ISOLATION AND CHARACTERIZATION OF LIGNOCELLULOSIC FIBRES FROM GIANT PANDA WASTE

by

GOH CHIU HUI

A report submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Natural Resources Science) with Honours

FACULTY OF EARTH SCIENCE

UNIVERSITI MALAYSIA KELANTAN

2017

DECLARATION

I declare that this thesis entitled “Isolation and Characterization of Lignocellulosic Fibres from Giant Panda Waste ” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : _____

Name : _____

Date : _____

ACKNOWLEDGEMENT

This project would not have been possible without the kind support and help of many individuals and organizations. I would like to extend my sincere gratitude to all of them.

First and foremost, I would like to use this opportunity to express my gratitude to my supervisor, Dr. Kamarul Ariffin B. Kambali @ Hambali. I would like to thank also my co-supervisor, Mrs. Nurul'ain Bt Mohd Kamal, for her invaluable comments, sharing of knowledge and advices in conducting my project throughout this period. Her guidance helped me in all the time of research and writing of this thesis.

My thanks goes as well as to all my lecturers and friends for their help, knowledge shared and support as it has been the core of my success in completing my project. Last but not least, my deepest appreciation and thanks to my family members for their continuous support. They have been the source of inspiration through their encouragement, financial support, help, patience and care during this project.

ISOLATION AND CHARACTERIZATION OF LIGNOCELLULOSIC FIBRES FROM GIANT PANDA WASTE

ABSTRACT

Giant Panda waste was used as a source for isolation of lignocellulosic fibres and its subsequent characterization was done. Non-cellulosic components such as lignin, hemicellulose, and pectin were removed from the fibres by subsequently washing, sodium hydroxide (NaOH) and sodium hypochlorite (NaOCl) treatment. Fourier transform infrared (FTIR) spectroscopy analysis showed that there were no significant variations in peak positions, signifying that the treatments did not affect the chemical structure of the fibres. The difference in peak intensity in treated fibres compared to untreated fibres indicated that hemicellulose and impurities on the fibre surfaces are partly removed by NaOH and NaOCl treatment. X-ray diffraction (XRD) analysis revealed that the natural structure of cellulose I polymorph was maintained irrespective of the treatment. X-ray analyses also showed that the treatments altered the crystallinity of the fibres where the bleached fibres had a higher crystallinity (28.62%) than that of untreated fibes (27.60%). A comparison of the curve of thermogravimetric (TGA) analysis and differential scanning calorimetry (DSC) for the treated and untreated samples is presented to demonstrate that the presence of treatment contributes to a better thermal stability for lignocellulosic fibres.

Keywords: Giant Panda waste, lignocellulosic fibres, NaOH, NaOCl, treated, untreated, FTIR, XRD, TGA, DSC

PENGASINGAN DAN PENCIRIAN GENTIAN LIGNOSELULOSA DARI SISA GIANT PANDA

ABSTRAK

Sisa Giant Panda telah digunakan sebagai sumber untuk pengasingan gentian lignoselulosa dan pencirian telah dilakukan. Komponen bukan selulosa seperti lignin, hemiselulosa dan pektin telah dikeluarkan daripada gentian dengan cara mencuci, rawatan NaOH dan rawatan NaOCl. FTIR analisis menunjukkan bahawa tidak ada perbezaan ketara dalam kedudukan puncak gentian, menandakan bahawa rawatan tidak menjejaskan struktur kimia gentian. Perbezaan dalam keamatan puncak dalam gentian dirawat berbanding gentian tidak dirawat menunjukkan bahawa hemiselulosa dan kekotoran pada permukaan gentian telah diasingkan sebahagiannya oleh rawatan NaOH dan NaOCl. XRD analisis menunjukkan bahawa struktur semula jadi selulosa I polimorf dikekalkan tanpa mengira jenis rawatan yang dijalankan. X-ray analisis juga menunjukkan bahawa rawatan mengubah penghabluran gentian di mana gentian terluntur mempunyai penghabluran yang lebih tinggi (28.62%) berbanding dengan gentian tidak dirawat (27.60%). Perbandingan lengkung TGA analisis dan DSC bagi sampel yang dirawat dan tidak dirawat dibentangkan untuk menunjukkan bahawa kehadiran rawatan menyumbang kepada kestabilan haba yang lebih baik bagi gentian lignoselulosa.

Kata Kunci: Sisa Giant Panda, gentian lignoselulosa, NaOH, NaOCl, dirawat, tidak dirawat, FTIR, XRD, TGA, DSC

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CrI	Crystallinity Index
Cu	Copper
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared Spectroscopy
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide
rRNA	Ribosomal Ribonucleic Acid
SNP	Single Nucleotide Polymorphisms
TGA	Thermogravimetric Analysis
XRD	X-ray Diffraction

LIST OF SYMBOLS

kg	kilogram
cm	centimeter
%	percentage
m	meter
μm	micrometer
°	degree
~	approximately
±	plus minus
mm	millimeter
MPa	megapascal
<	less than
bp	base pairs
°C	degree Celsius
g	gram
L	liter
min	minute
ml	milliliter
=	equal to
α	alpha
β	beta
θ	theta

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In recent years, there has been a growing interest for the use of renewable materials such as plant fibres, also known as ‘‘lignocellulosic fibres’’, due to the unique criteria of these fibres. These include abundant availability, renewability, biodegradability, as well as reduced weight, increased flexibility, greater moldability, reduced cost and sound insulation (Merkel *et al.*, 2014). Moreover, increasing environmental concerns have initiated a considerable interest in natural materials to produce “green” products. The rapidly rising environmental awareness, growing global waste problem, limited availability of crude oils, and high processing cost trigger the development concepts of sustainability and reconsideration of renewable resources (Nahar *et al.*, 2012).

Bamboo has been receiving considerable attention as a substitute for wood and other fibres, because its mechanical properties are very similar. The application of plant fibres as reinforcement allows the acquisition of composite products, which presenting smaller apparent specific mass and higher porosity, satisfactory values of traction and impact resistance, bigger control of cracking, besides ductile behavior during break (Junior *et al.*, 2014).

In this research, Giant Panda waste was used as the sample material for the isolation of lignocellulosic fibres. The primary goal of this research is to isolate lignocellulosic fibres that present in Giant Panda waste. In addition to this, the main objective is to study the effect of different treatments towards lignocellulosic fibres

extracted from Giant Panda waste. This work studied and characterized untreated lignocellulosic fibres sample washed with water, sample treated with sodium hydroxide (NaOH) and bleached sample treated with sodium hydroxide (NaOH) and also sodium hypochlorite (NaOCl).

The moisture content of lignocellulosic fibres submitted to different treatments was determined. Thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) were also employed to examine the surface, thermal and chemical properties of the lignocellulosic fibres isolated from Giant Panda waste.

1.2 Problem Statement

Giant Panda consume 15 to 30 kg of food per day (Viswanathan, 2010). They spend 10 to 16 hours daily consuming remarkable quantity of bamboo. Giant Panda have a simple stomach, a short, straight colon only 10 cm in length and a small gall bladder (Tarou *et al.*, 2005). Because of this type of simple digestive system, Giant Panda are only able to digest approximately 12-23% of the bamboo they consume (Tarou *et al.*, 2005). In relation to this, Giant Panda excrete up to 40 kg of waste each day (Viswanathan, 2010). Giant Panda diets consist mainly of bamboo and their faeces are mainly composed of undigested bamboo fragments since Giant Panda gut bacteria cannot efficiently digest bamboo. The Giant Panda gut did not harbour plant-degrading bacteria such as *Ruminococcaceae* and *Bacteroides* that are typically enriched in other herbivores (Xue *et al.*, 2015).

Giant Panda generate high volume of waste. However, not much research and utilization has been done on Giant Panda waste. Recently, studies on the utilization of lignocellulosic fibres have attracted the attention of scientists and technologies worldwide. They have become the focus of intense interest because of their tremendous advantages such as low density, biodegradability and excellent mechanical properties (Nahar *et al.*, 2012). Hence, this research aims to isolate lignocellulosic fibres from Giant Panda waste and to study the effect of treatment towards lignocellulosic fibres extracted from Giant Panda waste.

1.3 Objectives

1) To isolate lignocellulosic fibres from Giant Panda waste.

2) To study the effect of treatment towards lignocellulosic fibres extracted from Giant Panda waste.

1.4 Significance of Study

The main purpose of this research is to isolate lignocellulosic fibres from Giant Panda waste. Giant Panda spend up to 14 hours each day consuming about 15 to 30 kg of bamboo but only digest about 20% of what they eat (Viswanathan, 2010). Consequently Giant Panda defecate about 50 times or up to 40 kg of waste per day (Viswanathan, 2010). Giant Panda consumed almost exclusively of bamboo as their staple food, up to 95 to 99% of their diet (Julie *et al.*, 2007), contributing to its poor nutritional value and low dry matter digestibility due to low protein and high fibre and lignin contents of the diet. Giant Panda generate high volume of waste and their

faeces are mainly composed of undigested bamboo fragments since Giant Panda gut bacteria cannot efficiently digest bamboo (Xue *et al.*, 2015).

Giant Panda waste is thus a potential and valuable source of bamboo fibres or lignocellulosic fibres, that are biodegradable, sustainable and renewable (Roslan *et al.*, 2015). Thus, this research is essential to utilize the use of Giant Panda waste as a primary source of lignocellulosic fibres as well as to study the effect of treatment towards the lignocellulosic fibres extracted.

CHAPTER 2

LITERATURE REVIEW

2.1 Natural Fibres (Lignocellulosic Fibres)

Natural fibres or lignocellulosic fibres are one of the main renewable resource materials in the world. There are about 2000 species of useful fibre plants in all parts of the world that are applied widely. Now industrialists utilize the locally grown fibres as alternative for expensive synthetic fibres. Natural fibres basically composed of cellulose, lignin and hemicelluloses (Chirayil *et al.*, 2014). Pectin, pigments and extractives can be found in lower quantities. Thus, natural fibres are also called cellulosic fibres.

Natural fibres can also referred as cellulosic fibres since cellulose is their main chemical component, or they can be called lignocellulosic fibres when one takes into account that most fibres contain lignin, a natural polyphenolic polymer (Pukansky, 2005). The range of uses of natural fibres is very large, encompassing classical applications in the textile industry, as reinforcements in thermoplastic and thermoset polymer matrices and, more recently, as absorbent materials for heavy metals used in the treatment of industrial wastes, among other applications.

2.1.1 Fibre Source

The plants, which produce natural fibres, are classified as primary and secondary depending on their utilization. Primary plants are those grown for their fibre content while secondary plants are plants in which the fibres are produced as a

by-product. Jute, hemp, kenaf, and sisal are examples of primary plants. Pineapple, oil palm and coir are examples of secondary plants (Faruk *et al.*, 2012).

2.1.2 Fibre Type

There are six basic types of natural fibres. They are classified as bast fibres (jute, flax, hemp, ramie and kenaf), leaf fibres (abaca, sisal and pineapple), seed fibres (coir, cotton and kapok), core fibres (kenaf, hemp and jute), grass and reed fibres (wheat, corn and rice) and all other types (wood and roots) (Faruk *et al.*, 2012).

2.1.2.1 Coir (Coconut Fibre)

Coir husk fibres are located between the husk and the outer shell of the coconut. The fibres are strong, light and easily withstand heat and salt water. Coir is an abundant, versatile, renewable, cheap, and biodegradable lignocellulosic fibre used for making a wide variety of products. Coir has also been tested as filler or reinforcement in different composite materials. Furthermore, it represents an additional agro-industrial non food feedstock (agro industrial and food industry waste) that should be considered as feedstock for the formulation of eco-compatible composite materials. Coconut coir is the most interesting products as it has the lowest thermal conductivity and bulk density (Sen & Reddy, 2011). The addition of coconut coir reduced the thermal conductivity of the composite specimens and yielded a lightweight product. Development of composite materials for buildings using natural fibre as coconut coir with low thermal conductivity is an interesting

alternative which would solve environment and energy concern (Sen & Reddy, 2011). Coir fibres are more efficient and superior in reinforcement performance when compared to other reinforcement composites. However, the main limitations of coir fibres are high moisture content (Bongarde & Shinde, 2014).

