

# Structural Characterization and Physical Properties of Antimicrobial (AM) Starch-Based Films

Eraricar Salleh, Ida Idayu Muhamad, and Nozieanna Khairuddin

**Abstract**—Antimicrobial (AM) starch-based films were developed by incorporating chitosan and lauric acid as antimicrobial agent into starch-based film. Chitosan has wide range of applications as a biomaterial, but barriers still exist to its broader use due to its physical and chemical limitations. In this work, a series of starch/chitosan (SC) blend films containing 8% of lauric acid was prepared by casting method. The structure of the film was characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The results indicated that there were strong interactions were present between the hydroxyl groups of starch and the amino groups of chitosan resulting in a good miscibility between starch and chitosan in the blend films. Physical properties and optical properties of the AM starch-based film were evaluated. The AM starch-based films incorporated with chitosan and lauric acid showed an improvement in water vapour transmission rate (WVTR) and addition of starch content provided more transparent films while the yellowness of the film attributed to the higher chitosan content. The improvement in water barrier properties was mainly attributed to the hydrophobicity of lauric acid and optimum chitosan or starch content. AM starch based film also showed excellent oxygen barrier. Obtaining films with good oxygen permeability would be an indication of the potential use of these antimicrobial packaging as a natural packaging and an alternative packaging to the synthetic polymer to protect food from oxidation reactions.

**Keywords**—Antimicrobial starch-based films, chitosan, lauric acid, starch.

## I. INTRODUCTION

ANTIMICROBIAL (AM) packaging is a type of packaging, where the package is designed to release active agents to inhibit the growth of microorganisms inside the package. This type of packaging is in contrast with that involving the addition of chemical preservatives directly into the food

Eraricar Salleh is with the *Bioprocess Engineering Department, Faculty of Chemical Engineering and Natural Resources Engineering* Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia (corresponding author to provide phone: 607-5535543; fax: 607-5581463; e-mail: eraricar@fkkksa.utm.my).

Ida Idayu Muhamad is with *Bioprocess Engineering Department, Faculty of Chemical Engineering and Natural Resources Engineering* Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia (e-mail: idayu@fkkksa.utm.my).

Nozieanna Khairuddin is with *Bioprocess Engineering Department, Faculty of Chemical Engineering and Natural Resources Engineering* Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia (e-mail: nozieana@gmail.com).

matrix, where an excess amount of these synthetic additives is believed to be of concern. For the consumer, it seems safer when active agents are indirectly integrated in the food package. Moreover, consumers tend to accept products to which naturally occurring substances have been added more than those containing synthetic agents. This trend subsequently draws many researchers to integrate natural AM agents into food packaging materials. Many studies on AM packaging have been conducted, particularly in the last 10 years. Typically the AM agents are incorporated in a polymer matrix or coated on a polymer film where the AM layer is in direct contact with the packaged food [1].

Starch-based films exhibit physical characteristics similar to synthetic polymers: transparent, odorless, tasteless, semipermeable to CO<sub>2</sub> and resistant to O<sub>2</sub> passage. In order to improve the physical and functional properties of starch films, blending with other biopolymers, hydrophobic substances and/ or antimicrobial compounds has been proposed [2].

Chitosan is a natural 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. It is commercially available from a stable renewable source, that is, shellfish waste (shrimp and crab shells) of the sea-food industry. This biopolymer has revealed to be useful in formation of biodegradable films and preservation of foods from microbial deterioration. The potential of chitosan to act as food preservative of natural origin has been widely reported on the basis of in vitro trails as well as through direct application on real complex matrix foods [3].

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. Beuchat & Golden (1989) [4] suggested that fatty acids were bacteriostatic and may be potential microbial inhibitors in foods using a systematic approach with other antimicrobials. The incorporation of lipid compounds such as fatty acid to a starch film decreases the moisture transfer due to their hydrophobic properties. Fatty acids, such as lauric acid were found to be effective in limiting water vapor transfer through

edible film [5].

The objective of this research was to study the structural and physical properties of AM starch-based films incorporating with chitosan and lauric acid as AM agents.

## II. MATERIALS AND METHODS

### A. 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).

### B. Methods

#### 1) Film Preparation

A starch based film was formed using casting method. 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. All tables and figures you insert in your document are only to help you gauge the size of your paper, for the convenience of the referees, and to make it easy for you to distribute preprints.

#### 2) Structural Characterization

##### 2a) SEM

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.

