EFFECT OF CELLULOSE NANOWHISKERS' pH ON PROPERTIES OF STARCH-BASED BIONANOCOMPOSITES FILMS

NUNIN AKSORNTHONG

MASTER OF SCIENCE
IN
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Nunin Aksornthong

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Author	Nunin Aksornthong
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ABSTRACT

Bacterial cellulose nanowhiskers (BCNWs) were prepared by acid hydrolysis of bacterial cellulose (BC) using 50% (w/v) sulfuric acid at 50 °C. The effect of sulfuric acid hydrolysis time and pH adjustment on properties of the obtained nanowhiskers was investigated. Yield (%) of BCNWs decreased when increasing hydrolysis time. It was found that the 48 hours acid hydrolyzed BCNWs possessed the highest perfection of the crystal lattice or crystallinity. Transmission electron microscope (TEM) revealed that the continuous BC fiber network transformed into the isolated rod-like nanocrystals of the BCNWs with a diameter and length of averaged 28.18±2.0 nm and 637.61±147.10 nm, respectively. The sulfuric acid treatment leads to decreasing in the thermal stability of BCNWs confirmed by thermogravimetric analysis (TGA). This is due to the induced sulfate groups onto the BCNWs after acid hydrolysis. Additional pH adjustment by NaOH can significantly improve the thermal stability of the BCNWs. The pH of BCNWs was adjusted to 3, 5, 7 and they were used to reinforce in the starch matrix to prepare the bionanocomposites (with varied contents of 1, 5, 10 wt%) by film casting technique. With increasing BCNWs content, the bionanocomposites revealed a significant improvement in their crystallinity (confirmed by XRD), thermal stability (a increment

of 20-30 ^{o}C, confirmed by TGA) and water resistance. The highest water resistance was observed in the bionanocomposite films reinforced with 10 wt% BCNWs of pH 7. The mechanical properties of the films reinforced with BCNWs of pH 3 and BCNWs of pH 7 were not improved because of a poor interaction between BCNWs of pH3 and starch matrix and formation of large aggregates of BCNWs of pH 7 in the bionaocomposites structure. Finally, the films reinforced with BCNWs of pH 5 showed improved the mechanical properties possibly due to the optimum dispersion of BCNWs and sufficient interaction between BCNWs and the starch matrix in this system.

Keywords: Cellulose Nanowhiskers/Starch/Bionanocomposites/pH

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CHAPTER 1

INTRODUCTION

1.1 Background and Significance of the Research Problem

Composites are engineered or naturally occurring materials made from two or more constituent materials with significantly different physical or chemical properties which remain separate and distinct at the macroscopic or microscopic scale within the finished structure. Composites have gained popularity in high performance products that need to be lightweight, yet strong enough to take harsh loading conditions such as aerospace components. Composites are also used in a wide variety of applications; boat, scull hulls, bicycle frames and racing car bodies. Other uses include fishing rods, storage tanks, and baseball bats. Furthermore, there is considerable scope for tailoring their structures to suit the service conditions (Hull & Clyne, 1996).

In recent years, reinforcements from renewable resources such as natural fibers have attracted much attention to use for both thermoplastic and thermosetting polymer composites. Currently, more and more researchers are developing fully biodegradable composites, so called ‘green’ composites or biocomposites, which are composed of natural fibers and biodegradable polymer matrices. These composites are very attractive materials because of their environmental-friendly, sustainability and good mechanical properties (Wan et al., 2009).

Bionanocomposites in which the reinforcing material has nanometer dimensions are emerging to create the next generation of novel eco-friendly materials with superior performance. Bionanocomposites have found extensive applications in medicine, coating, packaging, automotive and so on. These materials have shown superior thermal, barrier and mechanical properties compared to today’s biocomposite materials (Hull & Clyne, 1996).

Cellulose is the most abundant biopolymer found in nature, as it is the major cell-wall component of plants, (Zhao et al., 2007) existing in a variety of living species such as plants, animals, bacteria, algae and some amoebas (Perez & Samain,

2010). Cellulose is a natural linear polysaccharide (homopolymer), in which D-glucopyranose rings are connected to each other with β-(1-4) glycosidic linkages (Baillie, 2004). Besides cellulose synthesized from plants, it is also secreted extracellularly by some bacterial species known as bacterial cellulose (BC). Plant cellulose and BC have the same chemical structure although they have different structural organization and mechanical properties (Sanz, Olsson, Lopez-Rubio & Lagaron, 2010). BC has found many applications in paper, textile, and food industries as well as a biomaterial in cosmetics and medicines due to its unique structure and properties i.e. high purity, high crystallinity, high mechanical strength and good biocompatibility (Rosa et al., 2010). Its high mechanical properties have also led to the use of BC as reinforcing agents in composite materials (Gea et al., 2011).

