

# Oyster Shell Disposal: Potential as a Novel Ecofriendly Antimicrobial Agent for Packaging: a Mini Review

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**Abstract** The management of oyster shell disposal is an ongoing challenge in the southern coast of Korea because of continuously dumping the oyster shell in environment. Oyster shell wastes could be a biocidal alternative after calcination using a heat treatment. Calcined oyster shell is normally obtained through thermally conversion of  $\text{CaCO}_3$  (main component in oyster shell (96%)) into  $\text{CaO}$ . This study provides a brief overview of oyster shell disposal and its potential as an antimicrobial agent with a focus on calcination process, antimicrobial mechanisms, and packaging applications.

**Keywords** Oyster shell disposal, calcination, antimicrobial activity,  $\text{CaO}$

## Introduction

The progressive trend in exploiting natural wastes to derive functional materials is continuously increasing owing to environmental-friendliness, cost-effectiveness, and convenient resource<sup>1)</sup>. Such compounds can provide various biocompatible materials by removing wastes from the environment. Therefore, taking the natural wastes back into environment by preparation of worthwhile materials and reusing them, can curtail the environmental issues of natural disposal.

Shell is a by-product of marine animals, which is widely produced by human diet. Oyster shells tend to be an environmental issue generally in the world and particularly in southern coast of Korea. There is an intense demand for oyster and its products in the market i.e. South Korea has dumped over 251706 tons of oyster shell wastes at 2005. The progressive demand for oyster shell in South Korea can lead to a big accumulation of dumping oyster shells in environment. The oyster shell disposal contributes to some environmental issues as follows: (i) accumulation of wastes in environment, (ii) water and marine pollution owing to illegal landfill and microbial activities, (iii) off-odor problem because of less-attention to cheap disposal, (iv) high expense of management, and (v) improper recycling and reusing such as fertilizer, which may increase the pH of soil<sup>2)</sup>. Southern coast of Korea is the main spot to mass-production of oyster shell disposal in the World<sup>3)</sup>. Accordingly, South Korea has dumped the

highest oyster shell per person at 2005 in the world (Table 1).

It has been predicted that over 300000 tons are annually dumped in South Korea, which is contributed to generate the big amount of  $\text{NH}_3$  and  $\text{H}_2\text{S}$  because of microbial decomposition<sup>4)</sup>.

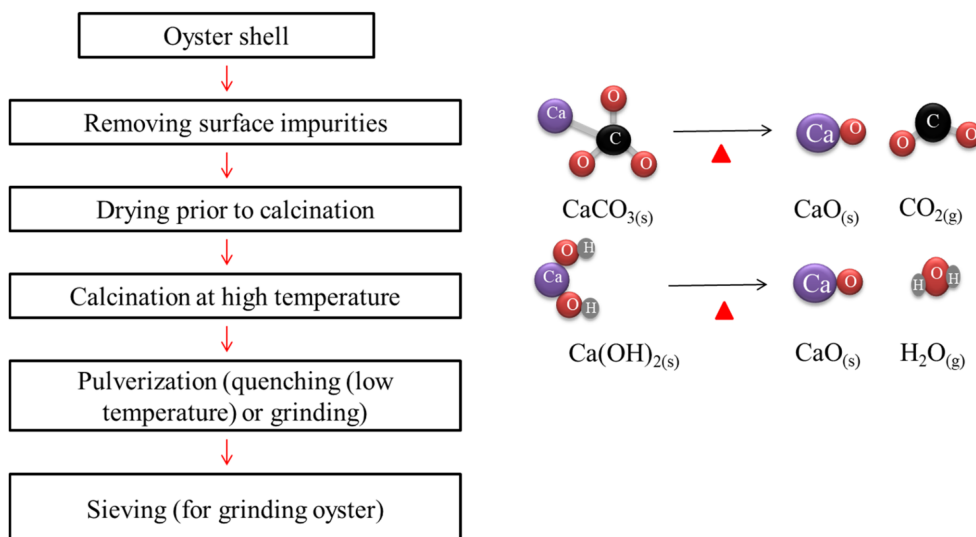
Numerous attempts have been directed to manage the oyster shells and prepare worthwhile materials with new properties<sup>2,5,6)</sup>. The marine shell is mostly composed of  $\text{CaCO}_3$ , which aragonite and calcite are the common forms<sup>7)</sup> despite vaterite and amorphous are also reported<sup>8)</sup>.  $\text{CaO}$ ,  $\text{Ca}(\text{OH})_2$ , and  $\text{CaCO}_3$ , calcium-based compounds, can be derived from marine shells, but  $\text{CaO}$  is more thermodynamically stable, which can be prepared by calcination of marine shells<sup>7,9)</sup>.  $\text{CaO}$  is widely used in various industrial and research setting owing to various functional properties such as catalyst, tissue engineering, and biological purposes<sup>7,9,10)</sup>. Biocidal activities of calcined oyster shell received much attention owing to strong antimicrobial activities, diverse biocidal mechanisms, and biocompatibility. Therefore, calcined oyster shell can be used as an antimicrobial alternative for wide applications such as food packaging<sup>6)</sup>. Such compounds can be incorporated in the packaging matrix as the direct food contact materials.

