Antimicrobial Characteristics of Heated Scallop Shell Powder and Its Application

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Received 23 February, 2011/Accepted 11 June, 2011

Scallop shells are used to make food additives and plastering and paving materials. However, most of the shell is considered commercial waste. In scallop-harvesting districts, large numbers of shells are heaped near the seaside, which creates problems such as offensive odors and soil pollution from heavy metals that leach out of the viscera. Therefore, new applications for scallop shells need to be developed. The main component of scallop shells is calcium carbonate (CaCO₃), which is converted to calcium oxide (CaO) when heated. Heated scallop shell powder (HSSP) possesses broad antimicrobial action against the vegetative cells of bacteria, spores, and fungi. HSSP applied to fresh vegetables and processed foods reduces the number of viable bacterial cells. 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 related pollution problem. This report is a review of the antibacterial activity of HSSP and its application for the control of microbes.

Key words : Scallop shell / Calcium oxide (CaO) / Antibacterial activity / Deodorization effect.

INTRODUCTION

In Japan, the shellfish catch in 2009 was approximately 8.7 x 10⁶ tons, of which 65% is scallops according to statistics from the Ministry of Agriculture, Forestry, and Fisheries of Japan (2010). The edible portion of the scallop constitutes only about 20% of the total weight, leaving nearly 4 x 10⁶ 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 districts, 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. Thus, research on applications of scallop shells has been conducted (Marcus et al., 1988; Ichikawa, 1993). The main component of scallop shells is calcium carbonate (CaCO₃), which is converted to calcium oxide (CaO) when heated. The antimicrobial activity of scallop shells originates from the generation of CaO.

Previous studies on the effects of ceramic powder on the growth of bacteria have been published. Twenty-six types of ceramic powder, such as metallic oxides and carbide, were examined for antibacterial activity; of these, ten types of powder inhibited bacterial growth (Sawai et al., 1995a). CaO, in particular, exhibited strong antibacterial and antifungal activity against a broad range of organisms (Sawai et al., 1995a, 1995b; Sawai and Yoshikawa, 2004).

The use of these materials in food processing, therefore, is expected to provide a source of minerals and prolong the shelf life of foodstuff. This review focuses on the antimicrobial activity of heated scallop shell powder (HSSP) and its application as a food additive. This report also discusses the benefit to the environment of finding uses for waste scallop shells.
ANTIBACTERIAL ACTIVITY OF HSSP

Antibacterial activity against vegetative cells of bacteria

Table 1 shows the results of the elemental analysis of scallop shell powder heated at 1000°C for 1 h. Calcium concentration was 70.8 wt%, and Mg concentration was 0.16 wt%. Other elements were present in very small amounts: P (0.073 wt%), Na (0.014 wt%), and Fe (0.003 wt%). The levels of Ag and Cu, known to be antibiotic (Woodward, 1965), were below the detection limits (<0.01 ppm). Shells contain small amounts of organic and inorganic compounds. During heat treatment, most organic materials vaporize and some inorganic materials undergo a phase transition. Weight loss of powder heated at 1000°C was 44 wt%. When all of the Ca (70.8 wt%) exists as CaO, shells heated at 1000°C contain 99% CaO by weight (Sawai et al., 2001a).

Figure 1 shows X-ray diffraction (XRD) patterns of scallop shell powder heated at various temperatures. Below 600°C, almost all of the diffraction peaks corresponded to CaCO₃ in the unheated powder. At 700 °C, the XRD peak of CaO was barely detected; however, at 800°C, CaO peaks were detected clearly. The CaO peaks became more distinct with increasing temperature, suggesting that Ca in HSSP exists in the form of CaO. The main component of unheated shells, CaCO₃, is decomposed to CaO by heat (CaCO₃ → CaO + CO₂) (Sawai et al., 2001a).

Figure 2 shows bacterial death caused by a slurry of shell powder heated at 1000°C. Bacteria were inoculated into a saline slurry of the heated scallop

| TABLE 1. Elemental analysis of scallop shell powder heated at 1000°C for 1 h |
|-----------------|----------|
| element         | wt %     |
| Ca              | 70.8     |
| Mg              | 0.16     |
| P               | 0.073    |
| Na              | 0.014    |
| Fe              | 0.003    |
| K               | ND       |
| Cd              | ND       |
| Pb              | ND       |
| Ag              | ND       |

ND: undetectable

FIG. 1. X-ray diffraction pattern of HSSP powder heated for 1 hr at (A) 1000°C, (B) 900°C, (C) 800°C, (D) 700°C, (E) 600°C, and (F) unheated (Sawai et al., 2001a).

