CHITOSAN STARCH BASED PACKAGING FILM ENHANCED WITH EMPTY FRUIT BUNCH CELLULOSE NANOFIBER

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To my beloved mother, my late brother and father….....

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ABSTRACT

Starch-based biopolymers exhibit low mechanical properties. Based on recent studies, it has been shown that the incorporation of nano fillers, such as cellulose nanofiber (CNF), into a polymer matrix can greatly improve its mechanical properties. Interestingly, the incorporation of CNF into antimicrobial packaging enhances the results of antimicrobial efficacy towards food shelf life. Cellulose nanofiber (CNF) from oil palm empty fruit bunch was prepared, through pretreatment, to remove non-cellulosic content and then underwent an hydrolysis process involving strong acid. Starch-based nanocomposite film was formed by the incorporation of 2, 4, 6, 8 and 10% CNF, per weight of starch, into the film matrix. The nanocomposite film appeared translucent and easy to handle however, the film became more opaque as the percentage of CNF was increased. The surface morphology of the film was observed using field emission scanning electron microscopy to analyze the effect of CNF addition on the film surface. Mechanical properties such as tensile strength, percentage of elongation and Young's Modulus were determined using a tensile test machine. Tensile strength of the starch-based film without CNF (as a control) was 3.1 MPa which increased to 4.68MPa with the addition of 2% CNF. However, the strength was gradually decreased with the addition of more than 2% CNF. Optimum Young's Modulus was also detected at 2% CNF incorporation. The percentage of elongation gradually decreased from 1.79% to 0.89% for film with 0% to 10% CNF loading, respectively. Influence of CNF addition in antimicrobial film with regard to its antimicrobial properties and food shelf life were also studied. Antimicrobial composite film was prepared by blending 1 to 9 ratio of chitosan per weight of starch while antimicrobial nanocomposite film was prepared by adding 2% of CNF and the control film was formed using starch only. The antimicrobial test showed that starch/chitosan with CNF gave 30% better bacterial inhibition towards Gram-positive bacteria *Bacillus subtilis* compared to the starch/chitosan control film. However, in Gram-negative bacteria *Escherichia coli* starch/chitosan with CNF composite packaging gave rise to a similar inhibition zone as the control starch/chitosan packaging. Results showed that nano-reinforced starch/chitosan packaging was able to retain moisture in a tomato 7 days longer than the control starch and starch/chitosan composites which gradually changed the tomato's colour which highlighted the slow ripening process and lessened mould infection. In conclusion, the addition of CNF to a starch-based matrix has shown a significant impact on the mechanical properties of the formed film as well as water barrier properties. It also embarked the potential of CNF as a filler for antimicrobial packaging as it enhances the results on antimicrobial properties and food shelf life.

ABSTRAK

Biopolimer berasaskan kanji menunjukkan sifat mekanikal yang rendah. Kajian terkini menunjukkan penambahan pengisi seperti gentian nano selulosa (GNS) ke dalam matrik polimer dapat menambahbaik sifatnya. Menariknya, GNS yang ditambah dalam pembungkusan antimikrob mampu meningkatkan keberkesanan antimikrob terhadap jangka hayat makanan. Gentian nano selulosa daripada tandan kosong buah kelapa sawit telah disediakan melalui pra-rawatan untuk membuang kandungan bukan selulosa dan kemudiannya menjalani proses hidrolisis dengan asid pekat. Filem komposit kanji nano telah diformulasikan melalui penggabungan 2, 4, 6, 8 dan 10% GNS per berat kanji ke dalam matriks filem. Filem komposit kelihatan lutsinar dan mudah dikendalikan. Walau bagaimanapun, filem itu menjadi lebih legap apabila peratusan penambahan GNS meningkat. Morfologi permukaan filem diperhatikan menggunakan mikroskop pengimbas penghantaran elektron untuk menganalisis kesan penambahan GNS ke atas permukaan filem. Sifat mekanik seperti kekuatan tegangan, peratusan pemanjangan dan Modulus Young ditentukan menggunakan mesin ujian tegangan. Kekuatan tegangan filem berasaskan kanji tanpa GNS adalah 3.1 MPa dan ia meningkat kepada 4.68 MPa dengan tambahan 2% GNS. Walau bagaimanapun, kekuatan itu secara beransur-ansur menurun dengan penambahan lebih daripada 2% GNS. Modulus Young yang optimum juga telah dikesan pada penambahan 2% GNS. Nilai peratusan pemanjangan pula berkurangan secara beransur-ansur daripada 1.79% kepada 0.89% bagi sampel dengan penambahan 0% hingga 10% gentian nanoselulosa. Pengaruh penambahan GNS dalam filem antimikrob ke atas ciri-ciri antimikrob dan jangka hayat makanan juga dikaji. Filem komposit antimikrob telah disediakan dengan menggabungkan nisbah berat 1 kitosan kepada 9 kanji. Filem komposit antimikrob nano pula disediakan dengan menambah 2% GNS dan filem kawalan telah dibuat dengan menggunakan kanji sahaja. Ujian antimikrob telah menunjukkan bahawa filem kanji/kitosan dengan penambahan GNS memberikan perencatan bakteria 30% lebih baik ke atas Gram-positif bakteria *Bacillus subtilis* jika dibandingkan filem komposit kitosan/kanji. Walau bagaimanapun, untuk bakteria Gram-negatif *Escherichia coli*, filem pembungkusan komposit kanji/kitosan dengan penambahan GNS memberikan zon perencatan hampir sama seperti filem komposit kanji/kitosan. Hasil kajian juga menunjukkan bahawa filem komposit kanji/kitosan nano dapat mengekalkan kelembapan tomato 7 hari lebih lama daripada filem kawalan kanji dan filem komposit kanji/kitosan, menukar warna tomato secara perlahan yang menunjukkan peranuman buah menjadi lambat dan mengurangkan jangkitan kulat. Kesimpulannya, penambahan gentian nanoselulosa ke dalam matriks berasaskan kanji telah menunjukkan peningkatan ketara ke atas sifat mekanik filem dan juga sifat kerintangan terhadap air. Ia juga menunjukkan potensi GNS sebagai pengisi untuk filem pembungkusan antimikrob kerana ia meningkatkan ciri-ciri antimikrob dan juga memanjangkan jangka hayat makanan.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
AM	-	Antimicrobial
AP	-	Active packaging
ASTM	-	American Society for Testing and Materials
CNF	-	Cellulose nanofiber
D.I	-	Deionized water
EFB	-	Empty fruit bunch
FESEM	-	Field Emission Scanning Electron Microscopy
FTIR	-	Fourier Transform Infrared Spectroscopy
OTR	-	Oxygen transfer rate
RH	-	Relative humidity
rpm	-	Revolution per minute
S/C	-	Starch/chitosan
SEM	-	Scanning Electron Microscopy
WVTR	-	Water Vapor Transmission Rate