##### 2b) X-Ray Diffraction

X-ray patterns of starch, chitosan and starch/chitosan blends film were analyzed using X-ray diffractometer (X' Pert PRO, PANalytical) in the angular range 5-40 ( $2\theta$ ) and operated at CuK $\alpha$  radiation ( $\lambda = 0.154$  nm) at a voltage of 40 kV and current of 30 mA. Prior to testing, the samples were

dried and stored in a desiccator. From the scattering spectrum, the effective percent crystallinity of films was determined as the ratio of the integrated crystalline intensity to the total intensity. Crystalline area was evaluated on the basis of the area of the main peaks (main d-spacing). Because of the complexity of the system, the calculated crystallinities are not taken as absolute, but rather used for comparative purposes.

#### 2c) Fourier Transform Infrared Spectrometry

The FTIR spectra of the films were recorded in an IR spectrometer (Nicolet Magna 4R 560, Minnesota, USA) in the range 4000-650  $\text{cm}^{-1}$ . The spectra obtained were used to determine possible interactions of functional groups between starch with lauric acid or chitosan.

### 3) Physical Properties

#### 3a) Film Transparency

Chitosan and lauric acid were used as antimicrobial agents. The effect of incorporation of antimicrobial agents on the transparency of the film was investigated by UV-vis spectroscopy (SHIMADZU UV-2550, Japan). Film samples were cut to 1 X 3 cm and placed on the internal side of a spectrophotometer cell to record the mode transmittance at wavelength from 400 to 800 nm.

#### 3b) Oxygen Transmission Determination (OTR)

Oxygen transmission rate is the rate at which oxygen gas permeates through a film at specified conditions of temperature and relative humidity. Values are expressed in  $\text{cc}/100 \text{ in}^2/24\text{hr}$  in US standard units and  $\text{cc}/\text{m}^2/24\text{hr}$  in metric (or SI) units. Measurements were performed on films with Ox-Tran model 2/21 comes with WinPerm<sup>TM</sup> permeability software using the method described in the standard ASTM D3985-05. OX-TRAN 2/21 system uses a patented coulometric sensor (COULOX<sup>®</sup>) to detect oxygen transmission through both flat materials and packages. Tests were carried out at 23°C temperature and 0% relative humidity using 20% oxygen as test gas. Aluminium foil masks, with an inner diameter of 5  $\text{cm}^2$ , were used to mount test pieces in the diffusion cell. The results were expressed for 100% oxygen.

#### 3c) Water Vapor Transmission Rate (WVTR)

WVTR tests were conducted using Mocon Permatran W3/33A following ASTM F1249-06. Flat film samples are clamped into the diffusion cell, which is then purged of residual water vapor using moisture-free carrier gas. This moisture-free carrier gas is routed to the sensor until a stable WVTR has been established. Absorbent material saturated with distilled water provides an atmosphere of 100% RH. Molecules of water diffusing through the film to the inside chamber are conveyed to the sensor by the carrier gas. The computer monitors the increase in water vapor concentration in the carrier gas and it reports that value on the screen as the water vapor transmission rate.

## III. RESULTS AND DISCUSSIONS

### 1. Film Appearance and Texture

In general starch-based film incorporated with lauric acid and chitosan obtained were compact, translucent and presented good flexibility and elastic than purely starch-based film was formulated and formed as can be seen in fig. 1. In addition they were easy to handle when dried off. After drying, the film facing the plate was shiny and the side facing the room was duller for the films to which lauric acid was added. The lower density of fatty acid and its hydrophobicity were probably responsible for phase separation during film dehydration [6]. Film thickness ranged from 0.03 to 0.04 mm; with an average  $0.0346 \pm 0.002$  mm. The oven-dried chitosan film had a pale yellow colour, which could be attributed to the preferential drying of the surface layers and formation of the mailard reactions product. The starch films, however, were white in colour and the blend films exhibited an intermediate colour [7]. Addition of starch content provided more transparent films while the yellowness of the film attributed to the higher chitosan content.



Fig. 1 A translucent starch-based film incorporated with lauric acid and chitosan

## 2. Structural Characterization

### 2a) SEM

SEMs of the surfaces of the pure starch film, chitosan film and the blend films are shown in fig. 2a-f. The pure starch film (fig. 2a) exhibit characteristic patterns on the film surface. These patterns represent the withered ghost granules of starch. Fig. 2f shows the surface of pure chitosan films to be relatively smooth, to be homogenous and to be a continuous matrix without 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 (fig. 2b-2e) also exhibit such patterns, the intensity of which reduced with the decreasing concentration of starch as revealed from fig. 2b to 2e. 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 [8].

