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

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Antimicrobial (AM) film applying a bio-switch concept is an active packaging concept that is now being commercialized in food packaging industries. By that, an investigation to determine a suitable formulation for starch-based film is done that consists of corn flour, tapioca flour, pure starch and soluble starch. Pure starch has the best film output which is translucent and elastic. AM compounds i.e. Lauric acid and Lysozyme were incorporated into formed film at different concentration. Incorporation of AM compound affects the appearance and texture of the film. Luria agar was prepared to culture *Escherichia coli* for inhibition test. The inhibition of *E.coli* from the AM film was observed in the broth and culture agar test. The inhibition effect increased with the increase in AM concentrations. Lysozymes-incorporated films show better inhibition than Lauric acid-incorporated films in both tests.

Keywords: Antimicrobial film; Bio-switch concept; Lysozymes; Lauric acid; Escherichia coli

ABSTRACT

Antimicrobial (AM) film applying a bio-switch concept is an active packaging concept that is now being commercialized in food packaging industries. By that, an investigation to determine a suitable formulation for starch-based film is done that consists of corn flour, tapioca flour, pure starch and soluble starch. Pure starch has the best film output which is translucent and elastic. AM compounds i.e. Lauric acid and Lysozyme were incorporated into formed film at different concentration. Incorporation of AM compound affects the appearance and texture of the film. Luria agar was prepared to culture *Escherichia coli* for inhibition test. The inhibition of *E.coli* from the AM film was observed in the broth and culture agar test. The inhibition effect increased with the increase in AM concentrations. Lysozymes-incorporated films show better inhibition than Lauric acid-incorporated films in both tests.

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INTRODUCTION

Food is mostly perishable products and relatively has short shelf-life. Because of that, the food safety becomes the essential issues of the purpose of food packaging. According to a short survey, the percentages of consumers who are "most confidence" in the safety of the food products decreased from 83% in year 1996 to 74% in year 2000. It might be because of the consumers nowadays are looking for a food packaging that not only could store the contents and avoid it from physicals abuse; it should also influence the freshness and taste and control the microbial activity. As a response to continuous changes in currents consumers' demands and market trends, active packaging has been introduced. Active packaging is a new functional packaging system with special interactive functions which were not used to exist in the packaging system. 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 (Han 2000; Appendini and Hotchkiss, 2002).Lysozymes and Lauric acid are two food-grades AM shown to be effective in food applications. Lysozyme is a lytic enzyme found in many natural systems. Previously research has reported the ability of lysozymes to inhibit the *B.licheniformis* growth (Boumans, 2003). However, its limited AM efficacy against Gram-negative bacteria restricts its application in the food industry. However Masschalck and Michiels (2003) recently reviewed several methods of extending the AM spectrum of lysozymes to Gram-negative bacteria, including denaturizing of lysozymes and modification by attachment of other compounds to lysozymes such as EDTA. Lauric Acid is colorless, needle-like crystals and slight odor of Bay Oil. Previous study shows that Lauric acid could reduce the population of *L.monocytogenes* (D.Paul et.al ,2000). Padgett et.al (1995) 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 and Lauric acid may be a good candidate in order to develop a new AM starch based film.

MATERIALS AND METHODS

Preparation of Antimicrobial Starch-Based Film

Starch-based films were prepared by dissolving 7.75 g starch in 100 mL distilled water with stirring. After the solution was completely dissolved, 2.9 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 17 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) and Lauric acid (Sigma-Aldrich) were added separately into beakers containing 17 mL film solution. 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 g/mL
1	None (Control)	-
2	Lysozyme	0.01
		0.04
		0.07
		0.2
3	Lauric acid	0.01
		0.04
		0.07
		0.2

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.

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 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 & DISCUSSION

Agar diffusion test

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. Figure 1 shows the agar plate contained the lysozyme-incorporated film, lauric acid-incorporated film in comparison to control film that contains no AM compound at all. Figure 2 shows the inhibition area versus concentration of antimicrobial compound. It shows that as the concentration of lysozyme and lauric acid in film increased, the inhibition area also increased respectively. Lysozyme shows a better inhibition compared to lauric acid.

