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EFFECT OF LAURIC ACID ADDITION ON THE MICROBIAL EFFICACY OF CHITOSAN-BASED FILM

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Abstrak. Kesan terhadap perubahan dinamik dalam pasaran dan permintaan pengguna telah mengalakkan pertumbuhan dalam bidang Pembungkusan Aktif Pembungkusan Aktif Anti-bakteria mengaplikasikan konsep 'bio-switch' yang mana ia dihasilkan dengan menggabung dan menyekat gerak agen anti-bakteria ke dalam pembungkus dan mengaplikasi konsep bio-suis. Mekanisma pembebasan anti-bakteria di antara partikel 'bio-switch' dan 'stimulus' terhadap kontaminasi mikrob telah di kaji. Objektif kajian ini adalah untuk menghasilkan filem berasaskan 'chitosan' yang digabungkan dengan agen antibakteria iaitu asid laurik. Filem kawalan dan filem LA berasaskan 'chitosan' yang diperolehi dari kajian ini adalah lutsinar, lebih fleksibel kehomogenan berbanding pencirian filem polyethylene di pasaran. Keberkesanan filem tersebut terhadap perencatan aktiviti mikrob telah dikaji. Zon cerah yang terbentuk di atas piring petri menunjukkan bahawa kombinasi gabungan anti-bakteria – anti bakteria tersebut telah memberi perencatan yang baik terhadap pertumbuhan bakteria *E. coli* dan *B. subtilis* pada kepekatan 0.625×10^{-2}M asid laurik. Pengenalan bahan polimer dan antibakteria yang terbaru dalam penghasilan pembungkusan aktif boleh memanjangkan jangka hayat makanan dan mengurangkan risiko keracunan makanan akibat kontaminasi dari bakteria dengan menghalang atau menyekat pertumbuhan kerosakan dan/atau patogenik mikroorganisme yang mencemari makanan.

Kata kunci: Pembungkus aktif; anti- mikrob; 'chitosan'; asid laurik; *Escherichia coli; Bacillus subtilis*

Abstract. In response to the dynamic changes in current consumer demand and market trends, the area of Active Packaging is becoming increasingly significant. An Antimicrobial Active Packaging can be made by incorporating and immobilizing antimicrobial agents into food packages and applying a bio-switch concept. Using this technique, the mechanism of antimicrobial release between the developed bio-switch particles and the stimulus of a microbial contamination can be studied. The objective of this research is to develop a chitosan-based film incorporated with antimicrobial agents consisting of lauric acid. The control and lauric acid chitosan-based film appearance achieved from this study were visually transparent, flexible homogeneity comparable to commercially polyethylene film characteristics. The effectiveness of the antimicrobial film towards inhibition of microbial activity was evaluated by zone inhibition on the agar plate test and liquid culture test. Clear zones formed on the film appearance showed the combination of both antimicrobial agents gives good inhibition to the growth of *E. coli* and *B. subtilis* with optimum inhibition rate at lauric acid concentration of 0.625×10^{-2}M. With the advent of new polymer materials and antimicrobials, the development of active packaging could prolong the shelf life of food and reduce the risk of food-borne illness caused by microbial contamination by restrain or inhibit the growth of spoilage and/or pathogenic microorganisms that are contaminating foods.

Keywords: Active packaging; Antimicrobial; chitosan; lauric acid; *Escherichia coli; Bacillus subtilis*

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1.0 INTRODUCTION

Now a day, the use of agricultural biopolymers that are effortlessly biodegradable not only would unravel these problems, but would also grant a potential new use in packaging industry. Antimicrobial packaging is a packaging system that is able to limit or prevent microbial growth that are contaminating foods. The new antimicrobial function can be achieved by adding antimicrobial agents in the packaging system and/or using antimicrobial polymers that satisfy conventional packaging requirements [1]. When the packaging system acquires antimicrobial activity, the packaging system (or material) limits or prevents microbial growth by extending the lag period and reducing the growth rate or decreases live counts of microorganisms. Antimicrobial packaging can extend the food shelf-life, thus improving the quality of the food. If the packaging materials have self-sterilizing ability because of their own antimicrobial activity, they may eliminate chemical sterilization of packages using peroxide and simplify the aseptic packaging process [1]. The self-sterilizing materials could be widely applied for clinical uses in hospitals, biological lab-ware, biotechnology equipment, and biomedical devices, as well as food packaging.