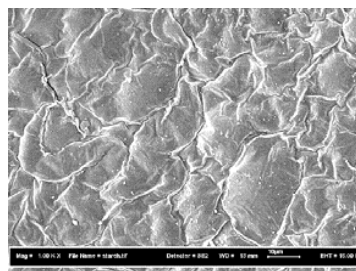


Fig. 2a: Starch pure film

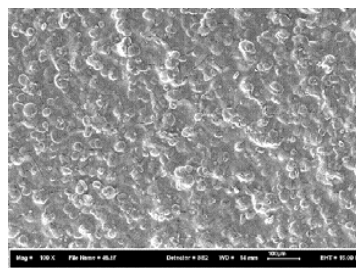


Fig. 2b: SC 4:6

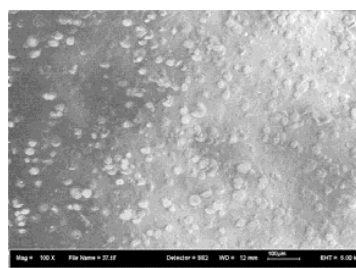


Fig. 2c: SC 3:7

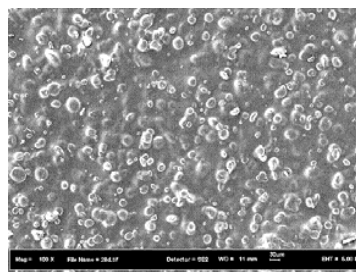


Fig. 2d: SC 2:8

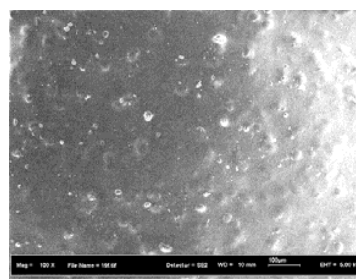


Fig. 2e: SC 1:9

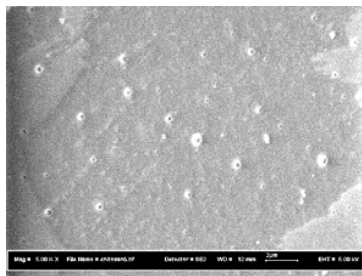


Fig. 2f: Chitosan pure film

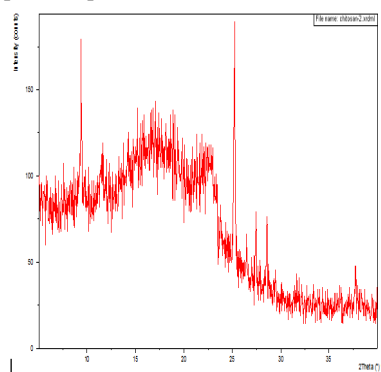
### 2b) X-Ray Diffraction

XRD analyses were carried out to determine the nature of materials whether the material is amorphous or crystalline. X-ray diffraction patterns of starch, chitosan and starch/ chitosan blend films SC ratio 1:9-9:1 are shown in figure 3a-k. The diffractogram of the chitosan film consisted of three major crystalline peaks at  $2\theta = 11, 15.4$  and  $20.1^\circ$ . The results demonstrated that, the chitosan film shows a two-phase morphology of the films i.e. in crystalline and amorphous state because of the presence of both sharp and diffuse diffraction peaks were observed in its x-ray diffraction pattern [9]. In the chitosan film, the intramolecular interactions between  $\text{NH}_3^+$  and hydroxyl groups in chitosan limited the molecular movement of the chitosan chain and reduced its crystallization. Chitosan films prepared by casting from aqueous acetic acid solution were in amorphous to partially crystalline form owing to the presence of the acetic acid solvent residue, which might have hindered the formation of inter- and intra-molecular hydrogen bonds in chitosan and resulted in less dense packing [7].