2.1.2.2 Sisal

Sisal is an agave, where the sisal fibre is a "hard" fibre extracted from fresh leaves of sisal plant *Agave sisalana*. The length of the sisal fibre varies between 0.6 and 1.5 m and its diameters range from 100 to 300 μm (Smole *et al.*, 2013). Cellulose content in sisal fibres is about 70% (Smole *et al.*, 2013). Sisal fibre is a kind of natural fibre which possesses high specific strength and modulus, low price, recyclability and high availability. Using sisal fibre as reinforcement to make sisal fibre reinforced polymer composites has aroused great interest of materials scientists and engineers all over the world. The tensile properties of sisal fibres are not uniform along its length. The fibres extracted from the root or lower parts of the leaf have a lower tensile strength and modulus. The fibres become stronger and stiffer at midspan, and the fibres extracted from the tip have moderate properties. The lower grade fibre is processed by the paper industry because of its high content of cellulose and hemicelluloses. The medium grade fibre is used in the cordage industry for making ropes, baler and binders twine. The higher-grade fibre after treatment is converted into yarns and used by the carpet industry (Smole *et al.*, 2013).

Table 2.1: Composition of a few natural fibres (Sen & Reddy, 2011)

Fibres	Microfibril angle ($^{\circ}$)	Cellulose (%)	Lignin (%)
Coir	30-49	43	45
Sisal	20-25	70	12
Bamboo	2-10	60.8	32.2

Table 2.2: Properties of a few natural fibres (Sen & Reddy, 2011)

Fibers	Density (g/cm^3)	Tensile Strength (N/mm^2)	Modulus of Elasticity (GPa)	Moist Absorption (%)
Coir	1177	95-118	8	93
Sisal	1370	347-378	15	110
Bamboo	1158	73-505	10-40	145

Coir fibres and sisal fibres presented low mechanical strength and low modulus of elasticity as compared to bamboo fibres. This has been explained due to the low cellulose content and high microfibrillar angle. From Table 2.1, it can be observed that bamboo has 60% cellulose and with a considerably higher percentage of lignin (~32%), its micro-fibrillar angle is relatively small (2°-10°). These facts about bamboo support its high tensile strength as shown in Table 2.2, the extremely high flexural strength, and rigidity of the fibre's polylamellate wall structure (Liu *et al.*, 2012). Since it is lightweight, durable, hardy, flexible, strong and renewable, treated bamboo has been widely used in construction, reinforcement and composite materials (Fu *et al.*, 2012). Specifically, the reason of the study to use bamboo fibres is because they have low density and high mechanical strength. The reason to use

Giant Panda waste for isolation of lignocellulosic fibres is that Giant Panda helps to facilitate the process as we do not have to carry out pre-processing of bamboo.

2.2 Bamboo

Bamboo (*Bambusa* Schreb.) is a perennial, giant, woody grass belonging to the group angiosperms and the order monocotyledon (Bahari & Krause, 2015). The grass family Poaceae (or Gramineae) can be divided into one small subfamily, Centothecoideae, and five large subfamilies, Arundinoideae, Pooideae, Chloridodeae, Panicoideae, and Bambusoideae. In distinction to its name, bamboos are classified under the subfamily Bambusoideae (Bahari & Krause, 2015). According to Anokye *et al.* (2016) there are about 60 to 70 genera and over 1200 – 1500 species of bamboo in the world. Giant Panda utilize over 60 species of bamboo, but 35 species comprise their main food source (Hu & Wei, 2004).

Bamboo is constituted primarily of structural carbohydrates (cellulose, hemicellulose, and lignin). Bamboo consisted 26-43% cellulose, 21-31% lignin and 15-26% hemicellulose (Mwaikambo, 2006). This highly indigestible structure poses a challenge or difficulty to Giant Panda, because they have limited ability to digest structural carbohydrates. A slower passage rate could limit intake but allow for better utilization of nutrients in bamboo. However, the passage rate of Giant Panda is very rapid within 5 to 11 hours (Julie *et al.*, 2007), with complete clearance of markers in less than 12 hours.

2.2.1 General Structure of Bamboo

Bamboo is divided into 2 major portions, the rhizomes and the culms. The rhizome is the underground part of the stem and is mostly sympodial or, to a much lesser degree, monopodial. The culm is the upper ground portion of the stem. It is the portion of the bamboo tree that contains most of the woody material. Most of bamboo culms are cylindrical and hollow, with diameters ranging from 0.635 to 30.48 cm, and height ranging from 30.48 to 3657.6 cm (Lipp-Symonowicz *et al.*, 2011). It is without any bark and has a hard smooth outer skin due to the presence of silica (Lipp-Symonowicz *et al.*, 2011). The culm is complimented by a branching system, sheath, foliage leaves, flowering, fruits and seedlings. Bamboo is distinguishable from one another by the differences of these basic features, along with the growth style of the culm, which is either strictly erect, erect with pendulous tips, ascending, arched or clambering (Lipp-Symonowicz *et al.*, 2011).

2.2.2 Chemical Composition of Bamboo

Bamboo belongs cellulose I crystalline structure, like that of cotton and ramie (Nayak & Mishra, 2016). The main chemical composition of bamboo is similar to that of wood, mainly cellulose (±55%), hemicellulose (±20%) and lignin (±25%), which accounting for over 90% of the total mass (Anokye *et al.*, 2016). The other constituents are protein, fat, pectin, tannins, pigments and ash. Compared with wood, however, bamboo has higher alkaline extractives, ash and silica contents (Anokye *et al.*, 2016). Bamboo contains other organic components in addition to cellulose and lignin. It contains about 2-6% starch, 2% deoxidized saccharide, 2-4% fat, and 0.8-6% protein (Rathod *et al.*, 2011). The chemical components are distributed throughout

the cell wall which is composed of primary and secondary wall layers. Chemical composition varies from plant to plant, and within different parts of the same plant. Chemical composition also varies within plants from different geographic locations, ages, climate, and soil conditions (Anokye *et al.*, 2016).

In general, the α-cellulose content in bamboo is 40-50%, which is comparable with the reported α-cellulose contents of softwoods (40-52%) and hardwoods (38-56%) (Nayak & Mishra, 2016). Cellulose contents in this range make bamboo a suitable raw material for the pulp and paper industry. The lignin content (20-26%) places bamboo at the high end of the normal range (11-27%) reported for non-woody biomass (Nayak & Mishra, 2016) and close to the ranges for North American softwoods (24-37%) and hardwoods (17-30%). The high lignin content of bamboo contributes to its high heating value and its structural rigidity with the latter making it a valuable building material (Li *et al.*, 2007).

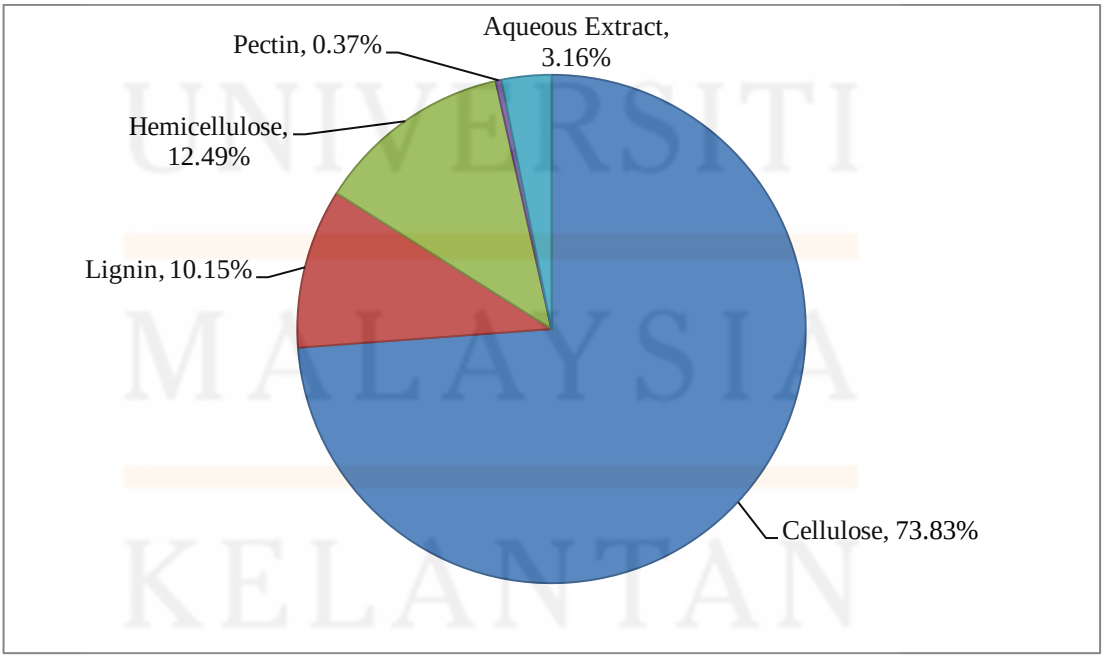


Figure 2.1: Chemical constituents of bamboo in percentage (Khalil *et al.*, 2012)

2.2.2.1 Cellulose

Cellulose makes up about 45% of the total wood dry weight. This lineal polymer is composed of D-glucose subunits linked by β-1,4 glycosidic bonds forming cellobiose molecules. Long chains called elemental fibrils are formed and linked together by hydrogen bonds and van der Waals forces. Hemicellulose and lignin cover microfibrils which are formed by elemental fibrils. The orientation of microfibrils is different in the different wall levels. Microfibrils are grouped together to constitute the cellulose fibre. Cellulose can appear in crystalline form, called crystalline cellulose. In addition, there are a small percentage of non-organized cellulose chains, which form amorphous cellulose. In this conformation, cellulose is more susceptible to enzymatic degradation (Chen, 2014).

2.2.2.2 Hemicellulose

Hemicellulose is a complex carbohydrate polymer and makes up 25–30% of the dry weight of wood. It is a polysaccharide with a lower molecular weight than cellulose. It consists of D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acids. Sugars are linked together by β-1,4- and occasionally β-1,3-glycosidic bonds (Pandey *et al.*, 2011). The major difference with cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. In contrast to cellulose, they are easily hydrolyzable polymers. They do not form aggregates, even when they are co-crystallized with cellulose chains.

2.2.2.3 Lignin

Lignin (along with cellulose) is the most abundant polymer in nature. It is present in the cellular cell wall, conferring structural support, impermeability, and resistance against microbial attack and oxidative stress. Structurally, lignin is an amorphous heteropolymer, non-water soluble and optically inactive. It consists of phenylpropane units joined together by different types of linkages. The polymer is synthesized by the generation of free radicals, which are released in the peroxidase-mediated dehydrogenation of three phenyl propionic alcohols: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p-hydroxyphenylpropanol), and sinapyl alcohol (syringyl propanol) (Welker *et al.*, 2015). Coniferyl alcohol is the principal component of softwood lignins, whereas guaiacyl and syringyl alcohols are the main constituents of hardwood lignins. The final result of this polymerization is a heterogeneous structure whose basic units are linked by C-C and aryl-ether linkages, with aryl-glycerol β-arylether being the predominant structure (Pandey *et al.*, 2011).

2.2.3 Uses of Bamboo Fibres

Bamboo is a natural fibre traditionally used to create various living facilities and tools. Their length varies from 1 to 5 mm (with an average of 2.8 mm) and the diameter 14-27 μm (average of 20 μm) (Lipp-Symonowicz *et al.*, 2011). The high strength with respect to its weight derives from fibres longitudinally aligned in its body meanwhile this is also attributed by its polylamelate wall structure that consists of alternating broad and narrow layers with different fibrillary orientation as shown in Figure 2.2 (Khalil *et al.*, 2012). Other than that, bamboo has 60% cellulose with high content of lignin and relatively small micro-fibril angle of 2–10° contribute to

this high strength of bamboo fibre (Khalil *et al.*, 2012). Bamboo fibres has a specialty of high strength and low density making it at par with glass in term of specific strength if compared to other natural fibres, while the specific strength of bamboo is 3 to 4 times to the specific strength of mild steel (Roslan *et al.*, 2015). Hence, bamboo fibre is known as "natural glass fibre" (Nahar *et al.*, 2012).

