MECHANICAL PROPERTIES AND ANTIMICROBIAL ANALYSIS OF ANTIMICROBIAL STARCH-BASED FILM

E. Salleh [1*] and I.I. Muhamad [2]
[1]Faculty of Chemical Engineering and Natural Resources Engineering, Universiti Teknologi Malaysia (UTM), 81310 Johor,
Malaysia
[2]Faculty of Chemical Engineering and Natural Resources Engineering, Universiti Teknologi Malaysia (UTM), 81310 Johor,
Malaysia
*corresponding author eraricar@fkkksa.utm.my

ABSTRACT

Antimicrobial (AM) packaging is one of the most promising active packaging systems. Increase demand in food safety, quality, convenience and environmental concerns associated with the handling of plastic waste has emphasized the importance in developing biodegradable and edible films from natural polymers, such as starch. Starch-based film is considered an economical material for antimicrobial packaging. This study aimed at the development of food packaging based on wheat starch incorporated with chitosan and lauric acid as antimicrobial agents. Chitosan has a widely been used in antimicrobial films, to provide edible protective coating, dipping and spraying for the food products due to its antimicrobial properties. Incorporation of chitosan and lauric acid as antimicrobial agent into starch-based film enhance physical and mechanical properties of starch-based film. The starch-based film also having antimicrobial properties that can extend shelf-life of the food packed. The antimicrobial effect of antimicrobial starch-based (AM) film was tested on *B. substilis* and *E. coli*. Inhibition of bacterial growth was examined using two methods, *i.e.* zone of inhibition test on solid media and liquid culture test (optical density measurements). The control (pure wheat starch) and AM film (incorporated with chitosan and lauric acid) were produced by casting method. From the observations, AM films exhibited inhibitory zones. Interestingly, a wide clear zone on solid media was observed for *B. substilis* growth inhibition whereas inhibition for *E. coli* was not as effective as *B. substilis*. From the liquid culture test, the AM films clearly demonstrated a better inhibition against *B. substilis* than *E. coli*.

Keywords: Biodegradable, Edible, Antimicrobial starch-based film, Chitosan, Lauric acid

1. INTRODUCTION

There has been growing interest in films made from natural biopolymers such as starch and chitosan as an alternative to solve waste disposal and undegraded polymers cause from synthetic polymers. Therefore, the use of agricultural biopolymers that are easily biodegradable not only would solve these problems, but would also provide a potential new use for surplus farm production.[1-3] Because of the environmental concerns and technological problems such as denaturing effects of thermal polymer processing methods, extrusion and injection molding, the incorporation of biopreservatives into biodegradable films is more suitable than incorporation into plastic films.[4-6]

Antimicrobial packaging has been touted as a major focus in the next generation of 'active' packaging.[7] Antimicrobial packaging is the packaging system that is able to kill or inhibit spoilage and pathogenic microorganisms 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. 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.[5] Antimicrobial packaging can extend the food shelf-life, thus improving the quality of the food.

In the food packaging sector, starch-based material has received great attention owing to its biodegradability, edible, wide availability as agricultural surplus raw material, abundant, can be produced at low cost and at large scale, nonallergic, easy to use and thermoprocessable. Several studies are concentrated on the development of starch-based materials for the above-mentioned reasons. [8] Starches are polymers that naturally occur in a variety of botanical sources such as wheat, corn, potatoes and tapioca. It is a renewable resource widely available and can be obtained from different left over of harvesting and raw material industrialization. [9] Besides, the preponderance of amylose in starch gives rise to stronger films. However, biodegradable products based on starch, possess many disadvantages, mainly attributed to the water solubility, brittle nature of starch films[10] and poor mechanical properties. [11] In order to improve mechanical properties and water resistance, starch can be modified by several methods such as blending with synthetic[10, 12, 13] or natural polymers. [14, 15] One of the effective strategies to overcome the poor mechanical properties, while preserving the biodegradability of the materials, is to associate starch with chitosan.

The scope of films made with starch combined with other polysaccharides was widened to include chitosan for several reasons. [16] First, chitosan is a biopolymer, cationic polysaccharide, produced by the partial deacetylation of chitin isolated from naturally occurring crustacean shells is one of the most abundant naturally occurring polysaccharide. [17] It is commercially available from a stable renewable source, that is, shellfish waste (shrimp and crab shells) of the sea-food industry. Second, chitosan forms good films and membranes. Chitosan films that were clean, though, flexible and good oxygen barriers were formed by solution casting. [18] Some of these films contained glycerol and had good tensile strength. They were readily biodegradable either in sea water or in soil. Third, the cationic properties of chitosan offer the film-maker an opportunity to take advantage of electrostatic interactions with other anionic polysaccharides. [16] In addition chitosan possess immense potential as a packaging material owing to its biodegradability, biocompatibility and antimicrobial activity, [19] and non-toxicity leading to extensively use over a wide range of applications. Besides, chitosan film has a potential to be employed as packaging, particularly as an edible packaging. Although chitosan films are highly impermeable to oxygen, they have relatively poor water vapor barrier characteristics. [20] Antimicrobials, antioxidants, nutrients, colorants and flavors can be possibly carried by chitosan-based films and released in a controlled manner. Chitosan can inhibit the growth of a wide variety of fungi, yeasts and bacteria. [21-24] For example, biodegradable and edible chitosan films were produced to protect foods from fungal decay and modify the atmospheres of fresh fruits. The functional properties of chitosan films are improved when chitosan is combined with other film-forming materials. [25]