This review briefly provides an overview of calcined oyster shell properties, applications, and preparation method with a focus on the antimicrobial activities, particularly in food packaging application. This study also reviews the antimicrobial

**Table 1.** Oyster shell production (FAO 2005)<sup>3)</sup>

Country	Production of oyster with shell (ton)
China	3,826,363
South Korea	251,706
Japan	218,896

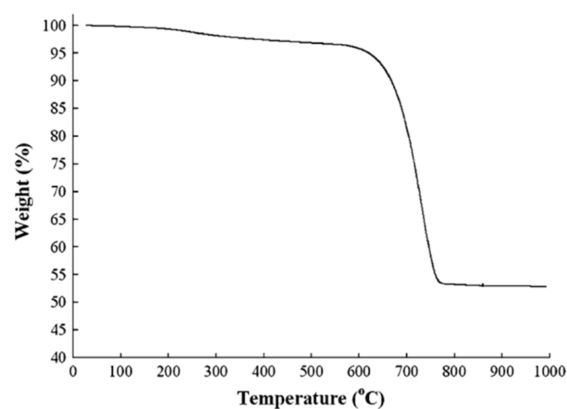
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**Fig. 1.** Schematic of calcination and chemical decomposition of marine shell using heat treatment.

application of calcined oyster shell as a natural and ecofriendly alternative in packaging science.

As shown in Figure 1, the calcination of marine shells is generally carried out based on the same protocol. The marine shells normally have some impurities, which should be removed prior to calcination through appropriate washing methods such as mechanical, heat treatment, alkaline/acid treatment, enzymatic hydrolysis, and high pressure<sup>8</sup>). Pulverization step is conducted to make powder using the thermal shock or mechanical grinding<sup>2</sup>). It is reported that quenching calcined shell at low temperature (i.e.  $-40^{\circ}\text{C}$ ) immediately after calcination led to uniform powders with small particles<sup>11</sup>). As mentioned earlier, marine skeletons are mostly composed of  $\text{CaCO}_3$  because  $\text{CaCO}_3$  is low soluble in sea water. Such compounds can be converted to  $\text{CaO}$  using a heat treatment by converting the chemical structure of  $\text{CaCO}_3$ . To calcinate the oyster shell using high temperature, the following changes can be expected during heat treatment (Figure 2): in an attempt, thermogravimetric analysis (TGA) exhibits a slight weight loss in range of 100 to 150 $^{\circ}\text{C}$ , corresponding to water inside the oyster shell, the second weight loss in range of 200 to 450 $^{\circ}\text{C}$ , corresponding to  $\text{Ca(OH)}_2$  and organic materials decomposition, and weight loss in range of 650 to 800 $^{\circ}\text{C}$  (sharp reduction), corresponding to  $\text{CaCO}_3$  decomposition as the main component. The resulting component after calcination is  $\text{CaO}$  and a small amount of traces such as  $\text{MgO}$  or  $\text{SiO}_2$ , and etc<sup>7</sup>). The  $\text{CaO}$  is thermodynamically stable, which can be used as catalyst, tissue engineering precursors, and biocidal agents. The calcination process using a clean method such as electric furnace can enhance the matter of environmental-friendliness of the natural wastes<sup>2</sup>). Therefore, thermal decomposition during calcination can provide the resulting compound ( $\text{CaO}$ ) to apply in aforementioned purposes.



