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Research Note

Microbial Inhibition and Migration of Nisin-incorporated Antimicrobial Polymer Coating on Paperboard

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Abstract Two kinds of polymer coating (acrylic polymer and vinyl acetate ethylene copolymer) added with 5% nisin were fabricated on the paperboards and tested in their antimicrobial activity against *Micrococcus flavus* ATCC 10240 inoculated into water contacting the coating at 10°C. Vinyl acetate ethylene copolymer giving faster and higher nisin migration presented the greater reduction in the microbial count than the other coating, which endorsed that the migrated nisin is mainly responsible for the microbial inhibition or destruction. There was also a slight effect of the coating polymer itself in the microbial inhibition.

Keywords Antimicrobial packaging, Nisin, polymer coating, Migration, antimicrobial activity

Introduction

Quality deterioration of perishable foods during storage mainly comes from microbial spoilage. These deteriorations conflict with the consumers' demands for high quality foods with ensured safety and extended shelf-life. As a way to solve these problems, concept of antimicrobial packaging has been proposed and developed[1-3]. Through the food-package interaction migratory or non-migratory, antimicrobial packaging delays or inhibits the microbial growth on the food surface preserving the microbial quality and extending the shelf life. Majority of antimicrobial food packaging studies reported 2 $\log_{10}$ reductions in microbial count by single application of antimicrobial materials, which is not satisfactory for ensuring safety and quality of packaged perishable foods[4]. Most of antimicrobial packaging developments are based on controlled release of active antimicrobial substances from polymer matrix to achieve their desired concentration on the food surface[5]. The antimicrobial substances incorporated into the whole matrix or coating layer is designed to be migrated into the food at controlled rate. For effective application of migration-controlled antimicrobial packaging, thorough understanding on the interaction between migration and microbial inhibition is desired[6].

In consideration of safety issues of antimicrobial agents used in antimicrobial packaging, natural substances or plant extracts have been widely studied for incorporation into packaging materials[4,7]. The most intensively studied antimicrobials include bacteriocins such as nisin, essential oils from spices and herbs, and chitosans [4, 8-10]. Even though migration of active substances from the packaging film or layer is recognized as a critical factor for attaining the desired microbial inhibition, its relation to antimicrobial effectiveness has not been studied sufficiently to elucidate its mechanism and kinetics. Even many studies were undertaken on antimicrobial packaging such as nisin-incorporated polymer, the results of these studies cannot be compared and analyzed because of substantial variations in the packaging matrix, fabrication methods, test microorganisms and test methods[3].

Therefore this study aims to look into the relationship between active agent migration and antimicrobial activity for an antimicrobial packaging system. As the antimicrobial packaging system, two types of nisin-incorporated polymer coating on paperboard were studied. It is understood that nisin incorporation is the most widely studied technique to fabricate the antimicrobial polymer structure for food packaging applications[10,11].

Material and Methods

A nisin as an antimicrobial agent was purchased from Sigma Chemical Co. (St. Louis, MO, USA). As a coating binder on a virgin paper board, two polymer emulsions of acrylic polymer (B-15J, solid content: 45.7%, pH: 6.3, viscosity: 45 cps, color: white) and vinyl acetate ethylene copolymer (Elavace 40724, solid content: 54.5%, pH: 4.4, viscosity: 2000cps, color: white) were used, and those coating binder were obtained from Reichhold Chemical Inc. (Research Triangle Park, NC, USA) and Rohm & Hass Co. (Philadelphia, PA, USA), respectively.

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A virgin paper board with thickness of 0.231 mm (Daehan Pulp Co., Chungwon, Korea) was used as a base material for coating. Prior to coating onto the paper, a nisin (St. Louis, MO, USA) was dissolved in 20% ethanol. The solution was then mixed with a coating binder to obtain a final 5% (w/w) of nisin coating medium, based on the dry weight of coating binders. The prepared coating mediums with nisin were coated manually on one side of the paper board by multiple uses of a No. 32 coating rod (RD Specialties Inc., Webster, NY, USA) The coating procedure was repeated to obtain the required thickness (≈3 mm). The nisin coated-paper was then dried at 60°C for 5 days to evaporate the ethanol and water in the coating binder. A hand-held micrometer (M120-25, Mitutoyo Co., Tokyo, Japan) was used for measuring the thickness.