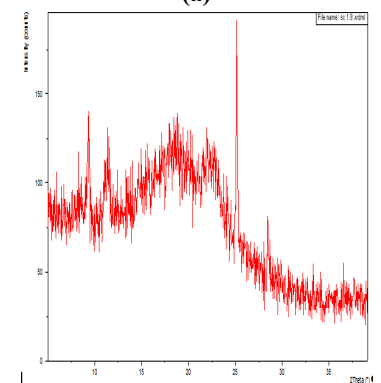
As can be seen in fig. 3k the starch pure film obtained after the gelatinization of starch had very low crystallinity with diffraction at around  $17^\circ\text{C}$  ( $2\theta$ ) and a few small peaks at around  $2\theta$  of  $15^\circ, 20^\circ, 22^\circ$  and  $24^\circ$ . Apparently in cases where the temperature is increased to  $45^\circ\text{C}$  for drying, the rate of nucleation is decreased and the overall rate of crystallization is low [10]; [11]. The crystalline structure was ascribed to spontaneous recrystallization or retrogradation of starch molecules after melting or gelatinization, which is similar to that often detected in the starchy food and thermoplastic material. When these two film forming components were mixed, three major chitosan peaks still observed for the low ratio starch to chitosan ratio 1:9 to 3:7 and the intensity of crystalline peaks was higher for SC ratio 1:9, SC ratio 2:8 and SC ratio 3:7 (fig. 3b, c and d), indicating that chitosan structure was not influenced by the addition of a small amount of starch. The increase in crystalline peaks with increase in chitosan content might be explained by the incidence of a molecular miscibility between these two components [12].

However, the crystalline peaks of the chitosan were suppressed when the starch ratio in the blends film was increased, in its place, a new broad amorphous peak, with greater intensity was observed (fig. 3e, f, g) demonstrating an interaction between these two components [13]; [14]. Fig. 3e,

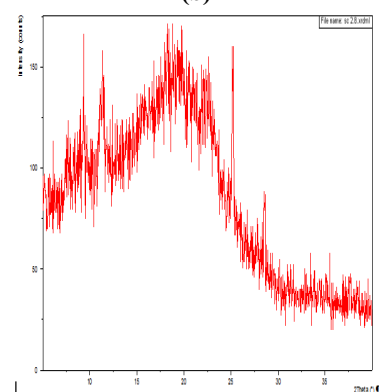
f and g depicted that SC ratio 5:5 and SC ratio 6:4 and SC ratio 7:3 are well mixed at the molecular level and consequently, the individual molecules cannot crystallize resulting in amorphous regions support good miscibility of the components blends in those ratios. The intra molecular interactions between the  $\text{NH}_3^+$  and hydroxyl groups in chitosan and starch chains and reduced its crystallizations [10]. Interestingly, chitosan peak reappeared with further addition of starch, starch to chitosan ratio 7:3-9:1 (fig. 3h, i and j). Reappearance of this peak suggests that there was a phase separation between the two main components of the blends films. This was consistent with the results of the TS and E values as both decreased noticeably at the highest starch ratio due to phase separation.



(a)



(b)



(c)

