Potential of textile hemp in biomedical and composite applications

by

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I am the author of the thesis entitled

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Abstract

Hemp is an eco-friendly plant in terms of growth characteristics. It requires less pesticides, fertilizers and herbicides than other crops. It can adapt to a variety of climate. Due to the fast growing speed, hemp can produce 250% more fibre than cotton and 600% more than flax in the same area of land. This research investigates the potential of textile hemp to be used in new applications, in particular, in biomedical and polymer-composite materials.

Powders made of plant fibres have found many applications in the food, pharmaceutical and personal care industries. However, the ability of hemp to be ground into fine powders has not been reported in scholarly journals. In the first part of the project, the fabrication of hemp powder was studied. A series of powder production facilities were employed, including a cutter mill, an attritor ball mill, a spray dryer and an air jet mill. For the wet milling in an attritor mill, the effects of key milling parameters such as milling time, fibre weight and water volume were investigated using orthogonal experiments. The production of ultrafine hemp powders with diameters of ~ 5 micron was demonstrated.

Hemp has been known to possess antibacterial properties. However, little scientific investigations have been conducted in the past on the antibacterial property of hemp. Using the hemp powders produced, the antibacterial properties of hemp powders and hemp plant extracts were studied. It was found that hemp powders showed antibacterial activities against gram-positive bacterium, *staphylococcus aureus*, but not against gram negative bacterium, *escherichia coli*. 
The formation of polymer composite fibres was investigated using the hemp powder and polypropylene. Such composite fibres are expected to have unique combinations of properties arising from both natural fibres and synthetic polymers. The combination of high mechanical strength arising from polypropylene and excellent dye and moisture absorption properties from natural fibres is attractive as a novel textile material. However, to date, there is no scholarly report on the fabrication of hemp-polypropylene composite fibres.

In this research project, composite fibres were extruded with PP polymer to make filament containing different percentages of hemp fibre powder. It was found out that hemp powder can mix well with polypropylene even without surfactants. The characterisation of compound filaments was carried out for mechanical and dye-absorption properties.
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Chapter 1  Introduction

1.1 Significance and research problems

Hemp fibre, as a natural bast fibre, is an important raw material in the textile industry [1]. Hemp is divided into two varieties including industrial hemp or textile hemp and drug hemp. The textile hemp is grown for industrial use, whilst the drug type hemp is used to produce recreational and medicinal drugs. Recently, the powder converted from natural fibres has become a new class of materials in many applications. Due to the possible antibacterial properties and cellulosit nature, hemp powders have the potential to be used in new applications, in particular, in biomedical and polymer-composite materials. This research investigates the potential of textile hemp to be used in new applications, in particular, in biomedical and polymer-composite materials.

The significance and novelty of this research are as follows:

- The fabrication of hemp fine powder was documented for the first time. Although the conversion of some bast fibres including kenaf and ramie fibres into fine powders have been often reported [2], the fabrication of hemp powder has rarely been documented in the past [3] and the details of the powder fabrication have not appeared in the literature.

- The antibacterial properties of hemp powders and hemp plant extracts from textile hemp were studied for the first time. It is widely claimed by textile retailers that fabrics made from hemp fibres exhibit a good antibacterial property. However, there are only a very small number of scientific reports on the antibacterial properties of hemp. In particular,
the antibacterial properties of textile hemp, that is used to make textile fibres, is largely unknown.

- The fabrication and characteristics of plant fibre powder blended polymer composite filaments were investigated for the first time using the hemp powder and polypropylene. Hemp is an important textile raw material which has some unique properties over man-made fibres. Clothes made from hemp fibres have good moisture absorption which makes them comfortable to wear, whereas synthetic fibres often have limited to no moisture absorption capacity. A synthetic fibre blended with hemp particles would be expected to exhibit a unique combination of the properties of both of the component materials. However, to date, there is no scholarly report on the fabrication of hemp-polypropylene composite fibres.

1.2 Aim and objects

The aim of this research is to investigate the potential of textile hemp to be used in new applications, in particular, in biomedical and polymer-composite materials. The specific objectives/tasks are:

1) To produce hemp powder by using a mechanical milling method. A series of powder fabrication facilities are employed in the production process, characterize hemp powder and compare the changes in properties after different milling stages.

2) To investigate the antibacterial property of hemp powder and plant extracts against common bacterial varieties.
3) To investigate the fabrication of polymer filaments blended with hemp powder and study the unique properties of the filament.

1.3 Outline of thesis

This thesis is structured with seven chapters as follows:

Chapter 1 gives an overview of the whole thesis and clarifies the objective of this research. The structure of this thesis is also outlined.

Chapter 2 provides a detailed literature review of the work related to the scope of this thesis. First, general descriptions of hemp plant, including growing conditions, classification, components, degumming methods and common applications, are presented. Next, the methods to prepare fine powders from plant fibres are reviewed. Common milling equipment and their advantages and disadvantages are discussed. Finally, antibacterial textiles and powder-blended fibres are reviewed as the potential applications of hemp powders.

In Chapter 3, the fabrication and characterisation of hemp powder are reported. Hemp powder was produced using mechanical milling methods consisting of a series of powder fabrication facilities including a cutting mill, an attrition mill and an air-jet mill. The effect of milling parameters on the resulting particle size was discussed. It is found that the volume of water was the most influential parameters for attrition milling among milling time, water volume and fibre weight. Under this condition, it was possible to produce fine hemp powders of approximately 5 μm in diameter. The particle size of air jet mill milled powder is reduced the particle size to approximately 3 μm. The produced powders are
characterized for their particle size, morphology, colour, surface area, chemical bonding, thermal and crystallographical properties.

Chapter 4 reports the investigation of the antibacterial property of hemp plant extract including male hemp leaf, male hemp bark, male hemp hurd, female hemp leaf, female hemp bark and female hemp hurd. The test organisms are common gram-positive strains including 2 strains of *Staphylococcus aureus* (ATCC 25923 and ATCC 29213), *Enterococcus faecalis* (ATCC 10100), *Streptococcus pyogenes* (ATCC 10096) and *Pseudomonas aeruginosa* (ATCC 27853). It is found that there is a strong correlation between the antibacterial property and the amount of CBD in the extracts. Ethyl acetate was found to be a suitable solvent to extract CBD from hemp plants. CBD is mainly existed in hemp leaf and, to a letter extent, in hurd. Hemp leaf and hurd extract showed antibacterial properties, while hemp bark extract in all tested organic solvents did not show antibacterial properties. The hemp plant extracts showed antibacterial properties only against *S.aureus* (ATCC 25923 and ATCC 29213). None of the extracts showed antibacterial properties against the other bacterial strains including *E.faecalis* (ATCC 10100), *S.pyogenes* (ATCC 10096) and *P.aeruginosa* (ATCC 27853).

Chapter 5 describes the evaluation of antibacterial property of raw hemp powder and degummed hemp powder. Gram-positive bacteria *S.aureus* (ATCC 25923) is used as the test bacteria. 100% cellulose powder is employed as control sample in the test. The antimicrobial activity of hemp powder was evaluated using the optical density method. Six different amounts of powder including 1mg, 2mg, 4mg, 8mg, 16mg and 32mg were tested. It is found that both raw hemp powder and degummed hemp powder have antibacterial properties against

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S. aureus. The antibacterial properties of degummed hemp powder starts to show its effect from 3h incubation, and the raw hemp powder starts to show antibacterial properties from 5h incubation. Moreover, a large amount of degummed hemp powder totally stopped the growth of S. aureus, while raw hemp powder showed inhibition of the growth of bacteria to a lesser degree. It is demonstrated that the degummed hemp powder have a stronger antibacterial properties than the raw hemp powder.

Chapter 6 describes the fabrication of polypropylene (PP) polymer filament containing different percentages of hemp powders. First, hemp powder was blended with PP to make master-batch chips, and then filaments were extruded by melt-spinning. Four different polymer/filler ratios were studied with powder concentration from 2 to 10%. The characterisations of filaments on tensile strength, morphology, colour, dye uptake ability, moisture absorption ability, thermal property and crystallinity are performed. It exhibited a good dispersion of powder within the filament. Filaments were delustered and changed in colour with the addition of hemp powder. Both dye uptake and moisture absorption ability of filament increased gradually with the addition of hemp powder into the filament. Filaments became more thermally stable with the addition of hemp powder. The addition of hemp powder into the filaments had a negative effect on the tensile property of filaments. The XRD result indicated that the addition of hemp powder into PP polymer can change the crystallinity index of filaments.

In Chapter 7, the conclusions drawn from the whole study together with the highlights of the thesis and the recommendations for further work are presented.
Chapter 2  Literature Review

2.1 Overview of hemp fibre

2.1.1 Bast fibre

Bast fibres are soft, woody plant fibres collected from the stems of dicotyledonous plants. Most of the bast fibres are obtained from herbs cultivated in agriculture [4]. Flax, ramie, jute, kenaf and hemp are common types of bast fibres [5]. The fibres are located between the epidermis and inner woody core. The fibres are usually separated from the stalk by degumming process. The advantages of bast fibres lie in their sustainability, origin and strength. Bast fibres are also characterized by fineness and flexibility. Bast fibres usually have higher tensile strength than other fibres [4]. Because of these reasons, bast fibres have many applications in high-quality textiles, ropes, yarn, paper, composite materials and burlap.

2.1.2 History of hemp

Hemp (*cannabis sativa*) is one of the earliest domesticated bast fibre plants in the history and it is one of the oldest cultivated fibre plants on earth. It has been grown for thousands of years [6]. Hemp is originated in the central Asia, but nowadays hemp could be found in many regions in the world, for instance, China, Russia, India, Pakistan, Japan, Canada, Chile, Turkey, Hungary, Romania and Poland. Currently China is the leading producer of hemp in the world, followed by Europe, Chile and Democratic People's Republic of Korea [7].
2.1.3 Growing conditions of hemp

Hemp has many advantages over other non-bast fibres. Hemp is an extremely fast growing plant [8]. Hemp can grow to a height of about 4-5 m with a yield as high as 12-14 tons of dry matter/hectare in one year [9]. Hemp can produce 250% more fibres than cotton and 600% more than flax in the same area of land [10]. Furthermore, hemp can adapt to a variety of climate. Although it can grow best in warm and moderately cool areas, the strong botanical properties of hemp make it adaptable for growing in many places all over the world.

Cultivation of cotton uses more than 10% of the world pesticide consumption and 25% of the world insecticide consumption. Moreover, it takes 1/3 pound of fertilizers to grow one pound of cotton [11]. Compared with cotton, hemp needs less pesticides, fertilizers and herbicides to grow [12]. As such, hemp is considered as more environmental friendly plant than other fibre plants.

The morphology of hemp plants vary largely depending on the species variety, agriculture conditions and harvested time [13]. The stem of hemp plants can grow up to 5.3 m in height and 20 mm in diameter. However, the optimum stem for textile fibre production is about 2 m in height and 5 mm in diameter [13].

2.1.4 Classification of hemp

Cannabis is the short term for hemp. Hemp is divided into two varieties, namely, *cannabis sativa* L. *subsp. sativavar* and *cannabis sativa* subsp. *Indica* [14]. *Cannabis sativa* L. *subsp. sativa var. sativa* is the variety grown for industrial use. Textile hemp is often used in fibre fabrication and hemp seed is used to
extract oil. *Cannabis sativa subsp. Indica* (marijuana), which is a drug type hemp mainly used to produce recreational and medicinal drugs [15].

Figures 2.1 and Figure 2.2 show these two kinds of hemp varieties respectively.

![Cannabis sativa L. subsp. sativa var](image1.png)

Figure 2.1 Cannabis sativa L. subsp. sativa var [16]

![Cannabis sativa subsp. Indica](image2.png)

Figure 2.2 Cannabis sativa subsp. Indica [16]

The major difference of these two hemp varieties lies in the morphology and Δ9-tetrahydrocannabinol (THC) content. THC is known to cause psychosis [17]. Textile hemp only contains a minute amount of THC, which is not sufficient to cause any physical or psychological effects. Generally, the THC content in
textile hemp is less than 0.3%. On the other hand, marijuana can have the THC percentage from 6% to 20% [15].

In the past, cultivation of cannabis plant was prohibited due to the presence of THC and the difficulty in distinguishing indica from the sativa species. Recently, the importance of the cannabis plant in fibre and seed production industries has been gradually recognised. Therefore, nowadays textile hemp has been allowed to grow in over 30 countries in the world. The cultivation of drug type cannabis plant, which usually contains more than 5% THC, is prohibited in most countries [18]. In Australia, only textile hemp that contains a THC percentage of less than 0.5% is permitted to be grown [18]. In Europe, the THC percentage should be less than 0.2% [19]. Both gas chromatography (GC) and high performance liquid chromatography (HPLC) are the common methods to identify and quantify the cannabinoids percentage in cannabis plant [20].

2.2 Composition of hemp fibre

2.2.1 Major components

Hemp generally contains about 67%-78.3% cellulose, 5.5%-16.1% hemicelluloses, 0.8%-2.5% pectin, 2.9%-3.3% lignin, and small amounts of fat and wax [21].

Cellulose is the main component of hemp. Cellulose makes up the framework structure of cell wall [22].

Hemicellulose, which is partly intermingled and oriented with cellulose, acts as the matrix substance between cellulose microfibrils [23]. Hemicellulose is not a
homogeneous substance and generally contains polysaccharides having a low degree of polymerisation.

Pectin is the main binder contained in primary wall and middle lamella of bast fibre [24]. Pectin comprises pectic acids and pectic salts in such forms as calcium, magnesium and iron salts.

Lignin is the encrusting substance to glue the fibre cells together which can provide rigidity to the cell wall. Lignin exists in the middle lamella and secondary layer of the bast fibre. The presence of lignin in hemp fibres generally causes poor fibre colour and lustre [25].

Fat and waxes are a complex mixture of aliphatic compounds, which can be found on the surface of the stem. Most of the compounds are wax esters consisting of fatty acids and fatty alcohols, hydrocarbons and derivatives [26].

2.2.2 Cannabinoids

*Cannabis sativa* contains unique chemicals called cannabinoids. The major cannabinoids include D9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinerol (CBG), cannabichromene (CBC) and cannabinol (CBN). Among these cannabinoids, THC is the constituent that possesses the psychoactive property. Non-psychoactive cannabinoids include cannabidiol (CBD), cannabinerol (CBG), cannabichromene (CBC) and cannabinol (CBN). THC is thermolabile and photolabile, and some THC can degrade into CBN after storage of cannabis plant [27]. It is reported that cannabinoids showed antibacterial property against a variety of methicillin-resistant *Staphylococcus aureus* (MRSA) strains [28]. Figure 2.3 show the Chemical structures of five main cannabinoids.
The cannabinoids in cannabis plants vary significantly due to some factors including environmental conditions of cultivation, harvest time and genetic influence [29].

As mentioned earlier, hemp plants are divided into fibre-type and drug-type, based on the phenotypic index. Phenotypic index, which is defined as the THC/CBD ratio or (THC + CBN)/CBD ratio, has been used to classify these types. The phenotypic index of drug type cannabis plants is above 1.0, whilst fibre type cannabis plants have a phenotypic index less than 1.0 [30-32].

Cannabinoid mainly exists in the epidermal glands of hemp. There are two types of epidermal glands including stalked and sessile [33]. The distribution of cannabinoid-producing glands varies depending on the plant part [33]. Textile fibres are normally extracted from the segment between the epidermis and inner woody core. However, the amount of cannabinoid in this plant part is largely unknown.
2.3 Common applications of hemp

In history, hemp was planted as a raw material for textiles, ropes and paper pulp [34]. Hemp is now an attractive source for fibres and is considered eco-friendly as it is renewable, has limited impact on the environment during growth, and is biodegradable at the end of life-cycle. Hemp has been used to produce paper, textiles, cordage of varying tensile strength, biodegradable plastics, construction, nutritional food and fuel [35].

2.3.1 Textile applications

Traditionally hemp has been both a crop plant and textile fibre plant. Hemp fibres are very versatile and they can be made into many items including clothing, furnishing cloths, ritual costumes and cordage [36, 37]. Hemp fibre is a desirable raw material in the textile industry. When compared to some other natural fibres such as cotton and wool, hemp is extremely durable and often stronger. These advantages also make hemp fibre a good textile material [38]. Hemp fibre can be spun and woven into a variety of fabrics. It can also be blended with other fibres such as cotton, ramie, linen and man-made fibres, to provide a better quality to fabric products. Since the agriculture of hemp plants do not need many pesticides, herbicides and fungicides, clothes made from hemp fibres do not have much residue of such hazardous chemicals and hence have less harmful impacts on human health than the textiles made from other natural fibres, such as cotton fibre. Clothes made of hemp fibres are good at blocking ultraviolet (UV) light to reduce the skin-damage from sunlight [39, 40].
2.3.2 Medical applications

Hemp has long been known to possess antibacterial properties. However, there have been little scientific investigations conducted on the antibacterial property of hemp in the past. It is generally believed that the antibacterial property of hemp mainly stems from cannabinoids [41].

It is reported that purified cannabinoids showed antibacterial property against a variety of methicillin-resistant *Staphylococcus aureus* (MRSA) strains [41]. It is also reported that the essential oils of textile hemp can significantly inhibit the microbial growth [42].

Another reason for the fabrics made from hemp fibres to possess antibacterial property is attributed to the micro-structure of hemp fibres, which makes it difficult for bacteria to adhere on the surface of fabric and also to penetrate into the fabric [43].

The antimicrobial properties of hemp make it useful in the personal care industries. Hemp extract is added into body care products such as shampoos and hair conditioners, lotions, massage oils, soaps, skin crèmes, sunscreen, and lip balm [44].

2.3.3 Other applications

In addition to textile and medical applications, hemp has a wide usage in other products. Oil extracted from hemp seeds is an important nutrition in food market [44]. Hempseed oil is rich in poly-unsaturated fatty acids, which makes it an excellent source of dietary oil. The high nutritional value of hempseed includes phytochemicals, vitamins and minerals [45]. Currently, hempseeds have a
market in functional food products and animal food. Hemp seeds have also been used in bird seed mix and as fishing bait. The application in fibre-reinforced composites is also an important one and the review of the technology is given in Section 2.7.

2.4 Degumming process on hemp fibre

2.4.1 Overview

For the production of textile fibres from natural plants, noncellulosic substances are normally removed to obtain the quality required for textile applications. Textile used hemp fibre need to contain a high amount of cellulosic substances. The removal of the noncellulosic substances, such as wax, pectin, lignin and hemicelluloses, is called a degumming process [46]. Clean white fibres can be achieved after the degumming process. There are a number of methods used for degumming. Some common degumming methods are explained in this section.

2.4.2 Field degumming

In field degumming, plant stems are spread on the ground, and pectins are attacked by pectinolytic microorganisms, mainly aerobic fungi [47]. Clostridia are also considered to be the major group of bacteria responsible for water degumming [48, 49].

This process requires no harsh chemicals and advanced facilities. As such, traditionally, field degumming was the method of choice for many years [49]. In fact, field degumming is still used widely to obtain fibres commercially for industrial use.
The disadvantages of field degumming includes low and inconsistent quality of degummed fibre, restriction to certain climatic regions, occupation of land for several weeks during degumming, weather-dependent nature, time-consuming process, and possible contamination of degummed fibres with soil [50].

### 2.4.3 Enzymatic degumming

Enzymatic degumming is capable of separating hemp fibre bundle into single fibres. It can produce comparably strong fibres which are suitable for high-quality textiles [51]. Enzymatic degumming of hemp fibres is of particular interest for reducing costs and the environmental impacts of the degumming process as compared to chemical degumming [5]. Various enzymes, such as hemicellulase, pectinase, xylanase and cellulase, have been employed to remove the noncellulosic substances. The suggested composition of the enzyme is a mixture of different ratios of pectinase, hemicellulase and cellulose. Scourzyme, a kind of enzyme containing pectinase and pectatase, is recommended for the cleaning of flax, hemp, linen and other natural bast fibres, as it does not apparently harm the bast fibres [52].

### 2.4.4 Chemical degumming

Traditionally, chemical degumming by alkali is an important and effective way to remove noncellulosic compounds in hemp. Currently, chemical degumming is the primary process used for the industrial production of bast fibres [53]. In chemical degumming, hemp fibres are soaked in a alkali solution and heated to a high temperature. Consequently, treated hemp fibres are rinsed thoroughly under running water to remove the residuals adhered to the fibres, such as
hemicelluloses and lignin. Finally, the fibres are dried to remove free water. Chemical degumming is quick to perform and can give a good result. However, it involves the usage of harmful chemicals, such as sodium hydroxide, which is not environmental friendly [46].

2.5 Powder fabricated from textile fibres

2.5.1 Overview

In recent years, there has been an increasing demand for powders fabricated from textile fibres. The powders converted from natural fibre have become a new class of materials in many applications.

For example, cotton powders have been used as low calorie additives in food and pharmacy industries. In the cosmetic industry, cotton powders have been applied as a thickener and an extender [54]. Protein powders such as those made from silk and wool have also been found in a wide number of applications in the market. For example, silk powder has been used as an essential ingredient in the cosmetic industry [55]. Wool powder was coated on cotton fabrics to modify the water and thermal transport property. Both silk and wool powders can be used for water treatment by binding heavy metal ions in waste water [56].