Besides, the choice of chitosan in preparing the antimicrobial packaging films was based on the fact that it has good film forming properties. The functional properties of chitosan films are improved when chitosan is combined with other film-forming materials. [15] Chitosan can also play an important role in the enhancement of starch-based film strength. [26]

In the last years much research has been done concerning the use of biodegradable films as a way of supporting antimicrobials in food products. Several researchers have previously reported on coating food contact surfaces with antimicrobial compounds. Nisin and lauric acid are two food-grade antimicrobials shown to be effective in food applications. Dawson et al. (2002), incorporated lauric acid and nisin singly and together into thermally compacted soy films. [27] Nisin and lauric acid films were equally effective in reducing *L. monocytogenes* in 1% peptone water after 48h exposure. However, the combination of nisin and lauric acid in corn zein cast films was found to be more effective in reducing *L. monocytogenes* in peptone water than when each used singly. [28] The advantage in having a film material carrying a biocide is that continued inhibition can occur during storage or distribution of the food product. A packaging material with a wide antimicrobial spectrum would be necessary and desirable for universal use to improve the storage stability of variety of foods. For this purpose, the incorporation of another antimicrobial agent into the packaging materials would be useful.

Lauric acid, a medium length- long chain fatty acid is found in the form of glycerides in a number of natural fats, coconut oil and palm-kernel oil. It offers advantages in food processing as it acts as a kind of preservative, staving off oxidation and spoilage. Lauric acid has been shown to have an antimicrobial effect against gram positive bacteria and yeasts. [29, 30] Beuchat & Golden (1989) suggested that fatty acids were bacteriostatic and may be potential microbial inhibitors in foods using a systematic approach with other antimicrobials. [29] Based on Padgett, Han & Dawson (2000), nisin instantaneously kills *L. plantarum* cells whereas lauric inhibits more slowly but steady inhibitory effect. [31] The incorporation of lipid compounds such as fatty acid to a starch film decreases the moisture transfer due to their hydrophobic properties. [32] Fatty acids, such as lauric acid were found to be effective in limiting water vapor transfer through edible film. [33-37]

The objective of this study was to improve antimicrobial efficacy of starch based-film incorporating with chitosan and lauric acid as antimicrobial agent. Mechanical and physical properties were characterized, and antimicrobial efficacy was asses against test strain of Gram-positive (*B. subtilis*) and Gram-negative bacteria (*E. coli*).

2. MATERIALS AND METHODS

2.1 Materials

Wheat starch and acetic acid (glacial 100%) that used to dissolve chitosan was purchased from Mersk (Malaysia). Medium molecular weight chitosan was from Sigma-Aldrich (Malaysia). Lauric acid was 99% pure purchased from Fluka Chemika (Malaysia) and glycerol as a plastisizer was bought from HmbG chemicals (Malaysia).

2.2 Film preparation

A starch based film was formed using casting process following previous work by Famá et al. (2004).[9] A control film, without lauric acid or chitosan was formed using mixtures of starch (5.0g), glycerol (2.5g) and water (92.5g).

Chitosan was dispersed in 400ml of distilled water to which 20 ml of glacial acetic acid was added to dissolve the chitosan. The solution of starch and chitosan with different mixing ratios [9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 starch/chitosan (w/w)] were prepared by adding glycerol (half amount of the starch) and 8% lauric acid (was added based on the percentage of starch (g fatty acid per g *starch).* The solution was mixed by gentle stirring with a magnetic stir bar until starch dissolved. The solution was then homogenized for about 15 min with addition of slow heating. Stirring and heating were ended when the solution reaches temperature of 80-86°C.

The 10 ml of the film forming solution was pipette and spread evenly into a petri dish bottom (100x 15 mm) and allowed to air-dry at room temperature overnight.

After casting, 5 measurements were made on each sample using an electronic micrometer (model Mitutoyo) and the mean thickness was calculated to the nearest 0.002 mm.

2.3 Testing Antimicrobial Effectiveness of AM Starch-Based Film

2.3.1 Agar Diffusion Method (Zone Inhibition Assay)

Antimicrobial activity test was carried out using agar diffusion method. Indicator cultures were *Bacillus subtilis* and *Eschericha coli*, representing Gram-positive and Gram-negative bacteria. One hundred microliters of the inoculum solution was added to 5 ml of the appropriate soft agar, which was overlaid onto hard agar plates.

Each film was cut into squares (1cm x 1cm) 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 48 h at 37 °C in the appropriate incubation chamber (aerobic chamber for *E.coli*). 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.[38]

2.3.2 Liquid Culture Test (Optical Density Measurements)

For the liquid culture test,[39] each film was cut into squares (1cm x 1cm). Three sample squares were immersed in 20 ml nutrient broth (Merck, Germany) in a 25 ml universal bottle. The medium was inoculated with 200µl of *Eschericha coli/ B. subtilis* 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 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 λ = 600nm using a spectrophotometer (Model UV-160, Shimadzu, Japan).

2.4 Mechanical Properties of AM Starch-Based Film

2.4.1 Tensile Properties

The tensile strength, % elongation and Young modulus values of the films were investigated using tensile machine Lloyd LRX materials testing machine (Lloyd Instruments Ltd, Fareham, UK) according to ASTM D 638-03. The gauge length and the crosshead speed were 9.53 mm and 10 mm/min, respectively. Films were cut into strips according ASTM D638 T.5 in dumbbell shape. The tests were carried out at 23 ± 2°C and 50 ± 5% RH. Each determination was taken from an average of five specimens.