Bamboo is considered a composite material because it consists of cellulose fibres imbedded in a lignin matrix. Cellulose fibres are aligned along the length of the bamboo providing maximum tensile flexural strength and rigidity in that direction (Rassiah & Ahmad, 2013). It has been found that bamboo fibre bundles have a potential ability to work as the reinforcement of polymer matrix and show higher tensile strength than jute fibres and improved the bio-composites from biodegradable polymers (Deshpande *et al.*, 2000). The tensile strength of bamboo is relatively high and can reach 370 MPa (Rassiah & Ahmad, 2013). Also, this makes bamboo an attractive alternative to steel in tensile loading application. Bamboo fibre, which is a cellulose fibre, can be easily blended with others natural fibres and it is recyclable under 100% sun light or at the soil with the microorganisms. Therefore, it is referred as a "natural green and economic fibre" and a 21st century material which also has antibacterial properties. The bamboo fibre-reinforced composite structures, with their mechanical properties, can benefit from the fibre's natural qualities and reasonable good physical properties (Nahar *et al.*, 2012).

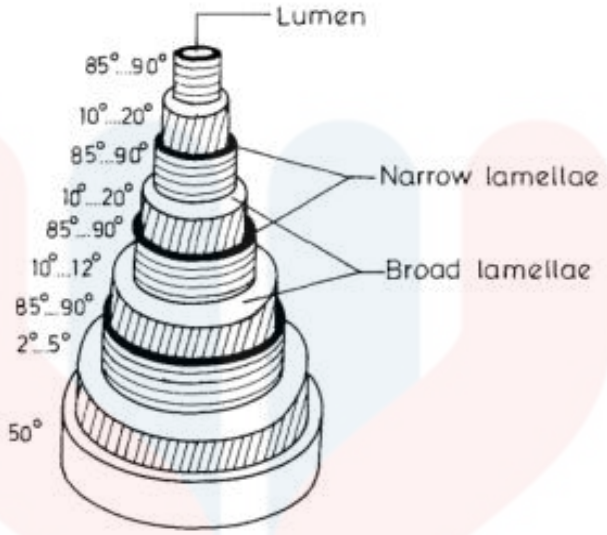


Figure 2.2: The polylamellate wall structure of a bamboo (Roslan *et al.*, 2015)

2.3 Giant Panda

Giant Panda (*Ailuropoda melanoleuca*) are phylogenetically classified as carnivores (Julie *et al.*, 2007). They are considered the "bear" species of family Ursidae within the order Carnivora. Nevertheless, their diets are dominated by highly fibrous bamboo leaves and stems, with the addition of fresh, soft bamboo shoots in spring and summer. Giant Panda consume more than 60 bamboo species across their range (Hu & Wei, 2004). These solitary, large-bodied mammals are extremely rare because they exist at low densities and are found only in highly-specific habitats in the mountains of southwestern China.

Despite being exclusively herbivorous, Giant Panda retain the simple stomach and short gastrointestinal tract typical of carnivores, and consequently need to consume large amounts of poorly digestible foods (Julie *et al.*, 2007). Adaptations

to reduce energy expenditure include large body size, a thick coat with oily, springy hairs, and minimization of calorically expensive activities.

2.3.1 Diet of Giant Panda

The diet of Giant Panda are primarily herbivorous, constituted almost exclusively of bamboo as their staple food, up to 95 to 99% of their diet (Julie *et al.*, 2007), with the remainder of the diet from various potential sources. They utilize over 60 species of bamboo, but 35 species comprise their main food source (Hu & Wei, 2004). Villagers in China have reported finding remains of small rodents such as bamboo rats (*Rhizomys* spp.), golden monkeys (*Pygathrix roxellana*), and musk deer (*Moschus* spp.) in the feces of wild pandas (Julie *et al.*, 2007). According to Julie *et al.* (2007), captive pandas consume supplements such as rice gruel, high-fibre biscuits, and food such as fruits and vegetables.

2.3.2 Digestibility of Giant Panda

Bamboo is a low nutrition or energy food, comprising 70–80% cellulose, hemicellulose and lignin, and 20–30% protein, soluble carbohydrate and fat (Wei *et al.*, 2015). Giant Panda digest a small proportion of this: 75–90% of the protein, only 27% of the hemicellulose and 8% of the cellulose (Wei *et al.*, 2015). The Giant Panda did not harbor putative cellulose-degrading phylotypes such as *Ruminococcaceae* and *Bacteroides* bacteria that are typically enriched in other herbivores, but instead, their microbiota were dominated by *Escherichia/Shigella* and *Streptococcus* bacteria. *Enterobacteriaceae* and *Streptococcus* have been identified

as predominant members of their gut microbiota by traditional culture-dependent methods (Xue *et al.*, 2015) and 16S rRNA gene clone library analysis (Wei *et al.*, 2007).

Members of the class Clostridia were common and abundant in the Giant Panda gut microbiota, but most of the members present were absent in other herbivores and were not phylogenetically related with known cellulolytic lineages. Therefore, the Giant Panda appear not to have evolved a gut microbiota compatible with their diet, which may adversely influence the coevolutionary fitness of Giant Panda (Xue *et al.*, 2015). The Giant Panda gut microbiota is low in diversity and highly variable across seasons. It also shows an overall composition typical of bears and entirely differentiated from other herbivores, with low levels of putative cellulose-digesting bacteria. The gut microbiota of Giant Panda is thus not well adapted to its highly fibrous diet, suggesting a potential link with their poor digestive efficiency (Xue *et al.*, 2015).

Metagenomic sequencing of three fecal samples from wild Giant Panda identified cellulolytic genes, suggesting that the Giant Panda gut microbiome might be able to digest cellulose in its bamboo diet (Zhu *et al.*, 2011). However, because of the particularly low digestibility of bamboo dry matter (<20%) due to low protein and high fibre and lignin contents, it was suggested that the Giant Panda may rely on utilization of bamboo cellular contents rather than the cell wall constituents (Xue *et al.*, 2015).

High levels of intake (up to 6% of body weight in dry matter) combined with fast rates of passage (8 ± 3 hours) allow Giant Panda to meet their nutritional needs even without digestion of plant structural carbohydrates. Unable to digest the cell

wall constituents efficiently, Giant Panda instead rely on utilization of cellular contents (Xue *et al.*, 2015). While Giant Panda uncomplicated digestive tract limits microbial digestion, it allows the successful strategy of permitting the unobstructed passage of large amounts of plant material, specifically, bamboo.

2.3.3 Adaptations to a Specialized Bamboo Diet

2.3.3.1 Morphological Adaptation

The forepaw of Giant Panda has evolved a pseudo-thumb: an enlarged radial sesamoid bone (Salesa *et al.*, 2006) that plays a critical role in grasping bamboo and facilitating feeding and is generally regarded a perfect example of adaptive evolution (Figure 2.3). Their skull is composed of dense compact bones and compared to other bears, has extremely expanded zygomatic arches (Figure 2.4) and well developed mandible structure (Figure 2.5) (Zhang *et al.*, 2007; Wei *et al.*, 2015), associated with zygomatic-mandibular muscle attachment. These structures facilitate mastication of bamboo, which is necessary for such a tough and fibrous food. The teeth are large and flat and have elaborate crown patterns, providing efficient crushing surfaces which enable effective mastication of coarse bamboo (Figure 2.6) (Wei *et al.*, 2015).

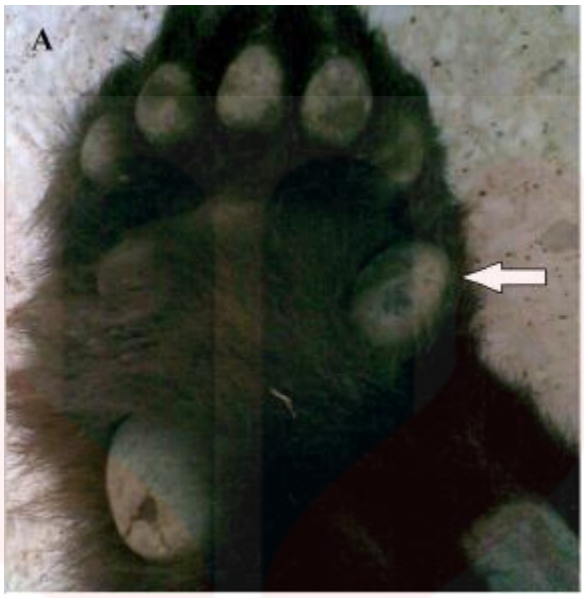


Figure 2.3: Adaptive morphological characteristics of Giant Panda: pseudo-thumb (denoted by the arrow) (Wei *et al.*, 2015)

Figure 2.4: Adaptive morphological characteristics of Giant Panda: skull (Wei *et al.*, 2015)

Figure 2.5: Adaptive morphological characteristics of Giant Panda: mandible (Wei *et al.*, 2015)

Figure 2.6: Adaptive morphological characteristics of Giant Panda: teeth (Wei *et al.*, 2015)

2.3.3.2 Ecological Adaptation

The Giant Panda has evolved a suite of optimal foraging, habitat use and activity rhythm strategies as adaptations to the low energy diet. Field research shows that it prefers the most nutritious bamboo shoots, tender leaves and first-year bamboo (Wei *et al.*, 2015), and chooses different bamboo species and bamboo parts at different times of the year in different mountain ranges (Zhang *et al.*, 2014). Nutritional analysis suggests that their foraging habits are tied to changes in the nutritional composition of bamboo and their dietary shifts are related to balances of nitrogen, phosphorus and calcium (Nie *et al.*, 2014). Giant Panda have also evolved a strategy of consuming a great deal of food per day (10-18 kg of leaves or stems, or about 38 kg of shoots) to maximize nutritional and energetic intake (Wei *et al.*, 2015). Moreover, the bamboo resource is widely available in their habitat and is easily sufficient to accommodate the actual population size.

Giant Panda have a clever way of utilizing their habitat. They often live in old-growth forest characterized by gentle slopes and a low density of fallen logs, shrubs and bamboo stands, because feeding and moving in an open microhabitat facilitates easier access to preferred bamboo and reduces energy expenditure (Zhang *et al.*, 2014). To further minimize energy expenditure, Giant Panda have an optimal activity rhythm whereby they often feed during two activity peaks, one near dawn and one at dusk, and 55% of the daily time is spent in foraging and 41% resting (Wei *et al.*, 2015). They often move only a short distance daily, between 300−500 m (Zhang *et al.*, 2014).

2.3.3.3 Genetic Adaptation

Adaptation to a specialized bamboo diet has also occurred at the molecular level. Giant Panda genome reveals that the umami receptor TAS1R1 gene has become pseudogenized due to a 2 bp insertion in exon 3 and a 6 bp deletion in exon 6 (Li *et al.*, 2010). The umami receptor senses components of meat and other protein-rich foods. Therefore, the loss of function of the TAS1R1 gene may have contributed to Giant Panda dietary switch (Li *et al.*, 2010). To test this hypothesis, Zhao *et al.* (2010) sequenced all six TAS1R1 exons of another individual and found another 4 bp deletion in exon 6, confirming pseudogenization of this gene. It is estimated that this pseudogenization occurred 4.2 million years ago, a timeline that matches the approximate date of the dietary switch in Giant Panda (Zhao *et al.*, 2010). For the bitter receptor gene, Zhao *et al*. (2013) detected significant positive selection in bitter receptor genes TAS2R49 and TAS2R3 in the Qinling Mountain population compared to non-Qinling populations based on whole-genome-wide SNP analysis, and the difference in selection may be associated with a higher bitter content (for example, alkaloids) in bamboo leaves (the main food of the Qinling Mountain population). Besides taste receptor genes, Jin *et al.* (2011) analyzed 166 major genes involved in the 'appetite-reward system' of Giant Panda and found a 12 bp deletion in the catechol-o-methyltransferase (COMT) gene, which likely results in loss of function in catecholamine metabolic pathways. This finding suggests that unusual metabolic processes may affect Giant Panda food choices.

