



**Characterizations, Properties and Degradation of
Empty Fruit Bunch Cellulose Nanocrystals-Chitosan
Composite Film**

by

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A thesis submitted in fulfillment of the requirements for the degree of
Doctor of Philosophy

**School of Bioprocess Engineering
UNIVERSITI MALAYSIA PERLIS**

2020

ACKNOWLEDGMENT

First of all, I would like to extend my special thanks and appreciation to my project supervisor, Associate Professor Dr. Sam Sung Ting, for his generous guidance, kindness and patience with me throughout the project. His wisdom and constructive comments have been of great value for me, not to mention his understanding and encouragement have provided a good basis for my conversion report. Similarly, I would like to thank Associate Professor Ir. Dr. Mohd Firdaus Omar, who gave me the helpful knowledge and valuable advice.

I also wish to thank all the technicians and PLVs from School of Bioprocess Engineering and School of Material Engineering, for their continuous guidance, cooperation and technical assistance during the preparation of my laboratory work. Special thanks to my lab mates, Chin Kwork Mern, Nurdiana binti Othman and Owi Wei Tieng for their kindness to share their knowledge related to the research. Not forgotten, my deepest thanks to the MyBrain15 scholarship provided by Ministry of Higher Education Malaysia.

Last, but not least, I would like to warmly thanks my parents, Gan Hwee Meng and Lim Sok Lian, for their dedication and the support during my postgraduate studies that provided the foundation for this work. Their support and encouragement were in the end what made this dissertation possible. I would like to express my gratitude and appreciation to my beloved, Soo Jian Aun who always support and encourage me throughout my PhD journey.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BNC	Bacterial nanocellulose
CH	Conventional heated
CI	Crystallinity index
CNC	Cellulose nanocrystal
CNF	Cellulose nanofiber
CV	Coefficient of variation
DES	Deep eutectic solvent
DSC	Differential scanning calorimetry
EFB	Oil palm empty fruit bunch
FTIR	Fourier transform infrared spectroscopy
GA	Glutaraldehyde
MC	Microwave cured
NB	Nutrient broth
NCBI	National Centre for Biotechnology Information
OD	Optical density
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PVA	Polyvinyl alcohol
R ²	R-Squared
RH	Relative humidity
RSM	Response surface methodology
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl radical
TGA	Thermogravimetric analysis
UV	Ultraviolet
WVP	Water vapor permeability
WVTR	Water vapor transmission rate
XC	Non-crosslinked
XRD	X-ray diffraction

LIST OF SYMBOLS

X_1	Acid concentration
X_2	Hydrolytic Temperature
X_3	Reaction Time
I_{002}	Highest peak intensity for the crystalline region
I_{AM}	Minimum peak intensity for the amorphous region
G	Change in weight
t	Time
A	Area exposed to the water vapor transfer
ΔP	Vapor pressure difference of water through the film at different RH
Y	Yield
T_{Max}	Maximum degradation temperature
T_m	Melting temperature

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Pencirian, Sifat dan Degradasi Selulosa Nanokristal Tandan Kosong Kelapa Sawit -Komposit Filem Kitosan