There are generally two types of methods to transform fibrous materials into powder; one is chemical and another is mechanical [57, 58]. In chemical treatment, fibres are first dissolved in solvents and subsequently particles are precipitated out [59]. This method usually involves the usage of chemicals and organic solvents which are harmful to humans and environment. Moreover, this method normally takes long time to produce particles. The principle of
A mechanical method is converting fibre into powder by applying mechanical force to grind fibrous materials into smaller size fragments. Compared with the chemical method, mechanical is less time-consuming and safer [60-62].

2.5.2 Mechanical milling

Mechanical milling technology is generally divided into two categories; media milling (ball milling) and non-media milling [63].

2.5.2.1 Media milling (Ball milling)

Ball mills are important devices which are widely used in the powder production industry to produce cement, silicates, fertilizer, ore powder, etc. Moreover, ball mills can also find their usage in mechanical alloying process to produce metal alloy powders [64]. Ball milling is a mechanical process. The mechanical energy to reduce big particles into small particles comes from two ways: collision energy and shear energy [65].

A ball mill commonly consists of a cylindrical chamber to grind or mix materials. The grinding chamber is partially filled with materials and grinding media. Common grinding media include ceramic balls, stainless steel balls and flint pebbles. There are three main considerations for the choice of grinding media; media size, media density and media hardness [66]. Zirconia is a common material used as the grinding media in the ball milling process, due to its excellent hardness. Shield gas that cannot react with the grinding materials can also be filled into the grinding chamber to prevent oxidation or explosive reactions that could happen in air [67]. Ball milling can be performed in both dry and wet milling conditions.
Ball milling has many advantages over other grinding methods [68]; the high degree of agitation generated by the ball milling process can break down the material easily. Ball milling process can be conducted at room temperature. Normally there is no gaseous emission and liquid effluents created in the ball milling process.

Shaker mills, planetary ball mills and stirred media mills (Attritors) are common media milling facilities [66, 68].

2.5.2.1 Attritor mill

An attritor generally consists of a milling chamber and rotating agitator blades. The chamber is filled with solid grinding media and raw materials. The vertical shaft of an attritor rotates at a high speed, and the milled sample and grinding media move along with the rotation of the vertical shaft [57, 58, 69]. Figure 2.4 shows an example of an attritor mill. In the milling process, vigorous friction occurs among the container wall, grinding media and milled sample, which breaks the sample into smaller fragments. Both container wall and grinding media have to be mechanically harder than the milled sample.

Attritor milling can be used either under dry or wet conditions. In general, attritor milling under wet conditions can give better milling efficiency than dry conditions, because the liquid can decrease the surface energy of particles and reduce particle aggregation [69].
2.5.2.1.2 Shaker mill

In shaker milling, raw materials and grinding media are filled in a vial. During milling, the vial is vigorously shaken to swing back and forth at a high speed. This creates collision events between the container wall, grinding media and milled sample, which breaks the sample into smaller fragments. Shaker milling generates more collision than friction [55, 70]. Figure 2.5 shows an example of a shaker mill.

![Shaker mill](image-url)
2.5.2.1.3 Planetary mill

Figure 2.6 shows a typical planetary mill. It has at least one grinding jar which is adhered to a sun wheel. The sun wheel and grinding jar move in opposite directions. The movement of the grinding jar makes the grinding balls in it move. However, there is a difference in the moving speed between the grinding jar and grinding balls, which cause the Coriolis force to release high energy for the raw materials to be milled into smaller particles [68].

Planetary mills are suitable to grind hard and brittle materials. It can be performed under both dry and wet conditions. The grinding can be finished in a short time and the particle size of final products can reach nano-scale [71, 72].

![Figure 2.6 Planetary mill](image)

2.5.2.2 Non-medial milling

Apart from media milling, non-media milling is another important milling technology. Cutting mill and jet mill are two main non-media mills. The major advantage of non-media milling over media milling is that it induces less contamination into the milled materials [74].
2.5.2.2.1 Cutting mill

Cutting mill is a kind of equipment that is generally employed in the initial milling step for particle size reduction of certain materials. Cutting mills are suitable for reducing the size of soft, medium hard, tough, elastic and fibrous materials.

![Figure 2.7 Cutting mill](image)

A typical appearance of a laboratory cutting mill is shown in Figure 2.7. It consists of a rotor with several cutting blades attached on the rotor. The cutting blade rotates at a high speed with the rotor to cut materials into small pieces. The mesh size of a sieve underneath the cutting blade can control the size of final products; raw materials are cut many times until it can go through the sieve. Both of the cutting blades and sieve have a variety of choices in order to match the mechanical properties of the material to be cut [75, 76].

Cutting mill is a very compact machine which can be fit on a lab countertop. It can pulverize a large variety of materials, even hard rock materials. Cutting mills do not involve high temperature; the milled material is only warmed up slightly in the milling process. Therefore, it is a good choice for grinding temperature-sensitive materials. The milling process can be finished in a short
time compared with other milling machines. However, the final product from cutting milling can have a very large particle size and size distribution.

### 2.5.2.2 Jet milling

Jet mills are widely used in the industry to produce polymer powders. Jet mills are also used for grinding inorganic materials. The applications of jet mills range from metallurgy to synthetic chemicals, paint and ink compounds, cosmetic and pharmaceutical products and food processing. Jet mill operation is usually expensive due to the large consumption of compressed air [77].

![Figure 2.8 Inside of jet mill](image_url)

Figure 2.8 shows the inside of a jet mill. The operation principle of jet milling process is that compressed air or gas impacts fine powders against each other through a vortex motion. Raw materials are fed into a compressed intake gas tube through a feeding funnel. This tube is connected to a cylindrical grinding chamber. A vortex was formed by compressed grind air or gas in the chamber. High speed rotation of this vortex causes particle-on-particle impacts in the grinding chamber. The centrifugal force generated by the vortex drives small particles to the centre of the vortex, and large ones to the perimeter. The fine particles are removed from the grinding chamber through an exit pipe. Jet mills
can produce powders with the average particle size of 1 to 10 microns with narrow size distributions [79, 80].

Jet mills have many advantages over other milling equipment. There is no contamination induced into the final products. The Joules Thompson cooling effect from the compressed air can avoid the harmful heating damage on organic materials. As such, jet mills are suitable for grinding heat sensitive materials. Jet mills require low maintenance, as moving parts are not easy to wear out [81, 82].

2.5.3 Production of powder from natural textile fibres

In the past, the production of fine powders from natural textile fibres has rarely been reported.

A method to produce cotton powder was proposed by Yuen and Cheng [54]. In their study, a mechanical method was used to mill cotton fibre into cotton powder. Cotton fibre went through three milling stages using rotary blades, an ultrasonic machine and a nano-colliding machine. 90% of the particles produced were smaller than 154nm. The crystal structure of cotton powder was unchanged after the treatment. The diameter of the powder was about 10~30 nm.

The production of silk powder and wool powder using mechanical milling was successfully demonstrated by Wang et al [57, 58, 69, 83-85]. The possible degradation of protein at a high temperature caused by milling was avoided by using wet milling. The milling was conducted using a combination of a chopper mill, an attritor ball mill, a planetary mill and an air jet mill. The particle size of silk powder thus obtained was less than 5μm in diameter.
There was only one report on the production of fine hemp powder [86]. Hemp fibre was ground into powder by mechanical action with ziroconia balls. However, the details of the hemp powder thus produced was not reported [86].

2.6 Antibacterial textiles

2.6.1 Needs for antibacterial textiles

In response to the continuously rising demand for hygiene and cleanliness of textiles, advanced textiles with antibacterial properties are being developed rapidly in recent years [87]. A large number of antimicrobial textile products have already appeared in the market. It is reported that the worldwide production of antibacterial textiles is 100,000 tons in year 2000 [88]. Antimicrobial textile is one of the fastest developing areas in the textile industry. It is shown that the production of antibacterial textiles is increasing at a rate of 15% per year worldwide [89]. Among antimicrobial textiles products, lingerie, socks, shoe linings and sportswear occupy about 85% of the total production [87]. Textile materials with antibacterial properties can also be used in many other applications, such as wound healing, bandaging and hygienic garments [90].

Some earlier researches on antimicrobial textile products have been reviewed by Williams, Joshi and Purwar et al. [91]. Although there are many reports and patents on antibacterial textiles, most of them are about the surface treatment of textiles with different antibacterial agents. There is no scholarly report about the use of the antibacterial property of natural fibres in the textile applications.
2.6.2 Current antibacterial agents and their problems

The common antibacterial agents used in textiles include chitosan, PHMB, metal and metal salts, N-halamine, peroxycids, triclosan and quaternary ammonium compounds (QACs) [87]. However, most of the antibacterial agents are toxic chemicals, they have side effects to both humans and environment. An ideal antibacterial product in textiles should be safe and environmental friendly [92].

Recently silver nanoparticles are widely used as an antimicrobial agent in the textile industry. Silver nanoparticles are coated on the surface of fabrics to make the fabric possess antibacterial property [93-95]. However, it is reported that nanoparticles on fabrics may penetrate into skin and, after entering the body, silver nanoparticle can be accumulated in liver, kidneys, bone marrow and spleen [91]. It can cause toxic effects to human body and damage DNA [96].

2.6.3 Antibacterial products made from plants

The extensive application of antibiotics has caused antimicrobial resistance effect to many bacteria strains. Therefore there is an urgent need for new antimicrobial agents to replace the existing antibiotics. In the last few years, natural plants that possess antimicrobial properties have attracted much attention. Both the plant itself and plant extract have the potential to limit the growth of some specific bacteria, and hence it can be used as a new generation of antibiotics [97]. Compared with traditional antibiotics, antibiotics made from plant materials are eco-friendly, biodegradable and may be non-toxic to human body [98]. If hemp products such as hemp fabrics, powders and extracts show antibacterial properties, they can be used as a new type of natural antibacterial
materials for many applications. Recently, hemp fabrics and clothing are sold with marketing claims as being antibacterial. However, antibacterial properties of hemp fabrics are not scientifically demonstrated despite its widespread marketing claims made by the textile manufacturers and retailers.

2.6.4 Methods of Antimicrobial Testing

2.6.4.1 Bacterial variety

Bacterial strains are divided into two categories: gram positive bacterial strains and gram negative bacterial strains [99]. *Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes* and *Pseudomonas aeruginosa* are typical gram positive bacterial strains commonly used in the antibacterial test. *Escherichia coli*, which is a gram negative bacterium, is often used as a test microorganism.

2.6.4.2 Agar Diffusion Test

Two types of antibacterial methods have been mainly used to determine the efficacy of antimicrobial textiles. These methods include the agar diffusion test and suspension test [100].

The agar diffusion test is a qualitative test. In the agar diffusion test, the test sample is laid on a nutrient agar plate which is inoculated over by the bacterial cells. The plate is then incubated at 37°C for 18–24 h. After the incubation time, the plate is taken out of the incubator immediately for the examination of the growth of bacteria around the edges of the test sample. The antibacterial property of the test sample is determined by the size of the so-called “zone of inhibition”. If the antimicrobial agent diffuses into the agar, a zone of inhibition
becomes apparent around the test sample. The agar test is easy to perform and suitable for a large amount of samples [101-103].

2.6.4.3 Suspension Test

The suspension test gives quantitative values about the efficacy/strength of antimicrobial property. Compared to the agar diffusion test, suspension test has its own advantages and disadvantages; the result obtained from the suspension test is more precise than the agar diffusion test. However, it takes longer to perform than the agar diffusion test [104, 105].

In the suspension test, a test sample is placed in a small volume (e.g. 5 ml) of growth media with bacterial inoculums in it. The test sample is incubated in the incubator at 37°C for up to 24 h. The optical density of growth media is measured at an interval of one hour by a spectrophotometer in the first 8 h of the incubation period. Appropriate control samples should be included in the suspension test. A sample without antibacterial property can be selected as the control. The control should be included in each experiment and go through the same procedure described above. The optical density of the growth media represents the concentration of the bacteria in the growth media. If the test sample possesses an antibacterial property, the growth media with the test sample is expected to show a lower optical density compared with the control sample [106].
2.7 Powder blended fibre

2.7.1 Overview

Textile fibres are divided into two types including natural fibres and man-made fibres [107]. Both of them have their own unique properties. For instance, natural fibres usually have good dye uptake and moisture absorption abilities. Clothes made from natural fibres have good handling and are comfortable to wear. Man-made fibres possess good tensile property, but they are often not good at moisture absorption. Because of this, fabrics made from man-made fibres may be uncomfortable to wear [108].

Blending of natural and man-made fibres allows the fabrication of a new type of advanced fibres. The blended fibres will simultaneously realize a high tensile property arising from man-made fibres and excellent dye and moisture absorption properties from natural fibres [109, 110].

Natural fibres are traditionally blended with man-made fibres using conventional blending facilities [111, 112]. However, the current technology has many drawbacks. For example, man-made fibres should be cut into staple fibres with a length similar to natural fibres, which is a time-consuming process [113]. Therefore, it is essential to develop a new technology to blend natural fibres with man-made fibres. Incorporating powders made from natural fibres into man-made fibres is one such approach. Natural-fibre powders can be added into man-made fibres during the extrusion process to produce composite fibres [109, 110]. This approach does not require the man-made fibres to be cut into staple fibres. Man-made fibres blended with natural-fibre powder can be fabricated using a melt extrusion process. First, polymer chips containing
natural-fibre powder need to be fabricated on the blending machine prior to the extrusion process. Polymer chips are used as the raw material for the next extrusion stage where the composite fibres are melt-spun. The final product is expected to have combined properties of the natural fibre and man-made fibre, with high tensile strength, good dye uptake and moisture absorption ability [109, 110].

2.7.2 Factors to influence powder blended fibre

Many factors can have an influence on the performance of powder-blended synthetic fibres:

1) Powder – polymer interface: Most of natural fibres have hydrophilic surfaces, whereas many of the petrol-based man-made textile fibres have hydrophobic nature. In order to obtain high degrees of powder dispersion and good adhesion of powder with the polymer matrix, the polarity of the surface/interface between powder and the polymer matrix should be matched.

2) Amount of powder: Higher amounts of powder-to-matrix ratio give the composite fibres more functionality that stems from the natural-fibre powder. However, higher amounts of powder reduce the overall mechanical strength of the composite fibres, because the breakage of the composite fibres normally occurs at the powder-matrix interfaces.

3) Particle size: In order to obtain a uniform composite structure inside the fibre, the average size of the particles and their agglomerates should be much smaller than the diameter of the composite fibre. When the powder size is too small, it becomes more difficult to form uniform dispersion of
the powder in the polymer matrix, because the chance of powder agglomeration becomes higher.

4) Relationship between the thermal decomposition temperature of natural-fibre powder and the melting temperature of polymer matrices: In order for the powder to be mixed during the melt-extrusion of composite fibres, the thermal decomposition temperature of natural-fibre powder should be higher than the melting temperature of the polymer matrix.

2.7.3 Previous research on powder composite filaments

To date, wool is the only natural fibre material used to fabricate composite fibres made from natural-fibre powder and man-made fibre [109, 110]. It is reported that wool powder was successfully blended with polypropylene through a melt spinning process. The particle size of wool powder was between 5µm and 10µm. The particles were surface modified to avoid the thermal degradation during melt spinning. The composite fibres exhibited a unique combination of properties arising from both wool and polypropylene, such as high mechanical strength, better dye uptake and moisture absorption, good elastic recovery and a warmth retention property.

However, wool is a protein fibre and it degrades rapidly at temperature over 135°C. The degradation results in colour change (yellowing). In addition, the thermal decomposition of protein starts from the temperature around 175°C, which is close to the melt-extrusion temperature of polypropylene. Thus incorporation of wool powder in polypropylene fibre is difficult to achieve.
2.7.4 Hemp powder blended fibre

Hemp is a cellulose-based natural fibre. Cellulose is thermally stable below 250°C and decomposes only above ~ 300°C [114]. Therefore, it is expected that hemp powder is easier to be incorporated into polypropylene than wool powder. Hemp-polypropylene composite fibres will have the unique properties similar to wool-polypropylene composite fibres with high mechanical strength, better dye uptake and moisture absorption, good elastic recovery and a warmth retention property. However, to date, there is no scholarly report on the fabrication of hemp-polypropylene composite fibres.
Chapter 3  Hemp powder: fabrication and characterization

3.1 Introduction

In recent years, there has been an increasing demand for powders fabricated from textile fibres. The powder converted from natural fibres has become a new class of materials in many applications. Protein powders including wool powder and silk powder have been also widely used in the market. For example, wool powder was coated on cotton fabric to modify water and thermal transport properties [115]. Silk powder was used as an essential ingredient in the cosmetic industry [55]. Both wool powder and silk powder can be used to bind heavy metal ions in waste water [56]. Cotton powder has been used as low-calorie additives in food and pharmacy industries. Cotton powder has also been applied as a thickener and an extender in the cosmetic industry [54].

Hemp powder has potential applications in many fields including composites, environmental remediation and biomedical materials. For example, inclusion of hemp powder in polymer composites will provide the host materials with additional functionality such as excellent dye absorption and moisture regulation abilities. Due to the increased surface area of fine powders, hemp powder may have a higher reactivity and enhanced absorbency properties, which is useful for heavy metal scavenging from waste water [86, 116].

As mentioned in Chapter 2, some research has been conducted on the fabrication of powder from natural textile fibres, such as cotton, wool and silk. There are generally two types of methods to transform fibrous materials into powder;
chemical method and mechanical method [57, 58]. In chemical methods, fibres are first dissolved in organic solvents and subsequently particles are precipitated out [59]. Yuen and Cheng [54] reported a solution method to produce cotton powder by agitating cotton fibres in a phosphoric acid solution. This approach usually involves the usage of chemicals harmful to humans and environment. Moreover, this method normally takes a long time to produce fine powders. The principle of mechanical method is converting fibres into powder by applying mechanical force to grind fibrous materials into smaller sizes. Compared with the chemical method, mechanical method is less time-consuming and environmentally safer [60]. The production of silk powder and wool powder using mechanical milling were successfully demonstrated by Wang et al [57, 58, 69]. In their experiments, wet milling was used to avoid possible degradation of protein at a high temperature caused by milling.

Hemp fibres have the potential to be fabricated into powder forms. However, although the conversion of some bast fibres including kenaf and ramie fibres into fine powders have been reported [117, 118], the fabrication of hemp powder has rarely been documented in the past [86]. The aim of this study is to investigate the fabrication of hemp powder using mechanical milling.

Without proper powder fabrication equipment, it is difficult to mill soft fibres into ultrafine powders. In the past, stone grinding and pan milling were applied to produce fine powders from bast fibres. In this study, a series of powder fabrication facilities including an attrition mill and an air-jet mill were employed. The produced powders were characterized for their particle size, morphology, colour, chemical bonding, thermal and crystallographical properties.
3.2 Experiment

3.2.1 Materials

Industrial degummed hemp fibre was used as the raw material for powder fabrication. The fibre was provided by CSM (Commins Stainless Manufacturing) farm in Whitton, NSW, Australia. The fibre was grown and harvested in April 2010 on the farm. The fibre was degummed by chemical degumming at 100°C with sodium hydroxide and sodium carbonate at Deakin University.

3.2.2 Powder fabrication procedure

In general, it is difficult to grind hemp fibres into ultrafine powders in a single operation. Therefore, a number of intermediate steps were introduced into the milling procedure. Three types of equipment were employed to produce hemp powder with fine particle sizes.

![Diagram of hemp powder fabrication](Diagram.png)

Figure 3.1 Schematic diagram of hemp powder fabrication.

Figure 3.1 shows the schematic diagram of milling sequence used in this study. In the first step, hemp fibres were first chopped into snippets with a length of 1 mm. In the second step, the snippets were ground in an attritor with milling
media (zirconia balls) of 5 mm in diameter. Ball milling was performed in wet conditions with deionised water. In the third step, the wet hemp powder was dried using a spray dryer. In the last step, the dried hemp powder was put through an air jet mill for further size reduction. The details of each step are described in the following section.

3.2.2.1 Pre-milling

A cutter mill, Pulvorizer 19 (Fritsch, USA) was used to chop hemp fibres. Figure 3.2 shows the photographic image of the cutter mill. There were five “V” shaped rotating blades mounted on the rotor. Other three blades were fixed on the cutter. A grid under these blades was designed to control the size of snippets. When the cutter was turned on, the rotor can rotate at 2888 rpm. These rotating blades work together with other three fixed blades to cut hemp fibres. The cutting action is repeated many times until the snippets become small enough to pass through the grid. The fibre snippets were collected in a drawer underneath the cutter. The cutter mill can cut hemp fibres into fibre snippets with a length of 1 mm [69, 83].

Figure 3.2 Cutter mill.
3.2.2.2 Attritor milling

An attritor, model 1S (Union Process, USA) was used in the second step of powder fabrication to mill fibre snippets into fine powders. Figure 3.3 shows the photographic image of the attritor. The 10 L container was filled with 20 kg yttrium-doped zirconium oxide grinding media with the diameter of 5 mm. The stirrer was set at 280 rpm according to the manufacturer’s recommendation. Cooling water with a temperature of approximately 18°C was circulated through the outside layer of the container to minimize the thermal degradation of hemp during milling [69]. Wet milling, i.e., milling of fibres in water, was also used to minimize the thermal degradation of hemp during milling.

![Attritor mill](image)

Figure 3.3 Attritor mill.

3.2.2.3 Spray drying

The wet powder slurry thus obtained was dried in a lab-scale spray dryer, B-290 (Buchi Labortechnik AG, Switzerland). Figure 3.4 shows the photographic image of the spray drier. The inlet temperature was set at 120°C, and the outlet
temperature was set at 70 °C, according to the manufacturer’s specification. The air flow rate was kept at 7-8 mL/min [69, 83].