2.4.2 Microstructure Studies by Scanning Electron Microscopy

Film surface morphology was examined using scanning electron microscopy. The dried film samples were mounted on a metal stub with double-sided adhesive tape .The morphological structures of the films were studied by a JSM-5600 LV scanning electron microscope of JEOL, Tokyo, Japan and the images were taken at accelerating voltage 5 kV and a magnification 100 times of origin specimen size.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Starch-Based Film Formation

In general a translucent starch-based film incorporated with lauric acid and chitosan presented good flexibility than purely starch-based film was formulated and formed as can be seen in figure 1. Film thickness ranged from 0.03 to 0.04 mm, with an average 0.0346 ± 0.002 mm.

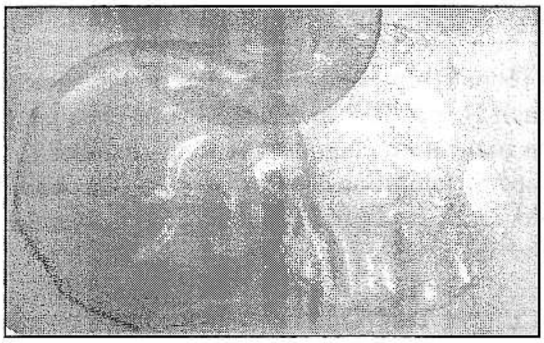
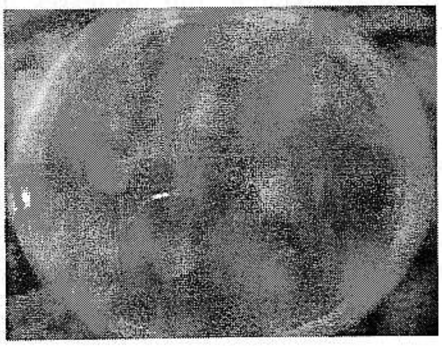


Figure 1: A translucent starch-based film incorporated with lauric acid and chitosan

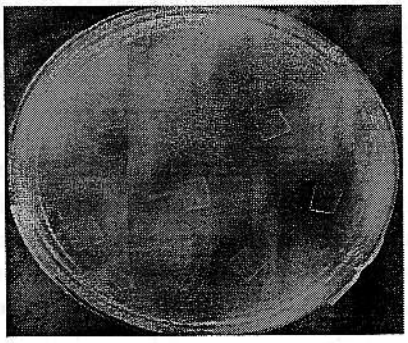
3.2. Antimicrobial Effectiveness of AM Starch-Based Film

3.2.1. Inhibition of *E. coli* and *B. subtilis* on Agar Plate Test

The details of antimicrobial effectiveness of starch-based film incorporated with chitosan and lauric acid are shown in figures 2- 5. The inhibitory activity was measured based on the average diameter of the clear inhibition zone. If there was no clear zone surrounding as revealed in figure 2, it was assumed that there was no inhibitory effect. After 24 hours incubation at 37°C for starch film only (S), there was no inhibition occurred for both *B. subtilis* and *E. coli*. Bacteria colonies also occurred at the top of film sample. On the contrary, chitosan film only (C), showed a better inhibition than starch film only for both *B. subtilis* than *E. coli* inhibition. However the effect was not as good as combination of chitosan and lauric acid as shown in figure 2.



a



b

Figure 2: Comparison of inhibition area of (a) control film and (b) AM incorporated film

Starch and chitosan different mixing ratio (S: C) 8:2, revealed the best inhibition on *B. subtilis* which is Gram- positive bacteria compared to other S: C ratios. In contrast, S: C ratio 9:1 showed a very good inhibition on *E. coli* (Gram-negative bacteria). From figure 3, the results indicated that S: C ratio 8:2 is the best formulation to inhibit both *B. subtilis* and *E. coli* effectively followed by S: C ratio 9:1. S: C ratio from 1:9-3:7 obviously more effective towards inhibition of *E. coli* than S: C 4:6-7:3.

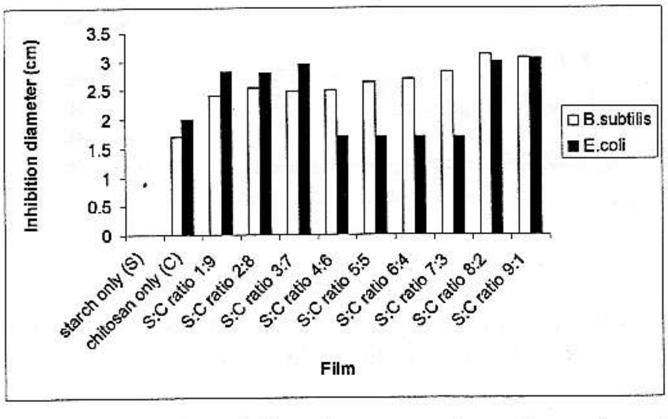


Figure 3: Inhibition of *B. subtilis* and *E. coli* on agar plates figure based on average zone diameter expressed as an area (cm) of inhibition zone

3.2.2 Liquid Culture Test (O.$D_{600\ nm}$ Measurement)

S: C ratio 8:2 is the most effective formulation to inhibit *B. subtilis* as can be seen in figure 4. Meanwhile, S: C ratio 9:1 is the best formulation to inhibit *E.coli* (figure 5). Although there were inhibition for both *B. subtilis* and *E.coli,* the antimicrobial starch-based film incorporated with lauric acid and chitosan were more effective against Gram-positive bacteria than the Gram-negative bacteria studied.

