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ANTIMICROBIAL ACTIVITY OF NOVEL PACKAGING MATERIAL MADE FROM BIOMASS PLASTICS AND SHELL (SCALLOP) POWDER

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ABSTRACT Plastics derived from biomass have recently obtained much attention from the public because they are synthesized from renewable raw materials. About 400 000 tons of scallops are produced every year in Hokkaido Prefecture of Japan. About half of that weight is shell and it is dumped as waste. It has been known that the baked shell powder has an anti microbe function. The function results from calcium hydroxide (Ca(OH)2) which is one of the components of baked shell powder. Ca(OH)2 is highly ionized and indicates strong alkali in water. Most microorganisms can not survive under such strong alkaline condition. The objective of our study was to develop new functional packaging film from biomass plastics and from the shell in order to encourage the use of biomass plastics and to utilize the shell as a valuable resource. A halo test was adopted to evaluate the anti microbe function of the film. The film was placed on a solid agar containing indicator microbe and the effect in terms of size of "no micro organisms placed (halo)" around the film was observed. The result showed that the halo did not expand around the film and there were no colonies under the film. In addition, the film was put into water to assess if the results were caused by Ca(OH)2. The result showed that the solution that was put in the film had a high pH value and indicated that Ca(OH)2 had dissolved from the film. It was concluded that the film has an effect of antimicrobial activity, but the effect can appear only under aqueous condition.

Keywords: Biomass Plastics, Antimicrobial activity, Freshness, Packaging film

INTRODUCTION In confronting the environmental crisis of carbon dioxide emissions and the exhaustion of oil on a global basis, plastics derived from biomass have been recently obtained much attention from the public, because they are synthesized from the raw materials derived from renewable resources such as corn. In the meantime, Scallop is one of the main marine products in Hokkaido Prefecture of Japan. It is produced about 400 thousand tons every year. Nearly half of the amount is shell, and it is mostly dumped as industrial waste. In order to utilize this waste as valuable resources, various usages have been examined, but the effective usage has not been developed. It has been known that this shell has antimicrobial activity. The effective usage which uses this function also has to be examined. Based on these backgrounds, the new functional film from biomass plastics and shell powder of scallop is developed. If this film also has antimicrobial activity, it will be advantageous to food packaging. To use such a film for food packaging can preserve freshness during through the distribution from production to consumption. In this study, therefore, the authors investigated the effect and the mechanism of antimicrobial activity of shell powder and the film.

METHODOLOGY

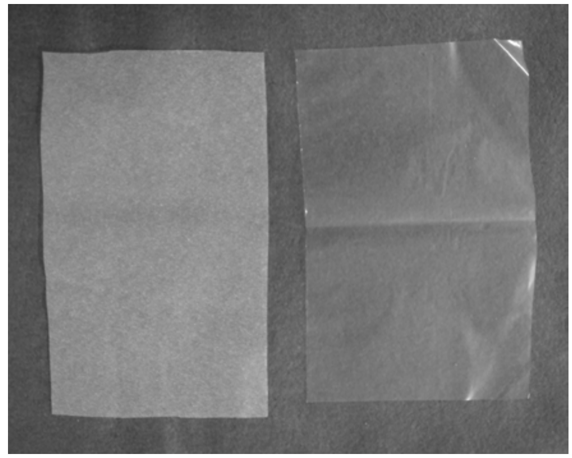

Figure 1. Appearance of the film
(Left) Film containing shell powder
(Right) Blank film

Materials The shell powder of scallop used in this study was baked at more than 900˚C, and it must consist of calcium carbonate ($CaCO_3$), calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$). The principal component of shell powder is $CaCO_3$. When $CaCO_3$ is baked at more than 900˚C, it becomes to CaO, then which changes to $Ca(OH)_2$ under wet environment. The types of film materials used in this study were Poly (lactic acid) (PLA) and Poly (butylene succinate adipate) (PBSA). PLA is bio-degradable and derived from biomass. PBSA is bio-degradable and derived from oil. Shell powder was mixed in these plastics. Mixing ratio of it was 10%. Average size of the shell powder which was contained to the film was 40μm. The film was produced by inflation technique. Considering that stability of producing the film, the types of plastics and mixing ratio of the shell powder was decided. Microorganisms used in this study as an indicator was *Klebsiella. oxitoca*.

