 |
| Sucrose     | 3.4                    | 0.1                | 1.00| N.D. | N.D. | N.D. | N.D.  | N.D.  | N.D.  |
|             | 17.1                   | 0.5                | 1.00| –    | –    | –    | 0.10±0.15 | 0.050±0.022 | N.D.  |
| Sorbitol    | 1                      | 0.054              | 1.00| N.D. | N.D. | N.D. | N.D.  | N.D.  | N.D.  |
|             | 1.8                    | 0.1                | 1.00| N.D. | N.D. | N.D. | N.D.  | N.D.  | N.D.  |
|             | 5                      | 0.27               | 1.00| N.D. | N.D. | N.D. | N.D.  | N.D.  | N.D.  |
|             | 5.5                    | 0.3                | 1.00| N.D. | N.D. | N.D. | N.D.  | N.D.  | N.D.  |
|             | 10                     | 0.54               | 1.00| N.D. | N.D. | N.D. | N.D.  | N.D.  | N.D.  |
|             | 18.2                   | 1.0                | 1.00| –    | –    | –    | N.D.  | N.D.  | N.D.  |

*HSSP (5mg/ml) and sugar were simultaneously added to 10 ml of pure water. Abbreviation: HSSP, heated scallop-shell powder.
N.D.: Not detectable (<2-log10 CFU/ml)
– : Not done

TABLE 3. Effect of sorbitol addition on scale formation in HSSP slurry and pH of the slurries*.

| Addition of sorbitol (%) | pH | Scale formation |
|--------------------------|----|-----------------|
| 0 (= HSSP only**)        | 12.5 | ++             |
| 1                        | 0.05 | 12.6            | +   |
| 2                        | 0.11 | 12.6            | +   |
| 3                        | 0.16 | 12.6            | +   |
| 4                        | 0.22 | 12.6            | +   |
| 5                        | 0.27 | 12.5            | -   |
| 10                       | 0.55 | 12.5            | -   |
| 15                       | 0.82 | 12.3            | -   |
| 20                       | 1.10 | 12.3            | -   |
| 25                       | 1.37 | 12.2            | -   |

*HSSP (5 mg/ml) and sugar were simultaneously added to 40 ml of pure water. HSSP slurry containing sugar was left at 25°C for 72 h. Abbreviation: HSSP, heated scallop-shell powder.
- : no scale formation
+ : scale formation
++ : scale formation (strong)

not change the antibacterial activity of HSSP against E. coli at all concentrations up to 10%. E. coli was confirmed at the sucrose concentration of 17.1% (=0.5 M) in 120 s, but below the detection limit at sorbitol concentration of 18% (=1.0 M) in 120 s. Because glucose and sucrose are readily used as substrates for the growth of microorganisms, in contrast to sorbitol, sorbitol was used in the subsequent study.

Scale generation and antibacterial activity of S-HSSP

Table 3 shows the effects of sorbitol concentration on the scale generation of HSSP slurry and the pH. The values of pH of the HSSP slurry hardly changed up to a sorbitol concentration of 10% and declined below 12.3 at a concentration of over 15%. The addition of sorbitol at a concentration of 5% or higher inhibited scale generation. Photographs of the scale generation associated with the HSSP slurry are shown in Fig.1. Scales were generated at a high level at the interface of the HSSP slurry without sorbitol on the inner wall of the beaker (Fig.1A). Rinsing with water could not remove them. Upon the addition of sorbitol at a concentration of 1%, the scales remarkably decreased (Fig.1B). Although there was almost no change in the effect of sorbitol up to a concentration of 4% (Figs.1C and 1D), no generation of scales was observed at 5% or more (Figs.1E and 1F).

Next, we examined the influence of the timing of sorbitol addition on the scale generation in HSSP slurry (Table 4). Adding sorbitol at the same time as or before HSSP inhibited the scale generation. However, when sorbitol was added 1 h after the addition of HSSP to water, its suppressive effect on scale generation did not appear. Conversely, when HSSP was added after
completely dissolving sorbitol in advance, the scale formation was suppressed even at a sorbitol concentration of 1%.