FIG. 2. Effect of the HSSP concentration in slurry heated at 1000°C on the bactericidal action against (A) E. coli, (B) S. Typhimurium, (C) B. subtilis, and (D) S. aureus (Sawai et al., 2001a).
shell powder slurry, and the number of viable bacteria was determined by conventional plate counting. Slurry temperature was 37°C. The ordinate is the ratio of bacteria colony-forming units (cfu) post-treatment \(N\) divided by non-treated cfu \(N_0\), and represents survival. The HSSP exhibited bactericidal action against both Gram-positive and Gram-negative bacteria, and an increase in powder concentration enhanced the bactericidal action (Sawai et al., 2001a).

With the logarithmic survival ratio and treatment time as the ordinate and abscissa, respectively, the populations of bacteria decreased in a nearly linear manner. For *Escherichia coli*, the curves became steeper with time. However, assuming that bacterial death by the HSSP follows first-order kinetics, the apparent first-order death rate constant \(k\) can be determined (Sawai et al., 2001a).

Figure 3 shows the effect of exposure temperature on the first-order death rate constant for *Staphylococcus aureus*. Powder heated at temperatures of 700°C or higher possessed bactericidal action against *S. aureus*. An increase in exposure temperature enhanced the antibacterial activity. The values of \(k\) of the powder heated at 1000°C were comparable to those of pure CaO. These results indicate that the antibacterial activity was due to the generation of CaO (Sawai et al., 2001a).

**Sporicidal activity of HSSP**

Some species of bacteria, such as *Bacillus* and *Clostridium*, produce spores in their stationary phase of growth. The spores are capable of long dormancy and are resistant to heat and many chemicals. Bacterial spores cause many serious problems

**FIG. 3.** Effect of the scallop powder heating temperature on the bactericidal action against *S. aureus*. ●, 1000°C; ◇, 900°C; ▲, 800°C; ■, 700°C; ○, CaO. (Sawai et al., 2001a)

**FIG. 4.** Sporicidal action of HSSP heated at 1000°C for 1 h against *B. subtilis* spores at 37°C at concentrations of ●: 1.4 mg/mL, △: 5 mg/mL, ●: 7.5 mg/mL, □: 10 mg/mL, ×: 100 mg/mL. (Sawai et al., 2003)

(Miyamoto et al., 1997; Andresson et al., 1998; Franciosa et al., 1999). Therefore, inactivation of bacterial spores is an important step in food processing. Thus, we examined the sporicidal effect of heated scallop shell powder on the spores of *Bacillus subtilis*.

Figure 4 shows the effect of the concentration of HSSP heated at 1000°C on the viability of *B. subtilis* spores. Powder slurry temperature was 37°C. HSSP treatment killed *B. subtilis* spores even at 37°C. Spore survival decreased with increasing powder concentration up to 10 mg/mL. No additional sporicidal effects occurred above that concentration. Survival rates for vegetative cells of bacteria treated with HSSP followed first-order kinetics (Fig. 1), with a linear relation between the logarithmic survival ratio and treatment time (Sawai et al., 2001a). In contrast, the survival curves for *B. subtilis* spores became steeper with time. The pH of saturated slurry powder at 37°C was 12.4. However, alkaline treatment involving NaOH and KOH solutions at pH 13 did not reduce spore survival. The HSSP possesses another antibacterial mechanism in addition to that of alkalinity (Sawai et al., 2003).

We investigated the inactivation of spores with and without germination. The decrease in the optical density of spore suspensions was measured after addition of the HSSP supernatant, and the germination
rate was determined based on the decrease in optical density. Germination was more rapid in supernatants from higher concentration HSSP slurries; the germination rate correlated with the decrease in spore viability. This result suggests that HSSP induces spore germination and kills spores that have lost resistivity (Sawai et al., 2007).

**Antibacterial mechanism**

Alkaline effects caused by the hydration of CaO are considered one of the primary mechanisms of the antibacterial activity of HSSP. Thus, the antibacterial activity of the HSSP was compared with that of an alkaline solution (NaOH). As shown in Fig. 5, the bactericidal action of HSSP depends on the pH of the powder slurry and is independent of the heating temperature. The bactericidal action was, however, larger than that of NaOH at the same pH (Sawai et al., 2001a). High pH has been shown to kill bacteria (Kinner and Moats, 1981; Pearson et al., 1987; Southam et al., 1987; Laird et al., 1991; Catalano and Knabel, 1994). Although many studies have attempted to elucidate the antibacterial mechanism at low pH (Cherrington et al., 1991; Slonczeski, 1992), there are few reports on the bactericidal mechanisms at high pH values.

