ANTIMICROBIAL PROPERTIES AND DEGRADATION WEIGHT LOSS AND MORPHOLOGY OF LLDPE/CHITOSAN BLEND

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Abstract- Chitosan has attracted much attention mainly because of its antimicrobial and metal-binding properties. Chitosan is is known to be the second most abundant biopolymer in nature and is the major component of the exoskeleton of crustaceans. Chitosan blends are of importance as they are cheap, sustainable and available widely. Antimicrobial films using Chitosan based material was blended with LLDPE in this study. Twin screw compounding machine was used to blend the material with LLDPE and antimicrobial additives. Three different formulations were prepared for an antimicrobial film Chitosan/ LLDOE blend. Antimicrobial test and weight loss was investigated to show antimicrobial and biodegradation effect of blend. Degradation of Chitosan/PE blends upon weathering for up to 2 weeks and 3 weeks for soil burial showed tensile properties variation. After exposure to environment and soil burial test, tensile strength, elongation at break, and Young’s modulus with different Chitosan content of 5 to 20% blend to LLLDPE. Chitosan/PE blends of 10% composition showed higher tensile strength, elongation at break, and Young’s modulus than other blends.

Index Terms- Antimicrobial agent, chitosan, composite film, LLDPE. Tensile strength, Weight gain/loss.

I. INTRODUCTION

Biodegradable materials is an alternative applications where biodegradability or the derivation of natural resources, particularly that used petroleum-based plastic, gives more advantages especially in short life-time application and when their recycling is less economical. Chitosan, Polyvinyl alcohol, Polylactic acid (PLA) are some of biodegradable materials used but they are not used for pure films for packaging. Strong packaging needs optimsied properties for strength and durability other than biodegrability.

Blending with conventional polymers are some of the technique in normal processing condition to produce film of high quality. Chitosan itself had proven to have antimicrobial properties. It had been used as film to protect fruits and edible foods by coating via dipping and spraying method. Chitosan were found to have antimicrobial properties (1) Polyethylene packaging is considered superior to any other material for this purpose and its use is widespread in the flexible packaging market.4. Vasile had recently reported to have blended LDPE/Chitosan prepared by melt processing recently (2).

Very few works had been done with the blending of PE and chitosan due to difficulty of processing. Flexible food packaging using chitosan and AM organic agent had not been exploited in industrial film processing. The biodegradation of chitosan need to be evaluated for blends of PE and chitosan. Hence, this research will investigate the properties of the use of chitosan as biodegradable component and conventional LLDPE as blended film for application in food packaging. In industrial food packaging, multilayer films are used widely in flexible packaging ; it serves to prolong the shelf life of food.

II. LITERATURE REVIEW

A. Antimicrobial Agents

Food can be easily attacked by bacteria, yeasts and fungi. The microorganisms can cause undesirable and can destroy the nutritional properties of food and also when the microbial growth, it can lead to food poisoning. In order to prevent the quality of food, antimicrobial packaging will be proposed on this research. Antimicrobial packaging is the packaging that able to kill the spoilage and pathogenic microorganisms that contaminating foods. There are two categories that involving in the antimicrobial polymer additives which are organic and inorganic system.

While there are two different effect of antimicrobial additives which are biocidal and biostatic. Moreover, biocidal are intended to destroy, prevent the action or controlling the effect on any harmful organism by chemical or biological means[4]. The biostatic is preventing the growth an organism (microorganism). Moreover, there are two different AM agents namely synthetics additive and natural AM agents. The natural AM agents have attracted attention as they are safe, from food sources by their potential action in food preservation. From the natural AM agents were classified by their sources to vegetal (herbs and essential oils (EO), animal (Iysozyme, lactoferrin), microbial (nisin, natamycin) or antimicrobial polymers (chitosan)[3].

At present, synthetic additives such zinc oxide are normally employed in the packaging industries to confer antimicrobial properties. Other examples include nano sytem and oxides. They proved to confer antimicrobial properties to plastics packaging but has It disadvantage to the human health[16].