Disinfection and preservation effects of S-HSSP treatment on lettuce

Table 5 shows the disinfection and preservation effects of each treatment (water-washing, NaClO, HSSP, and S-HSSP) on lettuce. The values upon storage on day 0 were used as the numbers of aerobes and coliforms immediately after each treatment. Although water-washing treatment caused a reduction of the coliforms of approximately one order of magnitude, the aerobe counts hardly decreased. Meanwhile, a significant reduction of more than two orders of magnitude for aerobes was confirmed following NaClO (200 mg/l), HSSP (0.5%), or S-HSSP [HSSP (0.5%) + sorbitol (1%)] treatment, and the coliforms could not be detected immediately after these treatments (p < 0.05). There was no significant difference in aerobe counts among the NaClO, HSSP, and S-HSSP treatments until the 5th day during the storage period, but the aerobe counts of lettuce treated with NaClO increased significantly on the 7th day of storage. On the other hand, HSSP and S-HSSP treatments could keep the coliform counts below the detection limit, even on the 7th day of storage. S-HSSP treatment showed no significant decrease in the effects upon the addition of sorbitol (p > 0.05) and exhibited disinfection and preservation effects equal to or higher than those of treatment with NaClO at 200 mg/l. Changes in color for the S-HSSP treatment were indistinguishable from those of other treatments in the visual range after treatment and during storage.

**DISCUSSION**

Inhibition of the scale generation in HSSP slurry

The addition of sugars inhibited the generation of CaCO₃ scales in HSSP slurry. In particular, the addition of sorbitol could suppress scale formation without

| Elapsed time from the addition of HSSP (h) | HSSP conc. (mg/ml) | Sorbitol conc. (%, w/v) | Scale formation |
|------------------------------------------|--------------------|-------------------------|-----------------|
|                                          | 5                  | 0.1                     | 12 h           |
| -0.1                                     | 5 50               | 0.1 1                   | + + + +        |
|                                          |                    |                         | 24 h           |
|                                          |                    |                         |                |
|                                          |                    |                         | 72 h           |

Abbreviation: HSSP, heated scallop-shell powder.
- : no scale formation
+ : scale formation
++ : scale formation (strong)
/ : not done
lowering the antimicrobial activity of HSSP. Several mechanisms were proposed to explain the interactions between cement and set-retarders (i.e., sugars or carboxylic acids). Some researchers have focused their studies on interaction with anhydrous surfaces. For Hansen (1960), adsorption onto anhydrous particles could occur and protect surfaces from initial attack by water. Suzuki and Nishi (1959) proposed that the retarding action of admixtures could be linked to the precipitation of insoluble calcium salts at the surface of anhydrous particles. The mechanism behind the influence of set-retarding additives, such as saccharide, on cement hydration has yet to be well elucidated (Peschard et al., 2006). If sugar addition causes the precipitation of insoluble calcium salts at the surface of anhydrous particles, our study result that sorbitol must be added prior to HSSP for the inhibition of CaCO₃ scale generation is reasonable. The decrease in pH of HSSP slurry with 15% sorbitol as shown in Table 3 was also reasonable.

Among the three sugars, the reason why the superiority of sorbitol was high is not clear. Takeuchi et al. (1997) report that the apparent adsorption amount of sorbitol to cement was higher than that of glucose.

**Mechanism of action of CaO and S-HSSP**

The main factor behind the antimicrobial activity of HSSP containing CaO as its main component is considered to be the alkaline effect of hydration of CaO (Sawai et al., 2001a). The addition of sorbitol did not influence the antibacterial activity of HSSP. The reason why sorbitol did not decrease the antibacterial activity of HSSP is not known at the present stage. The antibacterial activities of HSSP containing 1.0 M sorbitol at the same pH (12.3) was hardly declined (Table 2). Kawamura and Takeo (1989) also reported that the addition of sorbitol did not alter the antimicrobial activity of catechin against *Streptococcus mutans*, but there was no mention of the mechanism. Elucidation of these interactions will be a subject for further study.