**Fig. 2.** Thermal decomposition of oyster shell during heat treatment (Reproduced with permission from reference<sup>5</sup>).

### Characterization assays

Prior to and after calcination, characterization is normally conducted to evaluate the calcination yield and identification of resulting materials. The common characterization assays regarding the marine shells are as follows: X-ray powder diffraction (XRD) for crystallography and phase identification, X-ray photoelectron spectroscopy for surface elemental analysis, X-ray fluorescence (XRF) for evaluation of elemental and oxide composition, Fourier-transform infrared (FT-IR) spectrum for chemical bonding assessment, particle size distribution (PSD) for size measurement, and scanning electron microscope (SEM) for determination of microstructure and surface morphology. TGA for investigating the thermal decomposition of shells components during calcination. Further investigations are commonly used depending on the application. For example, inhibition zone end minimum inhibition concentration (MIC) assays are widely used to evaluate biocidal

potential of calcined marine shells<sup>12,13</sup>). In addition, the JIS Z 2801: 2000 standard can be used for investigating the antimicrobial efficacy of film containing marine shell powder.

### Chemical composition of oyster shell

Oyster shell, a marine skeleton, is mostly composed of  $\text{CaCO}_3$  (96%), organic materials, and some mineral traces<sup>14</sup>). The  $\text{CaCO}_3$ , the major component, can be converted into CaO, while organic materials cannot withstand high temperature (over 600°C) of calcination and decompose during heat treatment (calcination). It has been reported that calcined oyster shell is composed of CaO,  $\text{CaCO}_3$ ,  $\text{Ca(OH)}_2$ , and some mineral traces such as MnO,  $\text{TiO}_2$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{K}_2\text{O}$ ,  $\text{P}_2\text{O}_5$ ,  $\text{SiO}_2$ ,  $\text{Na}_2\text{O}$ , and  $\text{MgO}$ <sup>2,5</sup>). The mineral traces are common components among the marine skeleton, but  $\text{CaCO}_3$  and  $\text{Ca(OH)}_2$  after calcination might be related to entrap the  $\text{CO}_2$  and  $\text{H}_2\text{O}$  from the environment, resulting in generation of  $\text{CaCO}_3$  and  $\text{Ca(OH)}_2$  on the surface of CaO, respectively<sup>6</sup>). Therefore, calcined marine shell is mostly composed of CaO, and its functional properties are related to this compound.

### Antimicrobial mechanisms of oyster shell

Utilization of the natural wastes with functional properties tends to provide new compounds with an advanced property. Accordingly, application of natural resources, particularly waste products as biocompatible antimicrobial agents can enhance the human safety, and at the same time manage the wastes in the environment. The great attempts have been conducted to exploit marine shells as antimicrobial agents in the food packaging and food preservation<sup>6</sup>). Shell powder is prone to be a strong biocidal compound after calcination at high temperature. As mentioned earlier, calcination of shell powder can provide CaO from conversion of ( $\text{CaCO}_3$ ). It has been reported that the antimicrobial activity of calcined shell powder is related to CaO (main component after calcination)<sup>15</sup>). CaO is normally considered as an applicable antimicrobial agent in food and medical setting owing to strong biocidal activity, diverse antimicrobial mechanisms, and biocompatibility<sup>7,9,10</sup>). Therefore, antimicrobial activity of calcined oyster shell is also related to CaO. Antimicrobial activity of CaO is because of alkalinity of the environment as the primary mechanism as well as generating  $\text{Ca}^{2+}$  and reactive oxygen species (ROS) on the surface of CaO as the secondary mechanism<sup>15,16</sup>). The CaO can strongly increase the pH surrounding bacteria, thereby making an undesirable condition. Such change is the primary antimicrobial mechanism, following by secondary mechanism. CaO can dissociate in slurry phase and generate  $\text{Ca}^{2+}$ , resulting in the cationic environment. The secondary antimicrobial mechanism is related to generation of  $\text{Ca}^{2+}$ , and bond it with the cardiolipin (CL) (the main lipid in the cell membrane of bacteria)<sup>11</sup>). CL has negative surface charge, which can control the membrane