Fatty acids (FA) and their corresponding esters are one group of chemicals found in nature considered to have little or no toxicity, with proven antimicrobial activity [1,2]. Lauric acid is a type of fatty acid which is colorless, needle-like crystal and slight odor of Bay Oil. Lauric acid or dodecanoic acid is part of the class of organic compounds known as lipids, which are vital in the construction of cellular membranes and act as a source of food under starvation conditions. The molecular weight of the lauric acid is 200 ($C_{12}H_{24}O_2$). Contrary to popular beliefs, natural coconut and coconut milk are good for the health, mostly because of their high in lauric acid content. Lauric acid can be used as an antimicrobial agent. Besides, it is inexpensive, has a long shelf-life, non-toxic, safe to handle and can be incorperated in packaging. The objective of this research is to study the effects of lactic acid chitosan-based film on *E. coli* and *B. subtilis* growth?

2.0 METHODOLOGY

2.1 Preparation of Film

Various concentration (0.125×10^{-2}M; 0.25×10^{-2}M; 0.375×10^{-2}M; 0.5×10^{-2}M; 0.625×10^{-2}M) of lauric acid (99% pure, Fluka Chemika, Malaysia) and 2 g of chitosan (Sigma-Aldrich, Malaysia) was added into 1% (v/v) acetic acid (Merck, Malaysia) in separated beaker. Then 50% (w/w) glycerin (HmbG Chemicals, Malaysia) was added into each solution. Then each of them was heated and homogenized at 70°C for 40 minutes until a homogeneas solution obtained. Each film solution (20 ml) was cast into casting-glass plate. The film solutions were dried for 3 days under room temperature. The dried film can be peeled off from the plate for further testing.

2.2 Agar Plate Test

Antimicrobial activity test was carried out using the agar plate test. Indicator cultures were *Bacil lus subtilis* and *Eschericha coli*, representing Gram-positive and Gram-negative bacteria, respectively. Each film was cut into circle (1 cm diameter) and was placed on the bacterial lawns. Duplicate agar plates were prepared for each type of film and control film. The plates were incubated for 24 h at 37°C in the appropriate incubation chamber. The plates were visually examined for zones of inhibition around the film disc, and the size of the zone diameter was measured at two cross sectional points and the average was taken as the inhibition zone.

2.3 Liquid Culture Test

For the liquid culture test, each film was cut into circle (1 cm in diameter). Three samples were immersed in 20 ml luria broth (Merck, Germany) in a 25 ml universal bottle which was inoculated with 200 il of *Eschericha coli/ B. subtilis*. Then the samples were transferred into an orbital shaker and rotated at 150 rpm at 37°C. The culture was taken periodically (0, 4, 8, 12, 16, 20, 24 hours) during the incubation to obtain microbial growth profiles. The same procedure was repeated for the control starch-based film. The optical density was measured at λ = 600 nm using a spectrophotometer (Model UV-160, Shimadzu, Japan).

3.0 RESULTS AND DISCUSSION

3.1 Film Appearance and Texture

The control film and Lanz Acid (LA) chitosan-based film appearance achieved from this study were visually transparent, flexible homogeneity to commercially polyethylene film characteristics. At high concentration of the LA added, the film become harder and the LA fine granules begin to appear on top surface of the film. But at the lowest concentration, the film is smooth and LA was well incorporated into the blend. Hence, the selection of the most suitable concentrations is the best formulation to get optimum film properties. The film obtained has also influenced by glycerin. The film that does not contain the plasticizer is harder, brittle with pale whitish colour and need longer casting time. However the volume of glycerin needs to be fixed at the specified level. If it is too high or low, it induces the film appearance. Figures 1 (a) – (f) below show the AM-film appearance with various concentrations of lysozyme and Table 1 lists out the visual observation towards the film characteristics based on the film obtained.