B. Chitosan

Chitosan is biodegradable, biocompatible, non-antigenic, nontoxic, biofunctional and antimicrobial. It is also one of the most abundant natural polymer (polysaccharide) on earth after cellulose. The most important sources of chitin today are crustaceans such as shrimps. Chitosan is a linear polysaccharide of randomly distributed N-acetyl glucosamine and glucosamine units [6]. Its structure is as shown in Fig 1. Due to its antimicrobial property, many researchers had worked with

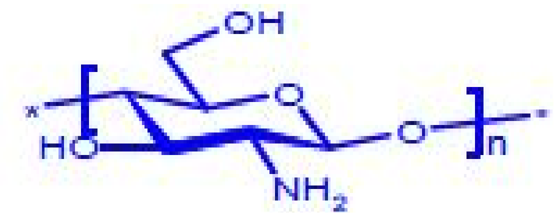

Fig 1 structure of chitosan upon treatment [8]

chitosan. However, chitosan suffers from a relatively poor mechanical properties mainly involving less flexibility or brittleness at room temperature. For wound dressing, use of chitosan seems promising (3). Antibacterial activity of konjac glucomannan/chitosan blend films and their irradiation-modified counterparts had been studied by Xuezhu (4) There are many reports on blending of chitosan with natural polymers such as konjac glucomannan (5) and with synthetic polymers such as poly (ethylene oxide) and PVA as membrane (6) and poly (lactic acid)) to improve chitosan properties. Several blends based on chitosan, have also been reported for biomedical applications such as bone and cartilage (7) Chitosan has strongly hydrophilic character and provides them with good barrier properties against gases and has biodegradability characteristic [8]. Chitosan have been used as coating on edible food through casting method as thin film to preserve foods [15]

C. Antimicrobial agent (AM)

Antimicrobial agent (AM) was of commercialisation in pharmaceutical and food industry. Previously, metal oxides were used (10) The interaction between polycationic nature of chitosan and AM can enhance the antimicrobial activity between these blend. AM is from organic nature and has ester groups and is safe for food application.

D. Linear Low Denstiy Polyethylene (LLDPE)

LLDPE was commonly used for production films, bags, tubes and so on. The combination between the LLDPE, chitosan and AM can be producing the production of film by melt processing via twin screw. This method can enhance the gas barrier properties at low relative humidities [5].

III. MATERIAL

Materials used in this research are LLDPE supplied by ETILINAS product code LL0209SA with a melting temperature of 130ºC and melt flow index of 2.0g/10min, manufactured by TITAN (M) Sdn. Bhd., Johore was used as a resin. Chitosan and antimicrobial(AM) agent are supplied locally in powder and liquid form respectively. Polyethylene are varied with chitosan composition 2%, 5%, 8% and 10% and fix amount of AM(5%).

IV. METHODOLOGY

Three samples of LLDPE /Chitosan blend films was prepared by mixing Anti Microbial Additives (AM) with chitosan and PE in the Twin Screw Compounding Machine at temperature between 158 to 165C . The compound are converted as compressed films. Table 1 below shows the Chitosan/PE blends formulation.

TABLE 1 Formulation of LLDPE and chitosan with AM

Sample	PE(g)	Chitosan(g)	NMA (g)
A	80	15	5
B	85	10	5
C	90	5	5

Testings conducted for compounded LLDPE/Chitosan blend were performed for unexposed and soil burial specimen. Burial tests were done soil of 10 cm depth in Malaysia yellow earth soil. Tensile Tests, ASTM D638 and Structural analysis were performed via FTIR . Weight loss were determined upon soil burial for different ??

Structural properties via FOURIER TRANSFORM INFRARED (FTIR) were performed for unexposed and soil burial samples. FTIR spectra of the films were recorded using an attenuated total refraction (ATR) method in an IR spectrometer (Bruker, Equinox 55, Japan). The films were applied directly onto the ZnSe ATR cell. For each spectrum, 128 consecutive scans at 4 cm^{-1} resolution were average raged.

Tensile properties were performed using dumb bell shape specimen with length, 70mm and gage length 4 mm following ASTM D638 with speed rate 20mm/min. Tensile specimen were loaded to a universal machine Instron -10kN.