LIST OF SYMBOLS

%E	-	Percentage of elongation
M_0	-	Weight of the sample at initial / before exposure
M_t	-	Total of weight after certain time of exposure
D, d	-	Diameter
l	-	Length, film thickness
r	-	Radius
t	-	Time
TS	-	Tensile strength
Y	-	Young's modulus
ρ	-	Density

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CHAPTER 1

INTRODUCTION

1.1 Background

Palm oil processing generates massive agro waste every year. Mun (2011) reported that along the year 2011, 78.53 million tons of solid waste consists of 21.34 million tons of oil-palm empty bunch, 7.73 million tons of mesocarp fiber, 4.46 million tons of palm kernel shell, and 45 million tons of oil palm fibers were produced in Malaysia. Even worse, pollution caused by non-biodegradable plastic packaging becomes out of control. Report shows that 150 million tons of non biodegradable plastic polymers were produce annually (Chrissafis *et al.,* 2005; Punrattanasin, 2001). The problems caused by the accumulation of plastic waste and under-utilized oil palm biomass could be both eliminated. Therefore, it is crucial to reduce plastic waste production. It is possible by developing biodegradable plastic using biomass from the empty fruit bunch (EFB).

EFB are composed of 45-50% cellulose and about equal amounts (25-35%) of hemicellulose and lignin (Deraman, 1993). Cellulose for instance, can be incorporated into polymer matrix as strenghthener as reported by previous researchers (John and Thomas, 2008). Furthermore, based on technological advances, cellulose fiber can be treated chemically and physically to a nano-size.

Nano material such as nanofiber has attracted massive attention because of its small size that interacts better with biodegradable polymer matrix. It is also more compatible when applied in flexible thin film. As initiated by Jonoobi *et al.,* (2011), the addition of cellulose nanofiber into biodegradable polymer matrix could enhance the physical and mechanical properties of the film. This explains that nano-size cellulose has a big potential in development of new and enhanced biopolymers.

Cellulose nanofiber could give excellent mechanical properties to the biodegradable plastic as it may exhibits comparable strength to petroleum based plastic. In its crystal region, cellulose nanofiber has high young's modulus approximately at 138Gpa (Sakurada *et al.,* 1962) also gives a very low coefficient of thermal expansion along longitudinal direction (Nishino *et al.,* 2004). All the characteristics mentioned make nanofiber as potential reinforcement materials for bio-plastic. The incorporation of cellulose nanofiber into the bio plastic such as starch-based packaging could enhance the physical characteristic of the film and able to control water vapor transmission rate as well as oxygen transmission rate which is important in food packaging application (Pinheiro *et al.,* 2013).

