ISOLATION AND CHARACTERIZATION OF CHITIN NANOWHISKERS FROM FERMENTED PRAWN WASTE AS FILLERS IN POLYLACTIC ACID COMPOSITES

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In the name of Allah, the Most Beneficent and the Most Merciful.

Dedicated to my beloved parents, Mohd Asri Bin Assan and Kamariah Binti Kadri; and my siblings Sofhian Sofhi, Suzian Yumi, Sujifarisya and Safaliza Azira,

for their endless support, love, concern, encouragement and continuous prayer for my success in completing this research.

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ABSTRACT

This study focuses on the application of chitin nanowhiskers (CHW) from different chitin sources to the development of CHW-reinforced poly(lactic acid) (PLA) nanocomposites. Chitin sources used in this study consisted of commercial chitin (CC), fermented chitin (FC) and treated fermented chitin (TFC) whereby FC and TFC were obtained from fermentation of prawn waste. PLA was chosen due to its advantages such as biodegradability and good mechanical strength. The CHWs produced by acid hydrolysis (CCHW, FCHW and TFCHW) were characterized using Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), x-ray diffraction (XRD) and thermogravimetric analysis (TGA). The FTIR analysis revealed that the acid hydrolysis process did not alter the chemical structure of chitin but changes its surface morphology. TEM images revealed that CHW particles obtained had rod-like shapes 10-20 nm in width and 300-500 nm in length. XRD analysis showed that CCHW has a higher crystallinity index (70%) compared to FCHW (46%) and TFCHW (68%). Thermal analysis indicated that CHW possess a good thermal stability. The effects of CHW on properties of PLA were investigated and the PLA/CHW nanocomposites were examined by FTIR, tensile test, TGA, and biodegradability and water absorption tests. Tensile strength for PLA/FCHW, PLA/TFCHW and PLA/CCHW increased with increasing filler content until it reached optimum value at 1 phr, 2 phr and 3 phr, respectively. Young's modulus for the nanocomposites increased with increasing filler content but elongation at break decreased significantly with increasing filler content for all types of nanocomposites. TGA results indicated that PLA/CHW nanocomposites displayed better thermal stability as compared to pure PLA. The biodegradability and water absorption of nanocomposites increased with increasing filler content.Polyethylene glycol (PEG) was used in view of improving ductility of PLA and PLA nanocomposites. The FTIR spectra of PLA/PEG and PLA/FCHW/PEG revealed that addition of PEG into PLA broaden the O-H peak significantly, indicating the formation of hydrogen bonds between PEG and PLA. PEG/PLA showed increment in tensile strength and elongation at break at optimum loading of 5 phr while Young's modulus decreased. Meanwhile, PLA/FCHW/PEG nanocomposites showed increment in tensile strength at an optimum loading of 1 phr while Young's modulus increased with increasing FCHW. The elongation at break decreased dramatically upon addition of FCHW.

ABSTRAK

Kajian ini memberi tumpuan kepada penggunaan nanowisker kitin (CHW) dari sumber kitin yang berbeza untuk membangunkan komposit poli(asid laktik) (PLA) bertetulang CHW. Sumber-sumber kitin yang digunakan dalam kajian ini terdiri daripada kitin komersial (CC) kitin ditapai (FC) dan kitin ditapai yang dirawat (TFC) di mana FC dan TFC diperoleh daripada penapaian sisa udang. PLA dipilih kerana kelebihannya misalnya kebolehan pereputan-bio dan kekuatan mekanikal yang baik. CHW yang dihasilkan dengan hidrolisis asid (CCHW, FCHW dan TFCHW) telah dicirikan menggunakan spektroskopi infra-merah transformasi Fourier (FTIR), mikroskopi elektron penghantaran (TEM), pembelauan sinar-x (XRD) dan analisis gravimetri terma (TGA). Analisis FTIR mendedahkan bahawa proses hidrolisis asid tidak mengubah struktur kimia kitin tetapi mengubah morfologi permukaannya. Imej TEM mendedahkan bahawa partikel CHW yang diperoleh mempunyai bentuk seperti rod dengan lebar 10-30 nm dan panjang 300-500 nm. Analisis XRD menunjukkan bahawa CCHW mempunyai indeks penghabluran yang lebih tinggi (70%) berbanding dengan FCHW (46%) dan TFCHW (68%). Analisis terma menunjukkan bahawa CHW mempunyai kestabilan terma yang baik. Kesan CHW terhadap sifat PLA telah disiasat dan komposit nano PLA/CHW telah diperiksa dengan FTIR, ujian regangan, TGA, dan ujian pereputan-bio dan penyerapan air. Kekuatan regangan bagi PLA/FCHW, PLA/TFCHW dan PLA/CCHW meningkat dengan peningkatan kandungan pengisi sehingga mencapai nilai optimum masing-masing pada 1 phr, 2 phr dan 3 phr. Modulus Young bagi komposit nano meningkat dengan peningkatan kandungan pengisi tetapi ciri pemanjangan takat putus menurun dengan ketara dengan peningkatan kandungan pengisi bagi semua jenis komposit nano. Keputusan TGA menunjukkan bahawa komposit nano PLA/CHW memaparkan kestabilan terma yang lebih baik berbanding dengan PLA tulen. Kadar pereputan-bio dan penyerapan air komposit nano meningkat dengan peningkatan kandungan pengisi. Polietilena glikol (PEG) telah digunakan untuk meningkatkan kemuluran PLA dan komposit nano PLA. Spektrum FTIR PLA/PEG dan PLA/FCHW/PEG mendedahkan bahawa penambahan PEG ke dalam PLA menambah luas puncak O-H dengan ketara, menunjukkan pembentukan ikatan hidrogen antara PEG dan PLA. PLA/PEG menunjukkan kenaikan kekuatan regangan dan pemanjangan takat putus pada muatan optimum 5 phr manakala modulus Young pula berkurangan. Sementara itu, PLA/FCHW/PEG menunjukkan kenaikan kekuatan regangan pada pemuatan optimum 1 phr manakala modulus Young meningkat dengan pertambahan FCHW. Pemanjangan pada takat putus berkurangan dengan ketara apabila FCHW bertambah.