Agar Diffusion Method (Antimicrobial Activity Test)

The antimicrobial activity of chitosan/polyethylene films was tested qualitatively and quantitatively by an inhibition zone method (Leceta et al., 2013). In this method, Escherichia coli were used as the bacteria for testing the antimicrobial activity of the films .

For qualitatively measurement of antimicrobial ativity, the films sample were punched to make a rounded disk (diameter=6mm) and the antimicrobial activity was determined using a standard nutrient agar. The plates containing the agar were examined for possible clear zones after incubation at 37^0C for 2 days. The quantitative antimicrobial activity of the films was determined by measuring the inhibition zone on the test pathogenic bacteria. Three different antibiotics were used to compare if any samples has the same inhibition zone as the antibiotics. The antibiotics used are Penicillin, Streptomycin and Tetracycline. Films samples were then cut into square pieces (0.5cm x 0.5cm) and placed in individual sterile flks to be used in the test for microbial inhibition. as Two Gram negative bacteria, Escherichia oli were separately cultivated in TS broth (Difco) at 30oC for 16h. Measurement is taken by calculating the area where the bacterial activity takes place. The growth of bacteria was measured in diameter (mm) using a Vernier caliper, and compared with the

Soil Burial Test (Degradability Test) And Environmental Exposure

Commercial soil-based compost as soil. Composting process produce microbial bacteria via enzymes to carbon dioxide and water. Biodegradation of plastics materials when exposed to soil is about similar. Tensile strength performance upon exposure will be used to investigate whether the biodegradation affects the tensile strength or vice versa. Figure 2. All samples are buried for 3 weeks.

Weight loss.

Films specimens were cut into 2 cm X 10 cm which was buried in the soil at a depth of 10 cm from the surface. Film weight and thickness, before and after the burial were noted and upon burial, it is subjected to the action of microorganisms. After the test, samples were removed, washed with distilled water and dried in the oven at 70°C for 24 h. Either any weight gain or loss will be recorded to determine degradation effect upon weight loss.

V. RESULTS AND DISCUSSION

Based on the data obtained, the diffusion of antibiotics in E. Coli bacteria shows that Penicillin does not work against E. Coli and only Streptomycin and Tetracycline that can be used to fight against bacteria E. Coli. While Tetracycline is the most suitable antibiotics against E. Coli bacteria. By the observation done through this antimicrobial growth of different antibiotics, we can compare and relate with the samples. This can be shown in the Table 3 as provided below.

Table 2. The diameter of antimicrobial growth for different antibiotics

SAMPLE	ANTIBIOTIC	DIAMETER (mm)
A	Tetracycline	0.12
B	Tetracycline	0.04
C	Streptomycin	0.04

The data obtained in Table 3 shows that Sample A has the most effective against E. Coli bacteria in the agar containing diffused antibiotic Tetracycline while are Penicillin, Streptomycin and Tetracycline. Films samples were then cut into square pieces (0.5cm x 0.5cm) and placed in individual sterile flks to be used in the test for microbial inhibition. as Two Gram negative bacteria, Escherichia oli were separately cultivated in TS broth (Difco) at 30oC for 16h. Measurement is taken by calculating the area where the bacterial activity takes place. The growth of bacteria was measured in diameter (mm) using a Vernier caliper.

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ANTIBIOTIC	DIAMETER (mm)	
Penicillin	0.00	0.00
Streptomycin	0.16	0.16
Tetracycline	0.27	0.28

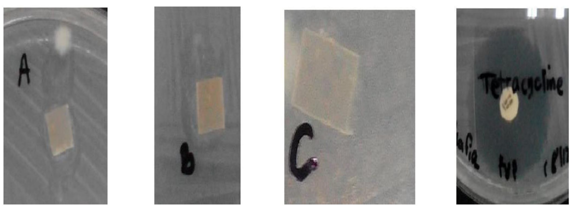

Fig 14. The antibacterial growth for every samples and antibiotic

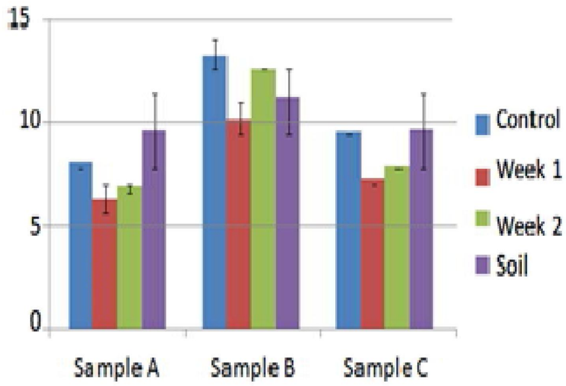

Fig 4. Tensile Strength (MPa) for different samples upon exposure to soil burial and sun exposure