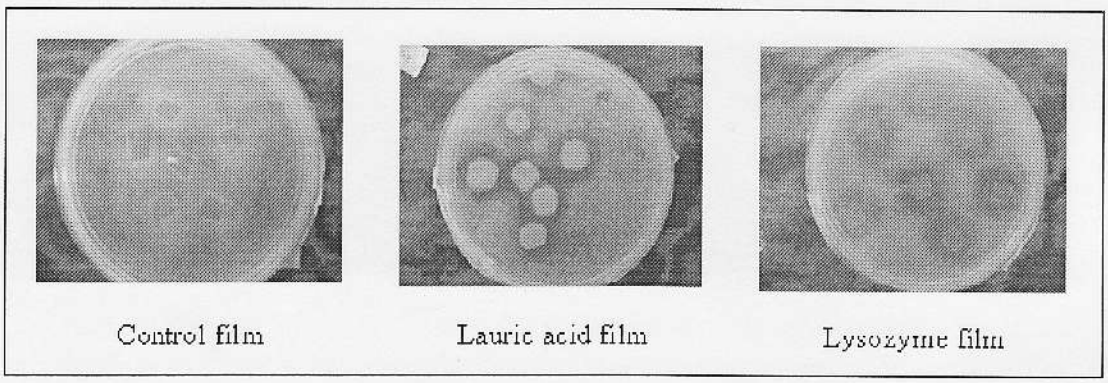

Figure 1. Inhibition area of AM-incorporated film in comparison to control film.

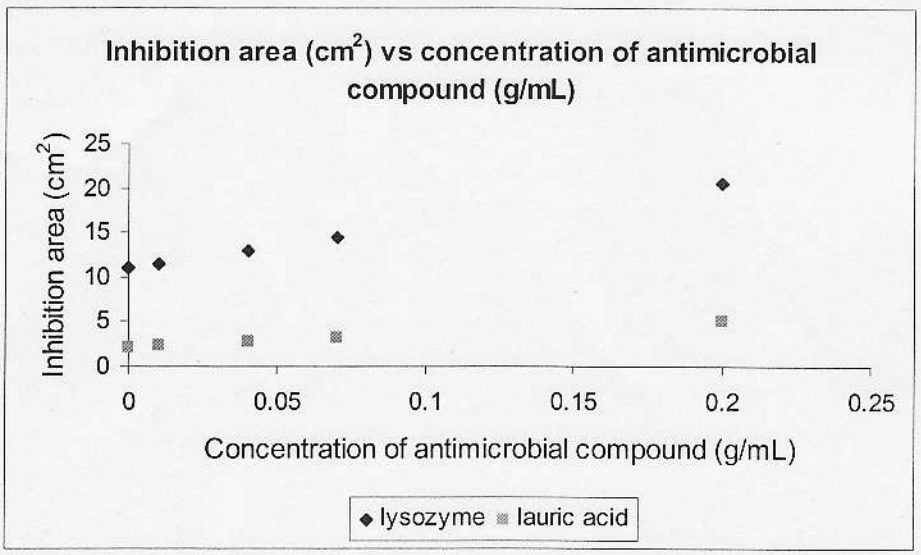

Figure 2. Graph inhibition area versus concentration of antimicrobial compound

Liquid Diffusion 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 lysozymes as AM in a liquid culture medium at 37°C. Clearly, as the concentration increased, the reading of OD decreased. It means that higher concentration of lysozyme in the film inhibits more *E. Coli* growth.

