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Inhibition of the Growth of Foodborne Disease-Causing Bacteria by Calcined Scallop Shell Powder

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Abstract

To make practical use of unused scallop shells, we ground the shells into a powder, calcined the powder, and then tested the powder's ability to inhibit the growth of foodborne disease-causing bacteria. Powder calcined at around 800°C showed strong bacteriostatic effect and completely inhibited cell growth of *Escherichia coli* O-157 : H7, *Staphylococcus aureus* and *Salmonella enteritidis* at concentrations greater than 0.4% (minimal concentration tested). $CaCO_3$, a major chemical constituent of scallop shells, changed to CaO after calcination at 800°C or higher. The calcined powder consisted of 97.5% Ca and 1.0% Na. The results suggest that the bacteriostatic effect of calcined powder prepared from scallop shells is due to the high alkalinity and the presence of trace elements.

Key words: Scallop shell, Calcined powder, Bacteriostasis, Foodborne disease-causing bacteria

Introduction

Scallop fisheries occur throughout Hokkaido, Japan. Harvested scallops are distributed and consumed in several forms, including fresh, frozen and dried. After the scallops are processed, many shells are discarded as industrial waste. Although some ideas have been proposed to make use of the shells, most shells continue to be discarded after processing. This study aimed to use a calcined powder derived from shells as a bacteriostatic agent against foodborne disease-causing bacteria. Because the bacteriostatic effects of aqueous suspensions of calcined powder depend on the calcination temperatures (Takama et al., 1999), we also describe the characteristics of the powder.

Materials and Methods

Materials

Dried scallop-shell powder was provided by the Tokoro Public Corporation of Industrial Promotion (Tokoro-cho, Hokkaido). The powder was heated in an electric furnace at 550°C or 800°C until the weight of the incinerated powder became constant. The incinerated powder was then kept in a desiccator until use in the subsequent experiments.

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Bacterial strains

The foodborne disease-causing bacteria, *Escherichia coli* O-157 : H7(provided by the Hokkaido Oshima Public Health Center, Hakodate, Hokkaido), *Staphylococcus aureus* IFO-15035, and *Salmonella enteritidis* IFO-3313 were used in this study.

Treatments

The basic treatment procedure was as follows ; an aqueous suspension containing a certain amount of the incinerated powder was autoclaved at 121°C for 15 min. After cooling to room temperature, 4.5 ml of the solution was added to 0.5 ml of a solution containing the test bacterial strain, which was previously cultivated and prepared to ca. 10^4 cfu/ml. After mixing in a tube for 5 min, 0.1 ml of the solution was inoculated on a general culture plate (a general agar culture for *E. coli* and *S. aureus* and an MLCB culture for *S. enteritidis*), and the number of colonies were counted after incubation at 37°C for 24 h.

Characterization of incinerated powder

Shell-powder samples were incinerated for 10 h at 500, 600, 700, 800 and 900°C. The mineral composition of the incinerated powder was analyzed using a fluoric X-ray diffractometer (Shimazu XRF-1700) ; the incinerated powder was shaped into a disk (ϕ 35 mm × 4 mm) and set on an Rh-target (X-ray tube) that was supplied to the instrument at a tube voltage of 40 kV and an electric current of 70 mA. The composition of the incinerated powder was determined by using an X-ray microanalyzer (JOEL, JDX-8020) with a Cu-target (X-ray tube) at a tube voltage of 40 kV and an electric current of 25 mA.

Results and Discussion

Bacteriostatic effect

Table 1 shows the effects of scallop-shell powders incinerated at 550°C and 800°C on the growth of *E. coli* as a representative foodborne disease-causing bacteria species. Powder incinerated at 550°C had little inhibitory effect, even when the powder formed 10% of the reaction medium. Powder incinerated at 800°C completely inhibited bacterial growth, even in a 0.4% suspension.

Characterization of incinerated powder

These results suggest that the bacteriostatic effect of incinerated powder is strongly linked with the incineration temperature. X-ray diffraction spectra in Fig. 1 show the compositions of powders incinerated at different temperatures. $CaCO_3$ peaks were recognized at incineration temperatures below 700°C, however, CaO peaks were recognized at temperatures over 800°C. This suggests that the conversion temperature of $CaCO_3$ to CaO occurs between 700°C and 800°C. Furthermore, CaO obtained by calcinating $CaCO_3$ converted to $Ca(OH)_2$ after standing for 1 month in a dessiccator (Fig. 2). Table 2 shows that the powder incinerated at 800°C for 10 h contained 97.5% Ca and 1.0% Na, indicating that the powder was a sort of calcined calcium.