Some reports have described the damage to the cell envelope caused by CaO or heated shell powder (Fransisc et al., 2011). By using scanning electron microscopy, Roy et al. (2013) revealed that microbial cell structure integrity was lost after treatment with CaO. Specifically, CaO appeared to cause immediate and widespread change in cell morphology after its addition. Ro et al. (2015) also reported that most of the cells exposed to CaO died or lost their cellular integrity and membranes. The effect of the alkalinity caused by the hydration of CaO is regarded as a main factor behind

| Treatments | Aerobe counts (log₁₀ CFU/g) | Storage (d) |
|------------|-----------------------------|-------------|
|            | 0*                          | 3           | 5           | 7           |
| Initial viable counts (log₁₀ CFU/g): 5.7 ± 0.1* | 5.4 ± 0.1* | 5.7 ± 0.7* | 6.4 ± 0.8* | 6.9 ± 0.5* |
| Water Washing (20 min) | 5.4 ± 0.1* | 5.7 ± 0.7* | 6.4 ± 0.8* | 6.9 ± 0.5* |
| NaClO (200 mg/l, 20min) | 3.4 ± 0.5* | 2.6 ± 1.3* | 3.7 ± 0.3b | 5.1 ± 0.2d |
| HSSP (0.5 w/v%, 20 min) | 3.1 ± 1.2b | 3.0 ± 0.1b | 3.2 ± 0.0b | 3.2 ± 0.9b |
| S-HSSP (0.5 w/v% + sorbitol w/v1%, 20 min)** | 3.1 ± 1.6b | 3.7 ± 0.3b | 4.6 ± 1.0b | 3.8 ± 0.2b |

| Treatments | Coliform counts (log₁₀ CFU/g)** | Storage (d) |
|------------|---------------------------------|-------------|
|            | 0*                              | 3           | 5           | 7           |
| Initial coliform counts (log₁₀ CFU/g): 5.0 ± 0.2a | 3.6 ± 0.6b | 5.5 ± 0.7a | 6.1 ± 0.9a | 6.2 ± 1.0a |
| Water Washing (20 min) | 3.4 ± 0.5b | 5.5 ± 0.7a | 6.1 ± 0.9a | 6.2 ± 1.0a |
| NaClO (200 mg/l, 20min) | N.D.a | N.D.a | 3.1 ± 0.8b | 3.5 ± 0.4b |
| HSSP (0.5 w/v%, 20 min) | N.D.a | N.D.a | N.D.a | N.D.a |
| S-HSSP (0.5 w/v% + sorbitol w/v1%, 20 min)** | N.D.a | N.D.a | N.D.a | N.D.a |

Storage on 0 day indicates the number of aerobes and coliforms immediately after the each treatment.

**Sorbitol was dissolved before HSSP to sterile pure water.
N.D.: Not detectable (< 2-log₁₀ CFU/g)
Different small letters or big letters within a table indicate significant differences, respectively (p < 0.05).
Abbreviations: HSSP, heated scallop-shell powder. S-HSSP, sorbitol-added heated scallop-shell powder.
the antibacterial mechanism. The bactericidal activity was, however, greater than that of NaOH at the same pH (Sawai et al., 2001a). Similarly, Capita et al. (2002) tested two alkaline treatments [trisodium phosphate (TSP) and NaOH at a pH of 12.5] as disinfectants against Listeria on chicken wings. The results showed that TSP and NaOH killed only 3.24 and 3.28 log CFU/g of Listeria cells, respectively. On the other hand, HSSP (0.5%) at the same pH killed 3.6-4 log CFU/g, and thus could be more effective against Listeria than TSP or NaOH (Cagri-Mehmetoglu, 2011). One possible reason for the high disinfection efficacy of CaO is that the pH of the thin water layer formed around the particles is much higher than that of the equilibrated solution (Dong et al., 2010; Sugiyama et al., 1995). However, the change in E. coli sensitivity induced by the CaO treatment was different from that in the case of alkaline treatment of NaOH (Mendonca et al., 1994; Sawai et al., 1997). The generation of active oxygen species such as superoxide anions has been observed from CaO powder slurry (Sawai et al., 1996). The change in sensitivity of E. coli treated with CaO is consistent with that caused by active oxygen treatment (Sawai et al., 1999). The results of Hewitt et al. (2001) obtained by using multi-parameter flow cytometry also support these conclusions.