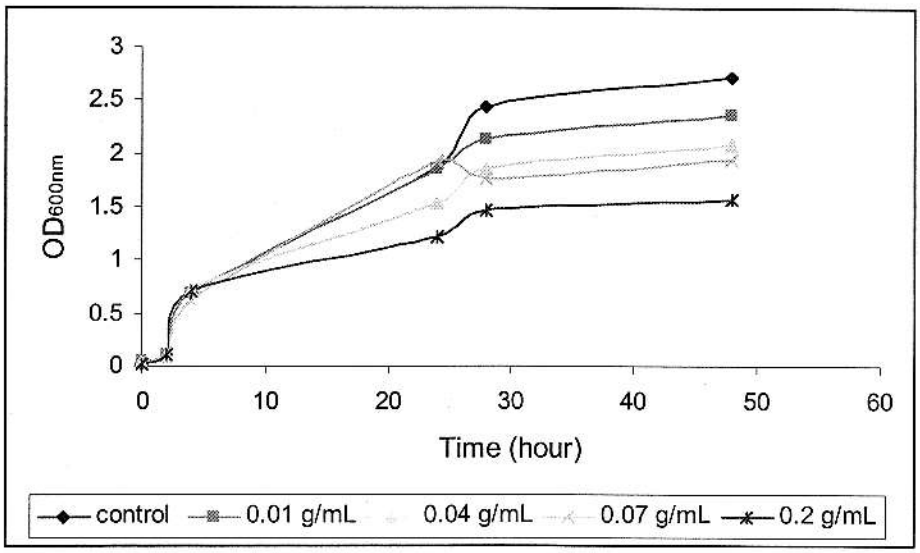

Figure 3. Inhibition of Escherichia Coli by the starch-based film containing lysozyme as AM in a liquid culture medium at 37°C

Figure 4 shows the inhibition of Escherichia Coli by the starch-based film containing lauric acid as AM in a liquid culture medium at 37°C. Similarly, it shows that when the concentration of Lauric acid in the film increased, the inhibition effects towards *E.coli* growth also increased. Lauric acid displays lower inhibition effect then lysozymes.

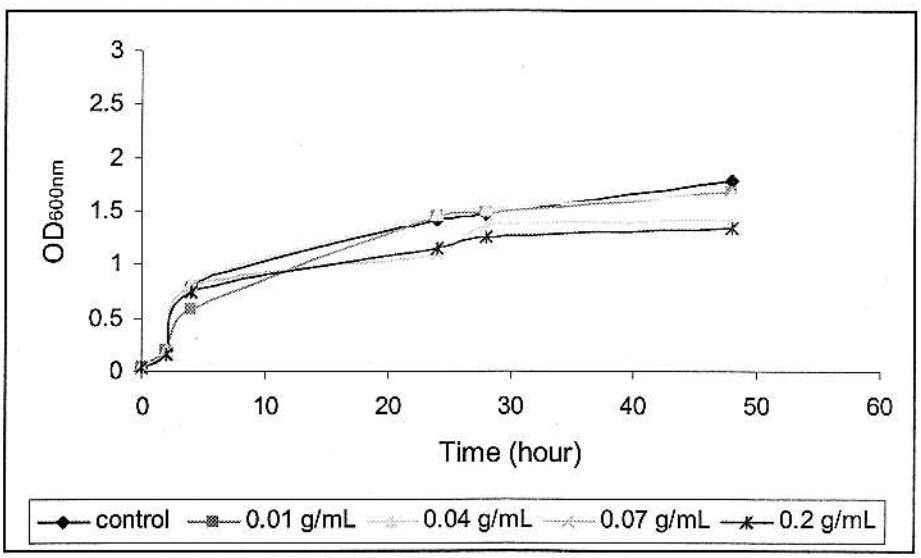

Figure 4. Inhibition of Escherichia Coli by the starch-based film containing lauric acid as AM in a liquid culture medium at 37°C

CONCLUSIONS

As a conclusion, the inhibition effects were observed by clear-zone formed on the agar during the agar diffusion test. In the liquid culture test, turbidity decreased from the inhibition reaction. Lysozyme-incorporated film effectively inhibits the growth of *E. Coli* better than Lauric acid-incorporated film.

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