Lauric acid alone only has antimicrobial effect against Gram-positive bacteria and yeasts.[29, 30] The incorporation of lipid compounds such as fatty acid to a starch film decreases the moisture transfer due to their hydrophobic properties.[32] Incorporation of chitosan, besides inhibit *E. coli* and increase the film effect on *B.subtilis* inhibition, it helps to enhance the antimicrobial starch-based film strength.[26]

In fact, one of the reasons for the antimicrobial character of chitosan it's 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.[40] In the Gram-positive bacteria, the major constituent of its cell wall is peptidoglycan and there is very little protein. The cell wall of Gram-negative bacteria also has an outer membrane, which constitutes the outer surface of the wall.[41] Study from Jiang, Bi, Wang, Xu & Jiang (1997), observed that from electron micrographs for Gram-positive and Gram-negative bacteria in the presence of chitosan show the cell membrane of Gram-positive bacteria was weakened or even broken, while the cytoplasma of Gram-negative bacteria was concentrated and the interstice of the cell were clearly enlarged. This study indicated that the mechanisms of the antimicrobial activity of chitosan were different between Gram-positive and Gram-negative bacteria. Additionally, the antimicrobial mechanism of chitosan might differ from that of other polysaccharides because there are positives charges on the surface of chitosan.[42]

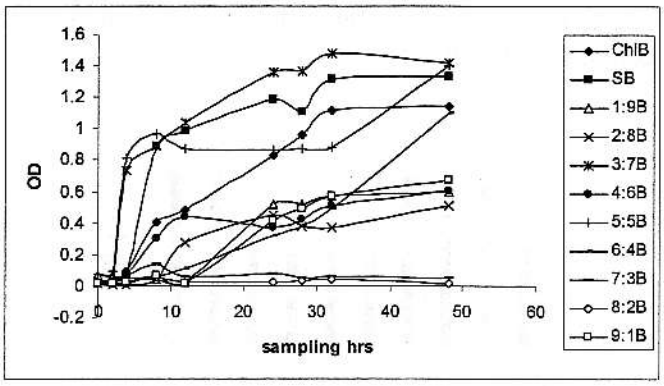


Figure 4: Inhibition of controls (starch only and chitosan only) and starch (S): chitosan (C) on *B. subtilis* in liquid culture test

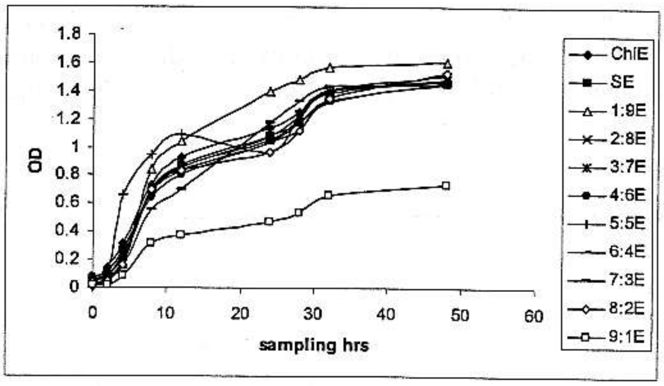


Figure 5: Inhibition of controls (starch only and chitosan only) and starch (S): chitosan (C) on *E. coli* in liquid culture test

3.3 Mechanical Properties of AM Starch-Based Film

3.3.1 Tensile Properties

The mechanical resistance of films was studied according to three parameters: tensile strength (TS), Young's modulus (Y) and percent of elongation (*E*). Freddi, Romano, Massafra & Tsukada (1995), observed that because polymer materials, such as films, may be subjected to various kinds of stress during use, the determination of the mechanical properties involves not only scientific but also technological and practical aspects. [43] The tensile strength (TS) value of the AM starch-based film incorporated chitosan and with 8% lauric acid films with different starch: chitosan (S:C) ratios are shown in figure 6. The tensile strength of the pure chitosan film was 36.7 MPa and much higher than of starch film (6.03 MPa). The results show that the preparation of AM starch-based film incorporated with chitosan and 8% lauric acid could improve the mechanical properties of the material. Both the addition of chitosan and lauric acid increase the AM starch-based film strength and stiffness. When the chitosan was added into the starch solution, there was a gradual increase in both tensile strength and Young's modulus (figure 6 and figure 8). The tensile strength of the resulting blend film increased with increase of chitosan content and reached a maximum point at about 90 wt % chitosan content. (S: C ratio 1:9), achieving 65.2 MPa. The significant increment in the tensile strength of the AM starch-based film incorporated with chitosan and lauric acid indicated the presence of intermolecular interactions in the blends films. Respectively, the Young's modulus value was 6252200 MPa at the maximum point of 90 wt % chitosan content.