**Disinfectant of heated shell powder for fresh produce**

S-HSSP treatment could effectively disinfect lettuce and preserve the growth of aerobes or coliforms during the storage of treated lettuce. Many researchers described the effectiveness of heated seashell powders for disinfecting fresh produce, as described in the Introduction section.

Fukuyama et al. (2009) examined the effects of the disinfectants NaClO and calcinated calcium on the food-borne pathogens (E. coli O157:H7) attached to shredded cabbage leaves. In their study, about 2.6 and 3.5 log reductions of E. coli O157 were achieved by treatment with NaClO (100 mg/l, pH 6.0, 10 min) and calcinated calcium (0.1%, 20 min), respectively without apparent deteriorative effects. The bacterial numbers in the treated cabbage did not increase during storage at 4°C. In addition, just after treatment, the evaluation of the smell and taste of the cabbage was satisfactory or was at a level similar to that of the control at day 3 of storage. Kim et al. (2011) also revealed that washing in HSSP at normal top water temperature was effective at reducing the microbial population in fresh-cut lettuce samples. The samples treated with calcinated calcium had good quality with low off-odor at the end of storage. Thus, HSSP is suggested to be an environmentally friendly sanitizer for the washing of fresh-cut iceberg lettuce without affecting sensorial quality. Yoon et al. (2003) also investigated the effect of CaO on fresh produce. In their study, CaO treatment for 10 min resulted in 2-3 log reductions in E. coli O157:H7, L. monocytogenes, and S. typhimurium inoculated on lettuce, radish, sprout, and apple slices. These results closely matched the results in our study, and provided evidence that HSSP has the potential to be used as a powerful antimicrobial agent for treating fresh produce.

Although we did not conduct a sensory evaluation of lettuce upon S-HSSP treatment in this study, there are no reports of negative results from the sensory evaluation of fresh vegetables treated with heated shell powder, as mentioned above. Furthermore, it is not expected that serious problems would be encountered because washing with water is performed after S-HSSP treatment.

Because results were only obtained with lettuce in this study, we need to analyze this approach with other types of food to prove the effectiveness of S-HSSP. Many sanitizers have been shown to be unable to remove high microbial loads, showing that a raw material with a low initial microbial count is required to guarantee product safety (Escalona et al., 2015; Inatsu et al., 2016). It is also important to determine these limits for S-HSSP, which is a subject for future study.

**CONCLUSION**

The inhibition of scale generation by adding sorbitol will further expand the opportunity to use HSSP for improving the microbial quality and shelf life of food. The use of HSSP in food processing provides a source of minerals and prolongs the shelf life of foodstuffs. Moreover, reducing the amount of scallop-shell waste would reduce the problems with pollution related to this. Furthermore, different methods to reduce and/or replace the use of chlorine have already been developed, and the heated seashell powder including CaO as the main component is also an attractive alternative for maintaining fresh produce. At present, given the need to dissolve sorbitol before HSSP, the coating of sorbitol on HSSP is under investigation for further improvement of the handleability and moisture absorbency of HSSP.

**ACKNOWLEDGEMENTS**

The authors would like to thank Mitsubishi-Kagaku Foods Co. for the gift of erythritol.

**REFERENCES**

Ahmed, S., Akand, N. R., Islam, M. T., and Bari, M. L. (2015)
Effectiveness of scallop powder ice in reducing bacterial load on fresh whole fish and in the melted ice water. LWT-Food Sci. Technol., 64, 270-274.