There are very few papers reporting practical applications which discuss the

Table 1. Effect of the calcined powder prepared from scallop shell on the growth of *Escherichia coli*

Concentration of calcined powder (%)	Colony count*/plate	
	Calcined temperatue	
	550°C	800°C
10.0	1,600	ND
8.0	1,670	ND
6.0	1,635	ND
4.0	1,755	ND
2.0	1,730	ND
1.2	ND	0
0.8	ND	0
0.4	ND	0
Control	1,790	860

ND: not determined, * mean value of duplicate measurements

control of bacterial growth using calcined material. Isshiki et al. (1994) reported that the preparation of 320 mesh calcined powder from the pearl layer of oyster shells using the Joule heat treatment effectively suppressed microbial growth. They also reported that the oyster shell calcined powder contained 60% Ca, that $CaCO_3$ in the powder changed to CaO and $Ca(OH)_2$, and that high alkalinization by

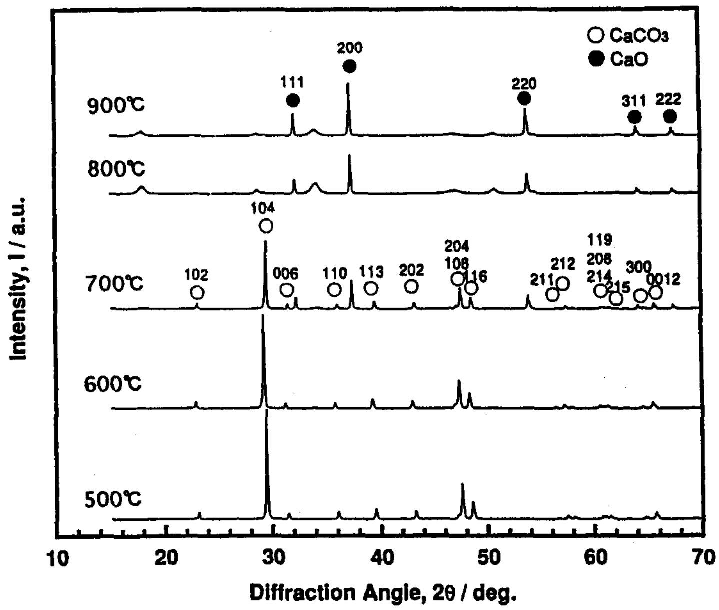

Fig. 1. X-ray diffractmetric spectra of scallop shell powders incinerated at different temperatures.
Numbers in Figure show interplanar spacings.

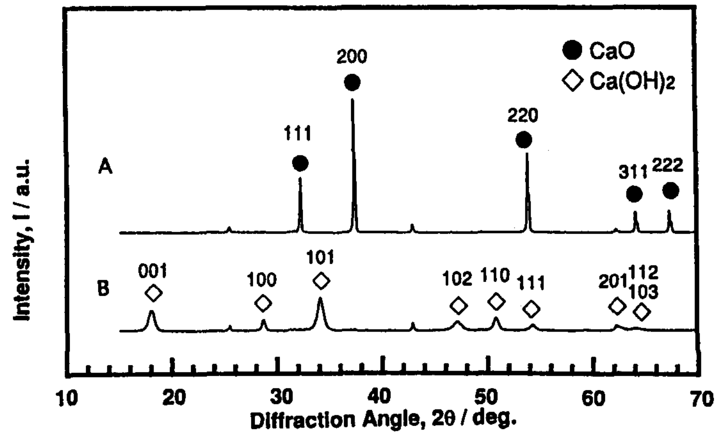

Fig. 2. Changes of product in calcined scallop-shell powder after standing for 1 month in a desiccator at room temperature.
A : just after incineration at 900°C for 10 h, B : 1 month later in a desiccator at room temperature.
Numbers in Figure show interplanar spacings.

Table 2. Mineral composition of calcined powder prepared from scallop shell (%)

Ca	97.50	Mg	–	Na	1.00	Cl	0.07
K	0.03	S	0.31	P	0.11	Sr	0.29
Fe	0.27	Si	0.23	Cr	0.14	Zn	–
Al	0.04	Br	–	Ni	0.01	Zr	–

– : not detected

those Ca-compounds in the medium should be the main cause of inhibition of the powder on bacterial growth.

The aqueous suspensions (0.05% or more) of calcined scallop-shell powder prepared at 800°C and above had pH levels higher than 12, and it seemed that the calcined scallop-shell powder inhibited bacterial growth by similar mechanisms to that of the oyster shell calcined powder.

Because the sanitary conditions must be maintained at all stages in food processing and product distribution to prevent consumers from catching foodborne diseases, effective treatments with bactericides are needed to inhibit bacterial growth in foods.

Bactericides registered as food additives in the Food Sanitation Law are classified in two categories : strictly regulated and not strictly regulated chemicals. The former group includes sodium chlorite, hydrogen peroxide and sodium hypochlorite, and the latter one includes high test hypochlorite.

When using bactericidal chemicals, care must be taken to avoid contaminating the food. As indicated in the Food Sanitation Law, the calcined materials from sea urchin shells, shellfish shells, bones, reef corals, milk whey, and hen egg shells have been approved as unrestricted food additives. These calcined materials are also used in calcium fortification. Therefore, they are regarded as safe, even when mixed with foods.

Recently, the importance of sanitary management of food environments has increased with the suppression of growth of microorganisms, including several kinds of parasites. This study shows that scallop shell calcined powder can inhibit the growth of foodborne dicease-causing bacteria. The numerous shells discarded in scallop fisheries and processing can now be used as antimicrobial material.

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