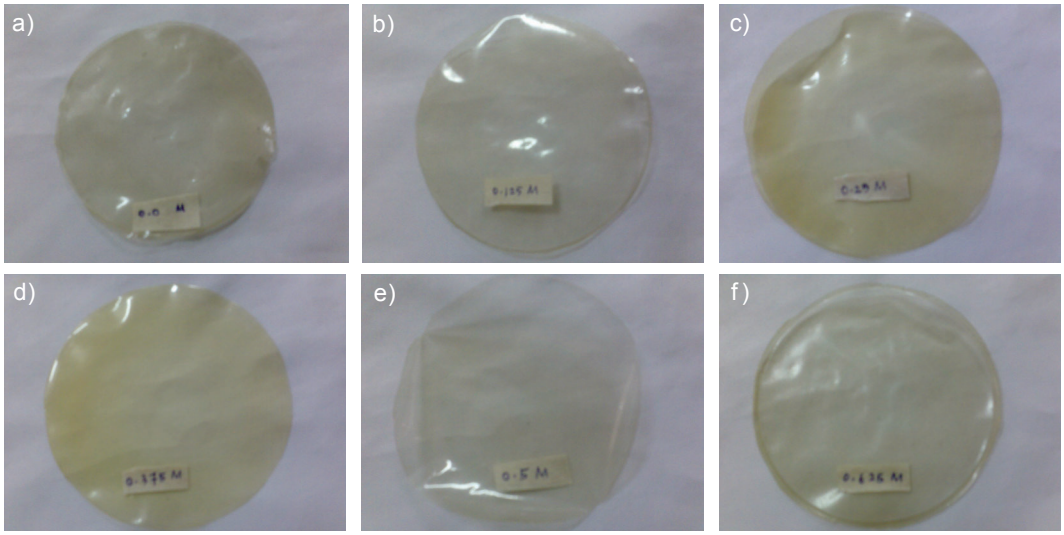

Figure 1 Film appearance after incorporated with LA a) 0 M (Control film); b) 0.125×10^{-2}M; c) 0.250×10^{-2}M; d) 0.375×10^{-2}M; e) 0.500×10^{-2}M; f) 0.625×10^{-2}M

Table 1 Films characteristics by visual observation

Lauric Acid Concentration ($M \times 10^{-2}$)	Pale yellow Colour	Flexibility	Transparently	Surfaces mooth
0.000	6	3	6	1
0.125	5	1	5	2
0.250	4	2	4	3
0.375	3	4	3	4
0.500	2	6	2	5
0.625	1	5	1	6

1 = strongly yes
2 = moderate yes
3 = yes
4 = no
5 = moderate no
6 = strongly no

3.2 Agar Plate Test

Antibacterial activity of AM film against two pathogenic bacteria was expressed in terms of zone inhibition. The agar diffusion test simulates wrapping of foods, and therefore can be used to estimate the amount of the antimicrobial agent migrates from the film to the food when the film contacts the contaminated surfaces [2-3]. All samples were examined for possible inhibition zones after incubation at 37°C for 24 hours. The inhibitory activity was measured based on the average diameter of the clear inhibition

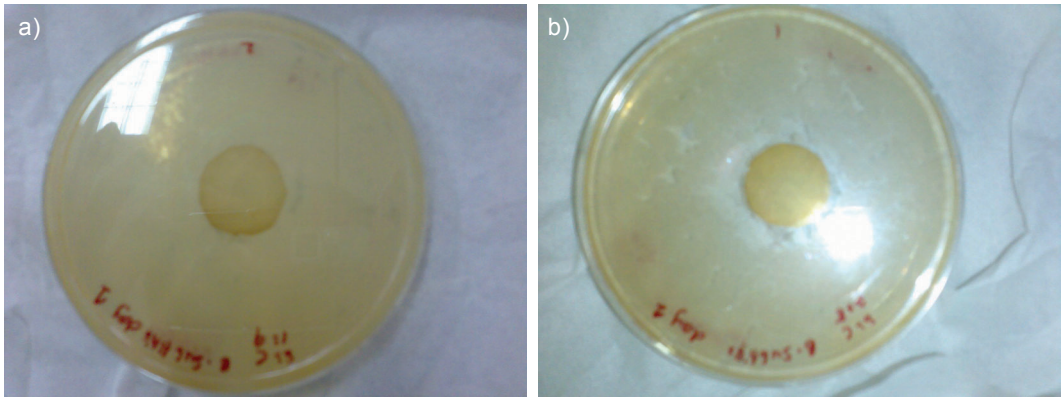

Figure 2 Comparison of inhibition zones between a) control film and b) LA chitosan-based film on plate containing *B. subtilis*