The subcellular distribution of the intermediary metabolic enzyme alanine:glyoxylate aminotransferase (AGT) is related with dietary choice, and AGT distribution tends to be peroxisomal in herbivores, mitochondrial in carnivores, and

both peroxisomal and mitochondrial in omnivores (Wei *et al.*, 2015). Birdsey *et al.* (2004) examined the subcellular distribution of AGT enzyme and the molecular evolution of AGT mitochondrial targeting sequence in the Giant Panda, and found that more AGT targeted to the peroxisomes and positive selection occurred on the AGT mitochondrial targeting sequence. This finding shows that molecular adaptation has occurred related with the bamboo metabolism. However, the molecular evolution study on the pancreatic ribonuclease gene (RNASE1) in carnivores, a digestive enzyme that plays an important role in foregut-fermenting herbivores, found only one RNASE1 gene copy and no gene duplication in the Giant Panda (Yu & Zhang, 2006), suggesting that the RNASE1 gene may be not important in bamboo metabolism for Giant Panda.

2.3.3.4 Co-Adaptation of Gut Microbiota

Research shows that 8% of the cellulose and 27% of the hemicellulose in bamboo is digested by Giant Panda (Wei *et al.*, 2015), but how this species digests cellulose has remained a puzzle for some time. Whole genome sequencing of Giant Panda found no specific genes responsible for the digestion of cellulose and hemicellulose (Li *et al.*, 2010), suggesting that gut microbes may play a role in digesting bamboo fibres. Zhu *et al.* (2011) combined 16S rRNA gene sequencing and metagenome analysis, and for the first time identified the microbe group and specific genes associated with the digestion of cellulose and hemicellulose in Giant Panda. They found 13 operational taxonomic units closely related to *Clostridium* groups I and XIVa which contain taxa known to digest cellulose, and recovered putative genes coding two cellulose-digesting enzymes (cellulase and beta-glucosidase) and

one hemicellulose-digesting enzyme (xylan 1,4-beta-xylosidase) in *Clostridum* group I. Their findings highlight that Giant Panda have adapted to a fibre-rich bamboo diet and maximize nutritional intake through symbiosis with specialist gut microbes. Due to the fact that bamboo fibres or lignocellulosic fibres can be found in abundant in Giant Panda waste where they are undigested, Giant Panda waste is thus a valuable source for lignocellulosic fibres.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

This project used Giant Panda waste as the sample material.

3.1.1 Chemicals

The chemicals that were used to conduct this research are sodium hydroxide (NaOH) and sodium hypochlorite (NaOCl).

3.1.2 Equipment

The equipment that were used are the blender, oven, water bath, electronic balance, digital calliper, pH meter, FT-IR Spectrometer (Nicolet iZ10), Thermogravimetric analyzer (Mettler Toledo TGA/DSC 2) and X-ray diffraction instrument (D2 Phaser, Bruker).

3.1.3 Apparatus

The apparatus that were used include beaker, volumetric flask and measuring cylinder.

3.2 Methods

3.2.1 Sampling

Giant Panda waste was obtained from Giant Panda named Xing Xing and Liang Liang from National Zoo of Malaysia.

3.2.2 Isolating Lignocellulosic Fibres from Giant Panda Waste

The Giant Panda waste was dried in oven at 120 ℃ for 2 hours to remove the excess water and for sterilization. The dried sample was washed with water for 3 times before it is filtered. The filtered sample was treated with NaOH (4g/L) at 80 ℃ for 20 minutes in order to remove the proteins and polysaccharides. The mixture was left for 20 minutes and allowed to cool down to room temperature. It was then neutralized to pH 7 by subsequent washing with water. The washed fibres were treated with NaOCl (10g/L) at 40 ℃ for 1 hour as a bleaching agent. After the treatment, the fibres were washed for 3 times and filtered before further drying at 105 ℃ for 2 hours prior to storage using zip lock bag. The length and width of fibres obtained were measured.

3.2.3 Physical Test

3.2.3.1 Percentage of Moisture Content

The percentage of moisture content was calculated using Eq. (1), where W_0 is the initial weight of sample before heating in oven and W_1 is the weight of sample after heating in oven (Spinac é *et al.*, 2009).

$$\text{Percentage of moisture content (\%)} = \frac{W_1 - W_0}{W_0} \times 100\% \qquad \text{Eq. (1)}$$

3.2.4 Characterization of Lignocellulosic Fibres

The fibres washed with water, treated with NaOH and treated with NaOCl were dried in oven at 105 ℃ and grounded into powder by using blender.

3.2.4.1 FTIR Analysis

Fourier transform infrared spectroscopy (FTIR) was performed using FT-IR Spectrometer (Nicolet iZ10) to obtain IR spectra. The FTIR spectra provide information regarding the characteristic functional groups of the fibres. The specimens were scanned for 16 times with 4 replications in the wavenumber range of 400-4000 cm^{-1} using KBr plate.

3.2.4.2 TGA Analysis

Thermogravimetric analyzer (Mettler Toledo TGA/DSC 2) was used to analyze the change in properties of the fibres as a function of increasing temperature. Thermogravimetric analysis (TGA) is a convenient method to measure the weight loss with rising temperature over time. The method is used for determination of the decomposition temperature (T_d) and the mass losses during heating. The samples were heated from 25 ℃ to 600 ℃ with heating rate of 5 ℃ /min by using American standard testing method (ASTM) D 3417 under nitrogen gas (N_2) atmosphere at 25 ml/min.

3.2.4.3 DSC Analysis

The samples were also tested using Differential Scanning Calorimetry (DSC) to determine the difference in the amount of heat required to increase the temperature of the samples. DSC uses the difference in enthalpies of a sample and a reference to determine the reverse heat flow at a certain temperature over time. The glass-transition temperature (T_g) and the melting point (T_m) were determined as the

inflection points of the transitions. The samples were heated from 25 ℃ to 600 ℃ with heating rate of 20 ℃/min according to ASTM D 3418 under nitrogen gas (N_2) atmosphere at 25 ml/min.

3.2.4.4 XRD Analysis

The fibres were evaluated by using X-ray diffraction (XRD) to determine the crystal structure. Diffraction patterns of samples were recorded by the X-ray diffraction instrument (D2 Phaser, Bruker). The samples were scanned in the angular range of 0-100° (2θ) using Cu Kα radiation.

The crystalline index of cellulose (CrI), was determined based on the empirical method (Jonoobi *et al.*, 2009),

$$\text{CrI (\%)} = \frac{I_{200} - I_{am}}{I_{200}} \text{ x } 100\% \qquad \text{Eq. (2)}$$

where I_{200} is the peak intensity corresponding to cellulose I, and I_{am} is the peak intensity of the amorphous fraction.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Percentage of Moisture Content

The Giant Panda waste was dried in oven at 120 ℃ for 2 hours. The dried sample was washed with water for 3 times before it is filtered. The filtered sample was then treated with NaOH (4g/L) at 80 ℃ for 20 minutes. The mixture was left for 20 minutes and allowed to cool down to room temperature. It was then neutralized to pH 7 by subsequent washing with water. The washed fibres were treated with NaOCl (10g/L) at 40 ℃ for 1 hour. After the treatment, the fibres were washed for 3 times and filtered before further drying at 105 ℃ for 2 hours.

Based on Eq. (1), the percentage of moisture content of untreated fibres is found to be 82.01%. Meanwhile, for NaOH and NaOCl treated fibres, the percentage of moisture content is calculated as 81.42% and 79.82% respectively. Hence, it showed that the percentage of moisture content for untreated fibres was the highest due to the fact that natural fibres exhibits highly hydrophilic properties with the presence of cellulose and lignin compared to the treated fibres (Ichetaonye *et al.*, 2015).

4.2 FTIR Analysis

FTIR helps to identify the functional groups present in the fibre, thereby highlighting the chemical differences among the fibre constituents. FTIR is also used to determine changes in functional groups that may have been caused by the treatments. The curves obtained for each type of treatment performed, the main IR

peaks and their corresponding functional groups are shown in Figure 4.1 and Table 4.1. These curves are the results of the vibrations that corresponded to the bands of these chemical elements in the range from 4000 cm^{-1} to 400 cm^{-1}. The spectra main characteristics were assigned to presence of lignin, hemicellulose and cellulose, natural fibres typical components.

Figure 4.1 implied some peaks related with C-O stretch part of hemicellulose, pectin and lignin compound in range 1300-1000 cm^{-1} and some peaks related with C-C stretch part in ring of lignin in range 1500-1400 cm^{-1}. Comparison of transmission intensity on the FTIR spectra for the fibres indicated that chemical treatments lead to decreasing of lignin and hemicelluloses content in the fibres.

A strong peak at 2894 cm^{-1} is related to the C-H stretching vibrations in hemicellulose and cellulose (Oliveira *et al.*, 2016). The 1320 cm^{-1} peak band is assigned for hydroxyl angular deformation (-OH) in the lignin aromatic ring (Rajeshkumar *et al.*, 2016). The 1242 cm^{-1} peak indicated C-O stretching vibration of the acetyl group in lignin (Xu *et al.*, 2013). The absorbance peak at 1160 cm^{-1} is attributed to the C-O-C asymmetric stretching in cellulose and hemicellulose (Oliveira *et al.*, 2016). Compared to the untreated fibres, these absorption peaks appear distinctly as a result of treatments.

Comparing all the spectra of different treatments, the peak at the frequency 1031 cm^{-1} is one of the dominant features in the spectrum. The sharp and strong band at 1031 cm^{-1} corresponded to the presence of C-O and O-H stretching vibration, which belonged to polysaccharide in cellulose (Mtibe *et al.*, 2015). A peak at 896 cm^{-1}, in all spectra corresponded to C-H rock vibrations of cellulose material. The peak at 833 cm^{-1} is due to β-glucosidic linkage (Zhang *et al.*, 2015).

Apparently, the chemical treatments on fibres did not cause changes in their structure, which could be evidenced by FTIR. Similarity in spectral profiles of untreated and treated fibres confirmed that the treatments did not significantly change the chemical nature of the fibres. Comparison of the curves showed that the locations of the peak positions of these samples have no significant deviation but an obvious difference in peak intensity, indicating that hemicellulose and impurities on the fibre surfaces are partly removed by the NaOH and NaOCl treatment although no new groups are introduced in the cellulose molecules. The weakening of peaks as detected in NaOCl treated fibres indicated the removal of the lignin after the treatment. The results showed that NaOCl treatment remarkably decreases certain chemical components, such as pectins and hemicellulose (Cao *et al.*, 2012).