ABSTRAK

Selulosa nanokristal (CNC) telah menarik banyak perhatian disebabkan sifatnya yang menarik. Tujuan kajian ini adalah untuk menilai sifat CNC-komposit filem kitosan dengan penambahan glutaraldehida (GA) sebagai agen sambung silang dalam keadaan pematangan yang berbeza. CNC telah berjaya diasingkan daripada tandan kosong kelapa sawit (EFB) dengan menggunakan hidrolisis asid sulfurik dan pra-perawatan pelarut eutektik alkali dan pelunturan. Parameter pemprosesan hidrolisis asid telah dioptimumkan dengan model Box-Behnken. Sebanyak 37.1% CNC telah dihasilkan menggunakan keadaan optimum kepekatan asid 60.0 wt% pada suhu 46.1°C selama 58.5 minit. Spektroskopi jelmaan Fourier inframerah (FTIR) menunjukkan penyingkiran benda asing yang tidak dikehendaki daripada EFB dengan pra-rawatan. CNC diameter purata dan panjang purata didapati adalah 6.78 ± 2.12 nm dan 160.06 ± 32.58 nm. Kehabluran serat EFB, selulosa yang dirawat dan CNC didapati adalah 38.7%, 51.2%, and 65.3% masing-masing. CNC menunjukkan kestabilan termal yang lebih rendah disebabkan kewujudan kumpulan sulfat pada permukaan CNC. Penambahan CNC dan GA sebagai agen sambung-silang dalam komposit filem CNC-kitosan telah dikaji. Pematut silangan komposit filem kitosan dilaksanakan dengan kaedah pematut silangan yang berbeza (pemanasan konvesional dan pematangan gelombang mikro). Kekuatan dan kekuatan modulus komposit filem CNC-kitosan telah ditingkatkan dengan penambahan CNC dan GA terutamanya sampel yang dimatang dengan gelombang mikro. Perubahan komposit filem CNC-kitosan dalam interaksi kimia telah dikaji dengan FTIR. GA terpaut silang CNC-kitosan filem komposit yang dimatang dengan gelombang mikro menunjukkan kestabilan termal yang lebih baik berbanding dengan komposit filem CNC-kitosan yang tidak terpaut silang dan GA terpaut silang komposit filem kitosan yang dipanaskan dengan kaedah konvesional. Hasil ini disebabkan oleh pembentukan struktur rangkaian yang stabil antara GA dan matriks kitosan. Tambahan pula, sifat-sifat berfungsi komposit filem seperti kebengkakan, kelarutan dan kebolehterapan wap air komposit filem telah dikurangkan dengan penambahan CNC dan GA. Walau bagaimanapun, keberkesanan pengurangan anti mikrob terhadap *Escherichia coli*, *Bacillus subtilis* dan *Saccharomyces cerevisiae* telah diperhatikan dalam komposit filem terpaut silang. Komposit filem CNC-kitosan terpaut silang didapati mempunyai kadar degradasi yang lebih rendah dibandingkan dengan komposit yang tidak terpaut silang. Bakteria pengurai yang dikenalpasti adalah *Enterobacter kobei* dan *E. roggenkampii* manakala kulat pengurai adalah *Ophiocordyceps heteropoda*.

Characterizations, Properties and Degradation of Empty Fruit Bunch Cellulose Nanocrystals-Chitosan Composite Film

ABSTRACT

Cellulose nanocrystal (CNC) has attracted significant attention due to its attractive properties. The aim of this research was to evaluate the properties of CNC/chitosan composite film with the addition of glutaraldehyde as crosslinker under different curing conditions. CNC has been successfully isolated from oil palm empty fruit bunch (EFB) using sulphuric acid hydrolysis preceded by alkaline deep eutectic solvent (DES) pre-treatment and bleaching. The processing parameters of acid hydrolysis were optimized using Box-Behnken Design. The results showed that the yield of CNC was 37.1%, under the optimal conditions of 60.0 wt% acid concentration at 46.1 °C for 58.5 minutes. Fourier transform infrared (FTIR) spectroscopy indicated the efficient elimination of unwanted impurities from the raw EFB fibers by pre-treatment. The average diameter and length of CNC were 6.78 ± 2.12 nm and 160.06 ± 32.58 nm, respectively. The raw EFB fibre, treated cellulose and CNC showed crystallinities of 38.7%, 51.2%, and 65.3%, respectively. The CNC had lower thermal stability, which was ascribed to the sulphate group present on the CNC surface. The addition of CNC and GA as crosslinker into chitosan composite films was studied. Crosslinking of chitosan composite films was carried out using different crosslinking methods (conventional heating and microwave curing). Tensile strength and modulus elasticity of chitosan composites were enhanced significantly by the addition of CNC and GA especially for microwave-cured samples. The changes in chemical interaction of the chitosan composite films were investigated by FTIR. The microwave-cured-GA-crosslinked chitosan composite films were more thermally-stable than non-crosslinked and conventionally-heated-GA-crosslinked chitosan composite films due to the formation of a more stable network structure between GA and chitosan matrix. Furthermore, the functional properties such as swelling, water solubility and water vapor permeability of composite films were reduced significantly by the addition of CNC and GA. However, the reduced antimicrobial efficacy of composite films against *Escherichia coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae* was observed in crosslinked composite films. The crosslinked chitosan composite films were also found to have a lower degradation rate than the non-crosslinked composites. The degrading-bacteria were identified as *Enterobacter kobei* and *E. roggenkampii* whereas the degrading-fungi was *Ophiocordyceps heteropoda*.

CHAPTER 1 : INTRODUCTION

1.1 Research Background

Plastics can be considered as one of the most versatile materials be known by human being. Plastic products have offered numerous benefits to the social and industry development owing to their ease of processing, low cost and excellent mechanical and barrier properties (Sangroniz et al., 2019). The annual worldwide plastic production has raised from approximately 1.5 million tons during the fifties to 348 million tons in 2017 (Ito et al., 2019). However, the synthetic plastics are highly durable and non-biodegradable. It can remain in the environment for years which resulted in severe environmental hazards. The tremendous amount of plastics consumption had raised the worldwide concern due to the potential health effect and environmental impacts arise from plastics pollution. The growing concerns on environmental devastation caused by the synthetic plastics has inspired the use of renewable resources for producing biodegradable and environmental-friendly films to replace the synthetic polymers.