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LIST OF ABBREVIATIONS

AFM	-	Atomic force microscopy
AMIMBr	-	1-allyl-3-methylimidazolium bromide
CC	-	Commercial chitin
CCHW	-	Commercial chitin nanowhiskers
CHW	-	Chitin nanowhiskers
CNF	-	Chitin naofibers
CNW	-	Cellulose nanowhiskers
DC	-	Decolourization
DD	-	Deacetylation
DM	-	Demineralisation
DMAc	-	*N,N*-dimethylacetamide
DP	-	Deproteinisation
ENR	-	Epoxidized natural rubber
EU	-	European Union
FC	-	Fermented chitin
FCHW	-	Fermented chitin nanowhiskers
FESEM	-	Field emission scanning electron microscopy
FPP	-	Flexible plastic packaging
HCl	-	Hydrochloric acid
HDPE	-	High density polyethylene
KOH	-	Potassium hydroxide
LAB	-	Lactic acid bacteria
LDPE	-	Low density polyethylene
LiCl	-	Lithium chloride
LP	-	Laminated polystyrene
MCC	-	Microcrystalline cellulose

MMT	-	Montmorillonite
$NaBH_4$	-	Sodium borohydride
NaClO	-	Sodium hypochlorite
NaOH	-	Sodium hydroxide
PDLA	-	Poly (D-lactide)
PE	-	Polyethylene
PEG	-	Polyethylene glycol
PETE	-	Polyethylene terephthalate
PHBV	-	Polyhydroxybutyrate-co-valerate
phr	-	Part per hundred
PLA	-	Polylactic acid
PLLA	-	Poly (L-lactide)
POM	-	Poly oxymethylene
PP	-	Polypropylene
PS	-	Polystyrene
PVA	-	Polyvinyl alcohol
PVC	-	Polyvinyl chloride
RPP	-	Rigid plastic packaging
SEM	-	Scanning electron microscopy
SSQ	-	Silsesquioxane-urethaneacrylate
TEM	-	Transmission electron microscopy
TFC	-	Treated fermented chitin
TFCHW	-	Treated fermented chitin nanowhiskers
TiO_2	-	Titanium dioxide
TGA	-	Thermogravimetric analysis
TOChN	-	TEMPO-oxidized α-chitin nanowhiskers
WF	-	Wood flour

LIST OF SYMBOLS

α	-	Alpha
β	-	Beta
γ	-	Gamma
g	-	Gram
µm	-	Micrometer
cm	-	Centimeter
mm	-	Millimeter
ml	-	Milliliter
θ	-	Diffraction angle (°)

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Synthetic polymers have become an essential and ubiquitous part of our lives, having wide applications in various fields including in agriculture, packaging and consumer products industry and in many medical applications (Luckachan and Pillai, 2011). Such widespread use confirms the success and the immense benefit of synthetic polymers. Although the present society acknowledges the uniqueness and advantages of using synthetic polymers, their setbacks are obvious. The lack of biodegradability of many synthetic polymeric materials has been showing its polluting effects on the environment. Therefore the need to continually focus on research and development in order to fulfil increasing stringent future requirements is of great priority.