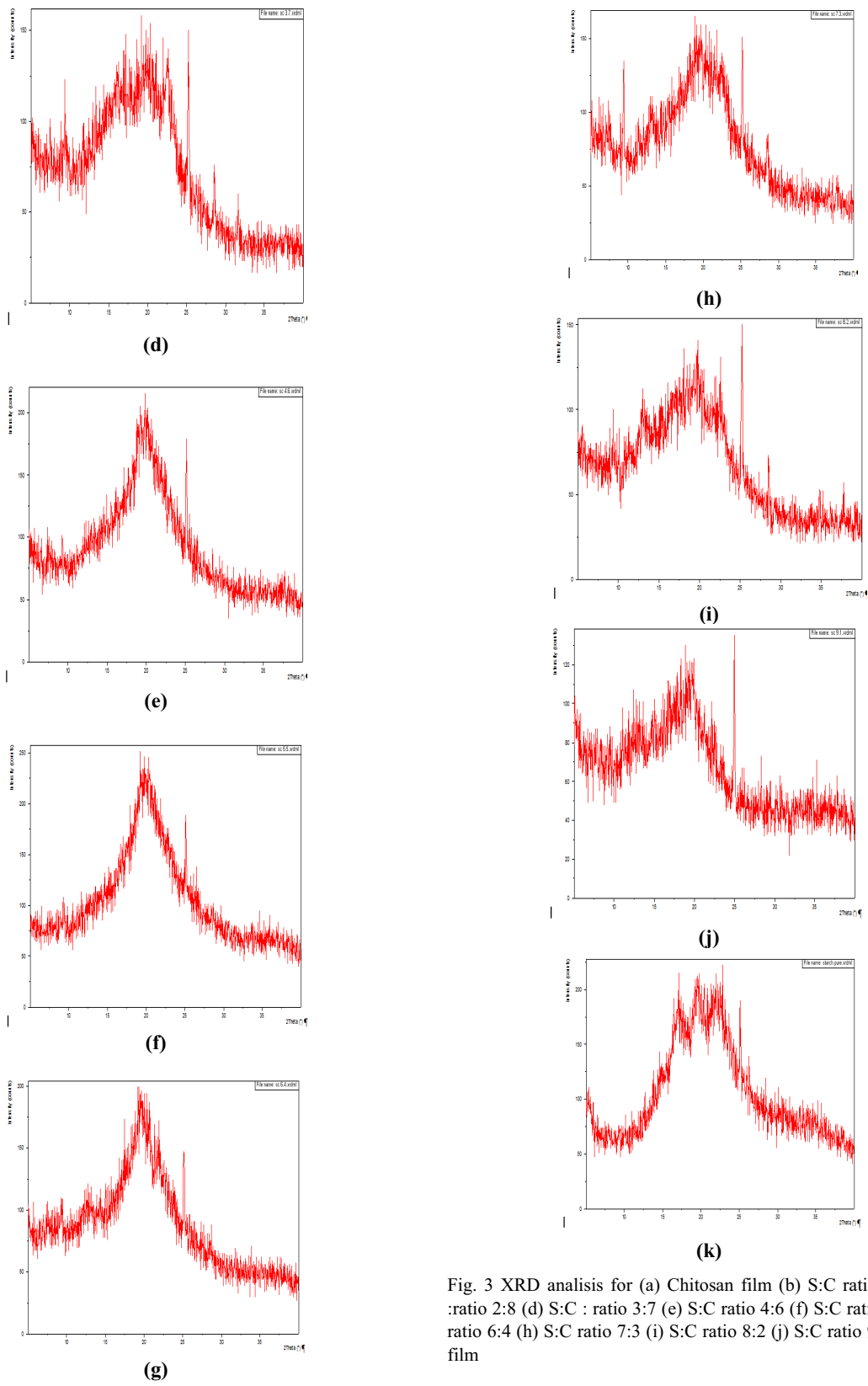


Fig. 3 XRD analysis for (a) Chitosan film (b) S:C ratio 1:9 (c) S:C :ratio 2:8 (d) S:C : ratio 3:7 (e) S:C ratio 4:6 (f) S:C ratio 5:5 (g) S:C ratio 6:4 (h) S:C ratio 7:3 (i) S:C ratio 8:2 (j) S:C ratio 9:1 (k) Starch film

### 2c) Fourier Transform Infrared Spectrometry

FTIR spectroscopy was used to study the interaction between the starch, chitosan and starch-and antimicrobial agents incorporated (chitosan and lauric acid). The infrared spectra of those films are presented in fig. 4-6. In the spectrum for starch film (fig. 4), the broad band at 3346.7 due to the hydrogen-bonded hydroxyl groups that contribute to the complex vibrational stretches associated with free inter- and intramolecular bound hydroxyl group, which make up the gross structure of starch. The sharp band at 2926.3  $\text{cm}^{-1}$  is characteristic of C-H stretches associated with the ring methane hydrogen atoms. The bands at 1658.7 and 1459  $\text{cm}^{-1}$  are assigned to the  $\delta$  (O-H) bendings of water and  $\text{CH}_2$ , respectively [15]. The bands from 763.7 to 1150.4 attributed to the C-O bond stretching. Fig. 5 revealed the IR spectra of chitosan at 3347  $\text{cm}^{-1}$  was the OH stretching, which overlaps the NH stretching in the same region. The peak at 2921  $\text{cm}^{-1}$  is typical C-H stretch. A small peak at 1633  $\text{cm}^{-1}$  was due to the C=O stretching (amide I), and the peak at 1580  $\text{cm}^{-1}$  and 1314  $\text{cm}^{-1}$  have been reported as amide II and III peaks respectively. The sharp peaks at 1413  $\text{cm}^{-1}$  correspond to the  $\text{CH}_3$  symmetrical deformation mode. The peak at 1148  $\text{cm}^{-1}$  indicates the saccharide structure and the band at 1072  $\text{cm}^{-1}$  was due to the C-O stretching vibration in chitosan vibrations [7]. Yin, et al. (1999) [13] reported that when two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic spectral peaks. Changes in the spectra as well as the shifting of peaks due to the hydrogen bonding between OH and  $\text{NH}_3^+$  of chitosan and OH group of starch was observed in the starch based film incorporated with chitosan and lauric acid (fig. 6). The blends film from SC ratio 9:1 to SC ratio 1:9 indicated the interactions were present between the hydroxyl groups of starch and the amino groups of chitosan [16]. In the blends film the amino peak of chitosan shifted from 1580  $\text{cm}^{-1}$  to peaks between 1575  $\text{cm}^{-1}$  and 1576  $\text{cm}^{-1}$  for SC ratios 1:9 to 9:1 with the addition of starch. Formation of 1575  $\text{cm}^{-1}$  to 1576  $\text{cm}^{-1}$  peak was due to the symmetric deformation of  $\text{NH}_3^+$ , resulting from the ionization of primary amino in the acidic medium [13]. The C-H symmetrical deformation mode at 1413  $\text{cm}^{-1}$  increased with the increase in the amount of starch added. This is probably due to interaction between C-H bonds of chitosan and starch with functional groups contained in lauric acid leading to covalent bonds and hence an increase in peak. The infrared spectral data support physical, mechanical and antimicrobial properties data of starch-based film incorporated with chitosan and lauric acid. Incorporation of chitosan and lauric acid as antimicrobial agent modified functional group of starch, therefore, they significantly changed physical and mechanical properties of AM starch-based film produced.