3.2.2.4 Air jet milling

A laboratory air jet mill (Sturtevant, USA) was employed in the last step of powder fabrication. Figure 3.5 shows the image of the air-jet mill. There is no blade in the mill to reduce the powder size. Instead, the powder experience repeated collision caused by high air pressure, which leads to the breakage and fragmentation of the powder. The air pressure in the air jet mill was set at 110 kg/cm². A powder hopper (K-Tron, USA) was used to feed hemp powder into the air jet mill with the feeding rate of 200 g/h [69, 83].
3.2.3 Characterization

3.2.3.1 Fibre diameter measurements

The diameter of hemp fibres were measured using an OFDA 2000 fibre-diameter profiler (BSC Electronics, Australia). Before diameter measurements, hemp fibres were cut into snippets and placed in a conditioned room at 20±2°C and 65±2% relative humidity for 24 hours. The measurements of fibre diameter were performed in scanning mode; OFDA 2000 uses a video microscope to capture the images of the individual fibres moving below the video camera. The instrument identifies fibres in the image and measures the diameter of each fibre to a resolution of 1 μm. At least eight hundred fibre snippets were measured in each scan. The mean diameter of hemp fibre was calculated by the instruments.

3.2.3.2 Particle size

A Mastersizer 2000 laser particle analyser (Malvern, USA) was employed to measure the particle size of hemp powders. Hemp powders were dispersed in distilled water and fed into a Malvern hydro 2000S side feeder. The refractive
index of hemp, 1.55, was used for the particle size measurements. The size results were calculated into volume-weighted size distributions by the analyser’s software version 5.22.

Some parameters including d(0.1), d(0.5), d(0.9), D[4,3] and D[3,2] were recorded. The volume median diameter d(0.5) is the diameter where 50% of the distribution is above and 50% is below; d(0.1) means 10% of the volume distribution is below this value; d(0.9) means 90% of the volume distribution is below this value. The volume weighted mean particle size D[4,3] is the volume moment mean, the surface weighted mean particle size D[3,2] is the surface area moment mean [119].

3.2.3.3 Colour

For colour measurements, powders were compressed into discs under a pressure of 2000 PSI by a 50 ton hydraulic shop press (SUNEX, USA) using a uniaxial compression method. Pressure was applied on powders for 5 min to form a disc of 16 mm in diameter. The colour of discs was then measured on a spectrophotometer SF600 Plus-CT (Datacolor, USA). CIE output results, DIN6167 Yellowness and Ganz-Griesser Whiteness indexes of powders were recorded.

3.2.3.4 Morphology

The Morphologies of particles and hemp fibre were observed under a Supra 55VP scanning electron microscope (Malvern, USA) at 5–10 kV accelerated voltage and 8–9 mm working distance. The cross section of hemp fibres was observed by embedding the fibres in a resin (TAAB, England) using a mould.
The resin was then dried at 60 °C in a fan oven for 2 days to make it solidified. The solidified resin was cut into 5μm on a microtome (CUT 5062 SLEE MAINZ, Germany).

The samples were mounted on a carbon tape that was placed on an aluminium stab. Gold sputter coating (coating thickness 10-15 nm) was applied to the samples prior to the measurements.

### 3.2.3.5 FTIR analysis

Fourier transform infrared (FTIR) spectroscopy measurements were conducted on hemp powders after different milling treatments. The FTIR spectra were recorded using a Vertex 70 spectrometer (Bruker, Germany). The samples were prepared using the standard KBr pellet technique. 1 mg hemp powder was blended with 100mg KBr powder and the mixture was pressed into a disc for FTIR measurements. A single-beam spectrum with a resolution of 2 cm$^{-1}$ of 32 scans between 600cm$^{-1}$ and 4000 cm$^{-1}$ was recorded on the KBr disc samples. Sample spectra were measured in absorbance mode. Three samples for each powder batch were measured. The average spectra of the three samples were used for the evaluation [120].

### 3.2.3.6 Measurement of thermal properties

Simultaneous thermo-gravimetric (TG) and calorimetric analyses (DSC) were performed with a STA 409 PC instrument (NETZSCH, Germany). About 10 mg of desiccated specimens were measured in a nitrogen atmosphere at a flow rate of 140 mL/min, using an alumina crucible. The samples were heated at a rate of 10°C/min over the temperature range from 25°C to 600°C in a ceramic crucible.
The readings were analysed using the NETZSCH Proteus-Thermal Analysis Software.

### 3.2.3.7 X-ray scattering analysis

X-ray diffraction (XRD) analysis was conducted to evaluate the effect of powder fabrication process on the crystallinity of hemp. XRD measurements were performed on X’Pert Power X-ray Diffraction (PANalytical, Netherlands) with Cu Kα radiation (λ = 0.154 nm) operated at 40 kV and 30 mA. Scanning rate was 0.04°/s. The spectra were analysed using the TracesV6 software [121]. The crystallinity index (CrI) of the fibres was calculated according to the Segal empirical method as follows [122]:

\[
CrI(\%) = \left( \frac{I_{002} - I_{am}}{I_{002}} \right) \times 100
\]  

(3.1)

Where \( I_{002} \) is the maximum intensity of the (002) lattice reflection of the cellulose crystallographic form at \( 2\theta = 22^\circ \) and \( I_{am} \) is the diffraction intensity of the amorphous portion represented at \( 2\theta = 18^\circ \).

### 3.2.3.8 BET surface area analysis

A Tristar 3000 nitrogen adsorption apparatus (Micromeritics, USA) was employed to measure Brunauer Emmett Teller (BET) surface area, pore volume and pore size of hemp powders. Prior to the measurements, hemp powder samples were conditioned in a vacuum oven at 40°C overnight [123]. Approximately 0.5 g of powder sample was placed in a glass analysis tube and degassed at 150°C for 60 min. The powder was then analysed at the liquid nitrogen temperature.
3.3 Results and Discussion

3.3.1 Fibre morphology

Figure 3.6 SEM images of hemp fibres.

Figure 3.6 (a) shows the surface morphology of a single hemp fibre and Figure 3.6 (b) shows the cross-section of hemp fibres. It was observed from Figure 3.6 (a) that the hemp fibre had a smooth surface. The small particles adhered to the surface were regarded as contaminants. The diameter of the fibre was distributed evenly along the whole fibre. The hemp fibres had a slight twist. It was noticed from Figure 3.6 (b) that the cross-sections of the hemp fibres are mainly oval in shape. The diameter was around 20μm.

3.3.2 Fibre diameter

Table 3.1 shows the OFDA result of the diameter of hemp fibres. In this table, four parameters are presented: Mean D is the mean diameter, SDD is the standard deviation, and CVD is the coefficient of variation.
Table 3.1 OFDA results of degummed hemp fibres.

The OFDA results showed that degummed hemp fibres had a mean diameter of 21.1μm with a standard deviation of 8.9μm, which was in good agreement with the SEM results. The CVD of chemical degummed hemp fibres was 42.3. Li et al. reported that hemp fibres had a mean diameter of 29.2μm with a standard deviation of 7.33 [124], and concluded that this kind of hemp fibres was suitable for being spun into yarns in traditional textile applications. The results of the present study showed a smaller mean diameter than Li et al. reported, which would make it more suitable for textile applications as a finer fibre provides more fibres in the yarn section.

3.3.3 Optimization of attritor milling process

In the attritor milling process, three factors (Fibre amount, water amount and milling time) were varied to determine the optimum milling conditions to obtain the smallest particle size in a shortest time. During the attritor milling, a small portion of the slurry (<20 mg) was collected at hourly intervals for particle size measurements. The total volume of the attritor’s cylinder was 20 L, and 20 kg of milling media occupied the majority of the space in the cylinder, which leaves about 5 L of the free volume for milling materials in the cylinder container. However, the cylinder was not able to be fully loaded, otherwise, the water would leak out of the cylinder during milling. Therefore, the amount of fibre and water should be less than 5 L in total. Hence, a preliminary study was conducted
with 200 g of fibres and 2 L of water. Attrition milling was carried out for 24 hours. Figure 3.7 shows the result of the milling of 200 g fibre in 2 L water.

![Figure 3.7 Particle size of 24h milling.](image)

Note that d(0.5) is the volume median diameter where 50% of the distribution is above and 50% is below. In this study, the average particle size is investigated, d(0.5) is selected to represent the particle size as it can represent the bulk particle size. It was noticed from Figure 3.7 that the particle size did not show substantial decrease after 6 hours of milling. Hence, the maximum milling time was fixed to 6 hours for all the following experiments.

Because a large amount of experiments were required to vary all the milling parameters, orthogonal experimental design [125] was applied in the attritor milling. The aim of the orthogonal experiment was to minimize the number of experiments and reach the same conclusion as that of a large number of experiments. Three key parameters, namely, fibre amount, water amount and milling time, were optimized by orthogonal experiment.
The orthogonal experimental design was based on the orthogonal table L_{9}(3^4) with four factors (A, B, C and D) and three levels. In the present experiments, fibre amount (A), water amount (B) and milling time (C) were selected as the three factors. The last factor (D) was set as blank. There were three levels in each factor; level 1, level 2 and level 3. In the factor A, level 1 to level 3 mean 100g, 200g and 300g, respectively. In the factor B, level 1 to level 3 mean 1L, 2L and 3L, respectively. In the factor C, level 1 to level 3 mean 2hours, 4hours and 6hours, respectively. The schedule of the orthogonal test was listed in Table 3.2.

<table>
<thead>
<tr>
<th>Number of level</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre/g</td>
<td>Water/L</td>
<td>Milling time/h</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2 Orthogonal table.

Table 3.3 shows the detailed experimental design and results. The numbers 1, 2 and 3 under the factor A, B and C columns indicate the levels of the factors. The particle size d(0.5) was selected as an index point to assess the milling performance under different factors and levels. It was observed from Table 3.3 that the total number of experiments would have been 27(=3^3) without the orthogonal experiment design. On the other hand, when the orthogonal experiment design was introduced into the experiment, the total number of experiments was decreased to 9.
<table>
<thead>
<tr>
<th>Test Number</th>
<th>Factor A</th>
<th>Factor B</th>
<th>Factor C</th>
<th>Particle size d(0.5)/\mu m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibre amount</td>
<td>Water amount</td>
<td>Milling time</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>12.0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>16.7</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>12.7</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>6.6</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>48.1</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>16.3</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>29.8</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>33.1</td>
</tr>
</tbody>
</table>

Table 3.3 Detailed scheme of orthogonal experiment.

In the column of particle size d(0.5), the lowest value $6.6 \mu m$ was obtained under the milling condition of 200 g fibre in 2 L water with 6 hours of milling time. The second lowest value was $8.5 \mu m$, which was achieved under the milling condition of 100 g fibre in 1 L water after 2 hours milling. In order to determine which milling condition has the strongest influence in the particle size, the orthogonal analysis was conducted using the particle sizes in Table 3.3.

<table>
<thead>
<tr>
<th>Particle size /\mu m</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(0.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_1$</td>
<td>2.759</td>
<td>2.810</td>
<td>5.758</td>
</tr>
<tr>
<td>$K_2$</td>
<td>4.669</td>
<td>3.498</td>
<td>3.996</td>
</tr>
<tr>
<td>$K_3$</td>
<td>5.303</td>
<td>6.422</td>
<td>2.977</td>
</tr>
<tr>
<td>$R$</td>
<td>2.544</td>
<td>3.612</td>
<td>2.781</td>
</tr>
</tbody>
</table>

Table 3.4 Orthogonal design-direct analysis.
Table 3.4 lists the result of direct analysis in the orthogonal experiment design. In Table 3.4, \( K_i (i = 1, 2 \text{ and } 3) \) represents the average effect of a factor at a level, i.e., the average value of particle size \( d(0.5) \) for the level \( i \) \((i = 1, 2 \text{ and } 3)\). For example, \( K_1 \) in the column A was the average particle size for the test number 1, 2 and 3 where the level of the factor A was 1. The definition of \( K_i \) can be expressed in the form of formulae as follows:

For factor A,

\[
K_1 = \frac{y_1 + y_2 + y_3}{3} \quad K_2 = \frac{y_4 + y_5 + y_6}{3} \quad K_3 = \frac{y_7 + y_8 + y_9}{3} \quad (3.2)
\]

For factor B,

\[
K_1 = \frac{y_1 + y_4 + y_7}{3} \quad K_2 = \frac{y_2 + y_5 + y_8}{3} \quad K_3 = \frac{y_3 + y_6 + y_9}{3} \quad (3.3)
\]

For factor C,

\[
K_1 = \frac{y_1 + y_5 + y_8}{3} \quad K_2 = \frac{y_2 + y_4 + y_9}{3} \quad K_3 = \frac{y_3 + y_6 + y_7}{3} \quad (3.4)
\]

Where \( y_i \) \((i = 1 \text{ – } 9)\) is the \( d(0.5) \) value of the test number \( i \) in Table 3.3. \( R \) in Table 3.4 was the range of \( K \), i.e., \( K_{\text{max}} - K_{\text{min}} \). \( R \) represents the influence of the factor on the result; the greater the \( R \) value, the stronger the influence.

The result of the orthogonal analysis showed that the factor B had the largest \( R \) value among the three factors. The \( R \) values of the factors A and C were similar to each other. The results indicated that the volume of water had the most notable influence on the experimental results. Both fibre amount and milling time were relatively unremarkable factors compared to the volume of water.
In order to analyse the influence of each factor, the so-called effect curves of the three factors were plotted in Figures 3.8, 3.9 and 3.10.

Figure 3.8 Average effect of the factor A (amount of fibre) on particle size $d(0.5)$. Figure 3.8 shows the influence of the amount of fibre on particle size $d(0.5)$. It was observed that the particle size decreased with the reduction of the amount of fibre per batch. The particle size reached the lowest point when the fibre amount was 100g. This result can be explained as follows: At a given milling time, the total number of ball-to-ball collision event during milling can be considered to be constant. Assume that one ball-to-ball collision contributes to the given degree of reduction in fibre/particle size, i.e., one ball-to-ball collision was equal to one size-reduction event that a fibre/particle experiences. Since the samples were constantly agitated in the mill, the size-reduction event was distributed evenly among the fibres/powder in the mill. Hence, when the total amount of fibres was smaller, each fibre experiences a larger number of size-reduction event. As a result, a smaller amount of fibres will result in smaller particle sizes.
Figure 3.9 Average effect of the factor B (amount of water) on particle size d(0.5). Figure 3.9 shows the influence of the amount of water on particle size d(0.5). It was observed that the particle size decreased with the reduction of the amount of water. The particle size reached the lowest point when the amount of water was the smallest (1 L). The result indicated that, even if the amount of fibre and milling time were constant, the amount of water influenced the particle size. This result is rather counter-intuitive. In order to analyse the effect of the amount of water, particle size d(0.5) was plotted against the amount of water that is normalized with the milling time, in Figure 3.10.
As evident in Figure 3.10, the particle size $d(0.5)$ was strongly dependent on the ratio between the amount of water and the milling time, irrespective of the amount of fibre.

This result may be explained in the following manner. At a given milling time, the total number of ball-to-ball collision event during milling can be considered as constant. The ball-to-ball collision event was not distributed among fibres/powder only but among the whole volume of the “slurry”, i.e., the mixture of water and fibre. When the amount of water was larger, more collision events were distributed to water rather than fibre/powder and thus the collision events do not contribute to the particle size reduction. On the other hand, a smaller amount of water will allow the collision events to be distributed more to fibre/powder and, as a result, leads to smaller particle sizes.

Since the density of hemp was higher than that of water, an increase in the amount of fibre in a fixed volume of water did not significantly increase the volume of the whole slurry. Hence, according to the above discussion, the
particle size \( d(0.5) \) was not strongly influenced by the solid-content (the ratio between the amount of fibre and the amount of water) in the slurry. In fact, as shown in Figure 3.11, \( d(0.5) \) did not have a strong correlation with the solid-content in the slurry.

Figure 3.11 \( d(0.5) \) as a function of the ratio between the amount of fibre and water. Milling times are indicated with different marks.

Figure 3.12 Average effect of factor C (milling time) on particle size \( d(0.5) \).
Figure 3.12 shows the influence of milling time on particle size $d(0.5)$. It was observed that the particle size decreased as the milling time was reduced. The particle size reached the lowest point when the milling time was 6 hour.

Milling for longer times increased the total energy input and the number of collision event, and hence further size reduction occurs as milling time is prolonged.

For the rest of the analysis, samples milled in the attrition milling under the optimized conditions of 100 g fibre, 1 L water and 6 hours of milling time were used.

### 3.3.4 Particle size

Figure 3.13 shows the volume-weighted particle size distribution of the hemp powders after chopping, attrition milling and air-jet milling. Table 3.5 lists the volume-averaged particle size of the hemp powders.
Particle size/μm  | Chopped hemp powder | Attritor milled hemp powder | Air jet milled hemp powder  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>d(0.1)</td>
<td>19.1</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>d(0.5)</td>
<td>87.3</td>
<td>4.6</td>
<td>3.2</td>
</tr>
<tr>
<td>d(0.9)</td>
<td>380.4</td>
<td>7.9</td>
<td>5.4</td>
</tr>
<tr>
<td>D[4,3]</td>
<td>144.4</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>D[3,2]</td>
<td>34.7</td>
<td>4.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Normalized size distribution width</td>
<td>d(0.9) – d(0.1) / d(0.5)</td>
<td>4.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 3.5 Particle size of hemp powders.

- d(0.1) is the diameter where 10% of the volume distribution is below this value.
- d(0.9) is the diameter where 90% of the volume distribution is below this value.

The difference in d(0.9) and d(0.1) represent the approximate breadth of particle size distribution. As shown in Figure 3.13 and Table 3.5, the particle size and size distribution decreased dramatically with the proceeding of powder fabrication through the 4 steps shown in Figure 3.1. Air jet milling reduced the mean particle size but only slightly.

The snippets had a large size distribution, in particular, a large difference between the volume-weighted mean particle size D[4,3] and surface-weighted mean particle size D[3,2]. This may be due to the particle-shape effect described as follows. When the shape of the particles is not spherical but elongated, the projection of the interaction area between the particles and the laser light, which is equivalent to the dipole moment contributing to the scattering of light, can have a wide range of lengths, despite the fact that the actual lengths of the
particles are uniform (Figure 3.14). In the laser diffraction particle sizer such as Malvern Mastersizer, the projection of the interaction area is detected as particle size.

![Figure 3.14 Interaction between the probe light and elongated particles in the laser-diffraction particle sizer.](image)

After attrition milling and air-jet milling, the size distribution and the difference between $D_{4,3}$ and $D_{3,2}$ became significantly smaller. This suggests that the particle shapes became more close to spherical. This is in good agreement with the results of SEM study as described later.

### 3.3.5 Colour

Figures 3.15(a) to (d) show the colours of the hemp fibre, chopped powder, spray dried powder, and air jet milled powder, respectively. Tables 3.6 lists the CIE output results of the chopped powder, spray dried powder and air jet milled powder, respectively.

The uniform CIELAB colour scale is a standard scale to compare colour values. The maximum value for $L^*$ axis is 100, which represents a perfect reflecting
diffuser. The minimum value for L* is zero, which represents black. Both a* and b* axes do not have specific numerical values. Positive a* is red, negative a* is green. Positive b* is yellow, negative b* is blue.

![Image of hemp fibre, chopped powder, spray dried powder, and air jet milled powder.](image)

(a) Hemp fibre  (b) Chopped powder  (c) Spray dried powder  (d) Air jet milled powder

Figure 3.15 Colours of powders.

<table>
<thead>
<tr>
<th></th>
<th>Hemp fibre</th>
<th>Chopped powder</th>
<th>Differences from hemp fibre</th>
<th>Spray dried powder</th>
<th>Differences from hemp fibre</th>
<th>Air jet milled powder</th>
<th>Differences from hemp fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>77.61</td>
<td>77.61</td>
<td>ΔL* 0</td>
<td>78.49</td>
<td>ΔL* 0.88</td>
<td>79.85</td>
<td>ΔL* 2.24</td>
</tr>
<tr>
<td>a*</td>
<td>2.39</td>
<td>2.39</td>
<td>Δa* 0</td>
<td>1.38</td>
<td>Δa* -1.01</td>
<td>0.11</td>
<td>Δa* -2.28</td>
</tr>
<tr>
<td>b*</td>
<td>10.71</td>
<td>10.71</td>
<td>Δb* 0</td>
<td>10.11</td>
<td>Δb* -0.60</td>
<td>10.00</td>
<td>Δb* -0.71</td>
</tr>
<tr>
<td>DE* with respect to hemp fibre</td>
<td>0</td>
<td>1.46</td>
<td>3.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle size/μm</td>
<td>87.3</td>
<td>4.6</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.6 CIE output result of hemp fibre and powders.

ΔL*, Δa* and Δb* indicate the difference between standard (raw hemp fibre) and milled samples in L*, a* and b*. The total colour difference DE* is an
overall difference between the $L^*$, $a^*$ and $b^*$ of the sample and standard, defined as

$$DE^* = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$  \hspace{1cm} (3.5)

Figure 3.16 showed the CIELAB colour space.

![CIELAB colour space](image)

Figure 3.16 CIELAB colour space [126].

It was observed in Table 3.6 that there was no colour change between the hemp fibre and chopped powder. Hence the chopping process didn’t have an influence on the powder colour.