Interests on biopolymers which are biodegradable and are synthesized from renewable resources are becoming more prominent among the academicians and researchers globally as indicated by the expanding literatures (Yates and Barlow, 2013; Gandini and Lacerda, 2015; Pretula *et al.*, 2016). The market share of biopolymers has increased in recent years in the plastic industry due to the environmental concerns and also the consciousness that petroleum resources are becoming limited (Nampoothiri *et al.*, 2010). In general, biopolymers from renewable resources can be classified into three categories; i) natural polymers (such as starch, protein, chitin and cellulose), ii) synthetic polymers from natural monomers such as polylactic acid (PLA) and iii) polymers from microbial fermentation such as polyhydroxybutyrate (PHB). The most promising bioploymer with bright prospect is PLA which is aliphatic polyester. It is currently considered as green eco-friendly material due to its biodegradable properties. In addition PLA also fulfills many requirements as a packaging thermoplastic material for general packaging applications (Nampoothiri *et al*., 2010).

To improve the properties of polymers such as mechanical and thermal, blending and modifications through incorporation of additives such as fillers were widely used. Natural fillers such as cellulose and chitin are becoming more important because of many advantages such as biodegradability compared to mineral fillers. Interestingly fillers in nano sizes known as nanofillers have received significant attention because they can improve the mechanical, thermal, optical, physical properties and fire retardancy of polymeric materials at low filler contents (Hong and Kim, 2013). One such example is cellulose-based nanofibers which have been used as reinforcing fillers in polymer nanocomposites (Azizi Samir *et al.,* 2005; Eichhorn *et al.,* 2010; Haafiz *et al.,* 2013; Arjmandi *et al.*, 2016).

Likewise, chitin nanowhiskers (CHW) has also shown to have high modulus and can therefore be used as nanofillers in reinforcing polymer nanocomposites. Chitin is one of the most abundant natural polysaccharides that exist in nature and is found in the outer skeleton of crustaceans such as shrimp, lobster, crab and in squid pens. Chitin and its derivatives chitosan are known to possess many desirable properties such as biocompatible, antibacterial and is an environmentally friendly polyelectrolyte (Struszczyk, 2006). Their industrial applications seem scarce which is largely due to high prices. Thus their applications are mostly used as speciality materials in medical applications. However the recent development in using cellulose nanowhiskers to improve the properties of nanocomposites has sparked new interest in exploiting CHW since both cellulose and chitin share similar glucopyranose backbone structure. Chitin differs from cellulose only at carbon-2 in the glucose unit where chitin has an acetylamino group instead of hydroxyl group.

Natural polymers such as chitin, starch and cellulose consist of both crystalline and amorphous regions. Chitin is found to have desirable mechanical properties due to their natural stacks of chitin nanocrystals or nanowhiskers. Various methods have been employed in production of CHW. One method that are been widely used is acid hydrolysis (Nair and Dufresne, 2003). The crystalline region in nanoscale size once isolated can be used as reinforcing nanofillers in polymer nanocomposites (Zeng *et al.*, 2012).

1.2 Problem Statement

Prawn waste is widely produced from prawn processing industries around Malaysia and its disposal is becoming an issue due to its high perishability. Thus its proper disposal and putting it to good use is becoming more urgent. In recent years, a biotechnological approach using lactic acid fermentation to purify chitin from crustacean waste is gaining popularity due to its environmentally clean approach and cheaper production cost. The other advantage of lactic acid fermentation approach as compared to chemical method is the ability to recover protein. Therefore, this approach has been used by several researchers to produce two industrially important products namely a protein liquid fraction and chitin solid fraction (Zakaria *et al.*, 1998; Nor e*t al.*, 2011). The protein fraction has successfully been converted into aquaculture feed (Nor e*t al.*, 2011). Finding good use of the chitin fraction produced through fermentation method will benefit prawn processing industries which discard about 50 % of the whole prawn as waste material (Zakaria *et al.*, 1998).

The study on the use of PLA as packaging materials for dairy, bakery and fresh food products has been reported. However several disadvantages such as high production cost and poor thermal stability limits its application in wider range of fields. The addition of fillers into PLA will reduce the cost and is expected to improve certain properties such as thermal stability, mechanical and biodegradability.