The percentage elongation (*E*) values of the AM starch-based film incorporated with chitosan and lauric acid are a measure of the flexibility of the film and were effected by the starch to chitosan ratios (S:C). The average *E* values of the films increased from 65.7 % to 79.57 % for the S: C ratio 5:5 as shown in figure 7. The increase in percentage elongation with increase in starch content is due to the reduction in the number of intermolecular cross-links and increase in the inter-molecular distance. [25]

Structurally, chitosan is similar to cellulose but contains an NH_2 group in the position of the C_2 hydroxyl group. Due to its linearity high molecular weight chitosan generally possesses the higher mechanical properties when compared with starch which contains branching amylopectin. As a result, the incorporation of chitosan into the starch-based film led to improvement in both tensile strength and Young's modulus due the reinforcement effect.[44]

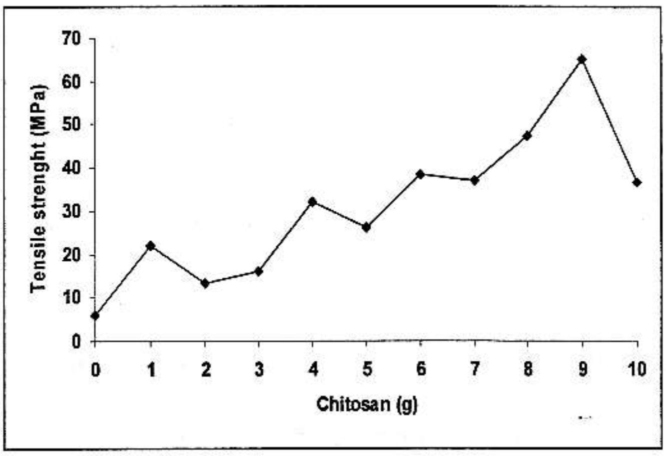


Figure 6: Effect of the film composition on tensile strength

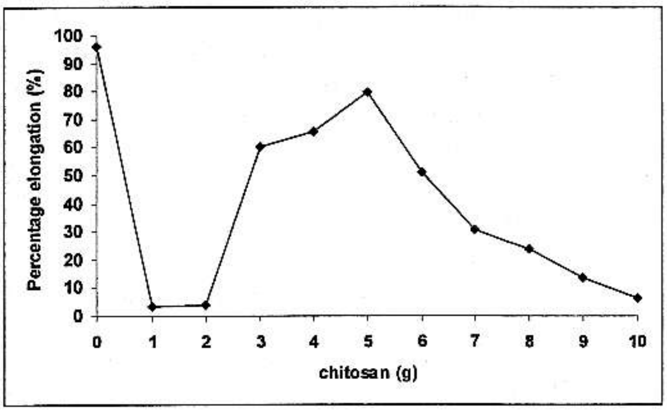


Figure 7: Effect of the film composition on percentage of elongation

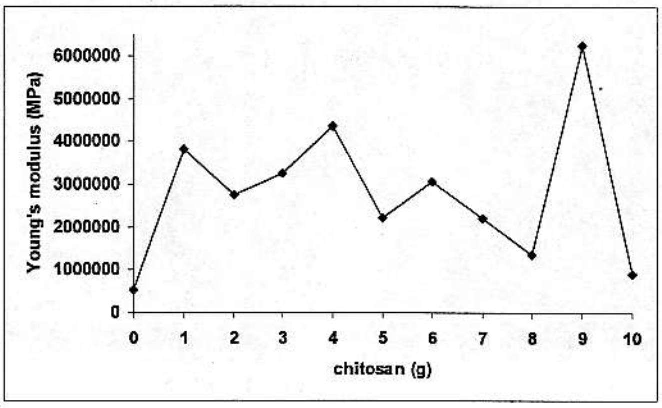


Figure 8: Effect of the film composition on Young's modulus

3.3.2 Microstructure Studies by Scanning Electron Microscopy

SEMs of the surfaces of the pure starch film, chitosan film and the blend films are shown in figures 9a-9k. The pure starch film (figure 9a) exhibit characteristic patterns on the film surface. These patterns represent the withered ghost granules of starch. Figure 9k shows the surface of pure chitosan films to be relatively smooth, to be homogeneous and to be a continuous matrix without any pores or cracks with good structural integrity. It was flat and compact with very sparsely distributed small particles without any phase separation. The blend films of starch-chitosan various ratio (figures 9b-9j) also exhibit such patterns, the intensity of which reduced with the decreasing concentration of starch as revealed from figures 9f to 9j. Chitosan microdomains were dispersed within the starch matrix in the blend films with relatively good interfacial adhesion between the two components and were similar to the surface cellulose/ carboxymethylated-chitosan blends. [45] As the amylose molecules are preferentially dissolved by water and are easily released from the starch granules, it can be supposed that the continuous region (matrix) correspond to a network structure consisting mostly of amylose and chitosan. [46]