functions such as transportation of nutrients into cell. The  $\text{Ca}^{2+}$  and CL binding results in imperfectness function of CL and change in the cell metabolism. CL cannot appropriately control the membrane function, resulting in starvation and metabolism imperfectness. Such changes may adversely contribute to cell wall integrity and cell wall rupture. The last mechanism may be related to generation of ROS ( $\text{HO}_2\cdot$ ,  $\cdot\text{O}_2$ , and  $\text{H}_2\text{O}_2$ ), following by generation of free radicals, which can strongly affect the cell integrity<sup>17</sup>). The generation of ROS is related to the presence of  $\text{O}_2$  in the slurry phase in which  $\text{O}_2^-$  can be generated through a simple electron reduction. The  $\text{O}_2^-$  also can generate other forms of ROS in the surface of CaO e.g.,  $\text{O}_2^- + \text{H}^+ \leftrightarrow \text{HO}_2\cdot$  and  $\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot\text{HO}$ <sup>16</sup>). The antifungal activities of CaO is also related to alkaline condition and ROS generation<sup>15</sup>). It has been also reported that high concentration of  $\text{Ca}^{2+}$  can be toxic for fungi because fungi cannot remove high concentration of  $\text{Ca}^{2+}$  from inside of the cell<sup>17</sup>). Therefore, alkalinity environment and high  $\text{Ca}^{2+}$  generation can make an undesirable condition for fungi, resulting in cell malfunction and cell dead<sup>10</sup>).

### Antimicrobial efficacy of oyster shell

The calcined oyster shell is prone to be applied in a bundle of applications to exploit its biocidal activities. As such, numerous attempts have been directed to exhibit antimicrobial efficacy of calcined oyster shell, particularly for food packaging<sup>18-24</sup>). As mentioned earlier, CaO is the major component for antimicrobial efficacy of calcined oyster shell, which can be used as an additive or can be incorporated in the polymer matrix. Finding an alternative for synthetic additive can improve the consumer safety and biocompatibility of products. In addition, preparation of functional compounds from waste, particularly antimicrobial agents may provide cost-effective and convenient materials.

Kim et al. (2007) extended the shelf life of tofu during storage time through addition of different contents of oyster shell powder (0.05, 0.1, and 0.2%). The oyster shell powder extended the shelf life of tofu up to 2 days through improving the sensory and quality of tofu. In addition, with increasing the content of oyster shell, the microbial number significantly reduced, which maintained the tofu quality and freshness<sup>18</sup>). In a similar attempt, addition of similar contents of oyster powder significantly improved the quality of kimchi during storage time. The oyster powder remarkably retarded the number of aerobic bacteria and maintained the number of lactic bacteria, resulting in longer shelf life of kimchi. Furthermore, addition of 0.05 % of oyster shell powder appropriately maintained the sensory and crispiness of kimchi<sup>19</sup>). Such biocidal activities led to prolong the shelf life of tofu and kimchi, which are related to antimicrobial effects of Ca based compounds in the oyster shell powder. It is implied that oyster shell powder can extend the shelf life of food items and maintain their quality

without any adverse effects on the sensory properties.