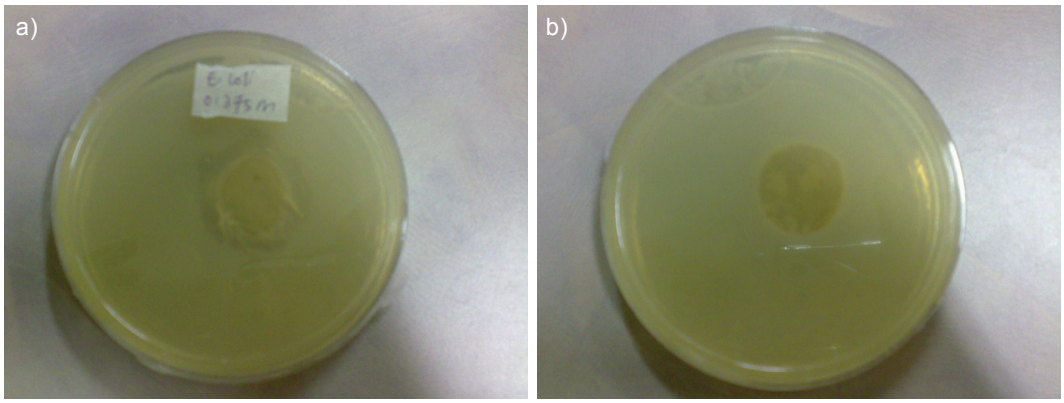

Figure 3 Comparison of inhibition zones between a) control film and b) LA chitosan-based film on plate containing *E. coli.*

zone. If there was no clear zone surrounding as revealed in Figures 2A-3A, it was assumed that there was no inhibitory effect [4]. The control films showed no inhibition area and colonies were formed all over the plate. However, LA chitosan-based film successfully inhibited the growth of both types of Gram-Positive bacteria *B. subtilis* and Gram-Negative *E. coli*.as shown in Figures 2B and 3B.

For this test, a measurement of inhibition zones on/around film squares on inoculated bacteria was determined. Figure 4 shows the plotted graph for calculated inhibition area for each plate test. It is shown that the clear zone expended is less than 5 mm diameter for inhibition of *E. coli* compared to *B. subtilis*. The result suggested that lauric acid is insufficient to retard the growth of gram negative bacteria, *E. coli*. The incorporation of lipid compounds such as fatty acid to a starch film decreases the moisture transfer due to their hydrophobic properties [10]. However, it shows a good inhibition effect on gram positive bacteria, *B. subtilis* where as the concentration of lauric acid increases the area of zone inhibition increase.

