STUDY OF AN ANTIMICROBIAL STARCH-BASED ACTIVE PACKAGING SYSTEM

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ABSTRACT

In response to the dynamic changes in current consumer demand and market trends, the area of Active Packaging (AP) is becoming increasingly significant. An Antimicrobial Active Packaging can be made by incorporating and immobilizing antimicrobial (AM) agents into food packages and applying a bio-switch concept. By that, the mechanism of antimicrobial release between the developed bio-switch particles and the stimulus of a microbial contamination can be studied. A starch-based film was prepared and incorporated with antimicrobial agents consisting of a combination of nisin and lysozymes. This film was then inoculated with the bacteria *Escherichia coli* and *Bacillus subtilis* to carry out the microbial contamination study. The changes in film appearance that indicate microbial growth inhibitory action by the AM agents were being examined. Clear zones formed on the film appearance showed the combination of both AM agents gives good inhibition to the growth of *E.coli* and *B.subtilis* with satisfying inhibition rate. With the advent of new polymer materials and antimicrobials, the development of AP could prolong the shelf life of food and reduce the risk of foodborne illness caused by microbial contamination.

Keywords: Active packaging; Antimicrobial film; Bio-switch concept; *Escherica coli*; *Bacillus subtilis*; Nisin; Lysozymes

INTRODUCTION

A current trend in the food industry is the manufacture of mildly preserved, healthy and easy-to-prepare products driven by consumer demands for fresh 'natural' convenience food. It is crucial to maintain food safety and quality while reducing costs and centralizing activities especially in longer distribution distances. The factors put high demands on the shelf life extending capacity of food packaging systems. A wide selection of antimicrobial substances, for example, organic acids, bacteriocins, antibiotics, fungicides, chelating agents, parabens and metal; have been considered to have possible antimicrobial activity when incorporated in or coated onto food packaging materials *(5,6)*. Nisin activity is restricted to Gram-positive bacteria but can be active against Gram-negative bacteria when combined with chelators and surfactants *(4)*. Larsen (1995) found that 15 mM EDTA was effective against *Escherichia Coli* when used in corn zein films with different nisin concentrations. Clemson University researchers, Dawson reported that nisin and lysozyme combined with EDTA when incorporated into the structure of corn zein film inhibited the growth of selected strains of Gram-positive and Gram-negative bacteria. The present article reports on the ability of starch-based film incorporated with those antimicrobials to inhibit growth of *E.coli* and *B.subtilis*.

MATERIALS AND METHOD

Preparation of Antimicrobial Starch-Based Film

Starch-based films were prepared by dissolving 8.35 g starch in 80 mL of 20% ethanol with stirring. After the solution was completely dissolved, 3.8 mL glycerin (HmbG Chemicals) was added as plasticizer and the mixture was heated slowly to a mild boiling. For antimicrobial incorporated films, antimicrobial agents were mixed with 10 mL of the film solution in a separated beaker just before casting. Five milliliters of the film mixture was pipetted into petri dishes (100 mm diameter by 15 mm depth). The petri dishes were placed for 24 hour in an oven (Memmert) set at 70°C.

Antimicrobial Incorporated Film

Ethylenediaminetetraacetic acid (EDTA) (HACH, Loveland, USA), lysozyme (Sigma-Aldrich, St. Louis, MO) and nisin (Sigma-Aldrich, Steinham) were added separately into beakers containing 10 mL film solution at concentration of 0.2 mL/mL, 0.07 g/mL and 0.0375 mg/mL respectively. Films were also formed by the combination of EDTA and lysozyme, EDTA and nisin, and nisin and lysozyme, using the concentrations as mentioned above. With each test, a control film was also formed with no antimicrobial added.

Table 1. ***Type of Antimicrobial Films***

Film	Antimicrobial
1	None (Control)
2	Nisin
3	EDTA
4	Lysozyme
5	Nisin + EDTA
6	Nisin + Lysozyme
7	EDTA + Lysozyme

Inhibition of *Escherichia Coli* and *Bacillus Subtilis* on Agar Plate Test

The strain selection represented typical spoilage organism groups commonly occurring in various kinds of food products. The strains were as follows: (1) *Escherichia Coli*, a conventional hygiene indicator organism, a Gram-negative rod belonging to the same family of *Enterobacteriaceae* as for example *Salmonella*. (2) *Bacillus subtilis*, a Gram-positive rod capable of forming heat-resistant spores. Spores and vegetative cells of *Bacillus* species are widely distributed in nature and are common for example in cereals. For the agar plate test, the starch-based films containing EDTA as antimicrobial agent were cut into six squares (0.5 cm x 0.5 cm). Six sample squares were then placed onto the plate spreaded with bacteria (0.1 mL per plate). The same tests were performed using other film containing, stated: nisin,

lysozyme, EDTA and nisin, EDTA and lysozyme, and nisin and lysozyme. Duplicate agar plates were prepared for each type of film and control film. The agar plates were incubated at 37°C for 48 hours.

Enumeration

For the liquid culture test, each film was cut into squares (1 cm x 1 cm). Three samples squares were immersed in 20 mL nutrient broth (Merck, Germany) in a 25 mL universal bottle. The medium was inoculated with 200μL of *Escherichia Coli* in its late exponential phase, and then transferred to an orbital shaker and rotated at 30°C at 200 r.p.m. The culture was sampled periodically (0, 2, 4, 8, 12, 24 hours) during the incubation to obtain microbial growth profiles. The same procedure was repeated for the control starch-based film. The optical density ($o.d._{600}$) was measured at $\lambda = 600_{nm}$ using a spectrophotometer (Model UV-160, Shimadzu, Japan).