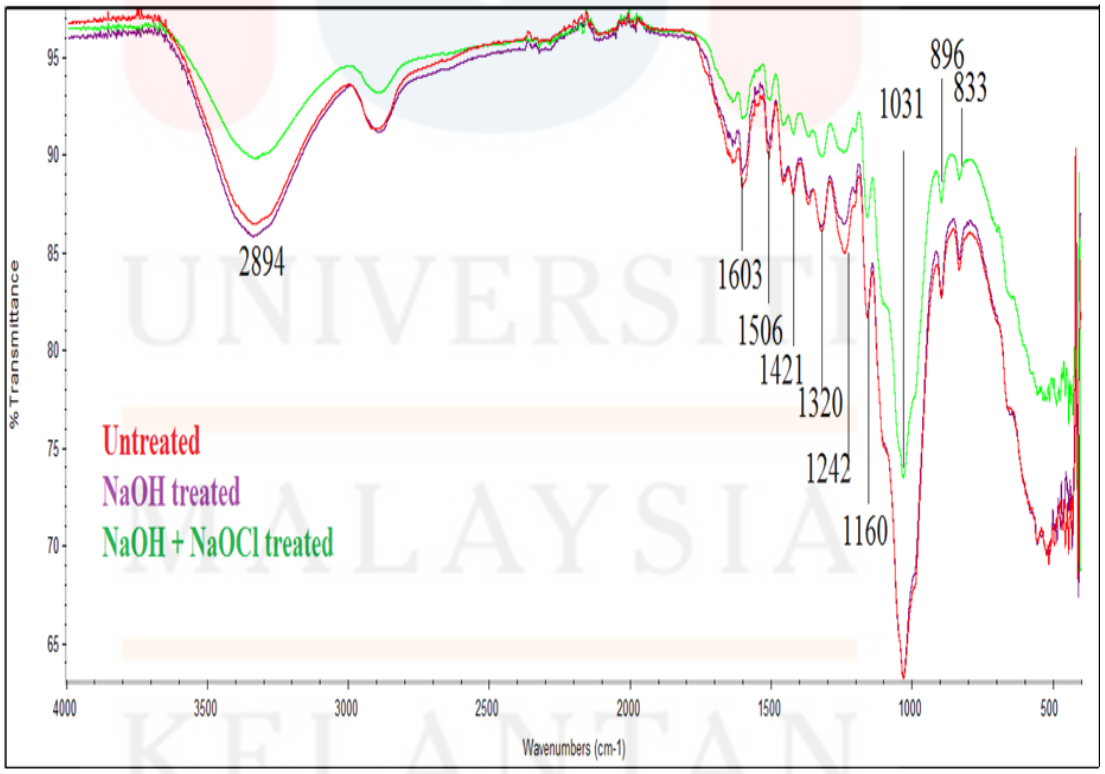


Figure 4.1: FTIR spectra for untreated, NaOH and NaOCl treated fibres

Table 4.1: Infrared transmittance peaks of fibre constituents (mean value) (Borchani *et al.*, 2015 ; Mtibe *et al.*, 2015 ; Oliveira *et al.*, 2016; Rajeshkumar *et al.*, 2016; Xu *et al.*, 2013; Zhang *et al.*, 2015)

Wavenumber (cm^{-1})	Assignment/ functional group	Polymer
2894	C-H stretching vibrations	Hemicellulose, cellulose
1603	C-O stretching, aromatic skeletal vibration	Lignin
1506	Aromatic ring vibration	Lignin
1421	Typical absorption peak	Cellulose
1320	O-H deformation in aromatic ring	Lignin
1242	C-O stretching vibration	Lignin
1160	C-O-C asymmetrical stretching	Cellulose, hemicellulose, lignin
1031	C-O and O-H stretching vibration	Cellulose
896	C-H rock vibration	Cellulose
833	β-glycosidic linkage	Cellulose, hemicellulose, lignin

4.3 TGA Analysis

The thermal stability of the untreated and treated fibres was investigated by TGA, and corresponding primary thermograms are shown in Figure 4.2. The degradation profiles of the three samples are distinctly characterized by three weight loss steps. Weight loss in the first step occurs below 100 ℃ due to moisture evaporation from the fibre structure. The corresponding mass loss is approximately 8%. Compared with the untreated fibres, NaOH and NaOCl treatment would decrease the hydrophilicity of the bamboo fibres (Zhang *et al.*, 2015). In the following thermal decomposition step, the temperatures are concentrated in the range

of 200 to 320 ℃, and the weight loss is mainly caused by the decomposition of hemicellulose and cellulose. Previous studies on hemp fibres reveal that cellulose is entirely decomposed under a higher temperature range (250-350 ℃) than that of hemicellulose (180-280 ℃) (Zhang *et al.*, 2015), thus the decomposition process occurs mainly on the cellulose, which in turn increases the overall degradation temperature of the treated fibres, leading to higher thermal stability. The third step ranging from 320 to 450 ℃ is derived from decomposition of lignin. Since lignin is more difficult to decompose, it has a lower decomposition rate and is degraded in a broad temperature range. Its decomposition usually extends to the whole temperature range, starting around 200 ℃ going up to 700 ℃ (Reddy *et al.*, 2013). When the temperature is above 450 ℃, the fibres are thoroughly degraded so that the residual mass remained unchanged with increasing temperature.

It can be seen that different treatments lead to a gradual removal of non-cellulosic materials, such as hemicellulose and lignin, from the raw fibres, and indicated that highly pure cellulose in natural fibres could enhance their thermal stability (Zhang *et al.*, 2015). The temperatures at the inflection points of the curves or the maximum rate of degradation occurred at 340 ℃, 360 ℃ and 350 ℃ for the untreated, NaOH treated and NaOCl treated fibres, respectively. This indicated the marginal difference in the thermal stability of the fibres, where NaOH treated fibres has the highest degradation temperature and is considered the most stable among others. The alkaline and bleaching treatments were believed to degrade the amorphous region in the cellulose and increase the degree of crystallinity (Jonoobi *et al.*, 2009). Consequently, the greater crystalline structure led to a high resistance towards heat and an increase in the maximum temperature for thermal degradation.

Hence, the thermal properties of the fibres is said to be increased in chemically treated fibre.

It was also noticed that the untreated fibres showed higher percentage of char residue at the end of thermal degradation as compared to the chemically treated fibres. This was due to the presence of lignin in raw samples (Saurabh *et al.*, 2016). The ash which remained at the end of the TG analysis for the untreated fibres is 23.34% of total sample weight. Meanwhile, the residue for NaOH and NaOCl treated fibres is 19.04% and 19.10% respectively. The higher amount of residue in the raw fibre as opposed to in the fibres after chemical treatments was due to the presence of ash as well as lignin, which have a very slow degradation rate (Jonoobi *et al.*, 2009).

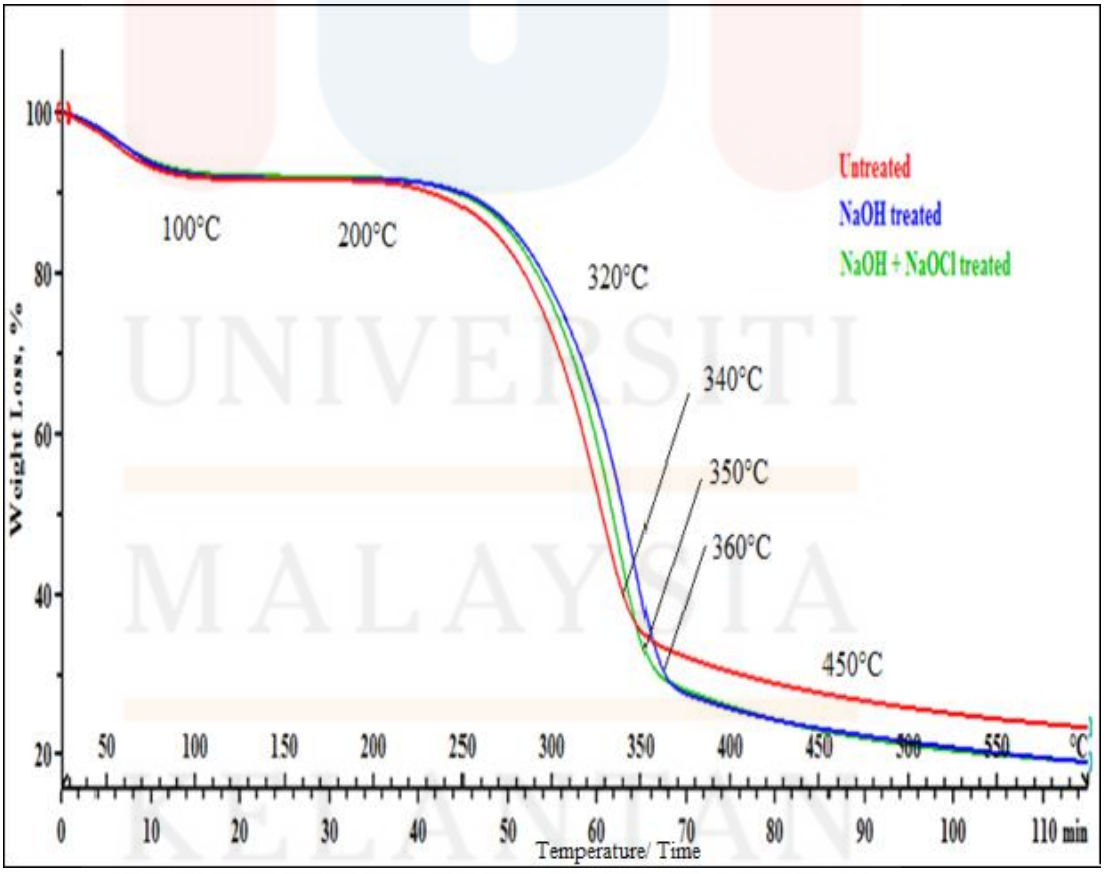


Figure 4.2: TGA curves for untreated, NaOH and NaOCl treated fibres

4.4 DSC Analysis

DSC analysis enables to identify the chemical activity occurring in the fibre as heat was applied. Figure 4.3 showed the thermal response of the untreated and chemically treated fibres as a function of temperature. Both untreated and treated fibres exhibited one broad endothermic peak at the temperature about 80 ℃. On the other hand, NaOH and NaOCl treated fibres showed another endothermic peak at temperature 374 ℃ and 361 ℃ respectively. The first endothermic peak corresponded to the evaporation of moisture absorbed by the fibre. Meanwhile, in the second endothermic peak region, some variations of thermal energy were observed with the effect of different treatments applied. This was due to the changes of moisture absorbed by the fibres after treatment (Zhang *et al.*, 2015). The region between 170-250 ℃ shows no exothermic or endothermic changes reflected that the fibres were thermally stable.

As compared in the figure, the DSC curve for untreated fibre showed an exothermic peak at around 370 ℃, which is derived mainly from the degradation or decomposition of hemicellulose. The endothermic peak at around 360-370 ℃ established in NaOH and NaOCl treated fibres is attributed to the degradation of cellulose. The phenomenon that the peak is weakened in NaOCl treated fibres leading to a gradual removal of binding materials, such as hemicellulose and lignin, from the raw fibres, which increases the relative amount of cellulose contents (Zhang *et al.*, 2015).

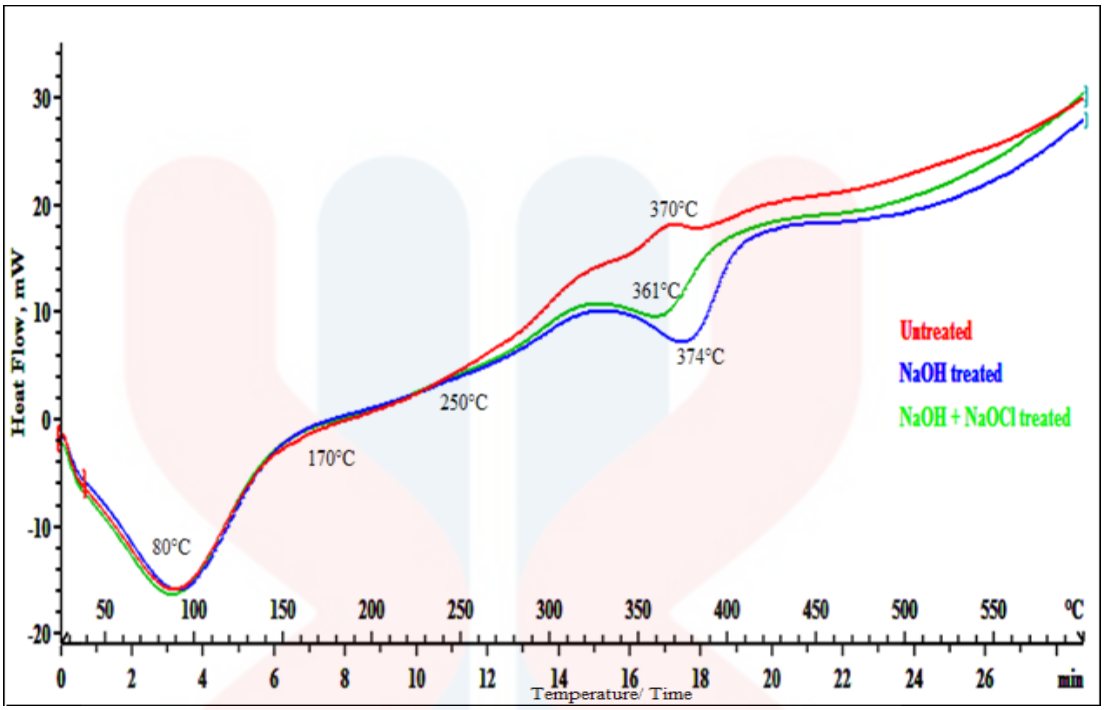


Figure 4.3: DSC curves for untreated, NaOH treated and NaOCl treated fibres

4.5 XRD Analysis

Cellulose has crystalline structure contrary to hemicellulose and lignin, which are amorphous in nature (Yuanita *et al.*, 2015). XRD analysis was completed to evaluate the crystallinity of the lignocellulosic fibres isolated from Giant Panda waste after different chemical treatments. The treatment performed on lignocellulosic fibres can affect the crystallinity of the cellulose. Hence the crystallinity of treated fibre can be examined and compared to untreated fibre to access the effectiveness of the treatment.