Bacterial death caused by pH and CaO treatment was examined, based on changes in antibiotic sensitivity. Cells injured sub lethally by stress became more sensitive than intact cells to antibiotics and inhibitors (Mackey, 1983). Using four types of antibiotics with different primary actions, stress-induced injury in E. coli was investigated using changes in sensitivity to antibiotics. Penicillin G (PCG), chloramphenicol (CP), nalidixic acid (NA), and rifampicin (RFP) were selected as the antibiotics, and agar containing the antibiotic was used as the selective medium. Stress-treated E. coli was pour-plated using selective medium and non-selective medium that did not contain an antibiotic. After incubation, the difference in colony counts of E. coli on the two media indicated sensitivity changes (i.e., cell injury). The antibiotic concentration in the selective medium (C_{MIC}) was considered at a maximum when the antibiotic had no effect on colony counts of intact E. coli cells. This concentration, C_{MAX}, is lower than the minimum inhibitory concentration (MIC), which is used commonly as an indicator of antibiotic efficacy. For uninjured cells, the number of colonies on the selective medium was the same as that on the non-selective medium. However, some injured cells form colonies on the non-selective medium but not on the selective medium. Seriously injured cells cannot form colonies on the selective agar containing a concentration lower than C_{MAX}. Therefore, by varying the antibiotic concentration within C_{MAX}, the degree of bacterial cell injury can be estimated. This method indicated that the patterns of sensitivity of E. coli to antibiotics depended on the type of stress, such as heating, far-infrared heating, UV irradiation, freezing, and acid treatment. This method is simple and effective when determining a mechanism of action (Sawai et al., 1997).

Although CaO powder slurry is alkaline, the change in E. coli sensitivity induced by the CaO powder slurry was different from that in the case of alkaline treatment (Sawai et al., 1997). E. coli treated with CaO had increased sensitivity to chloramphenicol and rifampicin. E. coli treated with alkaline solution, however, did not exhibit any increased sensitivities. Mendonca et al. (1994) reported that high-pH treatment did not damage cells of E. coli, Salmonella Enteritides, or Listeria monocytogenes, which grew equally well in both selective and non-selective media. In addition, Mendonca et al. (1994) proposed that high pH had an all-or-nothing effect. Our results agreed well with those of Mendonca’s study (Sawai et al., 1997). CaO powder possesses other antibacterial mechanisms in addition to alkalinity. For CaO and MgO, the generation of active oxygen species such as superoxide anions has been observed from a 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.,

**FIG. 5.** Comparison of death rate constants of E. coli after alkaline treatment (NaOH solution) and HSSP treatment at ●: 1000°C, ◆: 900°C, ▲: 800°C, ■: 700°C, and using ○: CaO, or ×: NaOH. (Sawai et al., 2001a)
1999). Thus, active oxygen also may be a primary mechanism of antibacterial activity. Results of Hewitt et al. (2001) support these conclusions through the use of multi-parameter flow cytometry.

**ANTIMUTAGENIC EFFECT OF CaO, THE MAIN COMPONENT OF HSSP**

Mutagens act on DNA and injure the base sequences of DNA. Many mutagens are also carcinogenic. CaO, which is the main component of heated scallop shell powder, is not mutagenic according to the Ames test done with *Salmonella* Typhimurium (Sawai et al., 1995c); instead, CaO exhibits antimutagenicity. Figure 6 shows the effect of CaO powder on the mutagenicity of methylglyoxal (MG), which is contained in coffee, toward *S. Typhimurium* TA102. The ordinate is the number of revertant *S. Typhimurium* TA102. Addition of CaO decreased the number of revertants and almost eliminated the mutagenicity of MG (Sawai et al., 1998). The antimutagenicity of CaO powder also has been detected against benzo[a]pyrene and 2-nitrofluorene (Sawai et al., 1995c). Therefore, HSSP containing CaO as the main component may be useful for the reduction of mutagenic activity in food as well as for food preservation and mineral supplements.