There was a slight colour change between the raw hemp fibre and spray dried powder. The spray dried powder became slightly brighter compared with the raw hemp fibre ($\Delta L^* = +0.88$). The difference in the $a^*$ value between the hemp fibre and spray dried powder was -1.01, which represented that the spray dried powder became greener compared with the raw hemp fibre. The difference in the $b^*$ value between the hemp fibre and spray dried powder was -0.60, which represented that the spray dried powder became bluer compared with the raw hemp fibre. The total colour difference $DE^*$ between the hemp fibre and spray
dried powder was 1.46. It was concluded that attritor milling and spray drying process had an influence on the colour of the powder.

The air-jet milled powder became brighter compared with the spray-dried powder. $\Delta a^*$ is -2.28, implying that the spray dried powder became greener compared with the raw hemp fibre. $\Delta b^*$ is -0.71, indicating that the spray dried powder became bluer compared with the raw hemp fibre. The total colour difference $DE^*$ between the hemp fibre and spray dried powder was 3.27. It was concluded that air jet milling process had an influence on the colour change of the powder.

<table>
<thead>
<tr>
<th></th>
<th>Hemp fibre</th>
<th>Chopped powder</th>
<th>Spray dried powder</th>
<th>Air jet milled powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellowness DIN6167</td>
<td>23.61</td>
<td>23.61</td>
<td>22.83</td>
<td>21.17</td>
</tr>
<tr>
<td>GanzGriesser Whiteness</td>
<td>53.58</td>
<td>53.58</td>
<td>54.05</td>
<td>56.42</td>
</tr>
</tbody>
</table>

Table 3.7 Yellowness DIN6167 and GanzGriesser Whiteness Index of hemp fibre, chopped powder, spray dried powder and air jet milled powder.

Table 3.7 lists the Yellowness DIN6167 and GanzGriesser Whiteness of the hemp fibre, chopped powder, spray dried powder and air jet milled powder. It can be observed that the chopped powder and raw hemp fibre had the same values in yellowness DIN6167 and GanzGriesser Whiteness. The spray dried powder had 22.83 and 54.05 in Yellowness DIN6167 and GanzGriesser Whiteness indices, respectively. The spary-dried powder was less yellow and more white in colour compared with the chopped powder. The value of Yellowness DIN6167 and GanzGriesser Whiteness of the air jet milled powder
were 21.17 and 56.42 respectively, indicating that the powder after air jet milling procedure became lighter in yellowness and darker in whiteness.

Since the sample temperature was kept below 50°C during milling, the colour change cannot be attributed to the degradation of hemp. The change in colour may have caused by the reduction of particle size. After attrition milling, the particle size decreased from 87.3 μm to 4.6 μm. After air jet milling, the particle size decreased from 4.6 μm to 3.2 μm. The yellowness of powder decreased with the decrease in particle size, while the whiteness increased with the decrease of particle size, due to the increase in light scattering with the decrease in particle size.

### 3.3.6 SEM

![SEM images](image)

(a) Hemp fibre

(b) Chopped hemp snippet
Figures 3.17 (a) to (d) show the SEM images of the samples milled under different milling facilities. It was noticed from the SEM images that, after the initial chopper milling stage, the long fibres were broken into short fibre snippets. The short fibre snippets became a powder form after attritor milling. The attritor milled hemp powder ranged from 2 $\mu m$ to 8 $\mu m$ in size, which is in good agreement with the mean particle size of around 5 $\mu m$ measured using a Malvern Mastersizer 2000 particle size analyser. The morphology of the hemp powder exhibited irregular shapes, due to the repeated fracturing caused by the milling balls.

After the air jet milling process, the powder size was further reduced to around 3 $\mu m$. The powder size ranged from 1 $\mu m$ to 6 $\mu m$. The powders became close to spherical shapes. Nonetheless, the reduction in particle size was not significant; the average size was reduced only from approximately 5 $\mu m$ to approximately 3 $\mu m$. This may be due to the following reason: During air-jet milling, hemp particles collide with each other. Since hemp is a rather soft material compared to zirconia milling balls, the fracturing of hemp particles by
the collision between hemp particles themselves is not expected to cause substantial fracturing of the particles. Instead, it will cause the breakage of bulged parts of the particles, resulting in slightly smaller size and more spherical shapes.

### 3.3.7 FTIR analysis

Figure 3.18 shows FTIR spectra of the hemp powder samples after the three milling stages, i.e., after chopping, attrition milling and air-jet milling. It was observed that there were no differences among these three spectra of hemp powder samples. All three curves showed similar peak positions. This indicates that the powder fabrication process had no influence on the chemical bonding in the hemp powders. The hemp fibre was converted into a powder form through a mechanical method, rather than a chemical method, and hence it was not expected to observe chemical structures to change after a series of milling stages.
3.3.8 TG analysis

Figure 3.19 TG curves of hemp fibre and powders.

The thermogravimetric analysis (TGA) and differential thermogravimetry (DTG) curves of hemp fibre, chopped powder, spray dried powder and air jet milled powder were presented in Figure 3.19 and Figure 3.20, respectively.
It was observed from TG and DTG curves that the thermal behaviours can be divided into two stages; the first stage was in the temperature range of 30°C–120°C, which can be attributed to the evaporation of physically absorbed water, and the second stage occurred between 250°C and 400°C was caused by the decomposition of the main component, cellulose.

The weight loss in these two stages for hemp fibres and three kinds of powders was listed in Table 3.8.

<table>
<thead>
<tr>
<th></th>
<th>Weight loss in the temperature range of 30°C–120°C</th>
<th>Weight loss in the temperature range of 180°C–600°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemp fibre</td>
<td>7.9%</td>
<td>70.1%</td>
</tr>
<tr>
<td>Chopped hemp powder</td>
<td>4.3%</td>
<td>72.2%</td>
</tr>
<tr>
<td>Attrition milled hemp</td>
<td>6.4%</td>
<td>69.3%</td>
</tr>
<tr>
<td>Air jet milled hemp</td>
<td>7.6%</td>
<td>64.6%</td>
</tr>
</tbody>
</table>

Table 3.8 Weight loss of hemp fibre and different kinds of hemp powders.

It was found that the second stage started earlier for the hemp powders after attritor milling and air jet milling compared to the raw hemp fibres, as the onset temperature of the thermal degradation shifted from around 320°C to 300°C (Figure 3.19). This indicates that the crystal structure of cellulose became slightly degraded after milling, possibly due to the mechanical force applied on the hemp powders. This result was in good agreement with the XRD result which showed a reduction in crystallinity index as discussed in the next section.
3.3.9 Crystallinity

The diffraction patterns of the raw hemp fibres and hemp powders after different processing stages are presented in Figure 3.21. The XRD spectra of the raw hemp fibre and chopped hemp powder showed similar patterns. Four well defined diffraction peaks at \(2\theta = 14.50^\circ, 16.10^\circ, 22.21^\circ, \) and \(33.81^\circ\) were characteristics of the cellulose-I crystal structure. These peaks corresponds to the \((110), (110), (200)\) and \((023)\) or \((004)\) crystallographic planes, respectively[127]. Hence the chopping process didn’t change the crystal structure of cellulose in the hemp fibres. On the other hand, in the XRD patterns of both hemp powders after attrition milling and air jet milling, the diffraction peaks at \(2\theta = 14.50^\circ\) and \(16.10^\circ\) disappeared and the peak around \(22^\circ\) showed considerable broadening. The result indicates that the cellulose crystalline structure was extensively damaged by the mechanical impacts induced to the powders during milling. The reduced onset-temperature of thermal
decomposition in the milled powders as shown in Figure 3.19 also supports the damaged crystal structure of the milled powders.

### 3.3.10 Surface area and pore size

Table 3.9 lists the BET surface area (m²/g), pore volume (mL/g) and pore size (Å) of the raw hemp fibres and powders after different processing stages. It was evident in Table 3.10 that there was an increase in the BET surface area, pore volume and pore size on the hemp powders after a series of powder fabrication processes. As powder fabrication steps progressed, the surface area became higher, pore volume became larger and pore sizes became larger. As described earlier, after powder production process, there was a significant reduction on particle size of hemp powder, which contributed to the increase of the surface area of the powders. The increase in pore volume and pore size may be attributed to the damage caused onto the crystal structure.

<table>
<thead>
<tr>
<th>Sample</th>
<th>BET Surface Area(m²/g)</th>
<th>Pore volume(mL/g)</th>
<th>Pore size(Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemp fibre</td>
<td>1.25</td>
<td>0.00315</td>
<td>95.</td>
</tr>
<tr>
<td>Chopped powder</td>
<td>1.37</td>
<td>0.00372</td>
<td>96.</td>
</tr>
<tr>
<td>Spray dried powder</td>
<td>2.59</td>
<td>0.00712</td>
<td>112.</td>
</tr>
<tr>
<td>Air jet milled powder</td>
<td>3.56</td>
<td>0.00954</td>
<td>121.</td>
</tr>
</tbody>
</table>

Table 3.9 BET Surface area(m²/g), pore volume(mL/g) and pore size(Å) of hemp fibre and powders.

### 3.4 Conclusion

Hemp fibres were successfully milled into ultrafine powders after three milling steps; cutter milling, attrition milling and air-jet milling. The fibres were firstly
cut in a cutter mill into fibre snippets with a length of around 1 mm. Secondly, the fibre snippets were mixed with water to be milled in an attritor mill. The milling conditions were studied for the amount of fibre, the amount of water and milling time, within the range of 100 – 300 g fibre, 1 – 3 L water and < 6 hours of milling time. It was found that, among the three parameters, the amount of water had the strongest influence onto the particle size. Within these variable ranges of the parameters, the optimum attrition-milling condition in terms of particle size (the smaller the better) was found to be 100 g fibre, 1 L water and 6 hours of milling time. Under this condition, it was possible to produce fine hemp powders of approximately 5 μm in diameter. After attrition milling, the wet powder slurry was passed through a spray dryer to remove the excessive water. The dried powder was milled in the air jet mill, which further reduced the particle size to approximately 3 μm.

The resulting hemp powders were characterised for morphology, colour, chemical bonds, crystallinity, thermal property, surface area and pore sizes. SEM results showed the changes in the morphology of powders during the powder production process, clearly showing the stages of the fibre to be pulverized into fine powders. The datacolor results showed that the milled powder had a higher whiteness index and lower yellowness index than the raw fibre due to the reduced particle size. FTIR results indicated that the milling process had no influence on the chemical bonding of the powder. However, XRD results showed that the cellulose crystalline structure was extensively damaged by the mechanical impact induced to the powders during attritor milling and air jet milling. BET results exhibited that the surface area, pore volume and pore size of hemp powder increased after powder fabrication. These
properties will have strong influence on the performance of the powders in various applications. For example, reduced crystallinity will be advantageous for adding dye and moisture absorption properties into synthetic fibres once hemp powder is incorporated in the fibres.
Chapter 4 Evaluation of antibacterial properties of hemp plant extract

4.1 Introduction

The use of plant extracts in the fields of food and health has attracted public attention in recent years. Some natural plants are known to have medicinal effects [128]. In fact, many plants have been used to treat diseases in traditional Chinese medicine for centuries [129]. It is also reported in scholarly journals that some plant extracts possess antibacterial properties against specific bacterial strains [130, 131]. Compared with artificial products, plant extracts are safer for human health. Plant extracts with antibacterial properties can be used as ingredients for antibiotics. It can also find applications in the cosmetics industry.

Hemp has attracted the attention of people in recent years as an important fibre. As explained in Chapter 2, hemp contains unique chemicals called cannabinoids. It was reported that purified cannabinoids showed antibacterial properties against a variety of methicillin-resistant *Staphylococcus aureus* strains [28]. Recently, hemp fabrics and clothing have been sold with marketing claims as being antibacterial. However, to date, there is no scientific evidence that cannabinoids are present in commercial hemp fabrics to cause antibacterial effects.

To the best of the author’s knowledge, there are only three scholarly papers on the antibacterial properties of hemp [7-9]. In 1958, Ferenczy et al. reported that the hemp plant contains unknown antibacterial substances which can be found...
in all parts of the plant, especially in the leaf and seed [132]. This antibacterial substance was readily extracted from hemp in alkali solutions and organic solvents, but not in water. The extracts shows antibacterial properties only against gram-positive bacteria, but not gram-negative bacteria, yeast and moulds [132]. The antibacterial properties of drug-type hemp (marijuana) were reported by Appendino et al. [91]. It was shown that extracts of marijuana leaves contained the five main cannabinoids, which showed antibacterial properties against a variety of methicillin-resistant *S. aureus* strains [41]. The antibacterial properties of textile-type hemp were reported by Nissen et al., where oils from the seeds of three textile hemp varieties including Carmagnola, Fibrano and Futura showed antibacterial properties against spoilage and food-borne pathogens, and phytopathogen microorganisms [42].

However, the antibacterial properties of other parts of textile hemp have not been investigated. Cannabinoids are mainly found in the epidermal glands of hemp, and the distribution of cannabinoid-producing glands varies depending on the plant part [133]. Textile fibres are normally extracted from the segment between the epidermis and the inner woody core. However, the amount of cannabinoids in this segment of the plant is unknown. Investigations on the antibacterial properties of extracts from different parts of textile hemp will give important information on the antibacterial efficacy of hemp fabrics.

The aim of this chapter was to investigate the antibacterial properties of plant extracts from different parts of textile hemp, against some common bacterial strains. In this study, six different hemp plant samples including male hemp leaf, male hemp bark, male hemp hurd, female hemp leaf, female hemp bark and
female hemp hurd were assessed for antibacterial properties. Figure 4.1 shows the location of hemp leaf, bark and hurd. Hemp hurd is the inside woody part of the stem. Bark is the outside layer of the stem.

![Hemp Leaf, Bark, and Hurd](image)

Figure 4.1 Hemp leaf, bark and hurd

The test organisms were common pathogenic gram-positive bacterial strains including 2 strains of *S. aureus* (ATCC 25923 and ATCC 29213), *Enterococcus faecalis* (ATCC 10100), *Streptococcus pyogenes* (ATCC 10096) and *Pseudomonas aeruginosa* (ATCC 27853). *S. aureus* infection can lead to a severe disease - staphylococcal scalded skin syndrome (SSSS) [134]. The infections caused by *E. faecalis* include endocarditis, bacteremia, urinary tract infections (UTI) and meningitis [135]. The diseases caused by *S. pyogenes* ranging from mild superficial skin infections to life-threatening systemic diseases [136]. *P. aeruginosa* can infect the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections [137].
4.2 Materials

4.2.1 Plant material

Hemp samples were collected from the CSM (Commins Stainless Manufacturing) farm in Whitton, NSW in April, 2010. The leaf, bark and hurd of each gender (male and female hemp) were collected separately. The leaf samples were dried at 60°C in a constant temperature oven for 12 hours, and then ground into powder by using a pulverizer 19 cutter mill. The bark and hurd samples were air dried for one week.

4.2.2 Chemicals

Organic solvents including ethanol, ethyl acetate, chloroform, hexane, petroleum ether of an analytical grade (Sigma, Castle Hill, Australia) were employed to extract hemp plant samples.

4.2.3 Equipment

A soxhlet apparatus (Sigma, Castle Hill, Australia) and a Buchi rotary evaporator (Buchi, Noble Park, Australia) were applied in the hemp plants extraction stage. Figures 4.2 and 4.3 show the soxhlet apparatus and rotary evaporator respectively.
A cutter mill, Pulverizer 19 (Fritsch, Goshen, USA) was used to chop hemp plants samples. Figure 4.4 shows the photographic image of the cutter mill.
4.3 Experiment

4.3.1 Extraction of samples

Air-dried plant samples were washed under running water and then dried at 60°C in a constant temperature oven. The clean plant samples were then ground into powder by a pulverizer 19 cutter mill and were put through a 368μm screen. Extracts were obtained with organic solvents (ethanol, ethyl acetate, chloroform, hexane, petroleum ether, in the sequence of decreasing polarity). These organic solvents were used separately to extract 5g of each plant sample using a soxhlet apparatus. The plant samples were extracted twice with each organic solvent. The extraction process was as follows: Firstly, the hemp samples were soaked in 300ml of organic solvent for 1.5 hours, then the extract in the solvent was removed from the soxhlet apparatus. Next, another 300ml of organic solvent was added into the soxhlet apparatus, and the samples were extracted for another hour. Finally, the extracts from these two steps were mixed together. The extract was evaporated to a thick slurry under a vacuum using a rotary evaporator. The thick slurry was dried at 40°C in a vacuum oven to remove the extra water. In the end, the dried extract was stored at 4°C until use.
4.3.2 HPLC analysis

Chromatographic analysis of hemp plant extracts was carried out on an Agilent Technologies 1200 Series liquid chromatography system, equipped with a solvent degasser system, quarter nary pump and an auto sampler (Agilent Technologies, Forest Hill, Australia). Analytical grade reagents were used in the analysis. Standard solutions of CBD were purchased from Sapphire Bioscience (NSW, Australia). The identification of CBD content in hemp plant extract was carried out by using CBD’s molecular weight of 314.46 g/mol. This work was done by Mr. Brendan John Holland in School of Life and Environmental Sciences, Deakin University.

4.3.3 Test Micro-organisms

The antibacterial activities of hemp plant extracts were determined against several common gram-positive bacteria. These included 2 strains of S.aureus (ATCC 25923 and ATCC 29213), E.faecalis (ATCC 10100), S.pyogenes (ATCC 10096) and P.aeruginosa (ATCC 27853). All bacteria were maintained as frozen stocks in 80% glycerol at -80°C. All bacterial growth media used in this study was from Oxoid, Thebarton, Australia. Table 4.1 below shows the growth conditions for these bacteria.

<table>
<thead>
<tr>
<th>ATCC number</th>
<th>Bacterium</th>
<th>Growth media</th>
<th>Plate media</th>
<th>Incubation temperature</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 25923</td>
<td>S.aureus</td>
<td>Tryptic soy</td>
<td>Tryptic soy agar</td>
<td>37°C</td>
<td>24hrs</td>
</tr>
<tr>
<td></td>
<td>S.aureus</td>
<td>broth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 25923</td>
<td>S.aureus</td>
<td>Tryptic soy</td>
<td>Tryptic soy agar</td>
<td>37°C</td>
<td>24hrs</td>
</tr>
<tr>
<td>ATCC</td>
<td>Organism</td>
<td>Media</td>
<td>Temperature</td>
<td>Time</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
<td>------------------------</td>
<td>-------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>10100</td>
<td><em>E. faecalis</em></td>
<td>Brain heart infusion +5% defibrinated sheep blood</td>
<td>37°C</td>
<td>24hrs</td>
<td></td>
</tr>
<tr>
<td>10096</td>
<td><em>S. pyogenes</em></td>
<td>Brain heart infusion +5% defibrinated sheep blood</td>
<td>37°C</td>
<td>24hrs</td>
<td></td>
</tr>
<tr>
<td>27853</td>
<td><em>P. aeruginosa</em></td>
<td>Nutrient broth</td>
<td>Nutrient agar</td>
<td>37°C</td>
<td>24-48hrs</td>
</tr>
</tbody>
</table>

Table 4.1 Growth conditions of bacteria

### 4.3.4 Media preparation

Tryptic soy broth powder, Nutrient broth powder, Tryptic soy agar powder, Nutrient agar powder and Brain heart infusion powder were purchased from Oxoid (Thebarton, Australia).

Tryptic soy broth was prepared by dissolving 30g Tryptic soy broth powder in 1 litre distilled water. Brain heart infusion was prepared by dissolving 37g Brain heart infusion powder in 1 litre distilled water. Nutrient broth was prepared by dissolving 13g Nutrient broth powder in 1 litre distilled water. Tryptic soy agar was prepared by dissolving 40g Tryptic soy agar powder in 1 litre distilled water. Nutrient agar was prepared by dissolving 28g Nutrient agar powder in 1 litre distilled water. These media were then sterilized by autoclaving at 0.1 MPa pressure and 121°C for 30 min. The growth media (Tryptic soy broth, Brain heart infusion, Nutrient broth) were cooled down, then stored at 4°C until use. 5% defibrinated sheep blood was added into the Tryptic soy agar to make the Tryptic soy agar +5% defibrinated sheep blood plate media. These three types of agar (Tryptic soy agar, Tryptic soy agar +5% defibrinated sheep blood, Nutrient agar,
agar) were poured into Petri plates in quantities of 10ml, and left on a flat surface to solidify. Both growth media and plate media were stored at 4°C until use.

4.3.5 **Bacteria preparation**

Frozen stocks of five bacterial strains were transferred to agar plates by the streaking plate method. Four different kinds of strips were streaked with a wire loop, from high density to low density. In between each streak, the loop was flamed on the burner until it became red to kill residual bacteria. The streaked plates were then incubated at 37°C for 24h. Three isolated colonies of the same morphological type were selected from each agar plate culture. The top of each colony was touched with a loop, and the bacteria transferred into a tube containing 4 to 5 ml broth medium. The broth culture was incubated at 37°C until the turbidity of 0.5 McFarland standard was achieved or exceeded (usually 2 to 6 hours). The absorbance at 625 nm was 0.08 to 0.10 for the 0.5 McFarland standard. The correct density of the broth culture was verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The turbidity of the actively growing broth culture was adjusted with broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10⁸ CFU/ml for all bacterial strains.