RESULTS AND DISCUSSION

Inhibition of *Escherichia Coli* and *Bacillus Subtilis* on Agar Plate Test

All samples were examined for possible inhibition zones after incubation at 37°C for 48 hours. Table 2 lists calculated inhibition area for each plate test. The control films (Plate 1) showed no inhibition area and colonies were formed all over the plate. Film containing EDTA (Plate 3) showed inhibitory growth of both *Escherichia Coli* and *Bacillus subtilis*. Film containing nisin (Plate 2) and lysozyme (Plate 4) alone showed no inhibition towards *B. subtilis* and *E. coli* respectively. This is not surprising since nisin had been regarded as being inactive against Gram-positive bacteria, while lysozyme was inactive against Gram-negative bacteria *(1, 2)*. Nisin and lysozyme combined with EDTA (Plate 5 & 7) inhibited growth of *B. subtilis* and *E. coli.* It is clear that nisin shows better inhibition on *B. subtilis* whereas lysozyme acts better on *E. coli.* This strongly supports the previous observation.

Table 2. ***Inhibition of Escherichia coli and Bacillus subtilis on agar plates expressed as an area (cm^2) of inhibition zone***

Film	*Bacillus subtilis* (48 hours @ 37°C)	*Escherichia Coli* (48 hours @ 37°C)
Control	-	-
Nisin	-	6.60
EDTA	13.77	17.97
Lysozyme	5.57	-
Nisin + EDTA	20.30	15.18
Nisin + Lysozyme	3.52	1.44
EDTA + Lysozyme	15.00	20.63

Liquid Culture Test

Figure 1 shows the inhibition of *E. coli* by the antimicrobial films in liquid culture test. Clearly, combination of lysozyme and EDTA shows the largest reduction of stationary growth phase. At the stationary growth phase, the cell concentration in the control medium ($o.d.600_{nm} = 1.175$) was 3 times higher than the cell concentration in the medium containing the starch-based film incorporated with nisin and EDTA ($o.d.600_{nm} = 0.412$). The results suggest no bacteriocidal effect on *Escherichia Coli* by the films containing single antimicrobial agent of nisin or combination of nisin and lysozyme.

From the above discussion, it can be concluded that single nisin is able to inhibit growth of Gram-negative bacteria such as *E. coli,* whereas single lysozyme is able to inhibit growth of Gram-negative bacteria such as *B. subtilis.* Whilst, nisin and lysozyme combined with EDTA enable inhibition of both bacteria growth. As a chelating agent, EDTA plays an important role for the antimicrobial to function in the film matrix.

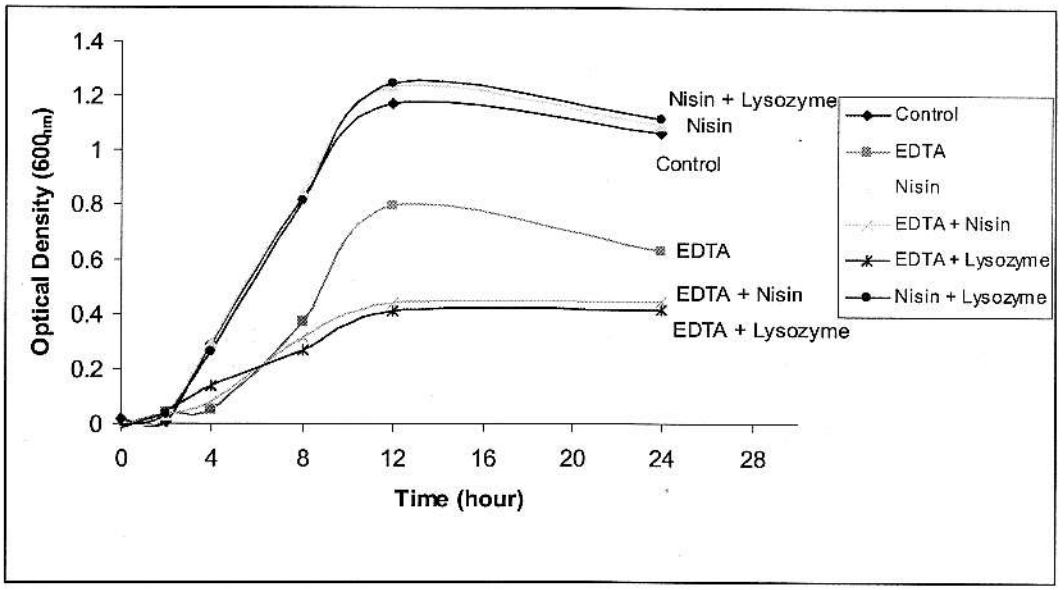

Figure 1. ***Inhibition of Escherichia Coli by the starch-based film containing antimicrobial agents in a liquid culture medium at 37°C***

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REFERENCES

1. N. J. Russel and G.W. Gould (1991). Food Preservatives
2. Paolo Appendini and Joseph H. Hotchkiss (2002). Review of Antimicrobial Food Packaging Innovative Food Science & Emerging Technologies, 3, 113-126.
3. Larsen, T. (1995). Incorporation of Nisin into Corn Zein Film. M. S. thesis. Clemson University, Clemson, South California.
4. K. L. Hoffman, I. Y. Han, and P. L. Dawson (2001). Antimicrobial Effects on Corn Zein Films Impregnated with Nisin, Lauric Acid and EDTA. *Journal of Food Protection*. 64:885-889.

5. Jari Vartiainen, Eija Skytta, Jouni Enqvist and Raija Ahvenainen-Rantala (2003). Antimicrobial and Barrier Properties of LDPE Films Containing Imazalil and EDTA . *Journal of Plastic Film & Sheeting*. 19:249-259.

6. Rooney, M.L. (ed.) (1995). *Active Food Packaging*, Chapman & Hall, London, UK.