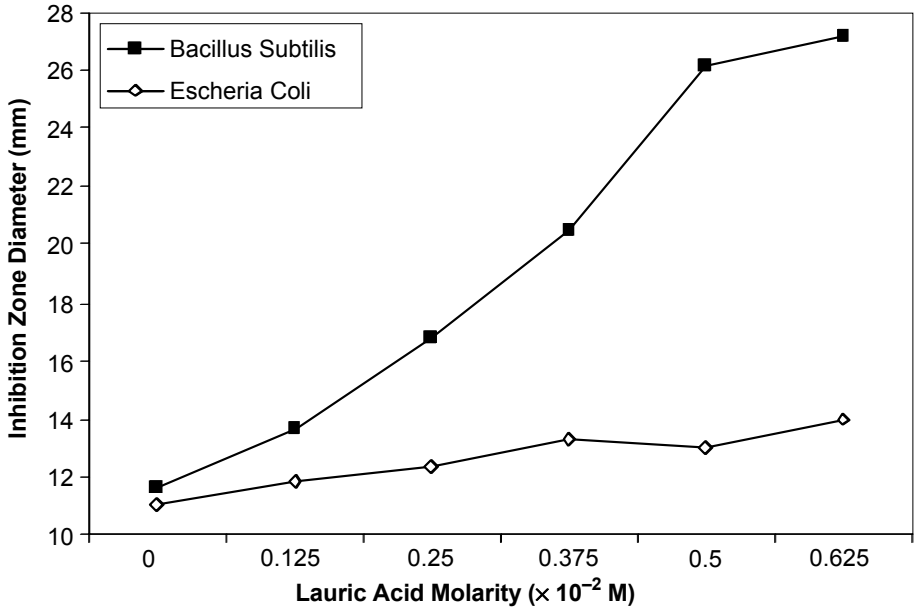

Figure 4 Comparison inhibition zone between E. coli and B. subtilis

3.3 Liquid Culture Test

Agar plate test also known as zone inhibition assay was performed to screen the antibacterial activity of all films formulations, in an effort to select film formulations with high antibacterial activity against test bacteria. The inhibitory zone in agar diffusion test can be affected by the solubility and diffusion rate of the test compounds in agar medium, thus agar diffusion test does not accurately reflect the antimicrobial effectiveness of the test compounds [5]. Therefore, the liquid culture test had been done to support the result of agar plate test also known as agar diffusion test. The liquid culture test determines the antimicrobial activity of the test compounds by viable count and provides information on microbial growth kinetics, thus being more sensitive than the agar diffusion method [5-6]. In this test the decrease in optical turbidity shows that the AM inhibits the bacterial growth.

3.3.1 Effect of lauric acid film on E. coli and B. subtilis

Figure 5 and 6 shows the inhibition of *E. coli* and *B. subtilis* by the LA chitosan-based films in liquid culture broth at 37°C respectively. It is shown that 0.625×10^{-2}M is the most effective concentration to inhibit *E.coli*. Lauric acid inhibition with the liquid culture test was sensitive compared to the zone assay (agar plate test). It may be due to the difference in the mobility of the bacterial cells within the two systems. The zone of inhibition assay allows little or no mobility of the non-motile *E. coli*, whereas the

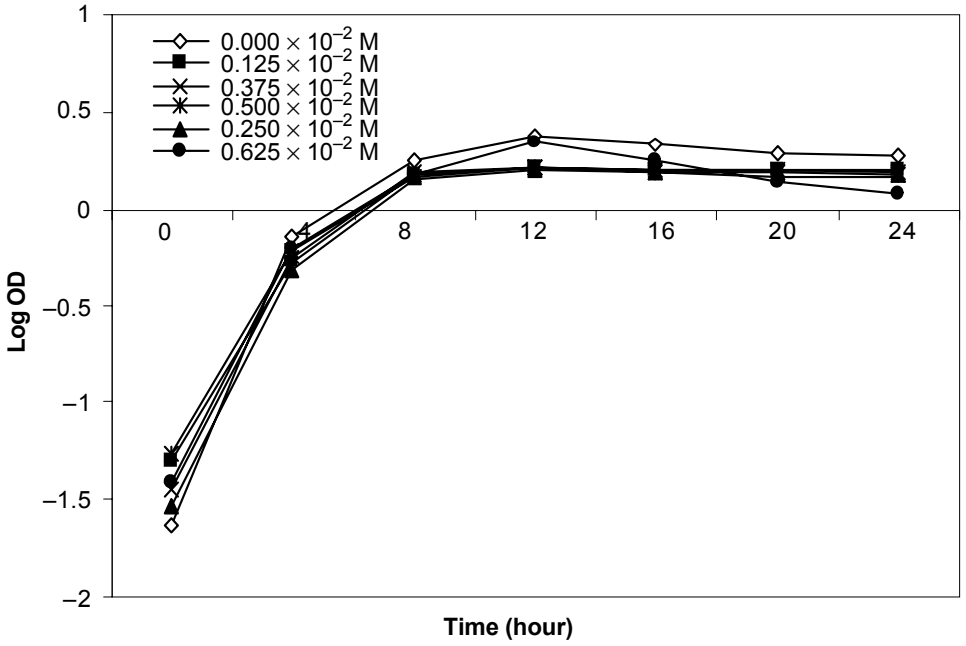

Figure 5 Inhibition of microbial growth by the LA chitosan-based film in a liquid culture medium containing *E. coli* at 37°C