4.3.6 **Disc diffusion assay**

The concentration of each extract was 5mg/ml for the 6 hemp plants samples. 5mg of dry powder extract was dissolved in 200ul DMSO using a vortex mixer,
then 800ul sterilized water was added to reach the final concentration. Two concentrations for cannabidiol (CBD) were used, one was 1mg/ml, another was 0.01mg/ml. 1mg CBD was dissolved in 200ul DMSO using a vortex mixer, then 800ul sterilized water was added to reach the 1mg/ml concentration. The solution with the CBD concentration of 1mg/ml was diluted 1:100 to reach the 0.01mg/ml CBD concentration. Due to the use of DMSO as a vehicle, its influence on antibacterial property also needed to be evaluated. 20% (v/v) DMSO was therefore selected as a negative control. Gentamicin, an antibiotic, was the positive control. 25ul of each solution was pipetted on a sterile 6mm diameter paper disc and air dried.

The broth culture was spread on the agar plate using a cotton stick. The air-dried disc paper was applied onto the incubated agar plate with a clockwise sequence of gentamicin disc, 1mg/ml CBD disc, 0.01mg/ml CBD disc, extract disc, 20% (v/v) DMSO disc. The plates were incubated at 37°C in the incubator upside down overnight. Microbial growth inhibition was determined by measuring the diameter of the clear zone of inhibition of growth around each disc, and recorded as diameter of inhibition zone (DIZ) in millimeters.

4.4 Results

<table>
<thead>
<tr>
<th></th>
<th>Ethanol</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Hexane</th>
<th>Petroleum ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polarity index[138]</td>
<td>4.3</td>
<td>4.3</td>
<td>4.4</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Female hemp leaf</td>
<td>0.83</td>
<td>0.83</td>
<td>0.21</td>
<td>~0.19</td>
<td>~0.19</td>
</tr>
<tr>
<td>Male hemp leaf</td>
<td>0.74</td>
<td>0.75</td>
<td>0.68</td>
<td>0.48</td>
<td>0.34</td>
</tr>
<tr>
<td>Female hemp bark</td>
<td>n.a</td>
<td>~0.03</td>
<td>~0.02</td>
<td>n.a</td>
<td>~0.03</td>
</tr>
</tbody>
</table>
Table 4.2 CBD content (mMole) in extracts and polarity number of the solvent used.

Table 4.2 shows the CBD content in each hemp plant sample extract by five organic solvents. Some results are below the experimental detection limit. Polarity data of the five organic solvents is included in the table. CBD content was determined by HPLC analysis, this work was done by Mr. Brendan John Holland in School of Life and Environmental Sciences, Deakin University.

HPLC is a reliable and accurate method of analysis, it is useful for sample profiling and to gain a comparison between samples of different origin.

Chromatographic analysis was carried out using an Agilent Technologies 1200 Series liquid chromatography system, equipped with a solvent degasser, quaternary pump and auto sampler (Agilent Technologies, Vic, Australia). Extract solutions were filtered through a 0.45 mm nylon membrane prior to HPLC analysis. All separations were carried out using an Agilent Eclipse XDB-C18 column (5 mm; 4.6mm_150mm) with an injection volume of 10 ml and mobile phase flow rate of 0.5ml/min. Quantitative results were obtained using peak areas.

Due to the missing data in other samples, extracts from female hemp leaf and male hemp leaf were selected to compare the extraction ability of five organic solvents. Hemp plant samples were extracted with organic solvents with different polarity. The CBD content decreased with the decrease in the polarity index of organic solvents from 4.3 for both ethanol and ethyl acetate to 0.01 for petroleum ether. This indicates that the extraction ability decreased with the reduction in the polarity index of the organic solvents. This is in contrast to the

<table>
<thead>
<tr>
<th></th>
<th>Male hemp bark</th>
<th>Female hemp hurd</th>
<th>Male hemp hurd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>~0.05</td>
<td>0.58</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>~0.03</td>
<td>~0.04</td>
<td>~0.06</td>
</tr>
<tr>
<td></td>
<td>n.a</td>
<td>~0.02</td>
<td>~0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~0.05</td>
<td>~0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n.a</td>
<td>n.a</td>
</tr>
</tbody>
</table>
general belief that CBD is insoluble in polar liquid [91]. Ethyl acetate appears to be the most suitable solvent to extract CBD out of plant samples. A CBD molecule has two OH- groups and that may be the reason why the solvents with mid-range polarity can extract CBD more than the solvents with extremely high or low polarity.

Ethyl acetate extract was selected to compare the CBD content variation among plant samples due to the missing data in other organic solvent extracts. We found that hemp leaf and hurd had higher CBD content than hemp bark. It is concluded that CBD is mainly prevalent in hemp leaf and hurd.

Figure 4.5 shows the antibacterial results of gentamicin, 0.1mg/ml CBD, 0.01mg/ml CBD and 20% (v/v) DMSO against *S.aureus* ATCC 25923, *S.aureus* ATCC 29213, *E.faecalis* ATCC 10100, *S.pyogenes* ATCC 10096 and *P.aeruginosa* ATCC 27853. In each figure, the paper disc with the largest zone of inhibition was the positive control gentamicin disc.

<table>
<thead>
<tr>
<th></th>
<th><em>S. aureus</em> ATCC 25923</th>
<th><em>S. aureus</em> ATCC 29213</th>
<th><em>E. faecalis</em> ATCC 10100</th>
<th><em>S. pyogenes</em> ATCC 10096</th>
<th><em>P. aeruginosa</em> ATCC 27853</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Gentamicin</td>
<td>16 ± 0.1</td>
<td>15 ± 0.1</td>
<td>14 ± 0.1</td>
<td>13 ± 0.1</td>
<td>20 ± 0.1</td>
</tr>
<tr>
<td>0.1mg/ml CBD</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.01mg/ml CBD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20% (v/v) DMSO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4.3 Inhibition zone of control discs (mm)

Table 4.3 shows the antibacterial result of gentamicin, 0.1mg/ml CBD, 0.01mg/ml CBD and 20% (v/v) DMSO. Data are presented as mean ± SEM value. The average diameter of inhibition zone for gentamicin was 16mm against
S. aureus ATCC 25923, 15mm against S. aureus ATCC 29213, 14mm against E. faecalis ATCC 10100, 13mm against S. pyogenes ATCC 10096 and 20mm against P. aeruginosa ATCC 27853 respectively. Based on the size of inhibition zone, it is concluded that gentamicin, as a positive control, showed strong antibacterial properties against these five bacterial strains.

The 0.1mg/ml CBD disc exhibited a 8mm diameter zone of inhibition against both S. aureus ATCC 25923 and S. aureus ATCC 29213, while it exhibited no zone of inhibition against E. faecalis ATCC 10100, S. pyogenes ATCC 10096 and P. aeruginosa ATCC 27853.

The 0.01mg/ml CBD and DMSO discs displayed no zone of inhibition against any bacterial strain.

4.4.1 Ethanol extracts
Figure 4.5 Antibacterial effect of ethanol extracts

<table>
<thead>
<tr>
<th></th>
<th>S.aureus ATCC 25923</th>
<th>S.aureus ATCC 29213</th>
<th>E.faecalis ATCC 10100</th>
<th>S.pyogenes ATCC 10096</th>
<th>P.aeruginosa ATCC 27853</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male hemp leaf</td>
<td>7.5±0.1</td>
<td>7.5±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.74</td>
</tr>
<tr>
<td>Female hemp leaf</td>
<td>7.5±0.1</td>
<td>7.5±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.83</td>
</tr>
<tr>
<td>Male hemp bark</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>~0.05</td>
</tr>
<tr>
<td>Female hemp bark</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>Male hemp hurd</td>
<td>7.0±0.1</td>
<td>7.0±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>Female hemp hurd</td>
<td>7.0±0.1</td>
<td>7.0±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
</tbody>
</table>

Table 4.4 Antibacterial effect of ethanol extract
Figure 4.5 (a)–(f) and Table 4.4 show the antibacterial effect of ethanol extracted hemp parts (male hemp bark, male hemp hurd and male hemp leaf, female hemp bark, female hemp hurd and female hemp leaf) against *S.aureus* ATCC 25923, *S.aureus* ATCC 29213, *E.faecalis* ATCC 10100, *S.pyogenes* ATCC 10096 and *P.aeruginosa* ATCC 27853.

Ethanol extract of male hemp leaf had a $7.5\pm0.1\text{mm}$ zone of inhibition against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, while it exhibited no zone of inhibition against the other three bacterial strains.

Similarly, ethanol extract of female hemp leaf had a $7.5\pm0.1\text{mm}$ zone of inhibition against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, but no zone of inhibition against the other three bacteria strains.

Ethanol extracts of male hemp hurd and female hemp hurd had a $7.0\pm0.1\text{mm}$ zone of inhibition against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, but no zone of inhibition against the other three bacteria strains.

Ethanol extracts of male hemp bark and female hemp bark had no zone of inhibition against these five bacteria strains.

Hemp plant contains unique chemicals called cannabinoids, and the antibacterial properties of hemp plant are associated with the cannabinoid chemicals contained in it. Hemp is known to contain a CBD content of greater than 0.3% by mass [5, 12, 14], and smaller amounts of other cannabinoids. As CBD is the most abundant cannabinoid in hemp, it is proposed that it is the cannabinoid which is responsible for anti-bacterial activity.
In Table 4.4, it is shown that hemp leaf has a much higher CBD concentration than hemp bark. CBD concentration in male hemp leaf and female hemp leaf are 0.74 and 0.83, respectively. However, CBD concentration in male hemp bark is 0.05, which is far less than that of hemp leaf. As CBD has been reported to be the substance that is responsible for the antibacterial property in hemp, we suggest that the antibacterial property of hemp leaf may be attributed to the CBD content in hemp leaf.

It is observed that hemp hurd has no CBD in it, however, both male and female hemp hurd show almost equal antibacterial properties as hemp leaf. The antibacterial properties of hemp hurd is not caused by CBD, it may be due to other chemical present in the hurd extract. The result may be explained on the basis of other active constituents present like tannins or other cannabinoids which may be present in greater amounts in the hurd part of the hemp plant.

### 4.4.2 Ethyl acetate extract

![Image](image_url)
Figure 4.6 Antibacterial effect of ethyl acetate extracts

<table>
<thead>
<tr>
<th></th>
<th>S. aureus ATCC 25923</th>
<th>S. aureus ATCC 29213</th>
<th>E. faecalis ATCC 10100</th>
<th>S. pyogenes ATCC 10096</th>
<th>P. aeruginosus ATCC 27853</th>
<th>CBD concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male hemp leaf</td>
<td>7.3±0.1</td>
<td>7.3±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.75</td>
</tr>
<tr>
<td>Female hemp leaf</td>
<td>7.4±0.1</td>
<td>7.4±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.82</td>
</tr>
<tr>
<td>Male hemp bark</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>~0.03</td>
</tr>
<tr>
<td>Female hemp bark</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>~0.03</td>
</tr>
<tr>
<td>Male hemp hurd</td>
<td>6.5±0.1</td>
<td>6.5±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.48</td>
</tr>
<tr>
<td>Female hemp hurd</td>
<td>6.4±0.1</td>
<td>6.4±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Table 4.5 Antibacterial effect of ethyl acetate extract

Figure 4.6 (a) to (f) and Table 4.5 show the antibacterial effect of ethyl acetate extracted hemp parts (male hemp bark, male hemp hurd and male hemp leaf, female hemp bark, female hemp hurd and female hemp leaf) against *S.aureus* ATCC 25923, *S.aureus* ATCC 29213, *E.faecalis* ATCC 10100, *S.pyogenes* ATCC 10096 and *P.aeruginosa* ATCC 27853. In each figure, ML means male leaf, FL means female leaf, MB means male bark, FB means female bark, MH means male hurd and FH means female hurd.

Ethyl acetate extract of male hemp leaf had a 7.3±0.1mm zone of inhibition against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, while it exhibited no inhibition against the other three bacterial strains.

Similarly, ethyl acetate extract of female hemp leaf had a 7.4±0.1mm zone of inhibition against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, but no inhibition against the other three bacterial strains.

The zone of inhibition for ethyl acetate extract of male hemp hurd was 6.5±0.1mm against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, but it did not show inhibition against the other three bacterial strains. Ethyl acetate extract of female hemp hurd had a 6.4±0.1mm against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, however, it had no zone of inhibition against the other three bacterial strains.

Ethyl acetate extracts of male hemp bark and female hemp bark had no zone of inhibition against these five bacterial strains.
It is observed from table 4.5 that CBD concentrations in male and female hemp leaf are 0.75 and 0.82, respectively. However, the CBD concentration in both male and female hemp bark is ~0.04, which is far less than that of hemp leaf.

It is found in our experiment that the higher concentration of CBD, the stronger the antibacterial property will be. Therefore, it is suggested that the antibacterial property of hemp leaf may be attributed to the high CBD concentration in hemp leaf.

### 4.4.3 Chloroform extract

![Chloroform extract images](a) (b) (c) (d)
Figure 4.7 Antibacterial effects of chloroform extracts

Table 4.6 Antibacterial effect of chloroform extracts

<table>
<thead>
<tr>
<th></th>
<th>S.aureus ATCC 25923</th>
<th>S.aureus ATCC 29213</th>
<th>E.faecalis ATCC 10100</th>
<th>S.pyogenes ATCC 10096</th>
<th>P.aeruginosa ATCC 27853</th>
<th>CBD concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male hemp leaf</td>
<td>6.8±0.1</td>
<td>6.8±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.71</td>
</tr>
<tr>
<td>Female hemp leaf</td>
<td>6.7±0.1</td>
<td>6.7±0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.68</td>
</tr>
<tr>
<td>Male hemp bark</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>~0.04</td>
</tr>
<tr>
<td>Female hemp bark</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>~0.04</td>
</tr>
<tr>
<td>Male hemp hurd</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>~0.05</td>
</tr>
<tr>
<td>Female hemp hurd</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>~0.04-0.05</td>
</tr>
</tbody>
</table>

Figure 4.7 (a) to (f) and Table 4.6 show the antibacterial effect of chloroform extracted hemp parts (male hemp bark, male hemp hurd and male hemp leaf, female hemp bark, female hemp hurd and female hemp leaf) against *S.aureus* ATCC 25923, *S.aureus* ATCC 29213, *E.faecalis* ATCC 10100, *S.pyogenes* ATCC 10096 and *P.aeruginosa* ATCC 27853. In each figure, ML means male.
leaf, FL means female leaf, MB means male bark, FB means female bark, MH means male hurd and FH means female hurd.

Chloroform extract of male hemp leaf has a 6.8±0.1mm zone of inhibition against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, while it exhibited no zone of inhibition against the other three bacteria strains.

Similarly, chloroform extract of female hemp leaf had a 6.7±0.1mm zone of inhibition against both *S.aureus* ATCC 25923 and *S.aureus* ATCC 29213, however, but no zone of inhibition against the other three bacteria strains.

Chloroform extracts of male hemp hurd, female hemp hurd, male hemp bark and female hemp bark had no zone of inhibition against these five bacterial strains.

It is observed from Table 4.6 that the CBD concentration in hemp leaf is much higher than hemp hurd and bark. CBD concentration in male hemp leaf and female hemp leaf are 0.71 and 0.68, respectively. However, CBD concentration in both male and female hemp bark and hurd is around 0.04, which is far less than that of hemp leaf. The antibacterial property of hemp leaf may be attributed to the high CBD concentration in hemp leaf.
4.4.4 Hexane extract

Figure 4.8 Antibacterial effect of hexane extracts
Figures 4.8 (a) to (f) and Table 4.7 show the antibacterial effect of hexane extracted hemp parts (male hemp bark, male hemp hurd and male hemp leaf, female hemp bark, female hemp hurd and female hemp leaf) against *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *E. faecalis* ATCC 10100, *S. pyogenes* ATCC 10096 and *P. aeruginosa* ATCC 27853. In each figure, ML means male leaf, FL means female leaf, MB means male bark, FB means female bark, MH means male hurd and FH means female hurd.

Table 4.7 shows that CBD concentration in hemp leaf is higher than hemp hurd and bark. CBD concentration in male hemp leaf and female hemp leaf are 0.47 and 0.14, respectively. However, both CBD concentration in male hemp bark and female hemp bark is 0.04, which is far less than that of hemp leaf. All of these hexane extracted hemp plant parts exhibited no zone of inhibition against these five bacterial strains. In the hexane extract, the highest concentration of CBD is found to be 0.47, which is lower than the three extractions above (ethanol 0.83, ethyl acetate 0.82, chloroform 0.71). This observation together
with the above results suggests that the concentration of CBD plays an important role in antibacterial property.

4.4.5 Petroleum ether extract
Figures 4.9 (a) to (f) and Table 4.8 show the antibacterial effect of petroleum ether extracted hemp parts (male hemp bark, male hemp hurd and male hemp leaf, female hemp bark, female hemp hurd and female hemp leaf) against \textit{S. aureus} ATCC 25923, \textit{S. aureus} ATCC 29213, \textit{E. faecalis} ATCC 10100, \textit{S. pyogenes} ATCC 10096 and \textit{P. aeruginosa} ATCC 27853. In each figure, ML means male leaf, FL means female leaf, MB means male bark, FB means female bark, MH means male hurd and FH means female hurd.

We can observe from Table 4.8 that the CBD concentration in hemp leaf is higher than hemp hurd and bark. CBD concentration in male hemp leaf and female hemp leaf are 0.34 and 0.20, respectively. However, CBD concentration in both male and female hemp bark and hurd is around 0.04, which is less than
that of hemp leaf. All of these petroleum ether extracted hemp plant parts exhibited no zone of inhibition against these five bacteria strains. In the petroleum ether extract, the highest concentration of CBD is found to be 0.34, which is lower than the three extractions above (ethanol 0.83, ethyl acetate 0.82, and chloroform 0.71). This observation together with the above results suggests that the concentration of CBD plays an important role in antibacterial property, which is consistent with the result obtained from hexane extraction.

4.5 Discussion

In this study, it has been shown that the extracts from hemp leaf and hurd exhibit antibacterial properties against *S. aureus*, whilst the extract from hemp bark showed no antibacterial property against *S. aureus*. As evident in Table 4.5, there was a strong correlation between the antibacterial property and the amount of CBD in the extracts. The results indicate the antibacterial efficacy of hemp may be primarily due to the presence of CBD, but there are also other antibacterial chemicals present.

The results have particular significance for the textile industry. As explained earlier, many textile retailers claim that hemp fabrics possess antibacterial properties, but there is limited scientific evidence to support the claims. Textile fibres are made from hemp bark, which did not show antibacterial properties against *S. aureus* nor the presence of a substantial amount of cannabinoids. The results of this study indicate that, if hemp fabric has antibacterial properties, it is not caused by cannabinoids.
Furthermore, the results of this research have an important implication for the textile industry. Traditionally, bark is the only part of hemp used to make textile fibres. The other plant parts including hurd and leaf are regarded as industrial waste. However, based on the findings of this research, hemp leaf and hurd can find applications to produce antibacterial agents. The results of this research will open up new ways of using waste materials in the hemp industry.

The results of this study also provide the opportunity to develop a new natural antibacterial agent. As explained in Chapter 2, the extensive application of traditional antibiotics has caused antimicrobial resistance effects to many bacterial strains. Therefore, there is an urgent need for new antimicrobial agents to replace the existing antibiotics. Hemp extract can be used as a new generation of antibiotics that eco-friendly, biodegradable and non-toxic to the human body [98]. For example, they can be potentially used as ingredients in the cosmetic industry and could be added into personal care products, such as shampoos & lotions, to enhance the antibacterial properties of these products. Many other industries including pharmaceutical, personal-care, industrial paint, and medical sectors may benefit from hemp extracts for antibacterial applications.

This study has some limitations, which are listed below:

- The extraction of hemp plant was performed only once on each plant sample. In order to study the reproducibility and increase the accuracy of experimental results, the extraction procedure is suggested to be repeated.
- This study did not address the fact that the amount of cannabinoids present is highly dependent on the hemp variety and growing conditions. The
growing conditions of hemp include the location, climate, fertilizer, irrigation, plant density, growing period, time of planting and harvesting. All of these growing conditions have a strong influence on the CBD content in hemp plants. For example, the young hemp plant has much less cannabinoids than a mature age plant [91]. The amount of cannabinoids in hemp also varies due to genetic influence [29]. The hemp plant in this project was obtained from CSM (Commins Stainless Manufacturing) farm in Whitton, NSW in April, 2010. This hemp was planted in August 2009 and harvested in April 2010, the growing period was eight months.

- This research only tested the antibacterial properties of hemp plant extract. It did not investigate the antibacterial properties of commercial hemp fibre and hemp fabrics.

### 4.6 Conclusion

This study has evaluated the antibacterial activity of hemp plant extract against common gram-positive bacterial strains including *S.aureus* (ATCC 25923 and ATCC 29213), *E.faecalis* (ATCC 10100), *S.pyogenes* (ATCC 10096) and *P.aeruginosa* (ATCC 27853).

We showed that the organic solvent ethyl acetate is a suitable solvent to extract the antibacterial chemical CBD from hemp plants. It is concluded that organic solvents with a polar index of approximately 4 have higher efficacy in extracting CBD out of hemp plant, than extremely polar or non-polar organic solvents.

The hemp plant extracts showed antibacterial properties only against *S.aureus* (ATCC 25923 and ATCC 29213). None of the extracts showed antibacterial
properties against the other bacterial strains including *E. faecalis* (ATCC 10100), *S. pyogenes* (ATCC 10096) and *P. aeruginosa* (ATCC 27853).

There was a strong correlation between the antibacterial property and the amount of CBD in the extracts. It was noted that the antibacterial chemical CBD mainly existed in hemp leaf and, to a letter extent, in hurd. Hemp leaf and hurd extract showed antibacterial properties, while hemp bark extract in all tested organic solvents did not show antibacterial properties.