The XRD profiles of the untreated and chemical treated (NaOH and NaOCl) fibres are shown in Figure 4.4. All samples displayed two reflection peaks of cellulose I at 2θ = 15° and 22° respectively. These are characteristic of cellulose I type of natural fibre (Saurabh *et al.*, 2016), indicating that chemical treatments did

not alter the natural cellulose structure in the lignocellulosic fibres. The peak at 2θ = 15 ° corresponded to the (1$\bar{1}$0) and (110) crystallographic planes and the peaks at 2θ = 22 ° corresponded to the (002) plane. For fibres with higher cellulose content, like cotton or flax, two peaks around 16 ° are observed, but for the sample fibres, only one broad peak was observed due to the presence of amorphous materials like lignin, hemicellulose and amorphous cellulose, which cover the two peaks (Spinac é *et al.*, 2009).

The reflection peak at 2θ = 22 ° became more intense upon chemical treatment, indicated the increase in crystallinity of the material. The untreated fibres as anticipated displayed a low crystallinity index (CrI) as compared to chemically treated (NaOH and NaOCl) samples. This could be due to the fact that the untreated lignocellulosic fibres contain non-cellulosic amorphous materials such as lignin, hemicellulose, pectin and waxes (Mtibe *et al.*, 2015). According to Mtibe *et al.* (2015), crystallinity index increases upon chemical treatment presumably due to the removal of non-cellulosic amorphous material induced by chemical treatments. In other words, crystallinity index increases due to the removal of amorphous phase and after treatment the material contains more crystalline cellulose region.

Crystallinity index increases from the untreated fibres to cellulose material obtained after chemical treatment. Crystallinity index for untreated fibres is found to be 27.60%, after NaOH treatment it increased to 28.27% and after bleaching it increased to 28.62%, showing a slight increase in CrI. The increased crystallinity following treated fibres compared to untreated fibres is thus due to the progressive removal of amorphous non-cellulosic fibre.

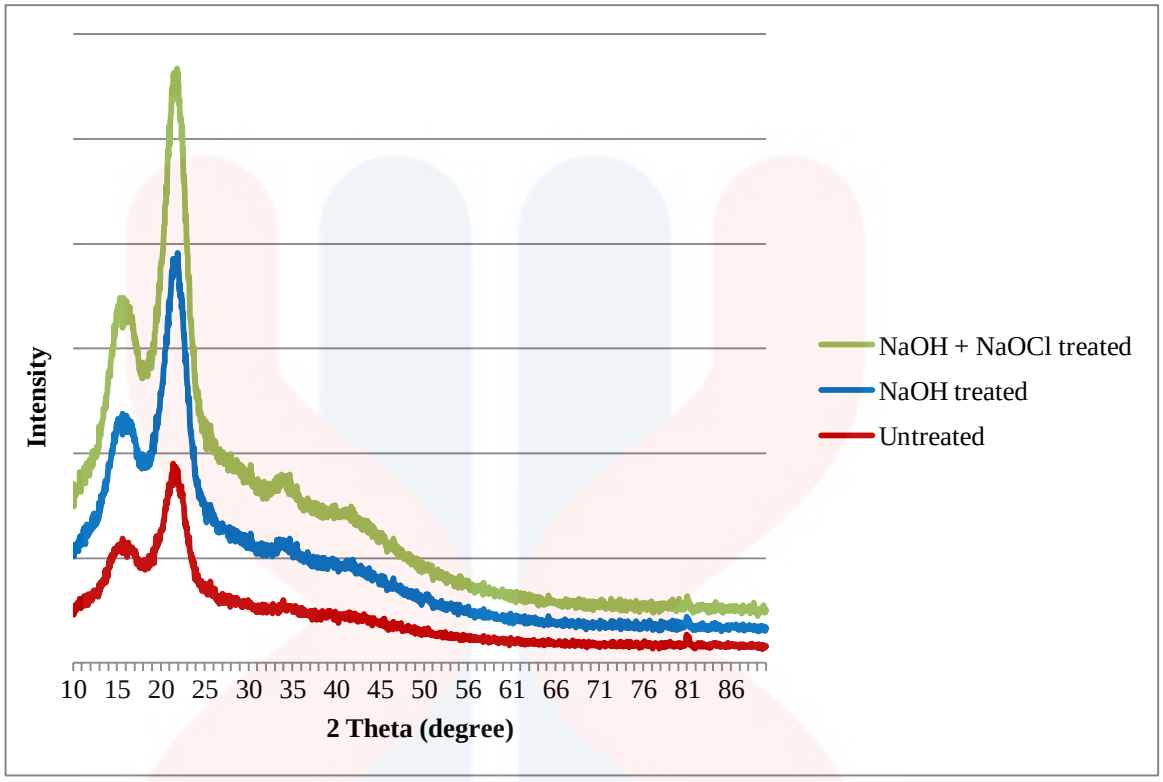


Figure 4.4: XRD curves for untreated, NaOH and NaOCl treated fibres

4.6 Comparison between untreated, NaOH and NaOCl treated fibres

In general, the modification of bamboo fibres will effectively removing the impurities and bond between fibres. This is due to the hydrophilic nature of bamboo fibres, where the different treatments required for improving interfacial surface adhesion. From the results obtained, the percentage of moisture content for untreated fibres was the highest (82.01%) as compared to NaOH treated fibres (81.42%) and NaOCl treated fibres (79.82%) since natural fibres exhibits highly hydrophilic properties with the presence of cellulose and lignin compared to the treated fibres (Ichetaonye *et al.*, 2015). The highest amount of residue in the untreated fibres (23.34%) as opposed to in the fibres after NaOH treatment (19.04%) and NaOCl treatment (19.10%) was due to the presence of ash as well as lignin, which have a

very slow degradation rate (Jonoobi *et al.*, 2009). On the other hand, untreated fibres displayed the lowest crystallinity index (CrI) of 27.60% as opposed to NaOH treated fibres (28.27%) and NaOCl treated fibres (28.62%) due to the fact that the untreated lignocellulosic fibres contain non-cellulosic amorphous materials such as lignin, hemicellulose, pectin and waxes (Mtibe *et al.*, 2015). Meanwhile, untreated fibres exhibited the lowest degradation temperature of 340 ℃ whereas NaOH and NaOCl treated fibres showed an increase in degradation temperature to 360 ℃ and 350 ℃ respectively. Hence, it can be said that the alkaline and bleaching treatments degraded the amorphous region in the cellulose and increase the degree of crystallinity (Jonoobi *et al.*, 2009). Consequently, the greater crystalline structure led to a high resistance towards heat and an increase in the maximum temperature for thermal degradation. The thermal properties of chemically treated fibres were improved where they showed a higher thermal stability. It is thus concluded that the presence of treatment contributes to a better thermal stability for the fibres.

Table 4.2: Comparison between untreated, NaOH and NaOCl treated fibres

	Untreated	NaOH treated	NaOH + NaOCl treated
Percentage of moisture content	82.01%	81.42%	79.82%
Percentage of residue remained	23.34%	19.04%	19.10%
Crystallinity Index (CrI)	27.60%	28.27%	28.62%
Degradation temperature	340 ℃	360 ℃	350 ℃

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Lignocellulosic fibres were successfully isolated from Giant Panda waste, which are abundantly found, through subsequently washing, NaOH and NaOCl treatment. This study aims to use its findings to understand the properties of isolated fibres with different treatments and as a basis for exploiting new natural reinforcements for designing superior green composites. In the present work, the effects of chemical treatments on the physical, thermal and chemical properties of lignocellulosic fibres isolated from Giant Panda waste are systematically analyzed by multiple measurements including FTIR, XRD, TGA and DSC. The results obtained would be useful for the understanding of the characteristics of bamboo fibre and its composites.

FTIR analysis confirmed that removal of hemicellulose and lignin can increase the relative amount of cellulose content in the treated fibres. Furthermore, no new groups are introduced in the cellulose molecules after treatment, as evidenced by spectra measurements. The removal of hemicellulose from fibre cells releases the internal constraint and the fibrils become more capable of rearranging themselves in a compact manner, leading to a closer packing of cellulose chains (Reddy *et al.*, 2013). This closer packing of the cellulose also improved the crystallinity of the fibre after different treatments as evidenced by XRD analysis. TGA-DSC testing revealed that surface treatment can influence chemical structure of bamboo fibres. Treated fibres exhibit higher thermal stability compared to untreated fibres, since the binding materials such as hemicellulose, pectin, and lignin can be diminished from the

bamboo fibres by chemical reaction. Experimental results also suggest that hemicellulose is the most reactive constituent and is more easily degraded than the cellulose and lignin. Cellulose exhibits better thermal stability and lignin is degraded in a wide range of temperatures.

5.2 Recommendations

To further enhance the mechanical, thermal, and tribological properties of the fibres to be used as fillers for different types of polymers, it is very crucial to understand well the properties of the fibres. However, there is inadequate information regarding the properties of lignocellulosic fibres isolated from Giant Panda waste since limited studies have been done on it. This has caused the limitation in understanding the characteristics of fibres obtained. Hence, to provide further and better understanding about lignocellulosic fibres obtained from Giant Panda waste, more in depth researches should be conducted. For instance, Scanning Electron Microscopy (SEM) can be carried out to study the morphology of the fibres before and after different chemical treatment. The concentration of the chemicals used can also be manipulated to study their effects on the treated fibres. Other than that, it is possible to use lignocellulosic fibres isolated from Giant Panda waste as reinforcement in thermoplastic or thermoset composites. For instance, thermoplastic pellets can be mixed with different fibre weight content to fabricate polymer composites. In accordance to this, mechanical testing such as tensile test can also be conducted to understand the mechanical properties of the composites. The suitability of the lignocellulosic fibres extracted from Giant Panda waste to be used as green material for composites can then be examined.

REFERENCES

Anokye, R., Bakar, S.E., Ratnansingam, J. & Awang, B.K. (2016). Bamboo Properties and Suitability as a Replacement for Wood. *PJSRR Pertanika Journal of Scholarly Research Reviews, 2(1),* 63-79.

Bahari, S.A. & Krause, A. (2015). Utilizing Malaysian Bamboo for Use in Thermoplastic Composites. *Journal of Cleaner Production, 110,* 16-24.

Birdsey, G.M., Lewin, J., Cunningham, A.A., Bruford, M.W. & Danpure, C.J. (2004). Differential Enzyme Targeting as an Evolutionary Adaptation to Herbivory in Carnivora, *Molecular Biology and Evolution, 21,* 632-646.

Bongarde, U.S. & Shinde, V.D. (2014). Review on Natural Fiber Reinforcement Polymer Composites. *International Journal of Engineering Science and Innovative Technology (IJESIT), 3(2),* 431-436.

Borchani, K.E., Carrot, C. & Jaziri, M. (2015). Untreated and Alkali Treated Fibers from Alfa Stem: Effect of Alkali Treatment on Structural, Morphological and Thermal Features. *Cellulose, 22(3),* 1577-1589.