Chitosan, a biopolymer which is obtained by N-deacetylation of chitin, is the second most abundant polysaccharide on nature after cellulose (Nechita, 2017). It has a similar chemical structure with cellulose. The development of chitosan-based film had been studied by many researchers. Its excellent properties such as film-forming capability, non-toxicity, biodegradability and anti-microbial activity have made it a potential candidate for the reduction of environmental and health problems often caused by synthetic polymers (Dehnad, Emam-Djomeh, Mirzaei, Jafari, & Dadashi, 2014). However, the high price as well as the brittleness of chitosan have restricted its

application (Kumar Gadghey & Sharma, 2017). Therefore, in order to overcome the limitations of chitosan, the reinforcement such as cellulose is required to enhance their properties and extend its applications.

Cellulose as one of the most abundant biomasses on the Earth, is an almost inexhaustible source of biopolymer for the production of environmental-friendly materials. Its biodegradability, renewability, low cost and low density make it an ideal environmentally benign bio-based materials for the reinforcement purpose (Mandal & Chakrabarty, 2014). The annual production of oil palm biomass in Malaysia is around 40 million tons. Among the oil palm biomass, empty fruit bunch (EFB) has occupied 280,000 tons of the biomass production (Fatah et al., 2014). However, only 10% from the total EFB production has been utilised and applied in agriculture sector as bio-fertiliser and in renewable energy generation while the remaining are wasted as agro-waste (Lani, Ngadi, Johari, & Jusoh, 2014). Utilisation of EFB as a cellulose source could resolve the problem of oil palm waste being left out or burned in the field and contribute to the economy by turning it into valuable products. Nevertheless, certain drawbacks such as high moisture absorption, tendency to form aggregates during processing, poor and mechanical properties had greatly reduced the potential of cellulose to be utilized as reinforcing fillers (Trache et al., 2016). New properties and functions are required for the next generation of cellulose based products and their engineering applications. Therefore, cellulose with at least one dimension in nanometre range which known as nanocellulose is developed.

Prior to the isolation of cellulose nanocrystals (CNC), pre-treatments are necessary to dissolve non-cellulosic components such as lignin and hemicellulose in order to increase the cellulose's accessibility for further treatments (Leite, Zanon, &

Menegalli, 2017). Recently, a new solvent which is known as deep eutectic solvent (DES) has appeared as an environmental-friendly alternative for the pre-treatment of biomass material. DES, also known as ionic liquids alternative, is a green solvent with similar physicochemical properties as ionic liquids (Das, Sharma, Mondal, & Prasad, 2016). DES is able to form the hydrogen bonds with lignin and hemicellulose by accepting and donating the protons, thus the solvation property can be improved. DES and ionic liquids share many native properties such as their biodegradability, good chemical and thermal stability, low toxicity, low vapor pressure and low flammability (E. L. Smith, Abbott, & Ryder, 2014). DES offers numerous favourable properties over the ionic liquid and conventional solvents yet overcome their disadvantages such as the ease of synthesis in which DES can be easily prepared from the mixtures of components and hydrogen bond donor compounds at moderate temperature and atmospheric pressure, good renewability, lower cost and higher sustainability (Vanda, Dai, Wilson, Verpoorte, & Choi, 2018). These properties propel DES to be one of the most promising for the production of sustainable biomaterial. A new type of alkaline DES, for example potassium carbonate (K_2CO_3) is used together with glycerol which acts as a hydrogen bond donor was found to have a high potential for different fields of applications. Naser et al. (2013) studied the properties of the combination of K_2CO_3 and glycerol as DES at different molar ratio. The results indicated that the DES with K_2CO_3 and glycerol at a molar ratio of 1:7 was the most suitable ratio for the application of chemical reaction and extraction at mid-range temperature (40°C and above). This research is a first attempt to explore the potential of K_2CO_3 -based DES as the pre-treatment for the isolation of CNC.

There are various methods for preparing CNC, with sulphuric acid hydrolysis being the most commonly used technique (Vasconcelos et al., 2017). Isolation of CNC

using sulphuric acid forms a negatively charged crystallite surface resulting from esterification by sulphate ions which lead to a more stable CNC dispersion. Isolation of CNC from renewable sources has earned the greatest interest lately due to its excellent mechanical properties (high specific strength and modulus), exceptional crystallinity, light weight and high aspect ratio compared to micro-sized cellulose (Flauzino Neto et al., 2016). The concept of using nanocellulose as reinforcement material in polymer matrix has been proven as the efficient approach for enhancing the tensile properties of biopolymers by forming the hydrogen bonding network between the filler and the matrix (Nair, Chen, Peng, Huang, & Yan, 2018).