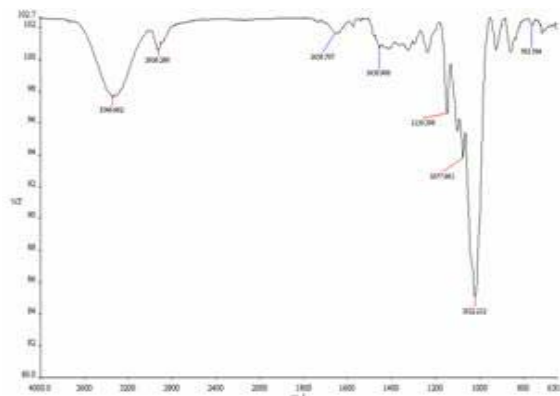


Fig. 4 FTIR of pure starch film

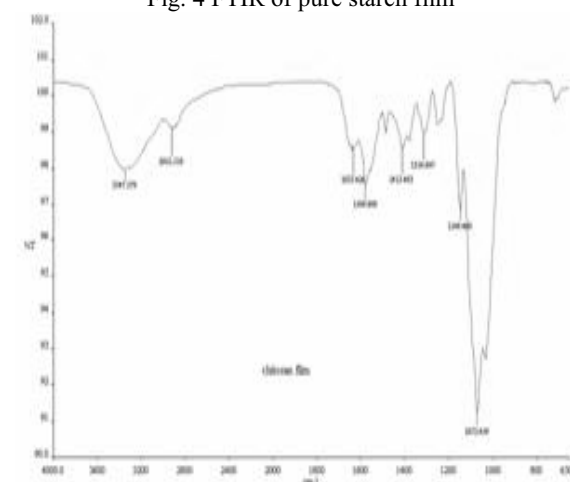


Fig. 5 FTIR of pure chitosan film

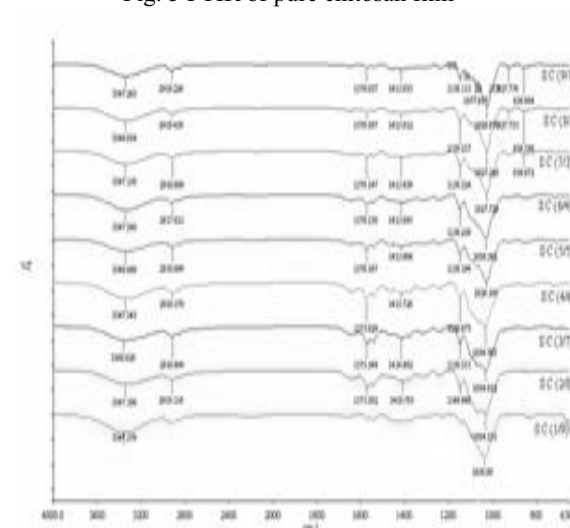


Fig. 6 FTIR of blend films SC ratio 9:1 to SC ratio 1:9

### 3) Physical Properties

#### 3a) Film Transparency

The percent transmittance values of film samples are presented in fig.7. From the figure the pure chitosan film due to the absence of light blockage particles exhibits the highest

percent transmittance followed by SC ratio 1:9, SC ratio 6:4, pure starch film and SC ratio 9:1. The larger the particle size the lesser the light transmission, therefore; the results of light transmittance further prove that the particle size of chitosan is smaller than starch-based film [17]. This result concurrent with the SEM results in fig. 2a and b shown that the withered ghost granules of starch and crystalline structure of starch compare to chitosan film which is flat and compact with very sparsely distributed small particles without any phase separation.