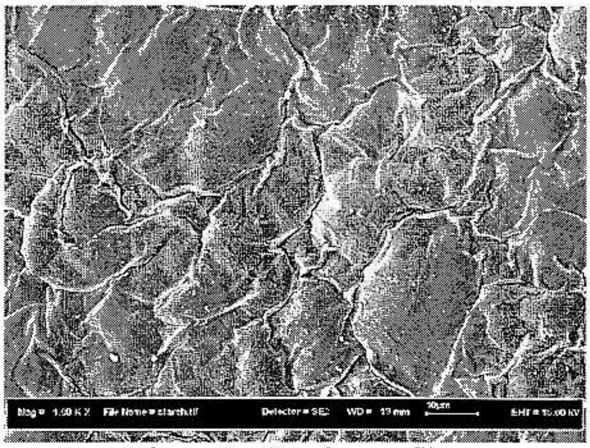
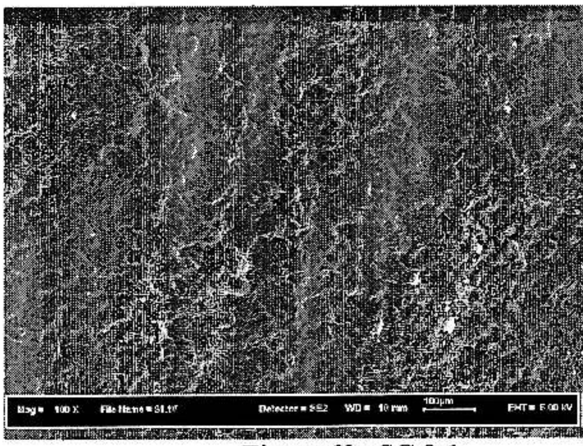


Figure 9a: Starch pure film



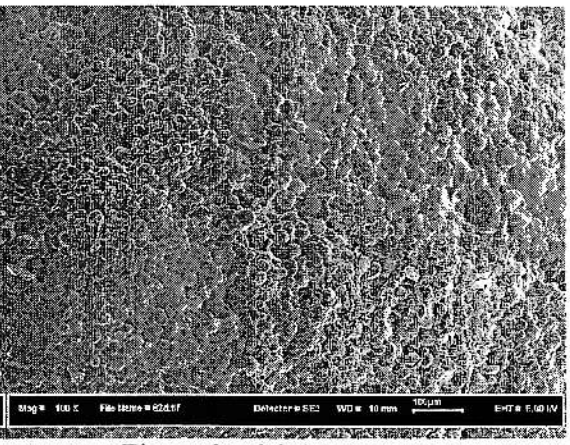
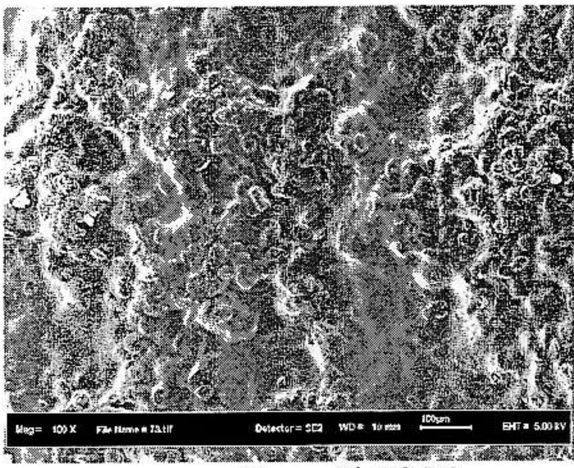


Figure 9b: SC 9:1

Figure 9c: SC 8:2



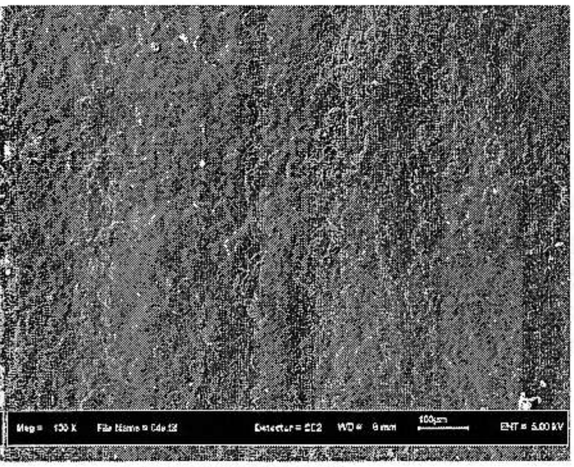
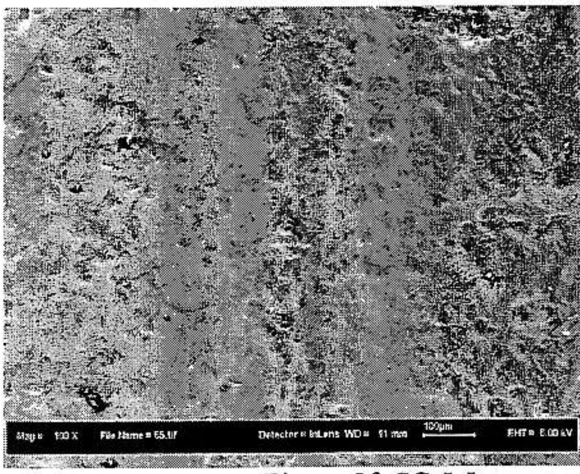