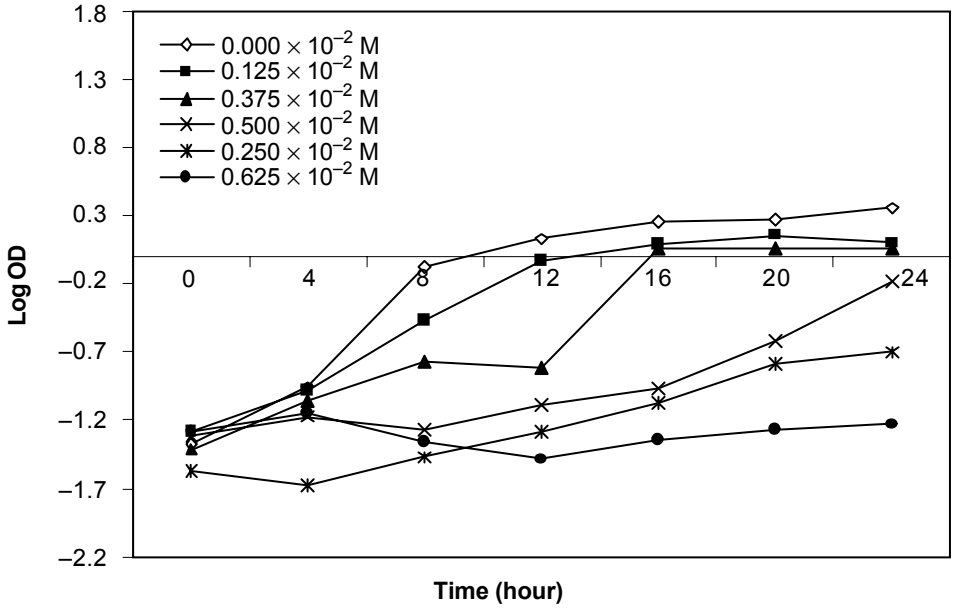

Figure 6 Inhibition of microbial growth by the LA chitosan-based film in a liquid culture medium containing *B. subtilis* at 37°C

liquid culture test uses a liquid broth under constant agitation which facilitates cell movement and exposure to the film despite the non-motile characteristic of the strain. The fluid nature and agitation of the broth may be have increased mobility of the bacteria and diffusion of the lauric acid resulting in a bacteriocidal activity not seen in the zone test on solid media [7].

Similarly, 0.625×10^{-2}M is also the most effective concentration of LA incorporated in chitosan-based film which inhibited the growth of *B. subtilis* (Figure 6). Although there were inhibition for both *E.coli* and *B. subtilis*, the LA chitosan-based film were more effective against Gram-positive bacteria than the Gram-negative bacteria studied. Lauric acid has been found to have an antimicrobial effect against gram-positive bacteria while the incorporation of chitosan as film based help inhibit gram-negative bacteria [5, 8]. In fact, one of the reasons for the antimicrobial character of chitosan is the positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms [9].

4.0 CONCLUSION

LA chitosan-based film exhibited good film forming property due to the presence of high density of amino groups and hydroxyl groups and inter and intra molecular hydrogen bonding. The chitosan and lauric acid showed interesting qualities in the field of antimicrobial packaging, due to antimicrobial activities of chitosan and lauric acid. Combination of chitosan and lauric acid as an active film showed obvious effects towards inhibition of *B. subtilis* and *E. coli* indicated that the film had synergistic antimicrobial effect. The LA chitosan-based film demonstrates more effective antimicrobial ability against *B. subtilis* than *E. coli*. At 0.625×10^{-2}M of lactic acid is the most effective concentration which had greater inhibition on both selected microbes.

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