A glass cylindrical cup with an inner diameter of 6.5 cm was attached to the nisin-added polymer coated paperboard using silicon sealant. Fifty milliliters of distilled water as an aqueous food simulant were poured onto the glass cup in contact with the polymer-coated paper, covered with glass lid and then stored at 10°C. A glass plate was placed on the bottom side of the paper sample in order to protect it from possible permeation and evaporation of water through the paper. One milliliter culture of *Micrococcus flavus* ATCC 10240 with a cell density about 10^9/mL was then inoculated into the distilled water in the glass cup. A 0.1 mL of samples taken periodically from the stored cell was diluted serially with sterilized distilled water and then was spread onto nutrient agar (Difco Laboratories, Detroit, USA) plate medium. The plate medium was incubated at 30°C for 2 days in order to measure the microbial count in colony forming units (CFU)/mL. The survival of microorganisms through time were overlaid and examined with progress of nisin migration to the water of the same configuration which had been reported by Kim et al.[11].

In order to directly examine the effectiveness of antimicrobial activity of nisin migrated from the polymer coatings on paper, 2 mL of water sample was taken from migration cell contacting the coatings for 7 days, and then added with 1 mL of *Micrococcus flavus* culture with cell density of 10^9/mL. These samples were stored for 6 hours and subjected to the microbial counting as described as above.

Results and Discussion

Fig. 1 shows the microbial inhibition of *M. flavus* ATCC 10240 in the water contacting the nisin-incorporated (5%) polymer coating at 10°C for 4 days. The distilled water contacting control polymer coatings without nisin did not support the growth of *M. flavus* probably due to absence of nutrients required for its growth. The coating binder, vinyl acetate ethylene copolymer, without nisin incorporation, showed a slightly greater microbial inactivation in the water. It is noted

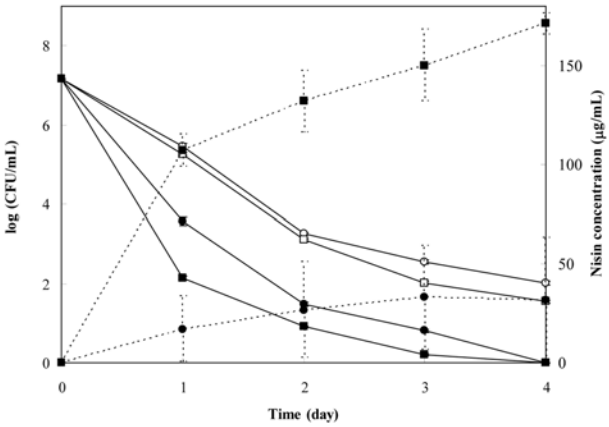

Fig. 1. Count of *Micrococcus flavus* ATCC 10240 in distilled water contacting the nisin-added polymer coating at 10°C. Solid lines are the microbial counts, and dotted lines are nisin concentration in the water reported by Kim et al. [11]. Vertical bars are standard deviations. ○: acrylic polymer coating only; □: vinyl acetate ethylene copolymer coating only; ●: acrylic polymer with 5% nisin; ■: vinyl acetate ethylene copolymer with 5% nisin.

that vinyl acetate ethylene solution for coating has a pH of 4.4, while acrylic polymer solution has a pH of 6.3. Acid migrated from vinyl acetate ethylene coating might have contributed somehow to the microbial inhibition.