The results of this study have important implications for the textile industry in which it raises questions to the commercial claim for hemp fabrics to be antibacterial and also it suggest the use of industrial waste (leaves and hurd) for value-added applications to produce a new type of natural antibacterial agents.
Chapter 5 Evaluation of antibacterial properties of hemp powder

5.1 Introduction

Microorganisms such as bacteria and fungi grow readily on textile products. In terms of clothing, the growth of these microorganisms can have a negative effect on the wearer. Therefore, there is a major requirement in the antimicrobial textile market [87]. A large amount of antimicrobial textile products have already appeared in the market. It is reported that the production of antibacterial textiles was 100,000 tons in 2000 all over the world [88]. Antimicrobial textiles is one of the quickest developing areas in the textile industry. It was shown that the production of antibacterial textiles is increasing at a rate of 15% per year worldwide [89]. Among these antimicrobial textiles products, lingerie, socks, shoe linings and sportswear occupy about 85% of the total production [87]. Textile materials with antibacterial properties can be also applied in many fields, such as wound healing and bandaging [90].

Surface treatment is one of the main methods in antibacterial finishing of fabric [139]. However, it always involves the application of potentially harmful chemicals which are not environmentally friendly. The use of silver nanoparticles has raised concerns regarding skin penetration [140]. Therefore, it is urgently needed to find an eco-friendly substance to replace these potentially harmful antibacterial agents.

Natural fibres are widely used in textile industry. Nowadays, natural fibre has found new applications in many other fields [141]. Among them, there has been
increasing demand for powder fabricated from textile fibres in recent years. It is reported that powder from natural fibres has become a new class of material in biomedical applications [142].

Hemp is an important natural fibre that may also be manufactured into powdered forms. Since hemp is reported to possess antibacterial properties [132], the hemp powder is also expected to exhibit antibacterial properties. However, there are a very limited number of scientific reports on the antibacterial property of hemp. Only two research papers were published on the antibacterial properties of medical and textile hemp since the 1960s [10,11].

It was reported by Appendino et al. that drug type hemp (marijuana) contains antibacterial cannabinoids and that the five main cannabinoids showed antibacterial properties against a variety of methicillin-resistant Staphylococcus aureus strains [41]. In the research of Nissen et al., three textile hemp varieties including Carmagnola, Fibranova and Futura were tested for antibacterial properties. Their study showed that essential oils of textile hemp, especially those of Futura, had antibacterial properties against spoilage and food-borne pathogens and phytopathogen microorganisms [42]. However, this research was about the hemp seed oil, not the hemp bark which textile fibre is normally made from.

In the previous chapter, it was shown that textile hemp contains CBD and has antibacterial properties against S. aureus. However, the antibacterial properties of hemp powder have not yet been investigated.
The aim of this study was to investigate the antibacterial properties of degummed hemp powder and raw hemp powders against gram-positive bacteria *S. aureus* (ATCC 25923).

### 5.2 Experiment

#### 5.2.1 Materials

##### 5.2.1.1 Media

Tryptic soy broth (TSB) powder and Tryptic Soy Agar (TSA) powder were purchased from Oxoid (Adelaide, Australia). Tryptic soy broth was prepared by dissolving 30g Tryptic soy broth powder in 1 litre distilled water. Tryptic soy agar was prepared by dissolving 40g Tryptic soy agar powder in 1 litre distilled water. These media were then sterilized by autoclaving at 0.1 MPa pressure and 121°C for 30 min. Tryptic soy agar was poured into Petri plates in quantities of 10ml, and left on a flat surface to solidify. Both growth media and plate media were cooled down and stored at 4°C until use.

##### 5.2.1.2 Microorganisms

The antibacterial activity of hemp powder was determined against gram-positive bacteria *S. aureus* (ATCC 25923), which was obtained from ATCC. All bacteria were maintained as frozen stocks in 80% glycerol at -80°C. Working cultures were grown and maintained on Tryptic Soy Agar.
5.2.1.3 Test sample

Two kinds of hemp powder including raw hemp powder and degummed hemp powder were tested for antibacterial ability. 100% cellulose powder (Sigma, Castle Hill, Australia) was selected as a negative control in the antibacterial test.

In the textile industry, hemp bark is used as a raw material to produce fibre. The raw hemp fibre was prepared by separating the bark from the hurd, which was performed by hand. Degumming process is to separate fibre bundle and remove non-cellulosic substances. Hemp fibre which went through degumming process is called degummed hemp fibre. The powder was prepared from the raw hemp bark according to the procedures discussed in Chapter 3, but without using jet-milling. (see 1.2.3.2)

5.2.2 Equipment

5.2.2.1 Incubator

An incubator (Bioline, SA, Australia) is essential for experimental work in microbiology to culture bacteria. The purpose of the incubator is to grow and
maintain microbiological cultures, as it can maintain optimal temperature, humidity and other conditions such as the oxygen and carbon dioxide content inside the incubator. The most commonly used temperature for bacteria such as *S.aureus* is 37°C, as these organisms grow well under such conditions. Figure 5.1 shows the incubator.

### 5.2.2.2 Autoclave

![Figure 5.2 Autoclave](image)

The autoclave (Tomy, NY, USA) is used to sterilize equipment and supplies, using high pressure and high temperature of 121°C. Figure 5.2 shows the autoclave.
5.2.2.3 Spectrophotometer

A spectrophotometer (Beckman, NSW, Australia) is a device to measure the optical density of broth culture. Figure 5.3 shows the spectrophotometer.

5.2.2.4 Plate spectrophotometer

The plate spectrophotometer (Bio Rad, Hercules, CA, USA) was a UV–visible absorbance spectrophotometer which was used to analyse the broth culture in microtiter wells or cuvettes. Figure 5.4 shows the plate spectrophotometer.
5.2.2.5 Vortex mixer

Vortex mixer (Ratek instruments, VIC, Australia) is a simple device to mix small vials of liquid. It has different speed settings and can be also set to run only when downward pressure is applied to the rubber cup. When a test tube is pressed into the rubber cup, a vortex is created in the inside liquid. Figure 5.5 shows the vortex mixer.

5.2.2.6 Plate shaker

The plate shaker (Sciquip, Merrington, UK) is used with microplates, in a variable speeds range from 40 to 1100 rpm. The running time is able to be set from 30 seconds to 5 minutes. Figure 5.6 shows the plate shaker.
5.2.3 Experimental methods

5.2.3.1 Chemical degumming of hemp fibre

Hemp fibre was prepared by degumming treatment on raw hemp fibre. Firstly, raw hemp was washed under running water at 40°C for 20 mins to remove dust on the surface. Hemp was then dried in a fan oven at 50°C until the excess water was evaporated. 200g Hemp fibres were soaked in 2L solution containing 300g sodium hydroxide (pH= 12) and heated to 80°C for 2 h, then they were removed from the solution. Consequently, treated hemp fibres were rinsed thoroughly under running water at 80°C to remove the residuals adhered to the fibres, such as sodium hydroxide, hemicelluloses and lignin. Finally, the fibres were dried in a fan oven set at 40°C for 10 h to remove free water. In the end, the dried hemp fibres were put into a glass container.

5.2.3.2 Hemp powder fabrication

Raw hemp powder and degummed hemp powder were produced by mechanical milling method which was described in Chapter 3. The powder fabrication process includes chopping, attritor ball milling and spray drying.

All powders possess a similar mean particle size, which was represented by $d(0.5)$. 100% cellulose powder had a mean particle size of 5$\mu m$, while raw hemp powder and degummed hemp powder had a particle size of 5.4$\mu m$ and 6.2$\mu m$, respectively. Figure 5.7 shows the particle size distribution of raw hemp powder and degummed hemp powder. Figure 5.8 and Figure 5.9 show the morphology of degummed hemp powder and raw hemp powder respectively, both of them have rough surface.
Figure 5.7 Particle size distribution of two kinds of powders

Figure 5.8 SEM image of attritor milled degummed hemp powder

Figure 5.9 SEM image of attritor milled raw hemp powder
5.2.3.3 Bacterial culture preparation

The *S.aureus* culture was prepared at the beginning of the experiment. The growth method for *S.aureus* culture was as follows:

One isolated colony was picked up from agar plate culture. A loop was employed to touch the top of the colony, the loop was then dipped in a tube which contained 5 ml of tryptic soy broth medium, the bacteria was transferred into the broth by stirring the loop.

The broth culture was then incubated in the incubator at 37°C. The OD value was measured after 4 hours until it achieved or exceeded the turbidity of 0.5 McFarland standard (usually 4 to 6 hours). If the optical density value was above 0.5 McFarland standard, the broth culture was adjusted with broth to obtain a turbidity of 0.5 McFarland standard.

0.5 McFarland standard optical density value of the broth culture was 0.095 to 0.10 at the wavelength of 625 nm, which means the culture contained approximately 1 to 2 x 10^8 CFU/ml for *S.aureus*. The optical density of the culture was measured on a spectrophotometer. Colony-forming unit (CFU) is a measure of viable bacterial or fungal numbers. The results are given as CFU/mL (colony-forming units per milliliter) for liquids, and CFU/g (colony-forming units per gram) for solids.

*S.aureus* culture with an OD of 0.1, which means the concentration of bacteria was 10^8 CFU/ml, was used in the following test.
5.2.3.4 Bacterial culture concentration

The initial bacterial culture concentration has a strong influence on the antibacterial experiment result, which makes it essential to select the correct initial concentration. Therefore, a preliminary experiment to choose the bacterial culture concentration to start with was performed prior the main experiment. In this preliminary experiment, a 96 well flat-bottom plate was used. Figure 5.10 shows the 96 well flat-bottom plate. These 96 wells were distributed into 8 rows and 12 columns on the plates, each well can contain a maximum volume of 300μl.

![96-well flat-bottom plate](image)

On the plate, wells in rows 1 and 2 were filled with Tryptic Soy Broth as control using a multichannel pipette. For the following two rows, from column one to column twelve, stock culture was seeded in different dilutions for two rows. These dilutions included 1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048. After that, the 96 well flat-bottom plate was placed on plate shaker with the shaking speed of 150rpm and incubated at 37°C in an incubator. An initial reading of optical density was performed on a plate spectrophotometer at a wavelength of 625nm, then another 6 readings were performed at hourly intervals, the last reading was performed at 24hours.
5.2.3.5 Antibacterial test on hemp powder

The antibacterial test was performed on the two types of hemp powder, and 100% cellulose powder was utilized as a negative control due to its non-antibacterial property. Six different amounts of powder including 1mg, 2mg, 4mg, 8mg, 16mg and 32mg were tested.

The antibacterial activity of hemp powder was evaluated using the optical density method. The antibacterial test on hemp powder was performed as follows.

Place powders into tubes

Pippet 5ml broth into control sample tubes

Adjust OD value of S.aureus culture to 0.1

Pippet 5ml diluted culture into test sample tubes

Pippet 150µl solution into 96 well plate and take an initial reading of OD value

Incubate test sample

Measure OD value at an hourly interval for 8 h
Firstly, powders were placed into each tube based on the powder concentration series. The same amount of powder concentration series was placed into the control sample and test sample groups. 5ml broth was pipetted into the control sample group as well. The *S.aureus* culture was adjusted to OD value of 0.1 according to the growth method mentioned above, which meant the concentration of bacteria was $10^8$ CFU/ml. The *S.aureus* culture was diluted in the ratio according to the preliminary test. 5ml diluted culture was pipetted into the 21 tubes of test sample group. Table 5.1, table 5.2 and table 5.3 below showed the antibacterial test on each kind of powder respectively.

150ul solution from the samples was pipetted into a 96 well flat-bottom plate, an initial reading of the optical density was performed on plate spectrophotometer at the wavelength of 625nm. These two kinds of samples were incubated at 37°C with a shaking speed of 200rpm. Optical density of these samples was measured by a plate spectrophotometer at the wavelength of 625nm at an hourly interval for 8 readings. This experiment was performed in triplicate.

<table>
<thead>
<tr>
<th>Control sample</th>
<th>Test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  broth</td>
<td>broth+bacteria culture</td>
</tr>
<tr>
<td>2  broth+1mg 100% cellulose powder</td>
<td>broth+1mg cellulose powder+bacteria culture</td>
</tr>
<tr>
<td>3  broth+2mg 100% cellulose powder</td>
<td>broth+2mg cellulose powder+bacteria culture</td>
</tr>
<tr>
<td>4  broth+4mg 100% cellulose powder</td>
<td>broth+4mg cellulose powder+bacteria culture</td>
</tr>
<tr>
<td>5  broth+8mg 100% cellulose powder</td>
<td>broth+8mg cellulose powder+bacteria culture</td>
</tr>
<tr>
<td>6  broth+16mg 100% cellulose powder</td>
<td>broth+16mg cellulose powder+bacteria culture</td>
</tr>
<tr>
<td>7  broth+32mg 100% cellulose powder</td>
<td>broth+32mg cellulose powder+bacteria culture</td>
</tr>
</tbody>
</table>
### Table 5.1 Antibacterial test on 100% cellulose powder

<table>
<thead>
<tr>
<th>Control sample</th>
<th>Test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  broth</td>
<td>broth+bacteria culture</td>
</tr>
<tr>
<td>2  broth+1mg raw hemp powder</td>
<td>broth+1mg raw hemp powder+bacteria culture</td>
</tr>
<tr>
<td>3  broth+2mg raw hemp powder</td>
<td>broth+2mg raw hemp powder+bacteria culture</td>
</tr>
<tr>
<td>4  broth+4mg raw hemp powder</td>
<td>broth+4mg raw hemp powder+bacteria culture</td>
</tr>
<tr>
<td>5  broth+8mg raw hemp powder</td>
<td>broth+8mg raw hemp powder+bacteria culture</td>
</tr>
<tr>
<td>6  broth+16mg raw hemp powder</td>
<td>broth+16mg raw hemp powder+bacteria culture</td>
</tr>
<tr>
<td>7  broth+32mg raw hemp powder</td>
<td>broth+32mg raw hemp powder+bacteria culture</td>
</tr>
</tbody>
</table>

### Table 5.2 Antibacterial test on raw hemp powder

<table>
<thead>
<tr>
<th>Control sample</th>
<th>Test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  broth</td>
<td>broth+bacteria culture</td>
</tr>
<tr>
<td>2  1mg degummed hemp powder</td>
<td>1mg degummed hemp powder+bacteria culture</td>
</tr>
<tr>
<td>3  2mg degummed hemp powder</td>
<td>2mg degummed hemp powder+bacteria culture</td>
</tr>
<tr>
<td>4  4mg degummed hemp powder</td>
<td>4mg degummed hemp powder+bacteria culture</td>
</tr>
<tr>
<td>5  8mg degummed hemp powder</td>
<td>8mg degummed hemp powder+bacteria culture</td>
</tr>
<tr>
<td>6  16mg degummed hemp powder</td>
<td>16mg degummed hemp powder+bacteria culture</td>
</tr>
<tr>
<td>7  32mg degummed hemp powder</td>
<td>32mg degummed hemp powder+bacteria culture</td>
</tr>
</tbody>
</table>

### Table 5.3 Antibacterial test on degummed hemp powder
5.2.3.6 Statistics method

Non-parametric statistics were used to compare treatment groups. This test was designed for distribution of data. Independent-samples Kruskal-wall test was used to analyse the powder dose influence on the OD value from 0h to 8h incubation. The significance level was set at 0.05.

5.3 Results

Figures 5.11 to 5.13 show the antibacterial test results. Figure 5.11 shows the antibacterial property of 100% cellulose powder, Figure 5.12 shows the antibacterial property of raw hemp powder and Figure 5.13 shows the antibacterial property of degummed hemp powder respectively. These figures show the optical density of bacteria culture in the solutions containing different powder concentrations in the first eight hours, measured at hourly intervals. The powder concentration in the solution varied from 0 mg to 32 mg.

The principle of the optical density method is that bacterial growth can be represented by the optical density of the solution. The number of bacterial in the solution is in direct proportion to the optical density of the solution, with increased bacterial numbers in the solution having a higher optical density value. The optical density of the bacterial culture is the value difference between test sample and control sample with media only.
Figure 5.11 Optical density of 100% cellulose powder suspensions as a function of incubation time.

Figure 5.11 shows the optical density curves of bacterial culture in the solution containing different percentages of 100% cellulose powder. The significance value of 100% cellulose powder antibacterial properties result is over 0.05 from 0h to 8h incubation. It means that it doesn’t have dose dependant inhibition of bacterial growth for 100% cellulose powder.
Figure 5.12 shows that the optical density curves of bacterial culture in the solution containing different percentages of raw hemp powder are different from each other. Compared with the optical density curve of bacterial culture solution containing no raw hemp powder, the solutions having higher raw hemp powder had lower optical density values. The significance value of raw hemp powder antibacterial properties result is less than 0.02 after 5h incubation. It means that dose dependant inhibition of bacterial growth starts to show its effect from 5h for raw hemp powder.
Figure 5.13 Dose dependent effect of degummed hemp powder (samples labelled with "*" have a significance p<0.05).

Figure 5.13 shows the optical density curve of bacterial culture solutions containing different amounts of degummed hemp powder. The optical density of bacterial culture solution containing degummed hemp powder is smaller in value than bacteria culture solution without degummed hemp powder. The significance value of degummed hemp powder antibacterial properties result is less than 0.03 after 3h incubation. It means that dose dependant inhibition of bacterial growth starts to show its effect from 3h for degummed hemp powder.
Figure 5.14 shows the optical density of the three kinds of powder at 8 h of incubation. 100% cellulose powder had a similar optical density regardless of the powder amount indicating no antibacterial properties. Both raw hemp powder and degummed hemp powder showed a decreasing trend in the optical density value as the amount of powder increased. Compared with raw hemp powder, the optical density of degummed hemp powder decreased to a lower level at a given amount of powder. The significance values for raw hemp powder and degummed hemp powder with the dose of 0mg, 1mg, 4mg, 8mg, 16mg and 32mg at the 8h incubation are less than 0.04. The results suggest that the degummed hemp powder used in this experiment has a stronger antibacterial property than raw hemp powder.
5.4 Discussion

This study aimed to investigate the antibacterial properties of textile hemp powder which is made from hemp bark. In this study, both raw hemp powder and degummed hemp powder had antibacterial properties against \textit{S.aureus}.

In the textile industry, hemp bark is used as raw material to produce hemp fibre. In the last chapter, it was shown that the extract of hemp bark did not show antibacterial properties against \textit{S.aureus}, potentially because the bark contained only low amounts of CBD. However, in this chapter, hemp powder made from bark showed antibacterial properties. As such, the antibacterial properties of the hemp powder may not be related to CBD content. The antibacterial property of the hemp powder is probably associated with the physical structure of the hemp powder. The rough surface of hemp powder makes it difficult for bacteria to adhere to [43]. It is noted from the SEM images in Figure 3.17 (in Chapter 3) that the surface of the attritor milled hemp powder was rough. The powder tested in this chapter was not air-jet milled. The surface roughness may have played a role in the antibacterial properties but further study is still needed to elucidate the reason.

It was found that degummed hemp powder appeared to have stronger antibacterial properties than raw hemp powder. Although not quantitatively analysed, the surface roughness of the degummed hemp powder appeared similar to that of raw hemp powder (Figure 6.8 and Figure 6.9). Hence it may not be possible to attribute the difference in the efficacy of antibacterial properties between these two samples to the surface roughness. The difference was possibly caused by the chemical residue in degumming treatment. During
the degumming process, sodium hydroxide was used. It is possible that there is still some residual sodium hydroxide on the surface of hemp fibre after degumming treatment. The residue of sodium hydroxide in the degumming process is difficult to remove with washing [46]. During the powder fabrication procedure, this residue may still adhere to the powder. As sodium hydroxide can kill bacteria, it could influence the antibacterial properties of the degummed hemp powder. Further study is needed to calculate the amount of sodium hydroxide residue on the powder.

There are other limitations with this study:

- Hurd as a contaminant: In this study, raw hemp powder was produced by mechanical milling of raw hemp fibre that came from the bark of the hemp plant. When the hemp plant was harvested, hemp hurd was attached to the bark. Most of the hurd was removed from the bark by a machine and the residual hemp hurd was removed by hand before the milling process. However, there may still have been some hemp hurd residue adhered to the surface of the hemp bark. In the last chapter, it was proved that the extract of hemp hurd had antibacterial properties against S. aureus, so the powder made from hemp hurd may also have antibacterial properties. The inclusion of hurd powder as a contaminant may have influenced the experimental result.

- The breadth of bacterial strain studied: In our study, S. aureus was selected as the test organism, as it is one of the most common pathogenic gram-positive bacterial strains. In order to increase the scope of this experiment, a number of other bacterial strains, such as E. faecalis, S.
pyogenes and P. aeruginosa could also be tested in the evaluation of hemp powder antibacterial properties.

- Particle attributes: The influence of particle size of hemp powder on antibacterial properties is worthy to be evaluated. In this study, one size hemp powder was tested. In future work, hemp powders with different particle sizes could also be tested for antibacterial properties.

Antibacterial hemp powder may be used as a new class of antibacterial agent in the future. For instance, it may replace the harmful antibacterial agents (such as silver) coated on the surface of fabric to produce antibacterial fabrics. Compared with other antibacterial agents which are based on chemical products, hemp powder is produced from natural and renewable raw material, so it possesses many advantages such as eco-friendliness, potential biodegradability, and non-toxic nature to humans.

Moreover, hemp powder could be widely used in the cosmetics industry. Some of the artificial preservatives in cosmetics industry are not good for human health[143]. Antibacterial hemp powders made from natural fibre may be suitable as a new generation of preservatives. They could be added into personal care products, such as skin care products and shampoo.