Bae, D. H., Yeon, J. H., Park, S. Y., Lee, D. H., and Ha, S. D. (2006) Bactericidal effects of CaO (scallopsHELL powde) on foodborne pathogenic bacteria. Arch. Pharmacal. Res., 29, 298-301.

Bari, M. L., Kusunoki, H., Furukawa, H., Ikeda, H., Isshiki, K., and Uemura, I. (1999) Inhibition of growth of Escherichia coli O157:H7 in fresh radish (Raphanus sativus L.) sprout production by calcinated calcium. J. Food Prot., 62, 128-132.

Bishop, M., and Barron, A. R. (2006) Cement hydration inhibition with sucrose, tartaric acid, and lignosulfonate: analytical and spectroscopic study. Ind. Eng. Chem. Res., 45, 7042-7049.

Bodur, T., and Cagri-Mehmetoglu, A. (2012) Removal of Listeria monocytogenes, Staphylococcus aureus and Escherichia coli O157: H7 biofilms on stainless steel using scallop shell powder. Food Cont., 25, 1-9.

Bodur, T., Yaldırık, G., Kola, O., and Cagri-Mehmetoglu, A. (2010) Inhibition of Listeria monocytogenes and Escherichia coli O157: H7 on frankfurters using scallop-shell powder. J. Food Saf., 30, 740-752.

Cagri-Mehmetoglu, A. (2011) Inhibition of Listeria monocytogenes and Salmonella enteritidis on chicken wings using scallop-shell powder. Poultry Sci., 90, 2600-2605.

Capita, R., Alonso-Calleja, C., del Camino Garcia-Fernandez, M., and Moreno, B. (2002) Activity of trisodium phosphate compared with sodium hydroxide wash solutions against Listeria monocytogenes attached to chicken skin during refrigerated storage. Food Microbiol., 19, 57-63.

Dong, C., Caireney, J., Sun, O., Maddan, O. L., He, G., and Deng, Y. (2010) Investigation of Mg(OH)2 nanoparticlles as an antibacterial agent. J. Nanopart. Res., 12, 2101-2109.

Escalaona, V. H., Hinojosa, A., Char, C., Villena, P., Bustamante, A., and Saenz, C. (2015) Use of alternative sanitizers on minimally processed watercress harvested in two different seasons. J. Food Process. Preserv., 39, 1267-1289.

Fransica, L., Zhou, B., Park, H., and Feng, H. (2011) The effect of calcinated calcium and chlorine treatments on Escherichia coli O157:H7 population reduction in radish sprouts. J. Food Sci., 76, M404-M412.

Fukuyama, S., Watanabe, Y., Kondo, N., Nishinomiya, T., Kawamoto, S., Isshiki, K., and Murata, M. (2009) Efficiency of sodium hypochlorite and calcinated calcium in killing Escherichia coli O157: H7, Salmonella spp., and Staphylococcus aureus attached to freshly shredded cabbage. Biosci. Biotechnol. Biochem., 73, 9-14.

Ghimire, K. N., Hai, H., Inoue, K., Ohto, K., Kawakita, H., Harada, H., and Morita, M. (2008) Heavy metal removal from contaminated scallop waste for feed and fertilizer application. Biosourc. Technol., 99, 2436-2441.

Ghio, V. A., Monteiro, P. J., and Demsetz, L. A. (1994) The rheology of fresh cement paste containing polysaccharide gums. Cem. Concr. Res., 24, 243-249.

Hansen, W. C. (1960) Actions of calcium sulfate and admixtures in Portland cement pastes. In Proc. the Symposium on Effect of Water-Reducing Admixtures and Set-Retarding Admixtures on Properties of Concrete, San Fransico, pp. 3-37, West Conshohocken, PA.

Hewitt, C. J., Bellara, S. R, Andreani, A., Nebe-von-Caron, G., and McFarlane, C. M. (2001) An evaluation of the antibacterial action of ceramic powder slurry using multi-parameter flow cytometry. Biotechnol. Lett., 23, 667-675.