Cao, Y., Chan, F., Chui, Y.H. & Xiao, H. (2012). Properties of Modified Flax Fibres. *BioResources, 7(3),* 4109-4121.

Chen, H. (2014). *Biotechnology of Lignocellulose: Theory and Practice*. Beijing: Chemical Industry Press.

Chirayil, C.J., Mathew, L. & Thomas, S. (2014). Review of Recent Research in Nano Cellulose Preparation from Different Lignocellulosic Fibers. *Review Literature and Arts of the Americas, 37,* 20-28.

Deshpande, A.P., Rao, M.B. & Rao, L. (2000). Extraction of Bamboo Fibers and Their Use as Reinforcement in Polymeric Composites. *Journal of Applied Polymer Science, 76,* 83–92.

Faruk, O., Bledzki, A.K., Fink, H-P & Sain, M. (2012). Biocomposites Reinforced with Natutal Fibers: 2000-2010. *Progress in Polymer Science, 37,* 1552-1596.

Fu, J., Li, X., Gao, W., Wang, H., Cavaco-Paulo, A. & Silva, C. (2012). Bio-processing of Bamboo Fibres for Textile Applications: A Mini Review. *Biocatalysis and Biotransformation, 30(1),* 141–153.

Hu, J. & Wei, F. (2004). Comparative Ecology of Giant Pandas in the Five Mountain Ranges of Their Distribution in China. Berkeley: University of California Press.

Ichetaonye, S.T., Madufor, I.C., Yibowei, M.E. & Ichetaonye, D.N. (2015) Physico-Mechanical Properties of Luffa aegyptiaca Fiber Reinforced Polymer Matrix Composite. *Open Journal of Composite Materials, 5(4),*110-117.

Jin, K., Xue, C.Y., Wu, X.L., Qian, J.Y., Zhu, Y., Yang, Z., Yonezawa, T., Crabbe, M.J.C., Cao, Y., Hasegawa, M., Zhong, Y. & Zheng, Y. (2011). Why does the Giant Panda Eat Bamboo? A Comparative Analysis of Appetite-Reward Related Genes Among Mammals. *PLoS One* 6:e22602.

Jonoobi, M., Harun, J., Shakeri, A., Misra, M., & Oksman, K. (2009). Kenaf Composition and Nanofibers. *BioResources, 4(2),* 626-639.

Julie, A.S., Jennifer, L.P., Heidi, A.B., Robert, S.S., John, R.O. & Brian, J.R. (2007). Determination of Bamboo-Diet Digestibility and Fecal Output by Giant Pandas. *Ursus, 18(1),* 38-45.

Junior, M.G., Novack, K.M. & Botaro, V.R. (2014). Bamboo Pulp Treated with NaOH and Benzophenone Tetracarboxylic Dianhydride as Reinforcement of New Polymerics Materials. *LatinAmerican Journal of Metallurgy and Materials, 34(2),* 196-208.

Khalil, H.P.S.A., Bhat, I.U.H., Jawaid, M., Zaidon, A., Hermawand, D. & Hadi, Y.S. (2012). Bamboo Fibre Reinforced Biocomposites: A Review. *Materials and Design, 42,* 353-368.

Li, X.B., Shupe, T.F., Peter, G.F., Hse, C.Y. & Eberhardt, T.L. (2007). Chemical Changes with Maturation of the Bamboo Species *Phyllostachys Pubescens*. *Journal of Tropical Forest Science, 19(1),* 6-12.

Li, R.Q., Fan, W., Tian, G., Zhu, H.M., He, L., Cai, J., Huang, Q.F., Cai, Q.L., Li, B. & Bai, Y.Q. (2010). The Sequence and *de novo* Assembly of the Giant Panda Genome. *Nature, 463,* 311-317.

Lipp-Symonowicz, B., Sztajnowski, S. & Wojciechowska, D. (2011). New Commercial Fibres Called 'Bamboo Fibres' – Their Structure and Proper. *Fibres & Textiles, 19, 1(84),* 18-23.

Liu, D., Song, J., Anderson, D.P., Chang, P.R. & Hua, Y. (2012). Bamboo Fiber and Its Reinforced Composites: Structure and Properties. *Cellulose, 19(5),* 1449-1480.

Merkel, K., Rydarowski, H., Kazimierczak, J. & Bloda, A. (2014). Processing and Characterization of Reinforced Polyethylene Composites Made with Lignocellulosic Fibres Isolated from Waste Plant Biomass such as Hemp. *Composites Part B: Engineering, 67,* 138-144.

Mtibe, A., Linganiso, L.Z., Mathew, A.P. & Oksman, K. (2015). A Comparative Study on Properties of Micro and Nanopapers Produced from Cellulose and Cellulose Nanofibres. *Carbohydrate Polymers, 118,* 1–8.

Mwaikambo, L.Y. (2006). Review of the History, Properties, and Application of Plant Fibres. *African Journal of Science and Technology, 7(2),* 120-133.

Nahar, S., Khan, R.A., Dey, K., Sarker, B., Das, A.K. & Ghoshal, S. (2012). Comparative Studies of Mechanical and Interfacial Properties between Jute and Bamboo Fiber-Reinforced Polypropylene-Based Composites. *Journal of Thermoplastic Composite Materials, 25,* 15-32.

Nayak, L. & Mishra, S.P. (2016). Prospect of Bamboo as a Renewable Textile Fiber, Historical Overview, Labeling, Controversies and Regulation. *Fashion and Textiles, 3(1),* 2.

Nie, Y.G., Zhang, Z.J., Raubenheimer, D., Elser, J.J., Wei, W. & Wei, F.W. (2014). Obligate Herbivory in an Ancestrally Carnivorous Lineage: The Giant Panda and Bamboo from the Perspective of Nutritional Geometry. *Functional Ecoology, 28,* 1-9.

Oliveira, J.A.R., Komesu, A., Martins, L.H. & Filho, R.M. (2016). Evaluation of Microstrutcture of A ça Í Seeds Biomass Untreated and Treated with H_2SO_4 and NaOH by SEM, RDX and FTIR. *Chemical Engineering Transactions, 50,* 379-384.

Pandey, A., Larroche, C., Ricke, S.C., Dussap, C.G. & Gnansounou, E. (2011). *Biofuels: Alternative Feedstocks and Conversion Processes.* London: Academic Press.

Pukansky, B. (2005). Interfaces and Interphases in Multicomponent Materials: Past, Present and Future. *European Polymer Journal, 41,* 645-662.

Rajeshkumar, G., Hariharan, V. & Scalici, T. (2016). Effect of NaOH Treatment on Properties of *Phoenix Sp.* Fiber. *Journal of Natural Fibers, 13(6),* 702-713.

Rassiah, K. & Ahmad, M.M.H. (2013). A Review on Mechanical Properties of Bamboo Fiber Reinforced Polymer Composite. *Australian Journal of Basic and Applied Sciences, 7(8),* 247-253.

Rathod, J.D., Pathak, N.L., Patel, R.G., Jivani, N.P. & Bhatt, N.M. (2011). Phytopharmacological Properties of Bambusa arundinacea as a Potential Medicinal Tree: An Overview. *Journal of Applied Pharmaceutical Science, 1,* 27-31.

Reddy, K.O., Reddy, K.R.N., Zhang, J., Zhang, J. & Rajulu, A.V. (2013). Effect of Alkali Treatment on the Properties of Century Fiber. *Journal of Natural Fibers, 10(3),* 282-296.

Roslan, S.A.H., Rasid, Z.A. & Hassan, M.Z. (2015). The Natural Fiber Composites Based on Bamboo Fibers: A Review. *ARPN Journal of Engineering and Applied Sciences, 10(15),* 6279-6288.

Salesa, M.J., Anton, M., Peigne, S. & Morales, J. (2006). Evidence of a False Thumb in a Fossil Carnivore Clarifies the Evolution of Pandas. *Proceedings of the National Academy of Sciences of the United States of America, 103,* 379-382.

Saurabh, C.K., Dungani, R., Owolabi, A.F., Atiqah, N.S., Zaidon, A., Aprilia, N.A.S., Sarker, Z.M. & Khalil, H.P.S.A. (2016). Effect of Hydrolysis Treatment on Cellulose Nanowhiskers from Oil Palm (*Elaeis guineesis*) Fronds: Morphology, Chemical, Crystallinity, and Thermal Characteristics. *BioResources, 11(3),* 6742-6755.

Sen, T. & Reddy, H.N.J. (2011). Application of Sisal, Bamboo, Coir and Jute Natural Composites in Structural Upgradation. *International Journal of Innovation, Maagement and Technology, 2(3),* 186-191.

Smole, M.S., Hribernik, S., Kleinschek, K.S. & Kreže, T. (2013). Plant Fibres for Textile and Technical Applications. *Advances in Agrophysical Research,* 369-398.

Spinacé, M.A.S., Lambert, C.S., Fermoselli, K.K.G & De Paoli, M.A. (2009). Characterization of Lignocellulosic Curaua Fibres. *Carbohydrate Polymers, 77,* 47-53.

Tarou, L.R., Williams, J., Powell, D.M., Tabet, R. & Allen, M. (2005). An Analysis of Bamboo Preferences in a Pair of Captive Giant Pandas (*Ailuropoda Melanoleuca*). *Smithsonian, 20008,* 1-29.

Viswanathan, V.K. (2010). What is Black and White and a Puzzle All Over? *Gut microbes, 1(3),* 129-130.

Wei, G., Lu, H., Zhou, Z., Xie, H., Wang, A., Nelson, K. & Zhao, L. (2007). The Microbial Community in the Feces of the Giant Panda (*Ailuropoda melanoleuca*) as Determined by PCR-TGGE Profiling and Clone Library Analysis. *Microbial Ecology, 54,* 194-202.

Wei, F., Hu, Y., Yan, L., Nie, Y., Wu, Q. & Zhang, Z. (2015). Giant Pandas Are Not an Evolutionary cul-de-sac: Evidence from Multidisciplinary Research. *Molecular Biology and Evolution, 32, 1,* 4-12.

Welker, C., Balasubramanian, V., Petti, C., Rai, K., DeBolt, S. & Mendu, V. (2015). Engineering Plant Biomass Lignin Content and Composition for Biofuels and Bioproducts. *Energies, 8(8),* 7654-7676.

Xu, F., Yu, J., Tesso, T., Dowell, F. & Wang, D. (2013). Qualitative and Quantitative Analysis of Lignocellulosic Biomass using Infrared Techniques. *Applied Energy, 104,* 801-809.

Xue, Z., Zhang, W., Wang, L., Hou, R., Zhang, M., Fei, L., Zhang, X., Huang, H., Bridgewater, L.C., Jiang, Y., Jiang, C., Zhao, L., Pang, X. & Zhang, Z. (2015). The Bamboo-Eating Giant Panda Harbors a Carnivore-Like Gut Microbiota, with Excessive Seasonal Variations. *mBio, 6(3),* 1-12.

Yu, L. & Zhang, Y.P. (2006). The Unusual Adaptive Expansion of Pancreatic Ribonuclease Gene in Carnivora. *Molecular Biology and Evolution, 23,* 2326-2335.

Yuanita, E., Pratama, J.N., Mustafa, J.H. & Chalid, M. (2015). Multistages Preparation for Microfibrillated Celluloses based on Arenga Pinnata "ijuk" fiber. *Procedia Chemistry, 16,* 608-615.

Zhang, S.N., Pan, R.L., Li, M., Oxnard, C. & Wei, F.W. (2007). Mandible of the Giant Panda (*Ailuropoda melanoleuca*) Compared with Other Chinese Carnivores: Functional Adaptation. *Biological Journal of the Linnean Society, 92,* 449-456.

Zhang, X., Wang, F. & Keer, L.M. (2015). Influence of Surface Modification on the Microstructure and Thermo-Mechanical Properties of Bamboo Fibers. *Materials, 8,* 6597–6608.