Methods

Evaluation of the antimicrobial effect of shell powder Indicator, *K.oxitoca* was added to the solution which contains shell powder in different concentration. This solution kept at 37°C for 24 hours. After then, the amounts of surviving microorganisms were determined by colony counting and results expressed as CFU/mL.

Evaluation of antimicrobial activity of the film There are no suitable methods to evaluate the effect of antimicrobial activity of film. But there are some official methods to investigate the effect of antimicrobial activity of paper, fiber, plastic plate and so forth. We applied these methods to evaluate the effect of the film.

First, we adopted 'Halo Test'. Generally, this method is adopted to evaluate the effect of antimicrobial activity of paper or fiber. In this method, test piece puts on agar containing microorganisms. After incubating in proper time and temperature, no microorganism area (halo) expand around test piece. The size of halo indicates strength of antimicrobial activity and the diffusion of antimicrobials from film. We placed the film which was cut into circle having 6mm diameter and sterilized the film by ethanol on a solid agar containing *K.oxytoca*. The agar plates are incubated at 35°C for 24, 48, 72 hours. After then, we observed if Halo expand the film or not.

Second, we adopted ‘Film Contact Method’. Generally, this method is adopted to evaluate the effect of antimicrobial activity of plastic plate and metal plate. In this method, bacterial culture is put on sample containing antimicrobials and blank sample. After incubating in proper time, temperature and humidity, viable cell in bacterial culture is counted. Strength of antimicrobial activity is evaluated by the difference of viable cell count between sample containing antimicrobials and blank sample. We placed the film which was cut into square having 5mm side and sterilized by ethanol in sterilized laboratory dish. Solution (0.4mL) containing *K.oxytoca* was put on the film. To spread the solution equally on the film, film which do not contain shell powder was put on the solution. The films are kept at 37°C and more than 90%RH for 24 hours. After then, the amounts of surviving *K.oxytoca* on the film were determined by colony counting.

Evaluation of dissolving shell powder from the film in water We put the film in water kept at 23°C to assess if shell powder dissolves from the film. The pH value of the solution was measured every 15 minutes by the pH meter.

Table 1. Antimicrobial effect between shell powder concentration and viability of *K.oxitoca*

Concentration (%)	Viable cell count (CFU/mL)
0.00	5.7×10^{9}
0.25	2.0×10^{8}
0.50	1.8×10^{7}
0.75	＜10
1.00	＜10
1.25	＜10

RESULTS AND DISCUSSIONS

Evaluation of the antimicrobial effect of shell powder As shown in Table 1, most of *K. oxytoca* cannot survive at more than 0.75% of shell powder in water. At less than 0.75% of shell powder in water antimicrobial activity to *K. oxytoca* was appeared. The pH value of the solution was increased to the concentration of shell powder up to 12.6 (Figure 1.). High pH value is derived from $Ca(OH)_2$ which is one of the component of baked shell powder. $Ca(OH)_2$ is high ionization and provides strong alkaline condition in solution. Most microorganisms cannot survive under such strong alkaline environment. From this result, we concluded that shell powder had the effect of antimicrobial activity.