Inatsu, Y., Ohata, Y., Ananchapattana, C., Bari, M. L., Hosotani, Y., and Kawasahi, S. (2016) Fate of Escherichia coli O157 cells inoculated into lightly pickled Chinese cabbage during processing, storage and incubation in artificial gastric juice. Biocontrol Sci., 21, 51-56.

Jeong, M. S., Park, J. S., Song, S. H., and Jang, S. B. (2007) Characterization of antibacterial nanoparticles from the scallop, Pinnopencten yessoensis. Biosci. Biotechnol. Biochem., 71, 2242-2247.

Jun, Y., Jeong, Y., Oh, J. E., Park, J., Ha, J. H., and Sohn, S. G. (2015) Influence of the structural modification of polycarboxylate copolymer with a low dispersing ability on the set-retarding of Portland cement. KSCE J. Civil Eng., 19, 1787-1794.

Kawamura, J., and Takeo, T. (1989) Antibacterial activity of tea catechin to Streptococcus mutans. Nippon Shokuhin Kagaku Gakkaishi, 36, 493-497. (In Japanese)

Kim, J. G., Nimitkeatkai, H., Choi, J. W., and Cheong, S. R. (2011) Calcinated calcium and mild heat treatment on storage quality and microbial populations of fresh-cut iceberg lettuce. Horticult. Environ. Biotechnol., 52, 408-412.

Kubo, M., Ohshima, Y., Irie, F., Kikuchi, M., and Sawai, J. (2013) Disinfection treatment of heated scallop-shell powder on biofilm of Escherichia coli ATCC 25922 surro-gated for E. coli O157:H7. J. Biomater. Nanobiotechnol., 4, 10-19.

Li, M., Yao, Z. T., Chen, T., Lou, Z. H., and Xia, M. (2014) The antibacterial activity and mechanism of mussel shell waste derived material. Powder Technol., 264, 577-582.

Mamun, A. A., Simul, H. A., Rahman, A., Gazi, N. N., and Bari, L. (2012) Prevalence of foodborne pathogens and effectiveness of washing or cooking in reducing microbiological risk of contaminated red amaranth. Agric. Food Anal. Bacteriol., 2, 222-231.

Marcus, J. M., Williams, A. D., and Heizer, D. D. (1998) Polynuclear aromatic hydrocarbon and heavy metal concentrations in sediments at coastal South Carolina marinas. Arch. Environ. Contami. Toxicol., 17, 103-113.

Mendonca, A. F., Amoroso, T. I., and Knabel, S. J. (1994) Destruction of gram-negative food-borne pathogens by high pH involves destruction of cytoplasmic membrane. Appl. Environ. Microbiol., 60, 4009-4014.

Oikawa, K., Asada, T., Yamamoto, K., Wakabayashi, H., Sasaki, M., Sato, M., and Matsuda, J. (2000) Antibacterial activity of calcined shell calcium prepared from wild surf clam. J. Health Sci., 46, 98-103.

Peschar, A., Govin, A., Pourchez, J. A. E., Fredon, E., Bertrand, L., Maximilien, S., and Guilhot, B. (2006) Effect of polysaccharides on the hydration of cement suspension. J. Eur. Ceram. Soc., 26, 1439-1445.

Ro, E. Y., Ko, Y. M., and Yoon, K. S. (2015) Survival of pathogenic enterohemorrhagic Escherichia coli (EHEC) and control with calcium oxide in frozen meat products. Food Microbiol., 49, 203-210.

Roy, A., Gauri, S. S., Bhattacharya, J., and Bhattacharya, J. (2013) Antimicrobial activity of CaO nanoparticles. J. Biomed. Nanotechnol., 9, 1570-1578.

Sawai, J. (2011) Antimicrobial characteristics of heated scallop shell powder and its application. Biocontrol Sci., 16, 95-102.