Among the blends film SC ratio 1:9 show the best optical transparence with 75% indicating the best miscibility between starch and chitosan among all blends. The lowest transparency value obtained for the SC ratio 8:2 can be attributed to the larger particle size of starch. However, compared to conventional low density polyethylene films with the transparency values 20-30 [18], all blends film were exceptionally more transparent, thus may be used as see-through packaging or coating materials. In general, films transparency was reduced as the antimicrobial were incorporated.

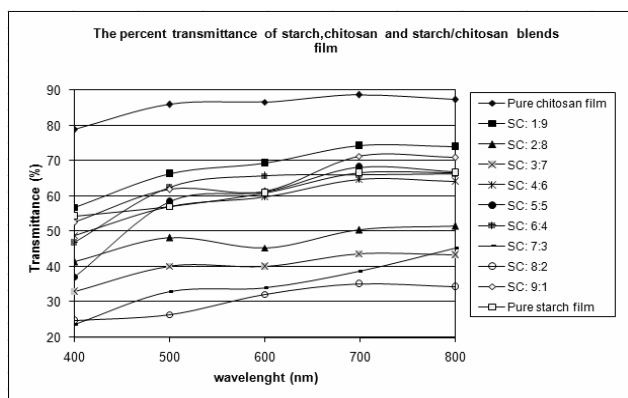


Fig. 7 The percent transmittance of pure chitosan film, pure starch film and chitosan/starch blend films.

### 3b) Oxygen Transmission Determination (OTR)

The results in fig. 8 showed that the oxygen transmittance rate  $0.5203 \text{ cc/m}^2/\text{day}$  obtained by chitosan control film exhibit the highest OTR value. SC ratio 6:4 and 5:5 are the lowest compare with other SC ratios with  $0.042$  and  $0.046 \text{ cc/m}^2/\text{day}$  respectively but overall all the films reveal low transmission rate values below  $50 \text{ cc/m}^2/\text{day}$  indicates that all the films had good oxygen barrier. AM starch based film showed excellent oxygen barrier. The low OTR in the blend film was attributed to the solute presumed to diffuse through the micro-channel or pore within the membrane structure. The water molecule thus fitted in the micro-channel may obstruct the movement of the gas molecule [18]. Obtaining films with good oxygen permeability would be an indication of the potential use of these antimicrobial packaging as a natural packaging and an alternative packaging to the synthetic

polymer to protect food from oxidation reactions. In addition, AM starch based films might retard fruit and vegetable respiration rates and extend their shelf life [19].

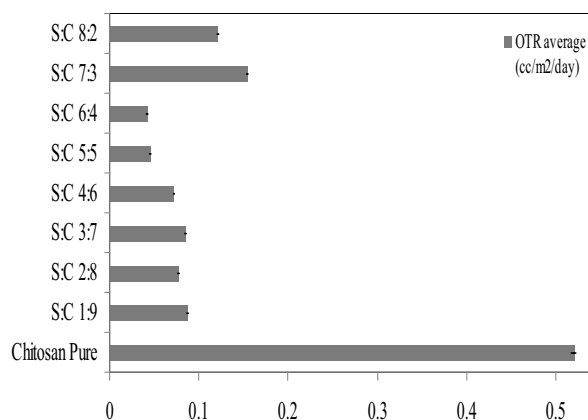


Fig. 8 OTR of control and starch/chitosan blended film

### 3c) Water Vapor Transmission Rate (WVTR)

Fig. 9 indicated that chitosan gained the highest WVTR value. The highest WVTR value is shown in this study for chitosan film due to stronger affinity of chitosan to water molecules [21]. Chitosan itself is a hydrophilic polymer, which absorbs water at ambient temperature while the lowest value of WVTR is for blends film SC ratio 6:4 followed SC ratio 5:5. From fig. 9, it is clearly observed that the WVTR value of the AM films decreases significantly as the chitosan and starch contents nearly in equal amount. Blends film SC ratio 1:9-4:6 and SC ratio 9:1 -7:3 results in increasing of WVTR value compare with SC ratio 6:4 at  $839 \pm 1.18 \text{ (g/m}^2/\text{day)}$  and SC ratio 5:5 at  $850 \pm 1.3 \text{ (g/m}^2/\text{day)}$  but yet still not over the permeability of the control film. This tendency could be explained by a higher hydrophilicity of biodegradable blends films when increasing starch or chitosan content over SC ratio 5:5 and 6:4.