Oikawa *et al.* (2000) calcined the marine shell such as oyster, scallops, clam, and roll to achieve antimicrobial agents. The antimicrobial evaluation of calcined shells exhibited that such powders possess strong biocidal activities against aerobic bacteria, particularly *Escherichia coli* (*E. coli*). It has been reported that the antimicrobial activities of shells are related to alkalinity environment caused by slurry calcined shells<sup>20</sup>. Calcined oyster shell powder showed an appropriate antimicrobial activity with retarding the microbial growth and reducing the number of *E. coli*.

Introducing the fillers into the polymer matrix can enhance the polymer performance, thereby increasing the applications. As such, addition of antimicrobial agents into polymer matrix can provide active packaging materials, which may extend the shelf life of fresh products and prevent foodborne disease. Tsou *et al.* (2019) prepared the propylene (PP) film containing calcined oyster shell to achieve an antimicrobial polymer. The results revealed that calcination of oyster shell powder significantly enhanced the antimicrobial efficacy. In addition, incorporating the calcined oyster shell into PP led to an augment on antimicrobial activity of PP, which was related to biocidal activities of calcined oyster shell powder. Notably, the addition of calcined oyster shell into PP did not present the cytotoxicity, indicating that such compound can be used in food packaging and biomedical purposes. Furthermore, the addition of calcined oyster shell relatively improved thermal and mechanical properties of PP<sup>6</sup>). The results proved that oyster shell powder is prone to be an alternative for commercial filler in packaging.

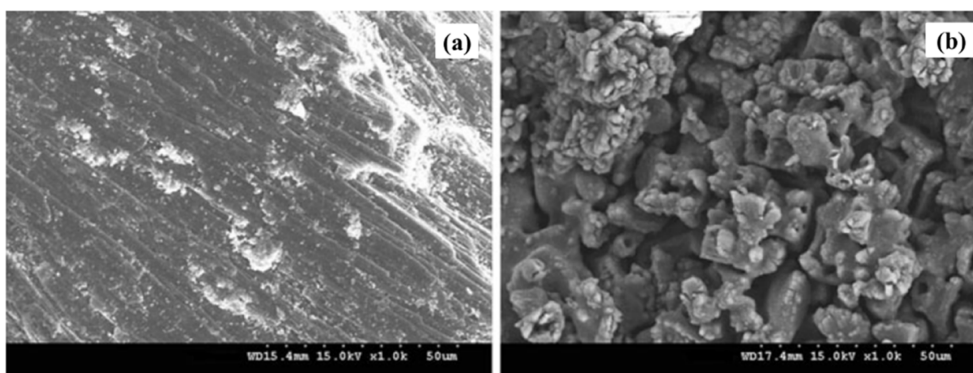
Addition of natural antimicrobial additive into processed and fresh food products can maintain the quality of food items without safety and biocompatibility concerns. Choi *et al.* (2014) prepared an antimicrobial agent to extend the shelf life of restaurant pork ham through addition of calcined oyster shell powder. Addition of calcined oyster powder strongly marinated the sensory quality of pork ham during storage. In addition, oyster shell powder extended the shelf life of pork

ham through retarding the microbial spoilage during storage time<sup>21</sup>).

Chen *et al.* (2015) prepared an antimicrobial agent using calcination of oyster, hard clam, and sea urchin to inactivate foodborne disease microorganism. The results showed that calcined oyster powder inactivated the microbial growth such as *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes*, *Salmonella typhimurium*, *Enterobacter aerogenes*, and *Proteus vulgaris*. Notably, the raw oyster shell powder did not present inhibitory against microbial growth, but after calcination, oyster powder showed a strong inhibitory ring against foodborne bacteria<sup>22</sup>). Such antimicrobial activities might be related to CaO, which was converted from CaCO<sub>3</sub> using calcination process. Therefore, calcined oyster shell could be an antimicrobial agent in food science and food packaging to extend the shelf life of food items and prevent the foodborne disease

Xing *et al.* (2013) provided the antifungal additive through calcination of waste shells and their pyrolyzed. This study calcined oyster and scallop shells using a heat treatment at high temperature (1050°C). The characterization (XRD) of resulting materials exhibited that the major component was CaO, which might be a strong biocidal agent. The antifungal activity of calcined shells against *Physalospora piricola* and *Rhizoctonia solani* was investigated in which both calcined shells showed strong antifungal activities. Notably, antifungal activities of calcined oyster shell was pronounced compared with scallop shell. In addition, non-calcined oyster shell powder also showed antifungal activity, which this inhibition might be related to alkalinity of CaCO<sub>3</sub> in the slurry phase<sup>23</sup>).

