PRELIMINARY STUDY OF ANTIMICROBIAL (AM) EFFECTS OF STARCH-BASED FILM INCORPORATED WITH NISIN, LYSOZYMES AND LAURIC ACID

Nozieana Khairuddin*, Ida Idayu Muhamad

Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.
e-mail: nozieana@yahoo.com

Abstract Active Packaging (AP) concept is a current trend in food packaging industries. An antimicrobial (AM) Active Packaging can be made by incorporating and immobilizing suitable 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, i.e. nisin, lysozyme and lauric acid. This film was then inoculated with the bacteria *Escherichia Coli* to carry out the microbial contamination study. The inhibition of *E. Coli* by the AM film was clearly observed in the broth and culture agar test. The decreased of optical density ($OD_{600\ nm}$) showed the inhibition of *E. Coli* growth. While, the clear zones formed on the film appearance showed that AM agents give good inhibition to the growth of *E. Coli* with satisfying inhibition rate. Lysozyme-incorporated film shows better inhibition than the other two in both tests.

Keywords: Antimicrobial film; Bio-switch concept; Nisin; Lysozymes; Lauric Acid; *Escherichia Coli*

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. In response to the dynamic changes in current consumer demand and market trends, the area of Active Packaging (AP) is becoming increasingly significant. The active food packaging is a specially designed food packaging system to preserve food quality, improve safety and prolong the shelf-life of the packaged food products. In general, active packaging provides several functions that do not exist in conventional packaging systems. The special active functions that distinguish it from conventional packaging systems include anti-microbial activity, oxygen scavenging ability, ethylene scavenging activity, moisture absorbing activity and many more. Among the active packaging technologies, the anti microbial (AM) packaging system is mainly chosen to be studied. Broad spectra of spoilage bacteria are tested to verify the effectiveness of antimicrobial packaging materials which contain chemical preservatives and natural antimicrobials. AM-enhanced packaging films have great potential for ensuring the safety of food surfaces through controlled release of AM substances from the carrier film structure to food surface. The AM compounds and their incorporation into packaging materials have been well reviewed by Appendini and Hotchkiss [1]. Previous study by Hoffman *et.al* [2] shows that Lauric acid could reduce the population of *L.monocytogenes*. Padgett *et.al* [3] found that films containing Lauric acid alone did not have a significant effect on *Lactobacillus plantarum* using a zone of inhibition method but successfully reduced the population when film was contact with a liquid broth. After realizing these previous successes, lysozymes, nisin and lauric acid may be among good candidates in order to develop a new AM starch-based film. Nisin activity is restricted to Gram-positive bacteria but can be active against Gram-negative bacteria when combined with chelators and surfactants [2]. Dawson [4] 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.*

Experimental

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. 0.2 mL/mL of Ethylenediaminetetraacetic acid (EDTA) (HACH, Loveland, USA) was added as chelating agent. 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 25°C.

Antimicrobial Incorporated Film

Lysozyme (Sigma-Aldrich, St. Louis, MO), nisin (Sigma-Aldrich, Steinham) and Lauric acid (Sigma-Aldrich) were added separately into beakers containing 10 mL film solution at concentration of 0.07 g/mL, 0.0375 mg/mL and 0.07 g/mL respectively. Table 1 below shows the concentration of each AM-incorporated film. With each test, a control film was also formed with no antimicrobial added.

Table 1. Type of Antimicrobial Films

Film	Antimicrobial	Concentration
1	None (Control)	-
2	Nisin	0.0375 mg/mL
3	Lysozyme	0.07 g/mL
4	Lauric acid	0.07 g/mL

Inhibition of Escherichia Coli on Agar Diffusion Test

The strain selection represented typical spoilage organism groups commonly occurring in various kinds of food products. The strain was Escherichia Coli, a conventional hygiene indicator organism, a Gram-negative rod belonging to the same family of Enterobacteriaceae as for example Salmonella. For the agar plate test, the starch-based films containing each 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). Duplicate agar plates were prepared for each type of film and control film. The agar plates were incubated at 37°C for 48 hours.

Liquid Culture Test

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 37°C at 200 r.p.m. The culture was sampled periodically (0, 2, 4, 8, 12, 24, 48 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

Figure 1 shows the mechanism of how the inhibition of microbial growth happened in both culture and liquid test. Microorganisms hydrolyze starch-based particles, causing release of the AM compound and resulting in inhibition of microbial growth.

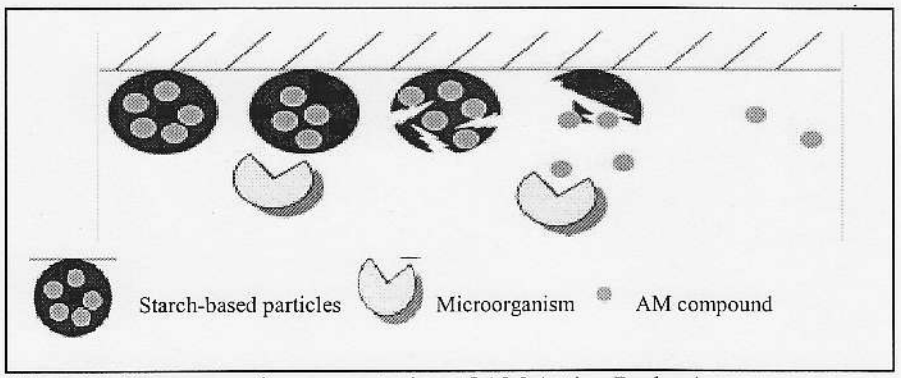

Figure 1. Diagrammatic representation of AM Active Packaging.