**APPLICATION TO FOOD PROCESSING AND ENVIRONMENT**

The use of processed food and the expansion of food distribution areas have resulted in increased utilization of minimally processed vegetables, which are commonly eaten raw. However, the bacterial contamination level in fresh vegetables can be high, and damage by cutting/slicing and secondary spoilage decreases vegetable quality. Bacterial food poisoning due to the consumption of fresh raw vegetables is increasing (Beuchat, 1996; Madden, 1992; Tauxe et al., 1997). Because fresh vegetables often are consumed without heat processing, reducing bacterial contamination is particularly important. In Japan, fresh vegetables usually are disinfected using a sodium hypochlorite solution. However, this practice raises safety concerns, such as harmful effects from residual chlorine and the effect of the generated gas on factory worker health (Hall et al., 1998; Kim et al., 1999). Many researchers have reported that the chlorination of water and foods causes the formation of potentially harmful chlorinated organic materials, such as trihalomethane (Bellar et al., 1974; Tomita et al., 1982). Hidaka et al. (1994) reported that treatment of soybean sprouts and cabbage leaves with sodium hypochlorite generated chloroform. Furthermore, Hirose et al. (1989) found that cyanogen chloride was formed by the reaction of peptides and proteins with hypochlorous acid in the presence of ammonium ions.

Cut vegetables (shredded cabbage) were treated with HSSP. The antibacterial effect of HSSP on shredded cabbage was compared with that of sodium hypochlorite. Figure 7 shows the antibacterial effect of HSSP on aerobe counts in shredded cabbage. Slurry temperature was 20°C. The cabbage sample was cut and used without washing. Initial viable aerobe counts were approximately $7 \times 10^4$ CFU/g. Elevated HSSP concentrations promoted microbial
death. At 1.0 g/l, a one-order magnitude reduction in the survival ratio was observed after a 20-min treatment. At 5.0 and 10 g/l, the survival ratio decreased by more than two orders of magnitude within 10 min, before becoming almost constant. We postulate that a fraction of bacterial cells are resistant to the heated shell powder and propose a model that accurately predicts the reduction in bacterial counts on shredded cabbage resulting from HSSP treatment (solid line in Fig. 7).

No coliform bacteria were detected within 5 min of treatment at 1.0-10 g/l. HSSP treatment did not produce any noticeable discoloration. For aerobic bacteria, the antibacterial effect of sodium hypochlorite at 200 mg/l and 20°C was almost identical to that of shell powder at 1.0 g/l and 20°C. However, after sodium hypochlorite treatment, coliform bacteria were detected at 10^2-10^3 CFU/g. During storage for 48 h at 4°C, aerobic counts on shredded cabbage increased after water-washing and sodium hypochlorite treatment, whereas counts did not increase after HSSP treatment. No change in color, such as browning, was observed. The decomposition of nutrients in vegetables by HSSP treatment was investigated by determining L-ascorbic acid concentrations in treated cabbage. Treatment at 5.0 and 10 g/l decreased L-ascorbic acid content by 30%, almost identical to that resulting from sodium hypochlorite treatment at 200 mg/l (Sawai et al., 2001b). Thus, HSSP is a useful alternative for hypochlorite that may reduce health risks to workers and consumers. The data indicate that large amounts of commercial shell waste can be recycled for use in the processing of foodstuff.

HSSP has been of interest not only because of its antimicrobial activity but also because of its deodorization effect and ability to reduce volatile organic compounds (VOC). Kishi (2002, 2006) investigated the effect of scallop shell powder on butanoic acid and acetic acid, substances that produce VOC. Scallop shells alone and building materials (for wall construction) containing scallop shell powder removed these acids. Yoshida et al. (2003) reported that heated scallop shells, which contain CaO as the main component, adsorbed chemicals such as formaldehyde, odoriferous compounds, and VOCs. Prevention of fungal contamination indoors and removal of VOCs by mixing antimicrobial substances into wall materials or paints may contribute to a safe living environment. With an aging society and an trend toward an increase in chemical allergies, many applications exist for heated shell powder. Understanding the characteristics and action mechanism of HSSP is necessary to expand its uses.

**CONCLUSION**

Organic antimicrobial agents, including antibiotics, have been detected in rivers in many parts of the world including Japan, and their effects on the environment have caused concern. However, scallop shells discarded from the harvest of scallop meat possess antimicrobial action and are safe for use in food. Even if the heated shell powder is discarded, the CaO it contains eventually reverts to CaCO3 by absorbing CO2 from air. Therefore, it does not cause environmental problems. The discarded shell powder can return to the sea after use in food through the river without causing pollution (Fig. 8). Scallop shells

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**FIG. 8.** Circulation of shells and shell materials.
are an antibacterial agent of the “circulating type.” This natural inorganic antimicrobial material shows promise as an environmentally safe material for food processing and medical applications.

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