Zhang, Z.J., Sheppard, J.K., Swaisgood, R.R., Wang, G., Nie, Y.G., Wei, W., Zhao, N.X. & Wei, F.W. (2014). Ecological Scale and Seasonal Heterogeneity in the Spatial Behaviors of Giant Pandas. *Integrative Zoology, 9,* 46-60.

Zhao, H.B., Yang, J.R., Xu, H.L. & Zhang, J.Z. (2010). Pseudogenization of the Umami Taste Receptor Gene Tas1r1 in the Giant Panda Coincided with Its Dietary Switch to Bamboo. *Molecular Biology and Evolution, 27,* 2669-2673.

Zhao, S.C., Zheng, P.P., Dong, S.S., Zhan, X.J., Wu, Q., Guo, X.S., Hu, Y.B., He, W.M., Zhang, S.N., Fan, W., Zhu, L., Li, D., Zhang, X., Chen, Q., Zhang, H., Zhang, Z., Jin, X., Zhang, J., Yang, H., Wang, J., Wang, J. & Wei, F. (2013). Whole Genome Sequencing of Giant Pandas Provides Insights into Demographic History and Local Adaptation. *Nature Genetics, 45,* 67-71.

Zhu, L.F., Wu, Q., Dai, J.Y., Zhang, S.N. & Wei, F.W. (2011). Evidence of Cellulose Metabolism by the Giant Panda Gut Microbiome. *Proceedings of the National Academy of Sciences of the United States of America, 108,* 17714-17719.

APPENDICES

APPENDIX A Weight of untreated, NaOH and NaOCl treated fibres before and after drying in oven

Untreated fibres washed with water

Oven-dried untreated fibres

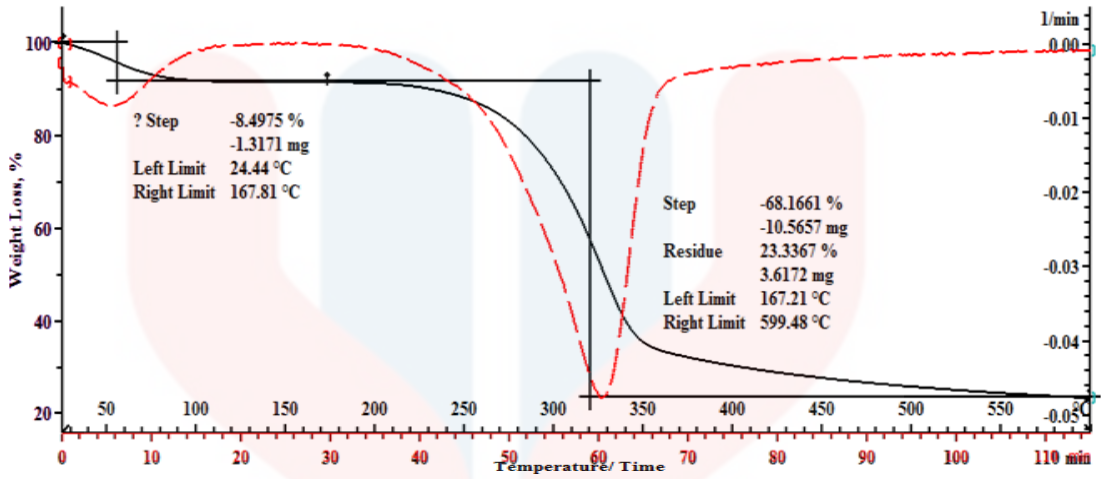
Fibres treated with NaOH

Oven-dried NaOH treated fibres

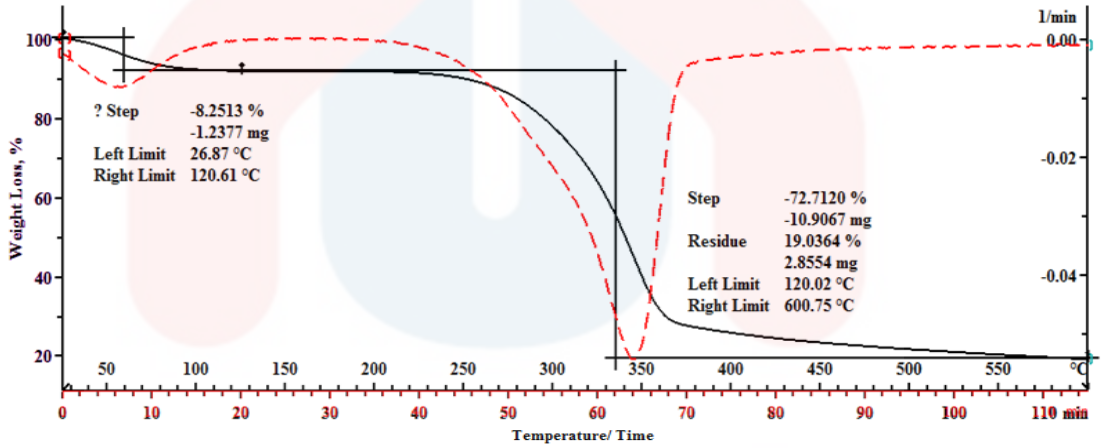
Fibres treated with NaOCl

Oven-dried NaOCl treated fibres

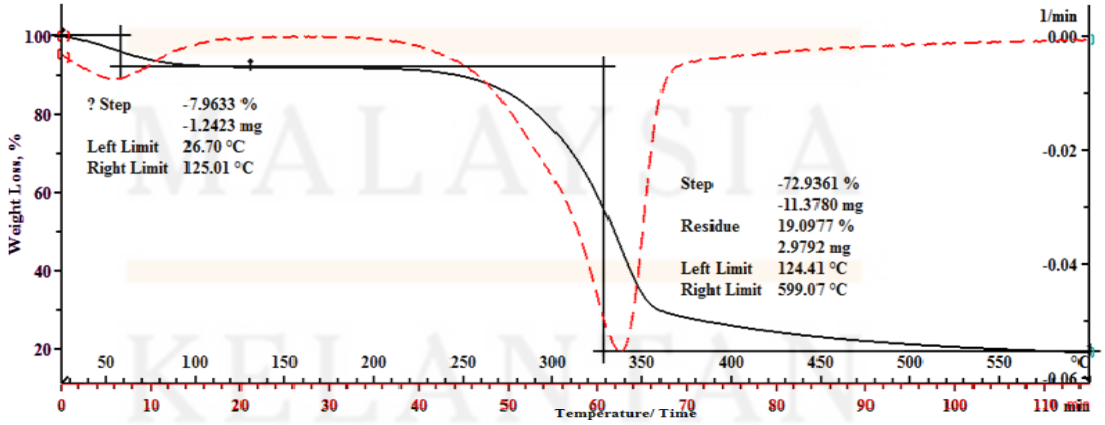
APPENDIX B TGA curves for untreated, NaOH and NaOCl treated fibres



TGA curve for untreated fibres

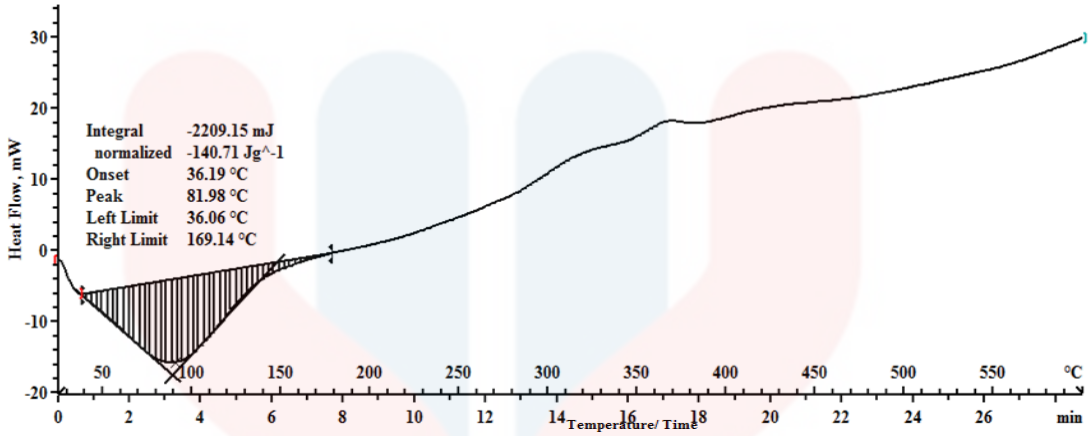


TGA curve for NaOH treated fibres

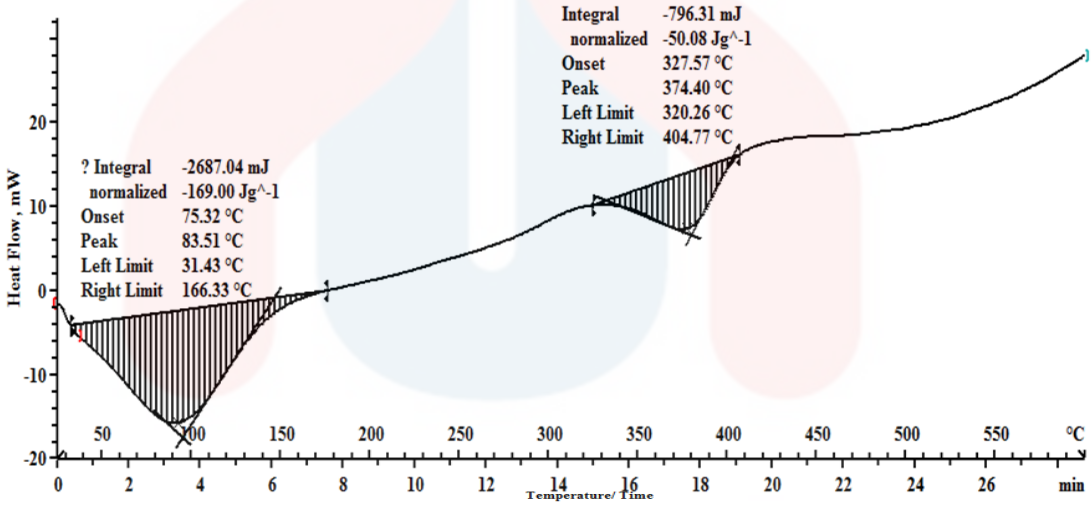


TGA curve for NaOH and NaOCl treated fibres

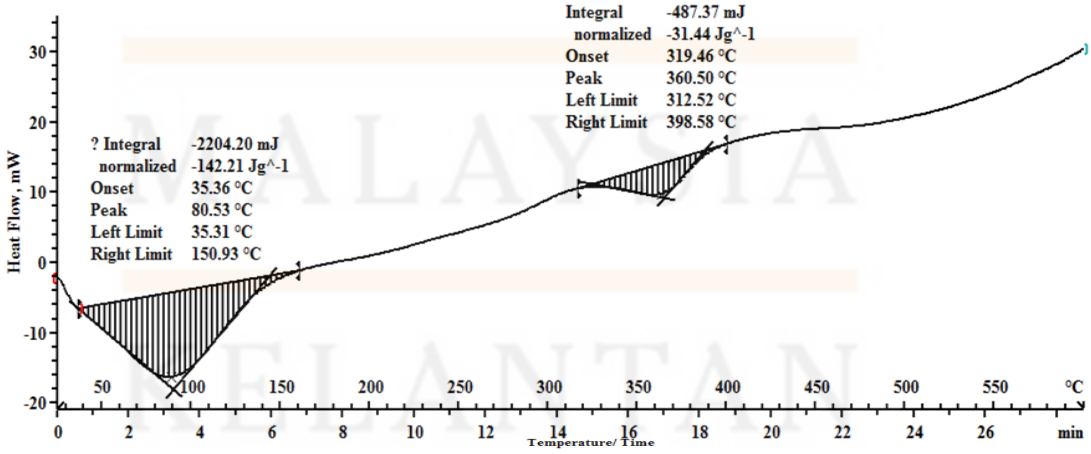
APPENDIX C DSC curves for untreated, NaOH and NaOCl treated fibres



DSC curve for untreated fibres



DSC curve for NaOH treated fibres



DSC curve for NaOH and NaOCl treated fibres