Agar Diffusion Test

Figure 2 shows the agar plate contained AM incorporated film in comparison to control film that contain no AM compound at all. From the observations, the AM-incorporated films showed clear zone formed on the agar plate after in contact with the microbe colonies. For this test a measurement of inhibition zones on/around film squares on inoculated bacteria was determined. Table 2 lists calculated inhibition area for each plate test. Lysozyme shows better inhibition on *E. Coli* followed by nisin and lauric acid.

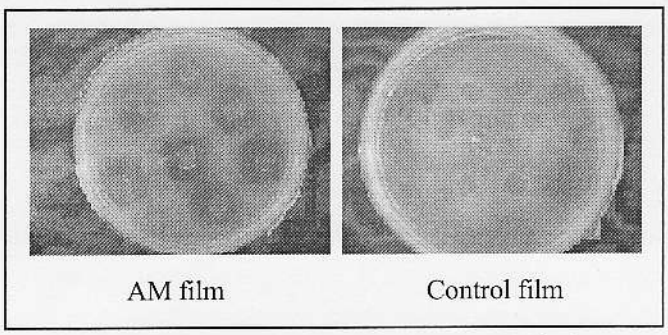

Figure 2. Inhibition area of AM incorporated film in comparison to control film.

Table 2. Inhibition of *E. Coli* on agar plates expressed as an area (cm^2) of inhibition zone.

Film	*Escherichia Coli* inhibition area (cm^2)
Control	-
Lysozyme	20.63
Nisin	15.18
Lauric acid	3.21

Liquid Culture Test

In this test the decrease in optical turbidity shows that the AM inhibits the bacteria growth. Figure 3 shows the inhibition of *E. Coli* by the starch-based film containing AM compound in a liquid culture medium at 37°C. Clearly, lysozyme shows the largest reduction of exponential growth phase. At the stationary growth phase, the cell concentration in the control medium (OD_{600nm} = 1.471) was about 3.5 times higher than the cell concentration in the medium containing lysozyme incorporated film (OD_{600nm} = 0.412). Broth culture exposed to lauric acid incorporated film had the lowest inhibition effect towards *E. Coli* growth.

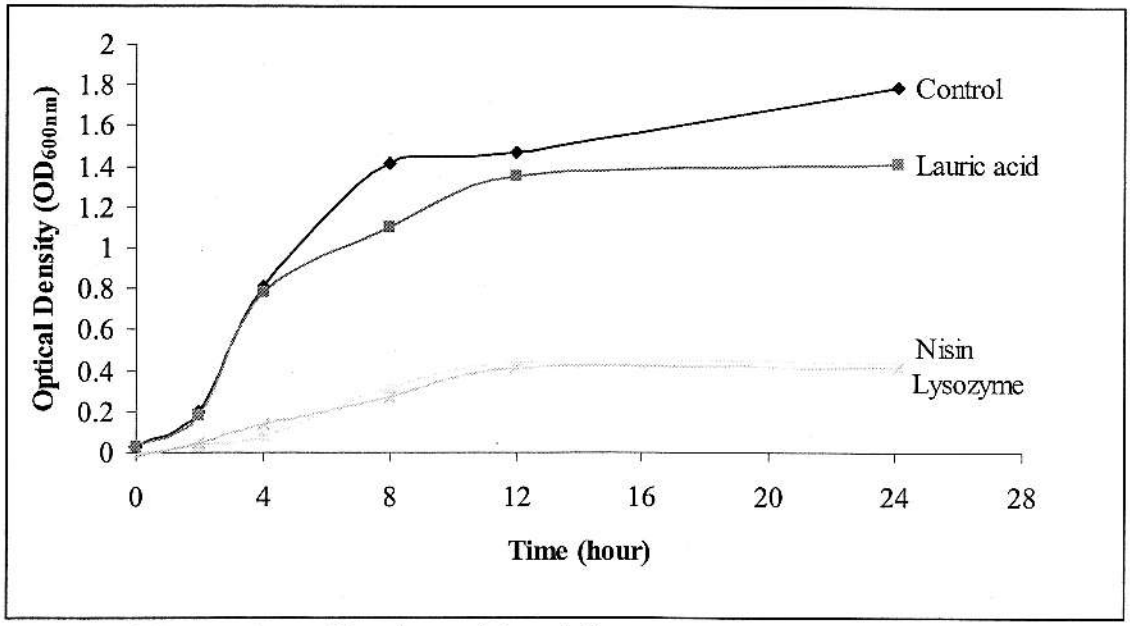

Figure 3. Inhibition of *E. Coli* by the starch-based film containing AM compound in a liquid culture medium at 37°C.

Conclusions

As a conclusion, the inhibition effects were observed by clear zone formed on the agar during agar diffusion test. In the liquid culture test, turbidity decrease resulted from the inhibition reaction. Lysozyme incorporated film effectively inhibits the growth of *E. Coli* better that nisin and lauric acid incorporated films.

Acknowledgement

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