Figure 9d: SC 7:3

Figure 9e: SC 6:4



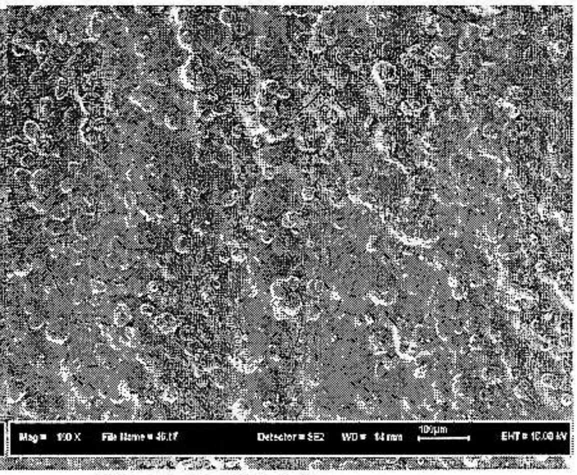


Figure 9f: SC 5:5

Figure 9g: SC 4:6

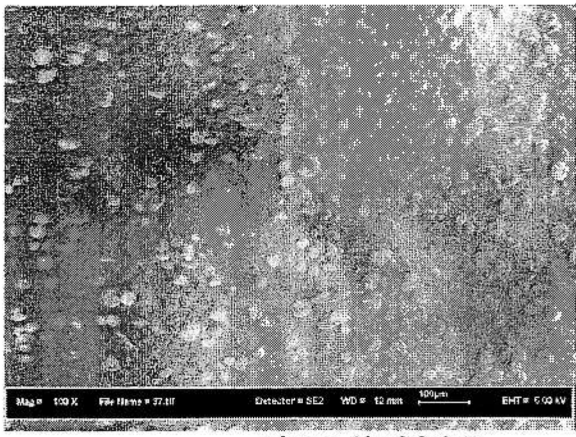


Figure 9h: SC 3:7

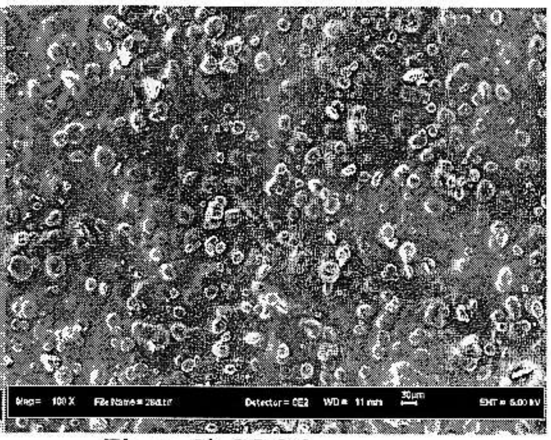


Figure 9i: SC 2:8

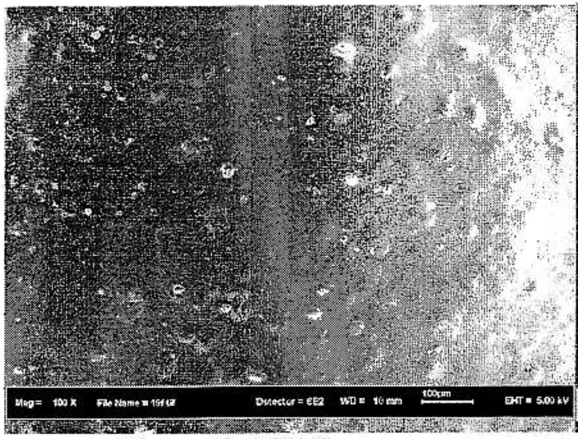


Figure 9j: SC 1:9

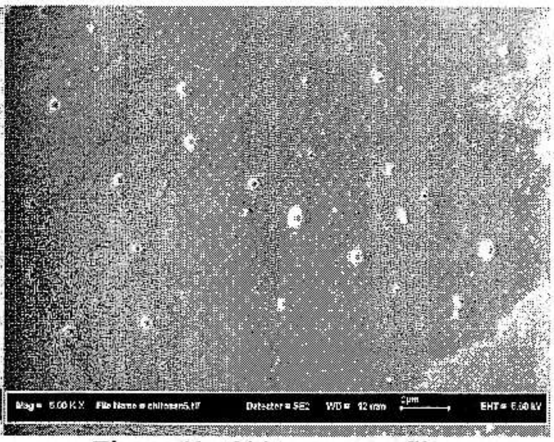


Figure 9k: Chitosan pure film

4. CONCLUSION

Antimicrobial starch-based film incorporated with chitosan and lauric acid was prepared successfully by casting technique. Antimicrobial starch-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. Incorporating chitosan and lauric acid into starch based film showed obvious effects towards inhibition of *B. subtilis* and *E. coli* indicated that the film had synergistic antimicrobial effect when chitosan and lauric acid were combined. The antimicrobial starch-based film demonstrates more effective antimicrobial ability against *B. subtilis* than *E. coli*. The solution of starch and chitosan with different mixing ratio (w/w) 8:2 and 9:1 were the most effective mixing ratio which had greater inhibition on both *B. subtilis* and *E. coli* than others solution as revealed in agar plate test and liquid culture test. The tensile properties of the antimicrobial starch-based film had been improved by the addition of chitosan. Chitosan reinforced the tensile strength and Young's modulus due to the structural similarity to starch and linearity. Surface morphology of antimicrobial starch-based film examined by SEM revealed that there was interaction and microphase separation between starch and chitosan molecules. These results suggested that these two film-forming components were compatible and an interaction existed between them. These antimicrobial starch-based films can be used to extend food shelf life.