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

Sawai, J., Kawada, E., Kanou, F., Igarashi, H., Hashimoto, A., Kokugan, T., and Shimizu, M. (1996) Detection of active oxygen generated from ceramic powders having antibacte-
Sawai, J., Kojima, H., Igarashi, H., Hashimoto, A., Shoji, S., and Shimizu, M. (1999) Bactericidal action of calcium oxide powder. Trans. Mater. Res. Soc. Jpn., 24, 667-670.

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

Sawai, J., Miyoshi, H., and Kojima, H. (2001c) Heated scallop-shell powder slurry treatment of shredded cabbage. J. Food Prot., 64, 1579-1583.

Sawai, J., and Shiga, H. (2006) Kinetic analysis of antifungal activity of heated scallop-shell powder against Trichophyton and possible application to the treatment of dermatophytosis. Biocontrol Sci., 11, 125-128.

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

Sawai, J., Shiga, S., Kojima, H. (2001b) Kinetic analysis of death of bacteria in CaO powder slurry. Int. Biodeterior. Biodegrad., 47, 23-26.

Shimamura, N., Irie, F., Yamakawa, T., Kikuchi, K., and Sawai, J. (2015) Heated scallop-shell powder treatment for killing and removal of Listeria sp. biofilm formed at low temperature. Biocontrol Sci., 20, 153-157.

Smith, B. J., Rawal, A., Funkhouser, G. P., Roberts, L. R., Gupta, V., Israelachvili, J. N., and Chmelka, B. F. (2011) Origins of saccharide-dependent hydration at aluminosilicate surfaces. Proc. Natl. Acad. Sci. USA, 108, 8949-8954.

Smith, B. J., Roberts, L. R., Funkhouser, G. P., Gupta, V., and Chmelka, B. F. (2012) Reactions and surface interactions of saccharides in cement slurries. Langmuir, 28, 14202-14217.

Sugiyama, K., Suzuki, T., and Satoh, T. (1995) Bactericidal activity of silicate-containing hydroxyapatite. J. Antibact. Antifung. Agent., 23, 67-71.

Suzuki, S., and Nishi, S. (1959) The effects of saccharides and other organic compounds on the hydration of cement. Semento Gijutsu Nempo, 13, 160-170. (in Japanese)

Takeuchi, T., Nagataki, S., Otsuki, N., and Tamugi, N. (1997) A fundamental study on chemical structures of organic compounds on set retardation. Doboku Gakkai Ronbunshu, 564, 5-87. (in Japanese)

Thammakarn, C., Satoh, K., Suguro, A., Hakim, H., Ruenphet, S., and Takehara, K. (2014) Inactivation of avian influenza virus, newcastle disease virus and goose parvovirus using solution of nano-sized scallop shell powder. J. Vet. Med. Sci., 76, 1277-1280.

Thamnakarn, C., Tsujimura, M., Satoh, K., Hasegawa, T., Tamura, M., Kawamura, A., Ishida, Y., Suguro, A., Hakim, H., Ruenphet, S., and Takehara, K. (2015) Efficacy of scallop shell powders and slaked lime for inactivating avian influenza virus under harsh conditions. Arch. Virol., 160, 2577-2581.

Thomas, N. L., and Birchall, J. D. (1983) The retarding action of sugars on cement hydration. Cem. Concra. Res., 13, 830-842.

Watanabe, T., Fujimoto, R., Kikuchi, M., Sawai, J., Yahata, S., and Satoh, T. (2014) Antibacterial characteristics of heated scallop-shell nano-particles. Biocontrol Sci., 19, 93-97.

Xing, R., Qin, Y., Guan, X., Liu, S., Yu, H., and Li, P. (2013) Comparison of antifungal activities of scallop shell, oyster shell and their pyrolyzed products. Egypt. J. Aqua. Res., 39, 83-90.

Yoon, J. H., Bae, Y. M., Jung, K. S., Heu, S., and Lee, S. Y. (2013) Combined effect of calcium oxide and sonication to reduce foodborne pathogens on fresh produce. Food Sci. Biotechnol., 22, 275-278.