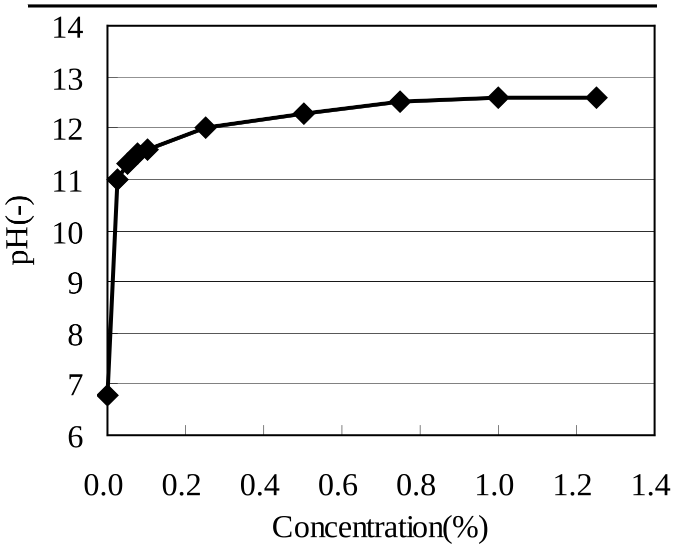

Figure 2. Value of pH in the solution and concentration of shell powder

Evaluation of the antimicrobial activity of the film In "Halo Test", halo did not expand around the film except under the film. Appearance of laboratory dish after incubation for 48 hours is shown in Figure 3. The results were the same after incubation for each hour. These results indicate that the films have antimicrobial activity, but strength of the activity could not investigate through this method. There are two possibilities to explain this result. One possibility is that the effect was limited. Other possibility is that solution which has a high pH did not diffuse in agar though $Ca(OH)_2$ was dissolved from the film. To investigate the strength of the activity, another way of evaluation should be done.

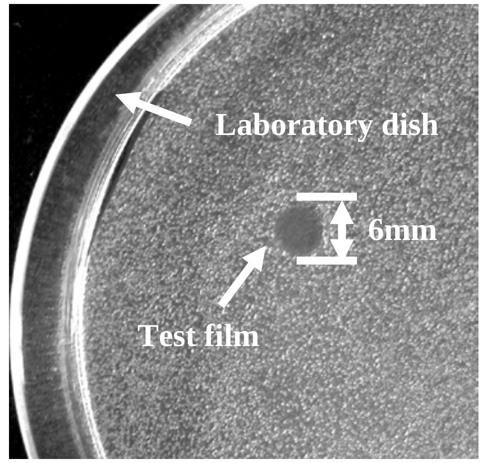

Figure 3. Result by Halo Test

The result by "Film Contact Method" is shown in Table 2. Much *K.oxytoca* was survived after incubating for 24 hours on blank film. On the other hand, almost *K.oxytoca* was killed after incubating for 24 hours on the film containing shell powder. These results also indicate that the film has antimicrobial activity.

Table. 2 Result by Film Contact Method

Sample	Viable cell count (CFU/sample piece)
Blank film	9.0×10^5
Film containing shell powder	<10

Evaluation of dissolving shell powder from the film in water The solution which was put in the film containing shell powder had a high pH value. Our results are shown in Figure 4. The pH value of the film was approaching to the pH value of shell powder. The results indicate that $Ca(OH)_2$ which is one of the component of baked shell powder dissolved from the film. The results also indicate that antimicrobial activity which was confirmed by "Halo Test" and "Film Contact Method" caused by baked shell powder. The results also indicate that this film needs wet environment to have the effect of antimicrobial activity.

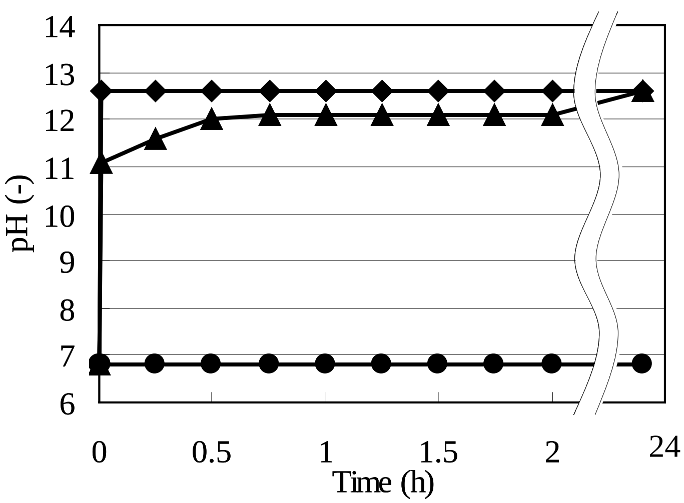

Figure 4. Value of pH in the solution which was put in shell powder (♦), film containing shell powder (▲) and blank film (●).

CONCLUSION In this study, we concluded that the film has an effect of antimicrobial activity, but the effect can appear only under wet condition. If this film is used for packaging of perishable food, antimicrobial activity can keep the food fresh for longer period.

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