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REFERENCES

1. Okada M, Progress in Polymer Science, **27**, 87-133 (2002).
2. Pavlath A E and Robertson G H, Critical Reviews in Analytical Chemistry, **29**(3), 231-241 (1999).
3. Scott G., Polymer Degradation and Stability, **68**, 1-7 (2000).
4. Appendini P and Hotchkiss J H, Innovative Food Science and Emerging Technologies, **3**, 113-126 (2002).
5. Han J H, Food Technology, **54**(3), 56–65 (2000).
6. Suppakul P, Miltz J, Sonneveld K and Bigger S W, Journal of Food Science, **68**(2), 408-418 (2003).
7. Brody A L, Food Technology, **5**, 82-84 (2001).
8. Maurizio A, Jan J D V, Maria E E, Sabine F, Paolo V and Maria G V, Food Chemistry, **93**, 467-474 (2005).
9. Famá L, Rojas A M, Goyanes S and Gerschenson I, LWT, **38**, 631-639 (2004).
10. Wu Q X and Zhang L N, Journal of Applied Polymer Science, **79**, 2006-2013 (2001).
11. Mathew S, Brahmakumar M and Abraham T E, Biopolymers, **82**, 176-187 (2006).
12. Arvanitoyannis I, Biliaderis C G., Ogawa H and Kawasaki N, Carbohydrate Polymers, **36**, 89-104 (1998).
13. Bhattacharya M, Journal of Materials Science, **33**, 4131-4139 (1998).
14. Coffin D R, Fishman M L and Cooke P H, Journal of Applied Polymer Science, **57**(6), 663-670 (1995).
15. Xu Y X, Kim K M, Hanna M A and Nag D, Industrial Crops and Products, **21** (2), 185-192 (2005).
16. Bangyekan C, Aht-Ong D and Srikulkit K, Carbohydrate Polymers, **63**, 61-71 (2005).
17. Wong D W S, Gastineau F A, Gregorski K S, Tillin S J and Pavlath A E, Journal of Agriculture Food Chemistry, **40**, 540-544 (1992).
18. Jeon Y J, Kamil J Y V A and Shahidi F, Journal of Agricultural and Food Chemistry, **50**, 5167-5178 (2002).
19. Hirano S, Seino H, Akiyama Y and Nonaka I, Biotechnology and bioactive polymers, Plenum Press, New York (1994).
20. Butler B, Vergano P and Testin R, Journal of Food Science, **61**, 953-955 (1996).
21. Rhoades J and Rastall B, Food Technology International, 32-33 (2000).
22. No H K, Park N Y, Lee S H and Meyers S P, Journal of Agricultural Food Chemistry, **51**, 7659-7663 (2003).
23. Tsai G J, Su W H, Chen H C and Pan C L, Fisheries Science, **68**, 170-177 (2002).

24. Sagoo S K, Board R and Roller S, Lett. Appl. Microbiol., **34**, 168-172 (2002).
25. Sidhu M, Brahmakumar M and Emilia T A, Biopolymers, **82**, 176-187 (2006).
26. Ban W, Song J, Argyropoulos D S and Lucia L A, American Chemical Society (2005).
27. Dawson P L, Carl G D, Acton J C and Han I Y, Poultry Science, **81**, 721-726 (2002).
28. Hoffman K L, Han I Y and Dawson P L, J. Food Protection, **64**(6); 885–9 (2001).
29. Beuchat L R and Golden D A, Food Technology, **43**, 134-142 (1989).
30. Kabara, J J, Medium-chain fatty acids and esters. In Antimicrobials in Foods, P M Davidson & A L Branen, eds., Marcel Dekker, New York, pp. 307-342 (1993).
31. Padgett T R, Han I Y and Dawson P L, J. Food Process Preserve, **24**, 423-432 (2000).
32. Coma V, Sebti I, Pardon P, Deschamps A and Pichavant H, J. Food Protection, **64**(4): 470-475 (2001).
33. Gennadios A, Weller C L and Testin R F, J. Food Science, **58**, 212-219 (1993).
34. Greener I K and Fennema O, J. Food Science, **54**, 1393-1399 (1989a).
35. Greener I K and Fennema O, J. Food Science, **54**, 1400-1406 (1989b).
36. Kamper S L and Fennema O, J. Food Science, **49**, 1478-1481, 1485 (1984).
37. Kester J J and Fennema O, J. Food Science, **54**, 1383-1389 (1989).
38. Padgett T R , Han I Y and Dawson P L, Journal of Food Protection, **61**(10):330-1335 (1998).
39. Chung D, Papadakis S E and Yam K L, International Journal of Food Science and Technology, **38**, 165-169 (2002).
40. Shahidi F, Arachchi J K V and Jeon Y J, Trends in Food Science & Technology, **10**, 37-51 (1999).
41. Zheng L N and Zhu J F, Carbohydrate Polymers, **54**, 527-530 (2003).
42. Jiang Y.-Y, Bi Y-Q, Wang Z-W, Xu L-Q and Jiang J-G., Application and explore, **2**, 22 (1997).
43. Freddi G., Romano M, Massafra M R and Tsukada M J, Journal of Applied Polymer Science, **56**, 1537-1545 (1995).
44. Kampeerapappun P, Aht-ong D, Pentrakoon D and Srikulkit K, Carbohydrate Polymers, **67**, 155-163 (2007).
45. Li Z, Zhuang X P, Liu X F, Guan Y L and Yao K D, Polymer, **43**, 1541-1547 (2002).
46. Jenkins P J and Donald A M, Carbohydrate Research, **308**, 133-147 (1998).

Fang, J.M, Fowler, P.A, Tomkinson, J, Hill, C.A.S (2002). Carbohydrate Polymer,47, 245-252.

Mano, J.F., Koniarova, D., Reis, R. L., 2003. Thermal properties of thermoplastic starch/synthetic polymer blends with potential biomedical applicability. Journal Material Science: Mater. Med. 14, 127-135.