In order to apply hemp powder as an antibacterial agent, there is still some work to be done. For example, the methodology to adhere hemp powder to fabric needs to be investigated, and the antibacterial properties of fabric coated with hemp powders still needs to be studied. The durability of fabric treated by hemp powder under sunlight and washing also need to be further investigated.
5.5 Conclusion

This study showed that both raw hemp powder and degummed hemp powder have antibacterial properties against *S.aureus*. The addition of degummed hemp powder into the solution at higher concentration appears to totally stop the growth of *S.aureus*, while raw hemp powder can inhibit the growth of bacteria to a lower degree. Degummed hemp powder appears to have stronger antibacterial properties than the raw hemp powder.
Chapter 6  Hemp powder and PP polymer blended filament

6.1 Introduction

Traditional man-made fibres, such as glass and carbon fibre, are widely used as reinforcement in the fabrication of composite materials. Due to the increasing price of traditional man-made fibres, natural fibres have replaced them to some extent and performed as reinforcement in composite material manufacturing [144]. Natural fibres utilised include flax, hemp, jute, sisal, kenaf, coir, kapok, banana, henequen and many others [145]. The advantages of natural fibres lie in their renewable source, low cost, low density and good mechanical properties, which makes them extensively used as reinforcements [146].

Due to the high tensile strength of hemp fibre, it has been selected as a reinforcement for composite materials [1]. Significant research has been conducted on hemp fibre reinforced composites although little has been undertaken on hemp powder filled filaments. Hemp powder has a large surface area and small size making it a good reinforcement material.

Hemp fibre, as a natural bast fibre, has some unique properties over man-made fibre. Clothes made from hemp fibre have good moisture absorption which makes them comfortable to wear whereas synthetic fibres often have limited to no moisture absorption capacity. A synthetic fibre blended with hemp particles would be expected to exhibit properties of both of the materials that it is made from. The addition of hemp powder to polypropylene (PP) could provide moisture absorption and dyeability.
Many factors can influence the performance of hemp powder filled melt spun filaments. The two key factors are the processing parameters and amount of powder added to the filament. The amount of powder added into filament will significantly influence filament properties and spinability [147]. Processing parameters selected to fabricate the filament also significantly influence the properties of the composite filament. In this study, hemp powder percentage was varied between 2% to 10%. Composite filaments were fabricated from PP by melt blending followed by melt extrusion.

Particle aggregation can influence the mechanical properties of a filament especially surface roughness and tensile strength. Surface modification of the powder by the addition of a compatibiliser has been shown to help achieve homogenous particle dispersion within a filament [109, 110, 147]. This chemical was used to coat the particle to provide a particle surface that had better miscibility with the polymer. 20,000 molecular weight polyethylene glycol (PEG 20,000) has been shown to be effective for this purpose for both wool and aluminium particles. In this work PEG 20,000 was used as a compatibiliser to help with dispersion and miscibility of the hemp particle within the PP.

The purpose of this study is to fabricate PP polymer filament containing different percentages of hemp powders. The main experiment is divided into two stages; melt blending of the hemp powder with PP to make a polymer chip and melt filament extrusion. Four different polymer/filler ratios were conducted with filler percentages from 2 to 10% investigated. Characterisations were performed on the filaments including tensile strength, optical microscopy,
colour analysis, dye uptake, moisture absorption, thermal analysis and X-ray diffraction (XRD).

6.2 Preliminary Experiment

6.2.1 Material

The polypropylene (PP) polymer used for these experiments was Moplen PP 183 (Basell, Australia). The compatibiliser used was 20,000 molecular weight polyethylene glycol (PEG20,000, Sigma Aldrich, USA). The PP was selected as it had a melting point (165°C) which was below the sublimation temperature of hemp powder. Hemp powder is produced according to the fabrication technology presented in chapter 2. The thermal decomposition temperature of cellulose is above 250°C which reduces its thermal degradation during extrusion.

Hemp powder characterisation

The hemp powder had a mean diameter of 6.04μm and a spread in the range from approximately 1.5 μm to 20 μm. Figure 6.1 shows the particle size distribution graph for the hemp powder. The hemp powder had a surface weighted mean diameter D[3,2] 4.85 μm, volume weighted mean diameter D[4,3] 6.71 μm. d(0.1) is 2.92 μm, d(0.5) is 6.04 μm, d(0.9) is 11.54 μm. The particle size displays a narrow bell shaped distribution with a tail skewed to the sub-micron side of the curve. Particle size was measured using a Malvern Mastersizer 2000 according to the method detailed in chapter 2 1.2.3.1 particle size.
Surface treatment of hemp powder

The addition of PEG 20,000 was done to the hemp powder before melt blending with the PP. The PEG was first made into a fine powder form by a grinding process detailed below before being thoroughly mixed with the hemp powder. The ratio of PEG 20,000 to hemp powder used was 0:1, 0.2:1, 0.4:1 and 0.6:1.

Preparation of PEG 20,000 powder

PEG 20000 powder was made by one pass grinding using a Pulverisette 19 rotary cutting mill (Fritsch, Germany). The sieve size at the base of the mill was 1mm and the PEG 20,000 was cooled to -80°C for 12 hours before grinding. After grinding the PEG 20,000 powder was sieved using a 0.5mm aperture standard sieve to remove large particles.

6.2.2 Blending procedure

Preparation of polymer chips

Polymer chips containing PP and hemp powder were blended in a yellow jacket twin screw melt blending line with twin screw side feeder (Wayne Machine and Die Company, New Jersey, USA). Gravimetric feed of the polymer was
performed by a single screw K-tronsoder (K-tron, New Jersey, USA) and the powder by a twin screw K-tronsoder (K-tron, New Jersey, USA). The hemp powder was mixed with PEG 20,000 sieved powder before placement in the gravimetric feeder. Four ratios of PEG 20,000 to hemp powder (0:1, 0.2:1, 0.4:1, 0.6:1) were conducted to determine the optimum ratio. Blended polymer chips containing hemp powder were then converted to filaments by melt extrusion.
Figure 6.2 Yellow jacket twin screw melt blending line geometry
Figure 6.3 Yellow jacket twin screw melt blending

Figure 6.4 Control panel of melt blending line

Figure 6.5 Water bath
Figure 6.6 Die zone

Figure 6.7 Single screw polymer feed
Figure 6.2 shows the geometry of Yellow jacket twin screw melt blending line. Images of each of the components have been given in figures 6.3-6.9. There are six individually controlled heating zones in total and the approximate locations of each of these are given in Figure 6.2. There was also a temperature controlled heater in the extrusion die (die zone). Table 6.1 shows the temperature settings
that were used for each zone. The speed of the twin screws were set at 200rpm. The twin screw speed of side feeder was set at 350rpm.

The blending line was cleaned before use by removal of the screws and wire brushing the residual polymer from the screw surface and barrel. The machine was purged for one hour using pure PP before blending was commenced. PP was feed into the main feeder and the mixture of PEG 20,000 and hemp powder was feed into the side feeder. Four different ratios of PEG 20,000 to hemp powder were conducted (0:1, 0.2:1, 0.4:1 and 0.6:1). Hemp powder was kept at 2% in the PP for each of these four different blending ratios. The gravimetric feed rate of the polymer and powder feed is listed in Table 6.2. The mixture of PEG 20,000 and hemp powder was added to the PP at zone 2 after the PP had been melted to enable better dispersion of the powder. The blended polymer was extruded through a 5.0mm die before water quenching and chipping into pellet form. Table 6.3 shows four kinds of polymer chips.

<table>
<thead>
<tr>
<th>Feed zone</th>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
<th>Zone 4</th>
<th>Zone 5</th>
<th>Zone 6</th>
<th>Die Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature/°C</td>
<td>193</td>
<td>193</td>
<td>194</td>
<td>196</td>
<td>196</td>
<td>202</td>
<td>202</td>
</tr>
</tbody>
</table>

Table 6.1 Temperature settings of feed zones

<table>
<thead>
<tr>
<th>PEG 20,000 to hemp powder ratio</th>
<th>0:1</th>
<th>0.2:1</th>
<th>0.4:1</th>
<th>0.6:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Polymer feed rate (kg/h) | 4.9 | 4.88 | 4.86 | 4.84 |
| Powder feeder rate (kg/h) | 0.1 | 0.12 | 0.14 | 0.16 |

Table 6.2 Feed rate of polymer and powder feeder
Polymer chip A  polymer chips with a PEG 20000 and hemp powder ratio of 0 to 1

Polymer chip B  polymer chips with a PEG 20000 and hemp powder ratio of 0.2 to 1

Polymer chip C  polymer chips with a PEG 20000 and hemp powder ratio of 0.4 to 1

Polymer chip D  polymer chips with a PEG 20000 and hemp powder ratio of 0.6 to 1

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer chip A</td>
<td>polymer chips with a PEG 20000 and hemp powder ratio of 0 to 1</td>
</tr>
<tr>
<td>Polymer chip B</td>
<td>polymer chips with a PEG 20000 and hemp powder ratio of 0.2 to 1</td>
</tr>
<tr>
<td>Polymer chip C</td>
<td>polymer chips with a PEG 20000 and hemp powder ratio of 0.4 to 1</td>
</tr>
<tr>
<td>Polymer chip D</td>
<td>polymer chips with a PEG 20000 and hemp powder ratio of 0.6 to 1</td>
</tr>
</tbody>
</table>

Table 6.3 Four kinds of polymer chips

### 6.2.3 Filament extrusion procedure

A yellow jacket single screw melt extrusion line (Wayne Machine and Die Company, New Jersey, USA) was used for extruding the composite fibres.

![Filament extrusion line](image)
Figure 6.11 Filament extrusion line

Figure 6.12 Control panel

Figure 6.13 Winder
The single screw melt extrusion line consisted of two heating zones and three heated die zones. Each of these zones had accurate temperature control. Screw speed was set at 30 RPM and the polymer was dried for 4 hours in an oven at 80°C before extrusion. The filament was drawn from an 18 hole spinneret and collected on a bobbin at 34 m/min.

Figure 6.10 shows the geometry of the filament extrusion line, Figure 6.11 shows the extruder, Figure 6.12 shows the control panel of extruder, Figure 6.13 shows the winder. Table 6.4 shows the temperature settings of each of the heating zones. Table 6.5 shows four kinds of filaments.

<table>
<thead>
<tr>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Die zone 1</th>
<th>Die zone 2</th>
<th>Die zone 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature°C</td>
<td>188</td>
<td>191</td>
<td>196</td>
<td>202</td>
</tr>
</tbody>
</table>

Table 6.4 Temperature settings of heating zones

<table>
<thead>
<tr>
<th>Filament</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filament A</td>
<td>Filament containing PEG 20,000 and hemp powder with a ratio of 0 to 1</td>
</tr>
<tr>
<td>Filament B</td>
<td>Filament containing PEG 20,000 and hemp powder with a ratio of 0.2 to 1</td>
</tr>
<tr>
<td>Filament C</td>
<td>Filament containing PEG 20,000 and hemp powder with a ratio of 0.4 to 1</td>
</tr>
<tr>
<td>Filament D</td>
<td>Filament containing PEG 20,000 and hemp powder with a ratio of 0.6 to 1</td>
</tr>
</tbody>
</table>

Table 6.5 Four kinds of filaments

6.2.4 Characterisation

6.2.4.1 Blended polymer

Microscopy images of polymer chips were taken by placing one polymer chip onto a glass microscope slide followed by heat treatment at 200°C for two hours in an oven. The polymer solution left on the slide was allowed to cool and
solidify before optical microscopy. A DP70 bright field reflected light optical microscope (Olympus, Japan) was used to characterise the morphology of the blended polymer.

6.2.4.2 Extruded filament

The filament was adhered to a glass slide using double sided tape at each end. Microscopy of the filament along its length was performed using a DP70 both bright field and dark field reflected light optical microscope (Olympus, Japan). A side light was used in the photo taking in dark field of microscope, the fibre was illuminated at a 70 degree angle with the fibre direction so that light travelled along the fibre and illuminated large particles within the fibre structure. Figure 6.14 shows the microscope and side light.

![Side light and microscope](image)

Figure 6.14 Side light and microscope

Tensile testing was performed using a SIFAN 2 (BSC Electronics, Australia). Single fibre tensile tests were performed according to the ASTM
method D3822-96. Filaments were conditioned at 20±2°C and 65±2% relative humidity for 24 hours before measurement. The test speed was 500 mm/min. The gauge length was set at 25 mm for each test. At least twenty filaments were measured for each batch of extruded filaments.

6.2.5 Result

6.2.5.1 Polymer chip

Figure 6.15 Polymer chips as blended

(a) Polymer chip A (0:1)
Figure 6.15 to 6.16 show images of the blended polymer containing 2% hemp powder and different ratios of PEG 20,000. In Figure 6.15, the polymer chips...
A(0:1), B(0:2:1), C(0:4:1) and D(0:6:1) are displayed from left to right. Figures 6.16 (a) to (d) show the melted polymer chips at 200 times magnification. There was no observed difference in the morphology of the polymer chip when the ratio of PEG 20,000 was varied. It was determined that the addition of PEG 20,000 was not necessary in the blending of hemp powders to provide particle dispersion as observed in other powder composite research.

6.2.5.2 Extruded filament

1.1.1.1.1 Morphological characterisation

![Figure 6.17 Filaments](image)

Figure 6.17 Filaments

(a) Filament (0:1) in bright field
(b) Filament A (0:1) in dark field

(c) Filament B (0.2:1) in bright field

(d) Filament B (0.2:1) in dark field
(c) Filament C (0.4:1) in bright field

(f) Filament C (0.4:1) in dark field

(g) Filament D (0.6:1) in bright field
Figure 6.18 shows the extruded filaments on each of the winder bobbins. Figures 6.18 (a) to (h) show the microscopic images of the filaments along their length. Each of these images was captured at 1000 times magnification. There is a slight surface roughness on each of the filaments showing the presence of particles protruding from the fibre surface. This surface roughness is the same for each of the filaments regardless of the amount of PEG 20,000. The filaments all show uniform diameter along the length with no necking of the filament or large lumps caused by agglomerated particles. Previous work has shown that when particle dispersion is good the filament has a uniform diameter whereas agglomeration often causes necking and lumps [147]. No agglomerated particles are visible within the transparent polymer filament.

It was observed from the dark field images that the addition of PEG 20,000 had no effect on particle distribution within the filaments. The addition of PEG 20,000 did not help to spread particles more evenly or reduce or increase agglomeration in the filament.
1.1.1.1.2 Tensile test result

(a) Diameter of filament A (0:1)

(b) Diameter of filament B (0.2:1)
Figure 6.19 Diameter of filament

(c) Diameter of filament C (0.4:1)

(d) Diameter of filament D (0.6:1)

Figure 6.19 Diameter of filament
<table>
<thead>
<tr>
<th></th>
<th>Filament (0:1)</th>
<th>Filament (0.2:1)</th>
<th>Filament (0.4:1)</th>
<th>Filament (0.6:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenacity (cN/tex)</td>
<td>49.61</td>
<td>44.27</td>
<td>32.01</td>
<td>29.60</td>
</tr>
<tr>
<td>SD</td>
<td>8.15</td>
<td>5.83</td>
<td>5.40</td>
<td>6.21</td>
</tr>
<tr>
<td>Strain (%)</td>
<td>172.35</td>
<td>222.37</td>
<td>255.10</td>
<td>368.05</td>
</tr>
<tr>
<td>SD</td>
<td>53.95</td>
<td>50.54</td>
<td>57.68</td>
<td>74.93</td>
</tr>
</tbody>
</table>

Table 6.6 Tenacity and strain of filaments

Figure 6.20 Fibre tenacity of filaments
Figure 6.21 Strain to break of filaments

Fibre tenacity was calculated using the Equation 6.1, the linear density was calculated using Equation 6.2 and strain was calculated using Equation 6.3.

\[
\text{Tenacity} = \frac{F}{LD}
\]  
(6.1)

Where \( F \) is the force to break in cN and \( LD \) is the linear density in tex (g/1000m).

\[
LD = \frac{\rho \times \pi \times d^2}{40}
\]  
(6.2)

Where \( \rho \) is the density of hemp (0.90 g/cm\(^3\)) in g/cm\(^3\) and \( d \) is the minimum diameter of the fibre tested in \( \mu m \).

\[
\text{Strain} = \frac{\text{elongation}}{\text{gauge length}} \times 100\%
\]  
(6.3)
Where elongation is the maximum extension under $F$, gauge length is the distance between two tensile mounting jaws.

Figures 6.19 (a) to (d) exhibit the diameter variations along the length of filaments. Table 6.6 display the tenacity and strain of four blended filaments. Figure 6.20 and 6.21 show the tenacity and strain trend respectively. The only orientation undertaken on the filament was from partial orientation caused by drawing of the filament from the spinneret. The draw off speed was the same for each of the filaments so fibre properties should be similar.

It was noticed that the addition of PEG 20,000 had very little influence on diameter variation within the filament. All four diameter versus length graphs showed similar variation along their length. Variations such as necking or particle lumps were not seen like that reported by Naebe et al [91].

It was observed that tenacity decreases with the addition of PEG 20,000 and strain increases with the addition of PEG 20,000. This was because PEG 20,000 has a lower molecular weight than the PP polymer it is being blended into. If PEG 20,000 was providing an improvement in the dispersion of hemp powder in the PP matrix it would be expected that the tensile properties would improve over the filament without PEG 20,000 present. As the opposite effect is seen it can be assumed that the PEG 20,000 is providing no improvement to hemp powder dispersion within the PP. It was concluded that the addition of PEG 20,000 has a negative effect on the tensile property of the filament and was not required when blending hemp powders within PP. The increase in breaking strain of filament was believed to be caused by the addition of PEG 20,000. The addition of short chain molecules of PEG into the polymer must allow
movement of the polymer chains within the filament rather than breaking of the chain structure. This polymer chain movement would also account for the lowering of the fibre tenacity.

6.2.6 Conclusion

The presence of PEG 20,000 had no effect on the uniformity of diameter along the length of the filaments. Agglomeration of the particles was not present in the filaments without PEG 20,000 present and the presence of PEG 20,000 had no change in particle dispersion. All filaments manufactured showed an even hemp powder distribution within the filament. The addition of PEG 20,000 has no influence on the hemp powder distribution along the filament. The addition of PEG 20,000 had a negative effect on the filament tenacity and elongation to break. It is not necessary to add PEG 20,000 during the blending process of hemp powders with PP to improve compatibility of the hemp particles.

6.3 Main experiment

6.3.1 Blending procedure

Based on the result of preliminary experiment given in part 2 of this chapter, it was found that the addition of PEG 20,000 provided no advantages for hemp powder dispersion within the PP matrix. Hemp powder blended directly with PP gave good dispersion and was found to be able to be used without the need of a compatibiliser. A second blending run was conducted to blend 2, 5, 7.5 and 10% hemp powder with PP. The parameter settings of the blending machine were the same as that outlined in part 2 of this chapter.
The feed rates of the gravimetric feeders for both polymer and powder have been listed in Table 6.7. Four different percentages of hemp powder in PP polymer were blended (2%, 5%, 7.5% and 10%). Table 6.8 shows the temperature settings of each of the heating zones and die zone.

<table>
<thead>
<tr>
<th>Polymer chip type</th>
<th>Main feeder(kg/h)</th>
<th>Side feeder(kg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2%</td>
<td>3.92</td>
<td>0.08</td>
</tr>
<tr>
<td>5%</td>
<td>3.8</td>
<td>0.2</td>
</tr>
<tr>
<td>7.5%</td>
<td>3.7</td>
<td>0.3</td>
</tr>
<tr>
<td>10%</td>
<td>3.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 6.7 Speed settings of main feeder and side feeder

<table>
<thead>
<tr>
<th>Feed zone</th>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
<th>Zone 4</th>
<th>Zone 5</th>
<th>Zone 6</th>
<th>Die zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature/°C</td>
<td>196</td>
<td>196</td>
<td>196</td>
<td>199</td>
<td>202</td>
<td>202</td>
<td>202</td>
</tr>
</tbody>
</table>

Table 6.8 Temperature settings of feed zone

### 6.3.2 Filament extrusion procedure

Filament extrusion was undertaken using the same settings and equipment as that outlined in part 2 of this chapter. Set temperatures of each of the heating zones and die zones are given in Table 6.9.

<table>
<thead>
<tr>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Die zone 1</th>
<th>Die zone 2</th>
<th>Die zone 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature/°C</td>
<td>188</td>
<td>191</td>
<td>196</td>
<td>202</td>
</tr>
</tbody>
</table>

Table 6.9 Temperature settings of zones and die zones
6.3.3 Characterisation of filament with varying percentages of hemp powder

6.3.3.1 Colour characterisation

Colour measurements were conducted on each of the filaments containing different percentage of hemp powder using a SF600 Plus-CT spectrophotometer (Datacolor, USA). DIN6167 Yellowness and GanzGrieoser Whiteness indexes of filaments were recorded.

6.3.3.2 Dye uptake property

The dye uptake property of filaments was performed on an Ahiba nuance laboratory dyeing machine (Datacolor, USA). Figure 6.22 shows the Ahiba nuance. The dye vessel used was 300ml with 1.0g of fabric and a 10:1 liquor ratio. The dye used was Cibacron Scarlet LS 2G and the wetting agent was Albegal FFA (Ciba Specialty Chemicals, Switzerland). The alkali used was a laboratory grade sodium hydroxide (NaOH) and sodium carbonate (Na₂CO₃) and the salt used was grade 5 sodium chloride (NaCl) (Sigma Aldrich, USA). The dye process is shown in figure 6.23.