The tensile strength test were performed on films of Sample A, B and C for 4 different types of testing conditions, namely control films, films that are exposed to environment for one week, films that are buried in the soil for 3 weeks. Tensile Strength, Young's Modulus and Elongation values were as depicted in Fig 4, 5 &6.

It was found that sample B has the highest stress at peak which is for 10% chitosan. Compared to other two formulation, this amount provide optimum intermolecular interaction between LLDPE and chitosan. This is brought about by better homogeneity where chitosan is well dispersed. Upon higher values, compatibility of chitosan and PE is greater as hydrophilic nature of chitosan had cause less interaction between PE and Chitosan due to greater incompatibility as envisaged by more aggregation of chitosan interacting with each other.

After exposing to the environment for 2 weeks, sample B shows the highest tensile strength which is ~12.26 MPa, compared to sample A which is ~6.91 MPa . This is because sample A has more chitosan than other compound. This led to the brittleness properties which affect the mechanical performance of the films. Next, the data for Young's Modulus are shown in the bar chart below.

Young's Modulus (N/mm2) above, for 2 weeks of environmental exposure, Sample B has the most brittle sample than sample A and C which is 255.94 N/mm2, while for 3 weeks of soil burial, Sample B has higher modulus than the others which is 220N/mm2 and Sample C has the lowest which is 187N/mm2. This is because the amount concentration of Chitosan in Sample C is low thus the brittleness properties of Chitosan affect the mechanical performance of the films.

The effect of weight gain or loss can be an indicator for polymer degradation. Hence, to investigate the effects of environment and soil on the films physically such as weight were monitored after 1, 2 and 3 weeks exposure.

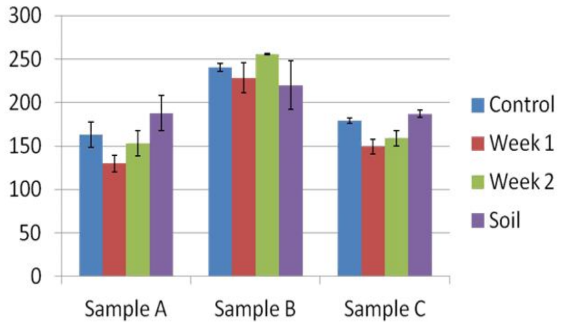

Fig 5 Youngs Modulus MPa of different samples upon soil burial for 3 weeks and sun exposure for 1 and 2 weeks

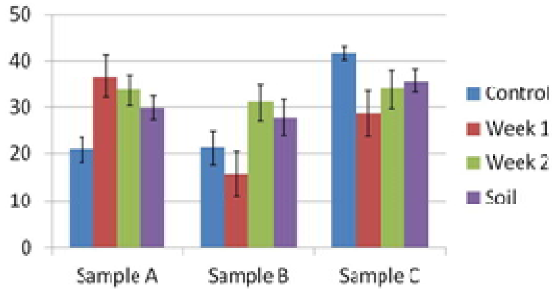

Fig 6 Elongation for different samples to different exposure condition.

For Elongation at Break (mm) above, the highest elongation at break obtained by a film is Sample A for week 1 which is 36.657 mm. For 2 weeks of environmental exposure, Sample C has the highest elongation at break than sample A and B which is 33.9mm, while for 3 weeks of soil burial, Sample C has ore elongation at break than the others which is 35.7mm and Sample B has the lowest which is 25.9 mm.

Chitosan introduce rigidity with reduced flexibility of polymer chain increase in elongation is imparted by lower chitosan content. This is because the amount concentration of PE in Sample C is higher and it has the ability to stretch and elongate.