Decrease in the viable counts of *M. flavus* ATCC 10240 in the water was greatly accelerated by its contact with the polymer coatings added with 5% nisin (Fig. 1). Particularly, nisin-incorporated vinyl acetate ethylene copolymer presented the pronounced antimicrobial activity compared to acrylic polymer. This evolution of microbial inactivation can be related to migration progress of nisin to the water. Both migration and microbial inactivation were fast during 1 day and slowed down thereafter. It seems evident that fast and highly migrated nisin contributed to the microbial count decrease, even though lower pH from vinyl acetate ethylene copolymer helped slightly the microbial inhibition. According to Kim et al. [11], nisin in vinyl acetateethylene copolymer coating has higher equilibrated migration (3.2 vs. 1.3%) and diffusion coefficient (9.3×10^{-12} vs. 4.2×10^{-12} m^2/s) than that in acrylic polymer at 10°C. According to Leung et al.[12], hydrophobicity of the polymer has great influence on the release of nisin from it.

Even though Fig. 1 showed that migration parallels the growth inhibition of *M. flavus* ATCC 10240, there is a need to directly verify that the migrated nisin is responsible for the microbial destruction. There might have been actions of nisin immobilized in the polymer coatings. For this purpose, 2 mL of 50 mL water samples having contacted the polymer coatings (5% nisin addition) for 7 days were mixed with 1 mL solution of *M. flavus* ATCC 10240 culture with cell density of 10^9/mL and then monitored in the microbial count. The con-

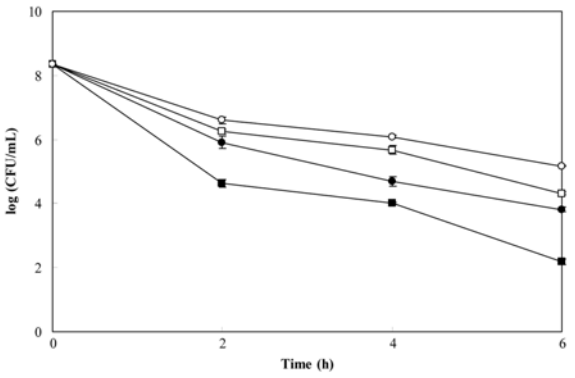

Fig. 2. Growth inhibition of *Micrococcus flavus* ATCC 10240 in 2 mL of distilled water at 10°C, which had contacted the polymer coating at 10°C for 7 days and then combined with 1 mL solution of *M. flavus* with ≈ 10^9 cells/mL. Nisin concentrations were 29.7 and 160.0 μg/mL in the distilled water samples having contacted acrylic polymer and vinyl acetate ethylene copolymer coatings, respectively. Vertical bars are standard deviations. ○: acrylic polymer coating only; □: vinyl acetate ethylene copolymer coating only; ●: acrylic polymer with 5% nisin; ■: vinyl acetate ethylene copolymer with 5% nisin.

tact with the vinyl acetate ethylene and acrylic polymer coatings for 7 days resulted in respective nisin concentration of 29.7 and 160.0 μg/mL in the water samples. As shown in Fig. 2, the food stimulant of water having contacted vinyl acetate ethylene coating with higher migration caused significantly higher microbial destruction than that of acrylic polymer. As with Fig. 1, the food stimulants without migrated nisin in the control coatings showed some degree of microbial destruction with greater microbial count decrease for vinyl acetate ethylene coating, which had a lower pH. However, the difference between two coating binders was much greater with nisin-incorporated ones, which confirmed the effect of migrated nisin on the microbial inhibition. Even though Figs. 1 and 2 confirmed that the migrated nisin is responsible for microbial destruction by the antimicrobial polymer coating, the results are qualitative. Further works with kinetic treatment is required for elucidating quantitatively the effect of the migrated active compounds on the antimicrobial behavior in the food.

This study has been conducted using distilled water as food stimulant for simplified analysis, and real liquid foods containing many soluble and non-soluble solids may have behavior different from the water. Further study is needed to elucidate the effectiveness of the migration-controlled nisin in real food systems and design the effective antimicrobial packaging incorporating nisin.

Conclusion

Growth inhibition of *Micrococcus flavus* ATCC 10240 in the aqueous food simulant contacting antimicrobial polymer coatings with nisin was found to be caused by nisin migrated. The polymer coating giving faster and higher migration resulted in greater decrease in the microbial count.

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