Increasing of chitosan or starch in blends film will leads to increasing the WVTR value due to both of those polysaccharide are hydrophilic. Blends film SC ratio 6:4 followed by SC ratio 5:5 had lowest permeability due to better integration in the film matrix, decreasing the free volume by creating more compact structure, lowered the WVTR. This might indicate the existence of intermolecular interactions and a decrease of the mobility of both starch and chitosan macromolecules when the mixing SC ratio were 6:4 and 5:5. Besides the increase of interactions between chitosan and starch (hydrogen bonding type), reduces the hydrophilic group as well as water vapour transmission rate ([14].

Overall all the WVTR values of the films were under  $1200 \text{ g/m}^2$  per day noted that all the films are acceptably low WVTR. The water resistance of starch film was ameliorated by the incorporation of hydrophobic materials such as fatty acids to enhance the film hydrophobicity [14]. Incorporation

of lauric acid in all blend film contributes to reduced water vapor permeability of the film because the hydrophobicity of the lauric acid regulates the water vapor transmission rate through the blend films. The cationic nature of chitosan makes it particularly suitable for impounding fatty acids. Thus, hydrophilicity is reduced. The formation of hydrophobic aggregates may be due to sorption capacities of chitosan towards fatty acids. Fatty acids absorbed by chitosan may be due to weak hydrogen bond or by physical interaction since the IR spectrum and TGA thermogram of chitosan is very similar to that of chitosan after treated with fatty acids [22]. Thus indicate that AM films decreases free sorption sites for water. A tough film, resulting from the interactions between chitosan, starch and lauric acid prevented water molecules from diffusing through the films, thus reducing the WVTR values. In spite of having an interaction between the two main components, the intensity of non-thermal mixing was lower than that obtained by intensive thermal mixing. The cross-linking caused by thermal mixing transformed the hydrophilic-hydrophilic blend into a more hydrophobic one [14]. High water resistance of a film is one of the most important properties from a food packaging point of view, especially for high water activity foods or foods coming into contact with high-humidity environments during transportation and storage [23].

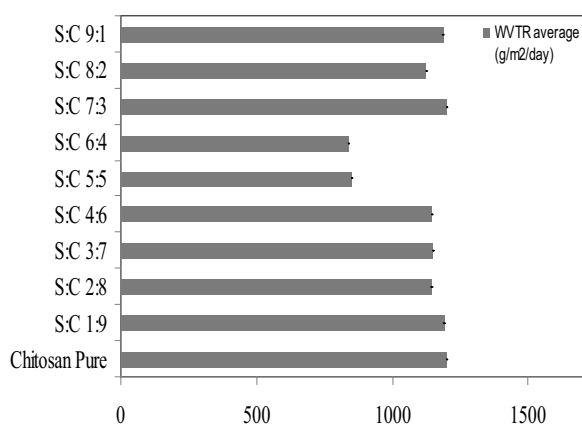


Fig. 9 WVTR of control and starch/chitosan blended film

#### IV. 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. Films formed by blending of two polymers usually result in modified physical properties compared to films made of the individual components. In summary AM starch based film incorporated with chitosan and lauric acid showed several interesting characteristics. The

physical appearance, transmittance, gas barrier and water resistance of AM starch based film were all improved compare to control (starch film).

Therefore, obtaining films with good oxygen permeability and desirable film physical properties would be an indication of the possible use of AM starch based film as an alternative secondary packaging and convenient use for consumers reducing food packaging waste. AM starch based films can be used for dried products and may have potential to be commercial.

#### ACKNOWLEDGMENT

The authors would like to thank the Ministry of Science and Innovation (MOSTI) Malaysia, the Ministry of Higher Education (MOHE) and Research Management Centre, Universiti Teknologi Malaysia for financial support of this project.

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