Furthermore, in food technology application, it is important to adjoin some additional features in the natural biopolymer. Thus, various additives such as chitosan are incorporated into the starch-based film to extend the food-shelf lives. In general, this study focused on enhancement of biodegradable starch-based plastic particularly in term of strength, increase food safety and finally reducing the depedency on petroleum based plastic by using inexpensive and abundant renewable resource with low technology production.

1.2 Problem Statements

In the past 20 years, the utilization of non biodegradable plastics in the world has enormously increased leading to a serious environmental issue (Chrissafis *et al.*, 2005). In addition, over dependence on synthetic plastic monomer from petroleum make this valuable source dangerously depleted. Hence, bio based film such as starch-based packaging film that totally biodegradable was introduced as an alternative in single use, short-life and disposable packaging (Narayan, 2006). Biopolymers such as starch-based polymer ought to be used in those applications where biodegradability and/or the derivation of natural resources gives added value, particularly for applications with a short life time such as food packaging (Avella et al., 2005). In some applications of food packaging, low barrier properties towards gas and moisture diffusion is desirable such as in packages for fresh fruits and vegetables whose shelf life is dependent on access to a continual supply of oxygen for sustained cellular respiration (Robertson, 2006). However, biopolymer from starch-based film naturally brittle and poorer mechanical properties than the conventional non-biodegradable plastic films used in the food packaging industries (Khalil *et al.*, 2012). Recent studies show that the filler with nano size was found to be impressively valuable in material reinforcement as it has significantly high surface charges that promote excellent adhesion with matrix *(*Flemming *et al.*, 2001). Thus, nano material such as cellulose nanofiber (CNF) from oil palm empty fruit bunch can be used as starch-based biopolymer reinforcement. In food packaging application, this finding could potentially beneficial for further prospects in active biodegradable packaging sector. Interestingly, previous finding showed that the addition of nano filler is able to control the release of the antimicrobial molecule, thus efficiently increase the food shelf life (Cozzolino *et al.*, 2012). Hence, the addition of nano material such CNF is found to be an applicable approach to enhance the mechanical and physical properties of the active biopackaging film at the same time improve the food packaging system to increase food shelf life.

1.3 Objectives of Study

The main objective of this study is to develop food packaging incorporated with EFB Cellulose nanofiber to enhance mechanical properties and food shelf life. The specific objectives of this study are:

1. To establish and characterize EFB cellulose nanofiber
2. To develop starch-based film reinforced with EFB nanofiber and characterize the mechanical and physical properties.
3. Incorporate the selected ratio of cellulose nanofiber with chitosan as antimicrobial agent, characterize the antimicrobial properties.
4. To apply active nanocomposite film as food packaging to study its effect on food shelf life and quality.

1.4 Scope of Study

This study delimited to these scopes. Extraction process was established using acid hydrolysis method and the chemical properties was validated using FTIR analysis (Fahma *et al.*, 2010). Starch-based active packaging film reinforced with EFB nano fiber was formulated by adding EFB cellulose nanofiber into starch-based film with different starch:EFB cellulose nanofiber ratio (0, 2, 4, 6, 8, 10 % per weight of starch). Physical and mechanical properties such as tensile strength, water uptake rate, films opacity was evaluated by using tensile machine-Lloyd LRX, Digital balance Intell-Lab LF Series, and UV-Vis Spectroscopy UV-160A, Shimadzu, Japan respectively. Then, the formulation that gave the best mechanical properties for stach/EFB cellulose nanofiber then selected to be incorporated with chitosan as antimicrobial agent. Microbial study of the films that been formed was evaluated by agar diffusion method and liquid culture test. The starch/EFB cellulose nanofiber film was applied as tomato wrap to study the significant of cellulose nanofiber incorporation as food packaging towards food shelf life. The visual observation (microflora appearances and colour) and weight loss was done daily for 28 days.

1.4 Significance of Study

Conventional and common plastic packagings are made from petroleum that is non-renewable sources. It is flexible, convenient and high in strength however, it is not biodegradable that uncontrollable accumulation will give serious environmental issues. For this reason, biodegradable film/plastic has been introduced to substitute petroleum-based plastic. However, the biodegradable plastic/film that made from starch is weak and easily swollen. The incorporation of EFB nano cellulose fiber could strengthen the plastic/film make it applicable for flexible packaging. Nanofiller such as cellulose nanofiber is relatively small could interfere efficiently with film matrix because it has larger surface are per volume. Hence, good interfacial adhesion between cellulose nanofiber and film matrix could provide mechanical and physical enhancement of the starch-based film. In food safety, application of active biodegradable packaging is commonly use to extend food shelf life. "Active" characteristic of the film was added by direct incorporation of chitosan as antimicrobial agent into the starch-based film matrix. However, study showed that native active packaging when applied on food, have certain limit as it tend to burst with fairly good properties on antimicrobial and food preservation. By any chance, additions of cellulose nanofiber into active packaging need to be done in order to determine whether it could give significant improvement on antimicrobial properties and food preservation.

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