Aside from adding reinforcing filler, the performance of chitosan can also be enhanced through crosslinking reaction. Crosslinking is an important procedure in the production of composite films as the properties such as mechanical strength are dependent on the crosslinking degree of composites (Falamarzpour, Behzad, & Zamani, 2017). The crosslinking reaction normally takes place between the functional group of crosslinker and the amine group of chitosan (Lusiana, Siswanta, & Mudasir, 2016). Among the crosslinkers, glutaraldehyde (GA) is the most common crosslinker used in chitosan matrix due to its high stability and proven ability in improving the tensile and thermal properties of chitosan films (Menegotto, Alan, Liliane, Pollo, & Cristina, 2018; Yeng, Husseinsyah, & Ting, 2015).

Most of the crosslinkers require heating for the crosslinking reaction to take place. The curing of film is generally performed by using conventional heating in which the thermal energy is directly transmitted to the film via conduction. Nonetheless, uneven crosslinking distribution could occur in which the surface of film has a higher temperature than the inside and result in the thermal gradient across the film which led to a non-

uniform stress transfer (Park, Choi, Choi, & Choi, 2017). Besides conventional thermal curing using hot air oven, microwave irradiation can also be employed as a potential alternative for the curing of composite films. Microwave heating involves the conversion of the electromagnetic energy to heat energy via the interaction between molecules with the electromagnetic field. It penetrates the film and generates heat in a volumetric manner (J. Sun, Wang, & Yue, 2016).

According to the literature, there are a number of studies reported regarding the enhancement of chitosan film properties using different crosslinkers and reinforcing fillers (Budianto, Muthoharoh, & Nizado, 2015; Kim, Nimni, Yang, & Han, 2005; V. Rubentheren et al., 2016; Yeng et al., 2015), however, to the best of our knowledge, there is no studies reported on the comparison of the influence of different crosslinking methods on the properties of CNC-chitosan composite films. Therefore, the aim of the present study is to fabricate the GA-crosslinked CNC-chitosan composite films with different CNC content using conventional heating and microwave curing methods. In this study, oil palm empty fruit bunch (EFB) is used as the raw material for the isolation of CNC through acid hydrolysis. The effect of different crosslinking methods on the morphological, tensile, thermal, barrier and biodegradation properties of GA-crosslinked CNC-chitosan composite films are investigated.

1.2 Problem Statement

The high oil palm production in Malaysia has led to a large amount of oil palm EFB. The large amount of EFB waste without economical value other than energy generation has been a major disposal problem. Besides, the disposal of EFB at plantation or designated site could resulted in the contamination of waterways and pollute the

environment as the oil residue in EFB could be leached by rain. EFB has a cellulose content around 40-50% which is relatively higher compared to other agriculture wastes. Therefore, the underutilisation of EFB, disposal problem and its high cellulose content make it an ideal source for the isolation of CNC. Increasing of public concerns regarding the environmental pollution by using non-biodegradable polymers throughout the world has led to the utilization of biodegradable and environment friendly polymer such as chitosan. Chitosan can be used as an alternative for the fabrication of biodegradable polymer composites to replace the synthetic polymers. However, chitosan is brittle and has poor mechanical properties. Therefore, CNC is introduced as the reinforcement filler to enhance its properties. Prior to the isolation of CNC, pre-treatment is needed to remove the unwanted impurities from the cellulose. Pre-treatment solvents such as deep eutectic solvent (DES) offers numerous favorable properties over ionic liquids and conventional solvents while overcoming their disadvantages such as high toxicity and low purity. The conventional pre-treatments such as sodium hydroxide pre-treatment could lead to the formation of salt and inhibitors during the pre-treatment. A large amount of water is required to wash the salts, which are incorporated into the cellulose and are cumbersome to remove. Although CNC and chitosan have similar molecular structures, direct addition of pristine CNC into chitosan matrix cannot produce composite film with excellent tensile properties. This is because the hydroxyl groups in CNC and chitosan have mostly formed intra- or intermolecular hydrogen bonds and difficult to develop new hydrogen bonds between two components by simple mixing which resulted in poor intermolecular interaction. Therefore, in order to overcome these problems, chemical crosslinking is needed to attain the desired compatibility. Crosslinking reaction can be induced by heat or microwave irradiation. Crosslinking reaction induced through conventional heating could lead to an uneven crosslinking distribution and cause a thermal gradient across the