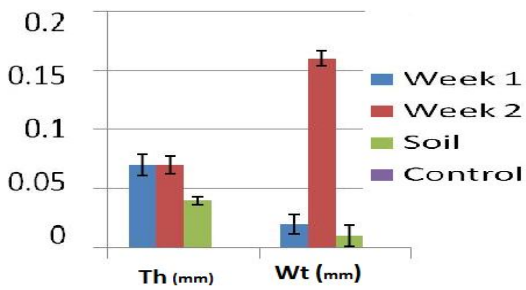

Fig 7 Thickness and Weight variation of specimens upon exposure to soil burial (3 wks) and sunlight (1,2weeks) for sample A

Generally, there is an increase in thickness for every sample exposed to almost all environment. This may result from the raining or high moisture from the environment where Chitosan highly absorb water. In week 2, Sample B has no difference in thickness while Sample A has the largest difference in thickness which is 0.02 mm. In terms of weight, Sample A also dominates the difference in weight which is 0.16 mm, followed by B having the lowest weight difference of 0.10 mm. Hence, it shows that when exposing to the environment, Sample B with the ratio 2:3 (Chitosan : PE) has the ability to withstand the effects from environment conditions.

With sample B of 10% Chitosan, the soil samples had underwent degradation process after 3rd week, as micorgrganism attck the polymer network structure causing it to disintegrate amd degrade.

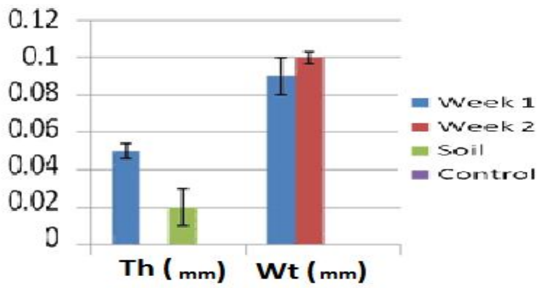

Fig 9 Thickness and Weight variation of specimens upon exposure to soil burial (3 weeks) and sunlight(1,2weeks) for sample B .

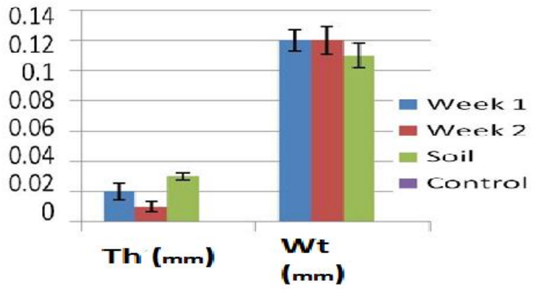

Fig 9 Thickness and Weight variation of specimens upon exposure to soil burial (3 weeks) and sunlight(1,2weeks) for sample C .

The microorganisms in the soil feed on the blends films resulting in the loss of their structural characteristics. The degradation of the blends films occurs due to either microbes or water. The water absorbed by the blends during the experiment as expected caused swelling of the blends and thus increased the thickness and accelerated biodegradation. The major constituents of Chitosan can be degraded by many bacteria and fungi in the soil environment. Microorganisms have very specific enzymes capable of hydrolyzing these polymers into digestible units so that the Chitosan structure is weakened and Chitosan strength is reduced.

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CONCLUSION

From the present study, it can be concluded that degradation affects the tensile properties of Chitosan/PE blends. The results obtained from the tensile tests after exposure to environment and soil burial test revealed that variation occurs in tensile strength, elongation at break, and Young's modulus with different levels of Chitosan content in the blends. The results pointed out that the Chitosan/PE blends of Sample B showed higher tensile strength, elongation at break, and Young's modulus than Chitosan/PE blends of Sample A and Sample C due to enhanced interfacial adhesion between the PE matrix and Chitosan. However, all the blends under study showed a reduction in the modulus properties with increase in Chitosan content as well as burial time as a result from the brittleness properties of Chitosan. The study has also indicated that blending PE with Chitosan with the ratio used in Sample A has higher antimicrobial ability.

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