One of the natural fillers that is widely used to reinforce PLA is cellulose nanowhiskers (Haafiz *et al.*, 2013; Arjmandi *et al.*, 2016). Haafiz *et al*. (2013)

reported that the incorporation of cellulose nanowhiskers (CNW) into PLA by solution casting method resulted in an increase in strength and modulus. In addition they also observed an increase in thermal stability and biodegradability of the PLA.

To date, while CNW/PLA nanocomposites have been widely reported (Haafiz *et al.*, 2013; Arjmandi *et al.*, 2016), the use of CHW as fillers in PLA is very much less reported. Rizvi *et al*. (2011) in their study on effect of adding CHW in PLA reported that the addition of CHW was found to decrease the thermal stability and tensile strength but increased the Young's modulus (Rizvi *et al.*, 2011). They reasoned for the reduced properties were due to the usage of gel-like suspension of CHW in melt blending process in which the composites may have higher chances of being hydrolysed during the high melt process temperature which affects the said properties. It would therefore be interesting to investigate the effect of CHW on mechanical properties of PLA nanocomposites if solution casting method is used and how it also affects thermal and biodegradability of PLA.

In this study, CHW from chitin obtained through fermentation of prawn waste is envisaged to be a potential material for PLA reinforcement due to its high Young's modulus (Nishino *et al*., 2006). In addition chitin obtained through fermentation method undergone the extraction of chitin process from prawn waste in a milder condition such as using low temperature and less chemicals since it is biologically processed, in contrast to commercial chitin which uses high temperatures and high volumes of acid and base. Due to the milder conditions, fermented chitin obtained is high likely to maintain its molecular structure. In view of this, it is interesting to evaluate the effect of using CHW from fermented chitin as fillers in PLA nanocomposites since the aspect ratio of fillers play an important role in determining the success of nanofillers (Siqueira *et al.*, 2010). To date, studies on CHW as fillers in PLA using CHW from fermented chitin has not been reported. Another added advantage is the utilisation of CHW from fermented chitin helps to reduce cost as it is a by-product of a process in which its main objective is to obtain protein for animal feed.

Although PLA has inherent good mechanical strength and modulus, the use of CHW is expected to improve further the mechanical strength and modulus of PLA but most likely at the expense of reducing ductility PLA nanocomposites. Many studies have reported that the brittleness of PLA has been overcome with the use of plasticisers such as PEG (Jacobsen and Fritz, 1999; Baiardo *et al.*, 2003; Chieng *et al.*, 2013) and glycerol (Petchwattana *et al.*, 2017). More extensive studies on the use of PEG in PLA nanocomposites have been carried out and the results have shown the effectiveness of PEG in enhancing the ductility of the composites (Paul *et al*., 2003; Pluta *et al*., 2006). Therefore, PEG will be used in this study to enhance the ductility of PLA/CHW nanocomposites to obtain more balanced properties.

1.3 Objectives of the Study

The objectives of this study are:

1) To produce and characterize CHW from different sources of chitin and investigate their potential as nanofillers in PLA nanocomposite.
2) To study the effect of sources of chitin and CHW content on mechanical, physical and thermal properties of CHW reinforced PLA nanocomposites.
3) To determine the effect of polyethylene glycol (PEG) content on mechanical, physical and thermal properties of CHW reinforced PLA nanocomposites.

1.4 Scope of Study

In order to achieve the above mentioned objectives, the study is focused on the following scope:

1) Three types of chitin were used; i) fermented chitin, ii) treated fermented chitin and iii) commercial chitin. Fermented chitin was prepared through treatment of prawn waste using a commercial starter culture EM.

2) CHW were produced through acid hydrolysis and characterized using the followings:

    a. Fourier Transform Infrared spectroscopy (FTIR)
    b. Scanning electron microscopy (SEM)
    c. Atomic force microscopy (AFM)
    d. Transmission electron microscopy (TEM)
    e. Thermogravimetric analysis (TGA)
    f. X-ray diffraction (XRD)

3) PLA/CHW and PLA/CHW/PEG nanocomposites prepared through solution casting were characterized for mechanical, physical and thermal properties.

1.5 Significance of Study

The use of CHW from fermented chitin as a reinforcement for PLA will benefit mankind since the production of chitin is through an environmentally friendly biological method. In addition the use of friendly microorganism during the fermentation method not only benefits in producing nutritional protein, it also produces chemically preserved chitin molecules due to the milder treatment conditions. No hazardous chemical waste is produced in this process as the liquid protein fraction is used as it is in aquaculture feed. The development of fermented CHW reinforced PLA composites with balanced mechanical, thermal and biodegradability properties will be useful as environmentally friendly packaging materials.

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