Figure 6.22 Ahiba nuance
30.0g/L of NaCl, 1% w/w dye and 1.0g/l of wetting agent were added to the filament in the dye pot at the beginning of dye process. The dye pot was heated up from room temperature to 30°C and kept at this temperature for 10 mins. The dye pot was then heated up to 80°C at a heating rate of 1°C/min and held at this temperature for 60 mins. The dye pot was then cooled to 70°C at a cooling rate of 3°C/min before the addition of 5.0g/l Na₂CO₃ and 2.0g/l NaOH. The temperature was held at 70°C for 60 mins. The filament samples were washed in cold water and then hot water until all unfixed dye was removed.

### 6.3.3.3 Moisture absorption

Moisture absorption property of the filaments was measured according to ASTM Doc 571 (1961): Moisture regains and commercial allowances for textile
materials. Approximately 5g of each filament were collected in a beaker and placed in a fan forced oven at 105°C. The filaments were removed from the oven and the mass recorded at a 15 minute interval until a constant stable mass was achieved. Weight measurement was conducted in a way to avoid moisture regain in the filament while away from the oven. Oven dried filaments were conditioned for 24 hours in an atmosphere of 20±2°C and 65±2% relative humidity.

6.3.3.4 X-ray diffraction

XRD analysis was applied in order to assess the influence of powder addition on filament crystallinity. X-ray diffraction was performed on a PANalytical’s X’Pertpower X-ray diffraction, the Cu Kα radiation (λ = 0.154 nm) was operated at 40 kV and 30 mA. X ray scan was over a 2θ range from 10° to 70°. The scanning rate was set at 0.1°/s and step size was kept at 0.05 respectively. Spectra were analysed using the TracesV6 software.

6.3.3.5 Microscopy test

Filament microscopy testing was conducted using the same method as that outlined in part 2 of this chapter.

6.3.3.6 Differential scanning calorimetry (DSC)

DSC measurement was performed on a DSC Q2000 differential scanning calorimeter (TA Instruments, USA) in the nitrogen atmosphere at a flow rate of 50mL.min⁻¹. The temperature range from -20°C to 300°C, the heating rate was set at 10°C min⁻¹.
6.3.3.7 Filament tensile testing

Filament tensile testing was conducted using the same method as that outlined in part 2 of this chapter.

6.3.4 Results

6.3.4.1 Colour measurement results

Figure 6.24 Filaments containing different percentages of hemp powders

<table>
<thead>
<tr>
<th></th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>C*</th>
<th>h</th>
<th>DE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PP filament</td>
<td>67.95</td>
<td>71.80</td>
<td>73.30</td>
<td>87.87</td>
<td>-0.28</td>
<td>2.95</td>
<td>2.96</td>
<td>95.41</td>
<td></td>
</tr>
<tr>
<td>Filament 12% hemp powder</td>
<td>65.39</td>
<td>68.86</td>
<td>67.62</td>
<td>86.43</td>
<td>0.24</td>
<td>5.14</td>
<td>5.15</td>
<td>87.29</td>
<td></td>
</tr>
<tr>
<td>Differences 1</td>
<td>-1.44</td>
<td>0.52</td>
<td>2.20</td>
<td>2.19</td>
<td>-0.55</td>
<td>2.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filament 25% hemp powder</td>
<td>65.49</td>
<td>69.16</td>
<td>64.96</td>
<td>86.58</td>
<td>-0.17</td>
<td>7.68</td>
<td>7.68</td>
<td>91.29</td>
<td></td>
</tr>
<tr>
<td>Differences 2</td>
<td>-1.29</td>
<td>0.11</td>
<td>4.73</td>
<td>4.72</td>
<td>-0.34</td>
<td>4.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filament 37.5% hemp powder</td>
<td>61.09</td>
<td>64.12</td>
<td>56.65</td>
<td>84.03</td>
<td>0.70</td>
<td>10.82</td>
<td>10.84</td>
<td>86.30</td>
<td></td>
</tr>
<tr>
<td>Differences 3</td>
<td>-3.84</td>
<td>0.98</td>
<td>7.88</td>
<td>7.88</td>
<td>-0.90</td>
<td>8.82</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.10 CIE output result of filaments

<table>
<thead>
<tr>
<th>Filament</th>
<th>410% hemp powder</th>
<th>54.78</th>
<th>57.18</th>
<th>47.50</th>
<th>80.28</th>
<th>1.45</th>
<th>13.57</th>
<th>13.65</th>
<th>83.90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differences</td>
<td>4</td>
<td>-7.59</td>
<td>1.73</td>
<td>10.63</td>
<td>10.69</td>
<td>-1.27</td>
<td>13.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.24 shows filaments containing different percentage of hemp powder. (a) is pure PP filament, (b) is filament containing 2% hemp powder, (c) is filament containing 5% hemp powder, (d) is filament containing 7.5% hemp powder, (e) is filament containing 10% hemp powder. It is observed from these images that with the increase of hemp powder percentage in the filaments, the filaments become deeper in colour. The colour change is from transparent white to beige.

Table 6.10 contains the CIE colour measurement results for each of the filaments. Differences are made in comparison with the pure PP polymer reference filament given in the first row of the table. DE* can be used to represent the total difference between pure PP control sample and the sample containing hemp powder.

The colour measurement results show that as hemp powder is added, the filament is delustered and changed in colour. The colour of the low percentage hemp powder containing fibres is similar to that of the hemp powder used to blend with the polymer. As the percentage of hemp powder is increased the colour becomes deeper than that of the hemp powder. This deepness of colour may be explained by thermal degradation of the hemp powder at the filament extrusion temperatures employed. At low concentrations of hemp powder this colour change is masked by the base colour of the PP.
6.3.4.2 Dye uptake property

Figure 6.25 Dyed filaments

Figure 6.25 shows the dyed filaments. It was observed that there was no dyeing of the pure PP filament when dyed with reactive dyes made from cotton. This is expected as the structure of the PP both resists penetration of dye into the fibre and the chemical structure provides no functional groups for the bonding of the dye to the fibre. All of the other filaments show the presence of dye absorption at various colour depths. Although not measured with a spectrophotometer, the filaments have a direct relationship of colour depth with hemp powder content. The filament with 2% hemp powder had the lightest colour, while, the filament with 10% hemp powder had the deepest colour. The dye uptake increases with the increase of hemp powder percentage in the filament. The presence of the hemp powder provides hydroxyl groups within the filament for the cotton reactive dyes to bond to. As dye molecules penetrate into the filament it was felt that the crystalinity of the PP must be changed by the presence of hemp powder to allow better moisture penetration. XRD measurements presented later in this chapter have shown that this was not the case with only limited changes in crystalinity. The positioning and possible interconnectivity of the particles is assumed to provide the pathway for dye into the fibre.
### 6.3.4.3 Moisture absorption

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Pure PP</th>
<th>2% hemp</th>
<th>5% hemp</th>
<th>7.5% hemp</th>
<th>10% hemp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone dry mass</td>
<td>5.0781</td>
<td>5.0768</td>
<td>5.017</td>
<td>5.089</td>
<td>5.0673</td>
</tr>
<tr>
<td>Conditioned mass</td>
<td>5.1022</td>
<td>5.1111</td>
<td>5.055</td>
<td>5.1318</td>
<td>5.1227</td>
</tr>
<tr>
<td>% moisture content</td>
<td>0.47</td>
<td>0.68</td>
<td>0.76</td>
<td>0.84</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Table 6.11 Weight change of filaments along with time

Table 6.11 displays the weight change of filaments along with time. The bone dry mass is recorded after drying for 120 minutes at 105°C. The conditioned mass is recorded after 24 hours at 20 ±2°C and 65±2% humidity. The moisture uptake is calculated as per equation 6.4

$$\text{Moisture content} = \frac{\text{Mass}_{\text{conditioned}} - \text{Mass}_{\text{BoneDry}}}{\text{Mass}_{\text{BoneDry}}} \times 100\% \quad (6.4)$$

Pure PP filament had the lowest moisture absorption of 0.47%, filament contains 10% hemp powder has the highest moisture absorption of 1.09%. The moisture absorption of other filaments ranges in between 0.68% and 0.84%. It is noted that the moisture absorption ability of filament increases gradually with the addition of hemp powder into the filament. PP filament is a synthetic textile fibre which is difficult to absorb moisture, hemp powder can absorb moisture easily. The addition of hemp powder in the filament can help it to absorb moisture. The more hemp powder is added, the higher moisture content value it is.
6.3.4.4 XRD result

Figure 6.26 X-ray diffraction of filaments

The X-ray diffractions of filaments are given in Figure 6.26. There are three major peaks observed on all filaments, these peaks were at 15.1°, 16.88° and 22.82° at 2θ diffraction angles, which indicates the presence of Type I cellulose in the filaments [148]. Since hemp powder is blended into polymer filament, the filament tends to become less crystallized. That is because PP polymer is a kind of long chain molecular with high molecular weight, which makes pure PP filament has the highest crystallinity index. The added hemp powder can break long chain molecular into smaller ones, which have an influence on the crystallization. The diffraction peaks were too broad to distinguish amorphous and crystalline components. As such, it was not possible to obtain meaningful % crystallinity values from the XRD patterns.
6.3.4.5 Microscopy test result

(a) Pure PP filament in bright field

(b) Pure PP filament in dark field

(c) Filament containing 2% hemp powder in bright field
(d) Filament containing 2% hemp powder in dark field

(e) Filament containing 2% hemp powder in bright field

(f) Filament containing 2% hemp powder in dark field
(g) Filament containing 5% hemp powder in bright field

(h) Filament containing 5% hemp powder in dark field

(i) Filament containing 5% hemp powder in dark field
(j) Filament containing 5% hemp powder in dark field

(k) Filament containing 7.5% hemp powder in dark field

(l) Filament containing 7.5% hemp powder in dark field
(m) Filament containing 7.5% hemp powder in dark field

(n) Filament containing 7.5% hemp powder in dark field

(o) Filament containing 10% hemp powder in dark field
(p) Filament containing 10% hemp powder in dark field

(q) Filament containing 10% hemp powder in dark field

(r) Filament containing 10% hemp powder in dark field

Figure 6.27 Microscopy images of filaments containing different percentage of hemp powder
Figure 6.27 (a) to (r) show the microscopy images of filaments containing different percentages of hemp powder. Fracture surfaces of cross section microtome samples did not accurately show dispersion however optical microscopy did and this is why microscopy images were reported. The pure PP filament exhibited a smooth surface and homogenous diameter along its length. The surface of the filaments containing hemp powder exhibited surface roughness indicative of hemp powders partially protruding from the filament. Filament containing 2% hemp powder has a smoother surface compared to other filaments which contain higher percentage of hemp powder. The number of protruding powders increased with the addition of hemp powder into filament, evident as increased surface roughness. The filaments exhibited good dispersion of powder within the filament however as the amount of powder increased in the PP extrusion became more difficult with frequent breakages during extrusion. This may be due to a build up of larger or agglomerated particles in the extrusion die. These larger particles were not evident in the filament and may have been infrequent within the blended polymer.

Dark field images can clearly show the particle dispersion condition along the filament, it was noticed that particle amount increased gradually in the filament with the addition of particles, the particles were dispersed evenly in the filament in most cases. In low particles content filaments there was no agglomeration of particles observed. Particle agglomeration was found in filaments containing higher percentages of particles, which started from 5%.
6.3.4.6 DSC result

Figure 6.28 DSC curves of filaments

Figure 6.29 DSC curves of filaments at around 160°C
The DSC curves of filaments in a nitrogen atmosphere are presented in Figure 6.28. It is shown that the DSC curve of filament present one peak of its major constituents (cellulose). It is observed that all DSC curves exhibit an endothermic peak at around 160°C, this peak is related to the melting of the polypropylene that occurs at about this temperature. The enlarged peak is shown in Figure 6.29.

The endothermal peak is moved to a higher temperature with the addition of hemp powder into the filaments, which mean the addition of hemp powder can raise the melting temperature of the polymer. It is noticed that filaments containing higher percentage of hemp powders have a higher temperature endothermal peak. It means there is a change in crystal structure in presence of Hemp powder. This result is in correspondent with the XRD result.

6.3.4.7 Tensile result

<table>
<thead>
<tr>
<th></th>
<th>Pure PP</th>
<th>2% hemp</th>
<th>5% hemp</th>
<th>7.5% hemp</th>
<th>10% hemp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenacity (cN/tex)</td>
<td>49.06</td>
<td>24.00</td>
<td>29.24</td>
<td>28.44</td>
<td>27.04</td>
</tr>
<tr>
<td>SD</td>
<td>8.28</td>
<td>3.16</td>
<td>4.99</td>
<td>3.38</td>
<td>2.532715</td>
</tr>
<tr>
<td>Strain (%)</td>
<td>180.53</td>
<td>485.77</td>
<td>398.04</td>
<td>402.18</td>
<td>335.84</td>
</tr>
<tr>
<td>SD</td>
<td>48.70</td>
<td>48.67</td>
<td>44.58</td>
<td>44.35</td>
<td>54.05</td>
</tr>
</tbody>
</table>

Table 6.12 Breaking force and extension of filaments
Table 6.13 display the tenacity and strain of the five blended filaments. The fibre tenacity and strain are shown in Figure 6.30 and Figure 6.31. It was noticed that
fibre tenacity decreased with the addition of hemp powder, while the tenacity of filaments containing different percentages of powder was similar. The strain of filaments increased since hemp powder was added, and there were not much difference in strain among filaments with various percentages of hemp powders. The decrease in tenacity was caused by the addition of powder. The arrangement of powder in filament happened in three cases; powder was adhered to the surface of the filament (Figure 6.32), powder was embedded within the polymer chains (Figure 6.32) and powders aggregated together to form a large powder bundle within the filament (Figure 6.32).

![Figure 6.32 Powder adhered to the filament surface.](image)

![Figure 6.33 Powder embedded within the filament.](image)
Figure 6.34 Aggregated powder within the filament.

PP polymer is a long chain molecule. Crystalline orientation of some of the polymer chains provides the tenacity of the fibre. The places where powders were added became weak points in the filaments, especially the powder was aggregated together. When the force was applied on filament, it would break from the weak points first. The decrease in tenacity is in correspondent to the XRD result, as it was shown in XRD result that the addition of hemp powder made filament crystallinity index decrease. The more crystalized filament was expected to have a higher tenacity. It was concluded that the addition of hemp powder into the filaments had a negative effect on the tensile property of filaments.

The increase in strain of filament was also attributed by the addition of hemp powder. As the crystalinity of the polymer was changed to be more amorphous the ability for polymer chain extension before break was increased. This caused an increase in the strain observed. As the filaments containing different percentages of hemp powder show a similar tenacity value, the strain is similar among these filaments.
6.4 Conclusion

According to the result of preliminary experiment, hemp powder was directly blended with PP polymer in the polymer chips fabrication stage without PEG 20000. Four ratios of hemp powder in PP (2%, 5%, 7.5% and 10%) were successfully extruded into filaments.

Colour measurement showed that the filaments were delustered and changed in colour with the addition of hemp powder. The more hemp powder added, the deeper the change in colour observed. Dye uptake increased with the increase of hemp powder percentage in the filament. 10% hemp powder filament shows the best dye uptake property. The moisture absorption ability of filament increases gradually with the addition of hemp powder into the filament. XRD result indicated that the addition of hemp powder into PP polymer changes the crystallinity index of the filaments. Filaments containing hemp powder become less crystallized. Optical microscopy images showed that filaments exhibited good dispersion of powder within the filament however as the amount of powder increased in the PP extrusion became more difficult with ends frequently breaking. DSC result showed that the addition of hemp powder can raise the melting temperature of the polymer, the filaments became more thermally stable with the addition of hemp powder. Tensile test showed that the addition of hemp powder into the filaments had a negative effect on the tensile property of filaments.
6.5 Chapter conclusion

This study has significant influence in the filament production field. The addition of hemp powder into filament has some advantages. The polymer filament itself is hard to absorb moisture and dye. The filaments containing renewable hemp powder have higher moisture absorption ability and dye uptake ability than pure PP filament. Moreover, the addition of hemp powder into filament can also reduce the input of polymer into the filament. However, the disadvantages include that the filament can become discoloured with the addition of hemp powder and the tensile properties are significantly reduced.
Chapter 7 Conclusion

7.1 Summary

7.1.1. Fabrication and characterization of hemp powder

In this research, the fabrication of hemp fine powder was documented for the first time. Hemp fibres were successfully milled into ultrafine powders after three milling steps, namely, cutter milling, attrition milling and air-jet milling. The fibres were firstly cut in a cutter mill into fibre snippets with a length of around 1 mm. Secondly, fibre snippets were milled in an attritor mill with water. The optimum milling conditions were studied for the amount of fibre, the amount of water and milling time, within the range of 100 – 300 g fibre, 1 – 3 L water and < 6 hours of milling time. It was found that, among the three parameters, the amount of water had the strongest influence in the particle size. Within these variable ranges of parameters, the optimum attrition-milling condition in terms of particle size (the smaller the better) was 100 g fibre, 1 L water and 6 hours of milling time. Under this condition, it was possible to produce fine hemp powders of approximately 5 μm in diameter. After attrition milling, the wet powder slurry was passed through spray dryer to remove the excessive water. The dried powder was milled in the air jet mill, which further reduced the particle size to approximately 3 μm.

The resulting hemp powders were characterized for morphology, colour, chemical bonds, crystallinity, thermal property, surface area and pore sizes. SEM results indicated the morphology of powders during the powder production process, clearly showing the stages of the fibre to be pulverized into
fine powders. The datacolor result showed that the milled powder had a higher whiteness index and lower yellowness index than the raw fibre. FTIR results indicated that the milling process had no influence on the chemical structure of the powder. XRD results showed that the cellulose crystalline structure was damaged by the mechanical impact induced to the powders during attritor milling and air jet milling. BET result exhibited that the surface area, pore volume and pore size of hemp powder increased after powder fabrication. These properties will have influence on the performance of the powders in various applications.

7.1.2. Antibacterial properties of hemp powder and hemp extract

In this research, the antibacterial properties of hemp powders and hemp plant extracts from textile hemp were investigated for the first time. Ethyl acetate was found to be one of the most suitable solvent to extract CBD from hemp plants. The extracts from hemp leaf and hurd showed antibacterial properties against *S. aureus*, but not against all the bacteria such as *E. faecalis*, *S. pyogenes* and *P. aeruginosa*. The extract from hemp bark showed no antibacterial property against *S. aureus*. There was a strong correlation between the antibacterial property and the amount of CBD in the extracts. It was found that CBD is mainly contained in the leaf and hurd, but not in the bark where textile fibres are made. The results indicate the antibacterial efficacy of hemp extract may be primarily due to the presence of CBD.

Both raw hemp powder and degummed hemp powder have antibacterial property against *S. aureus*. The degummed hemp powder was bacteriocidal, totally stopping the growth of bacteria at a high concentration. On the other
hand, raw hemp powder showed a bacteriostatic property, inhibiting the growth of bacteria to certain extent but not totally stopping the growth of bacteria. It is concluded that degummed hemp powder has a better antibacterial property than raw hemp powder. This maybe attributed to the residual chemical (NaOH) that is used for degumming treatment on the hemp fibre.

The outcomes of the investigation will open up new applications of hemp textile fibre (bark) and waste materials in the hemp textile industry (leaves and hurds) in the medical, pharmaceutical and cosmetic industries, as a new natural antibacterial agent ways of using.

7.1.3. Polypropylene/hemp composite fibres

In this research, the fabrication and characteristics of plant fibre powder blended polymer composite filaments were investigated for the first time using the hemp powder and polypropylene. Hemp powder was directly blended with polypropylene polymer in the polymer chips fabrication stage without surfactants. Then, the polymer chips were melt-extruded into filaments. It was demonstrated that the addition of hemp powder provided new properties to polypropylene fibres such as dye uptake and moisture absorption. The thermal stability of polypropylene was also enhanced by the addition of hemp powder. However, the addition of hemp powder induced some negative effects such as delustering and yellowing, reduction in tensile strength and crystallinity. This study demonstrated a new application of hemp powder as a functional additive in synthetic-polymer filaments.
7.2 Future work

From the results of this thesis, several recommendations for future work are discussed below:

• For the powder production, other types of milling equipment, such as bead mills, shaker mills, and planetary mills can also be investigated for their suitability to grind hemp into fine powders.

• For the antibacterial properties of hemp plant extract, the antibacterial properties of textile hemp grown in different growing period should be investigated.

• For the antibacterial properties of hemp powder, the influence of particle size should also be considered. Hemp powder with different particle size can be tested for antibacterial properties. In order to increase the scope of this experiment, a number of other bacterial strains, such as *E. faecalis*, *S. pyogenes* and *P. aeruginosa* could also be tested in the evaluation of hemp powder antibacterial properties.

• The colour measurement values (K/S) of dye uptake property of PP/ hemp powder blended filaments is suggested, as it would give a clearer observation about dye uptake, for example, depth of shade.

• The antibacterial property of hemp powders and solvent extracts have been evaluated, the antibacterial property of PP/hemp powder blended filaments is also going to be tested in the future.

• The application of hemp powder for water-treatment should be investigated. High absorption properties of hemp fine powders may be beneficial for
absorbing organic wastes from water. Due to the high number of OH groups of the surface of hemp powder, certain heavy metal ions may also be effectively absorbed on hemp powders.
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