Jung *et al.* (2010) utilized the oyster shell powder as an antimicrobial additive to prolong the shelf life of Gat Kimchi during storage time (80 days 5°C). Oyster shell powder reduced the number of inoculated bacteria (lactic acid bacteria, yeast, and *E. coli*) during storage time and appropriately maintained the quality of Gat Kimchi compared with control. Despite the oyster shell powder extended the shelf life of Gat Kimchi, there was a limitation regarding its content in Gat



**Fig. 3.** Surface microscopic evaluation of (a) none-calcined and (b) calcined oyster shell (Reproduced with permission from reference<sup>5</sup>).

Kimchi. It has been reported that oyster shell powder showed high pH compared with other samples, which might be related to an alkalinity environment (primary antimicrobial mechanism) caused by calcium based compound in oyster shell powder. Therefore, oyster shell powder reduced the microbial number and extended the shelf life of Gat Kimchi<sup>24</sup>.

### Safety and biocompatibility

The progressive trend to find natural biocidal alternative may contribute to some challenges such as human safety or environmental issues. Despite deriving functional materials from natural wastes can probably provide safe compounds, the characterization in detail are required to understand chemical, physical, and biological effects of new materials on human body and environment. As such, care must be taken to ensure new materials can meet the safety requirements. It has been reported that Ca based compounds are biocompatible and safe for human because such compounds are widely used in industrial, medical, and food setting such as sugar refining, tissue engineering, and antimicrobial purposes<sup>7,9,10</sup>. To evaluate the biocompatibility of calcined oyster shell, *in-vitro* cytotoxicity of such compound in the PP polymer matrix was conducted using a microscopic assessment of fibroblast cell growth. The results showed that cells were appropriately grown in the presence of different contents of calcined oyster shell, indicating that such compound may not show any cytotoxicity against human body. The number of proliferation of cells was relatively similar in all samples, which might possess appropriate biocompatibility<sup>6</sup>. The direct contact materials in food packaging should not led to safety hazards. CaO derived from calcination of oyster shell can be used as a direct additive in food items or can be introduced into polymer matrix. In addition, utilizing the natural materials, particularly wastes may contain some impurities and pollutants such as heavy metal or toxic materials. Therefore, care must be taken to ensure required characterizations are conducted to detect hazards prior to application.

### Conclusion

Preparation of natural biocidal compounds is an attractive alternative for synthetic antimicrobial agents in packaging and food preservation. As such, finding such compounds through utilizing natural wastes can provide ecofriendly materials with an appropriate biocompatibility. Calcination marine shells, particularly oyster shell through a clean method can produce the CaO as an antimicrobial agent. Oyster shell disposal tends to be an environmental issues in the southern coast of Korea owing to continuously dumping in the landfills. Calcination of oyster shell using a heat treatment can convert the CaCO<sub>3</sub> (main component in oyster shell) into CaO. CaO can inactivate the microbial growth and reduce the microbial number through

some mechanisms such as alkalinity condition as well as generation of Ca<sup>2+</sup> and ROS. The addition of calcined oyster shell into food packaging can maintain the quality of packed food items and prolong their shelf life until the products reaches the consumers. Deriving CaO from natural-based resources can obviate the safety concern regarding cytotoxicity or carcinogenicity owing to high compatibility with human body and lack of toxicity. The characterization of resulting materials can provide sufficient information regarding the calcined oyster shell prior to application. Therefore, calcination of oyster shell disposal can provide an emerging biocidal compound to apply in the food packaging.

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