Recently, nano meter-sized cellulose crystals commonly referred to as whiskers, nanowhiskers or nanofibrils have gained interest to use as nanocomposite's reinforcement. They can be obtained from various natural fibers and some sea animals. The extracted of cellulose nanowhiskers from renewable resources show high aspect ratio, large surface area, exceptional mechanical properties (high specific strength and modulus), and environmental benefits (Rosa et al., 2010). Different approaches have been applied to prepare cellulose nanowhiskers, all of them lead to different types of nanofibrillar material, depending on the cellulose raw material, its pre-treatment, and the disintegration process itself. Sulfuric acid hydrolysis of cellulose is a well-known process used to remove amorphous regions (Sanz et al., 2010). Sulfuric acid produced sulfate ester group on aqueous suspensions and leads to good dispersion of cellulose nanowhiskers which were negative charge on cellulose nanowhiskers (Wada, Kuga & Okano, 1998). The surface charges on cellulose nanowhiskers led to their effective separation for reinforcing in composites. However, this reaction significantly decreases the thermal stability of cellulose whiskers. Since typical processing temperatures for thermoplastics rise above 200 ^{o}C, the thermal stability of these crystals is a key factor for them to be used as effective reinforcing materials. The thermal stability of cellulose whiskers can be recovered by neutralization step of the sulfuric acid groups with strong bases such as sodium hydroxide (Rosa et al., 2010). However, with neutralization, the degree of nanowhiskers' dispersion would be reduced. Therefore, this work aims to study the

effect of pH adjustment of cellulose nanowhiskers on both, its thermal properties and dispersion degree in the bionanocomposites.

Cellulose and starch are two common carbohydrates and the most abundant natural polymers. Starch is an energy storage material occurring as granules in some plants and microorganisms (Baillie, 2004). The size and shape of starch granules depends upon the source. Starch contains about 70-80% amylopectin, a highly branched polymer with a weight-average molecular weight of 10^7-10^9 and 20-30% amylose, a linear polymers (molecular weight of 10^5-10^6) (Baillie, 2004; Janssen & Moscicki, 2009). Amylose and amylopectin consist of glucopyranosis molecules, yet the structural differences between these two polymers determine their different properties (Alavi, 2003; Janssen & Moscicki, 2009). Amylopectin consists of α-1,4 bonded glucose segments, linked by α-1,6 bonds at the branching sites. Estimates are that around 4–6% of bonds in a standard amylopectin molecule appear to be α-1,6 links, which results in over 20,000 branchings in a molecule, although the branching are not large (Janssen & Moscicki, 2009; Mitrus, 2006). The most commonly employed starch types are those derived from maize, wheat, and potato (Baillie, 2004). With its availability, cheapness, biodegradability, and biocompatibility, starch is considered as a promising raw material for developing novel environmentally friendly bionanocomposite materials. However, starch is greatly hindered by its intractable nature, brittleness, water sensitivity, and poor mechanical properties. It has been found that using reinforcing materials in a starch matrix is an effective method to improve performances of the starch-based biocomposites. (Woehl et al., 2010).

The objectives of this research is firstly, to prepare bacterial cellulose nanowhiskers (BCNWs) to use a reinforcement and, secondly, to study the effects of bacterial cellulose nanowhiskers' pH and also BCNWs content on structure and properties of the bionanocomposite films. The morphology, crystallinity, thermal and mechanical properties of BCNWs and bionanocomposite films were characterized by transmission electron microscopy (TEM), Scanning electron microscopy (SEM), thermogravimetric analysis (TGA), X-ray diffraction (XRD), and tensile testing, respectively. Moisture absorbtions behavior of bionanocomposite films was also determined.

1.2 Research Objective

To study the effects of BCNWs' pH and content on structure and properties of the bionanocomposite films.

1.3 Scope of Research

1.3.1 Study the effect of acid hydrolysis time on yield (%), morphology, thermal property and crystallinity of the BCNWs.

1.3.2 Study the effect of pH adjustment on thermal property of the BCNWs.

1.3.3 Preparation of the starch-based bionanocomposite films reinforced with BCNWs of pH 1, 3, 5 and 7 at contents of 1, 5 and 10 wt% by film casting technique.

1.3.4 Characterizations of the bionanocomposite films by XRD, SEM, tensile test, TGA and moisture absorption technique.

1.4 Thesis Outline

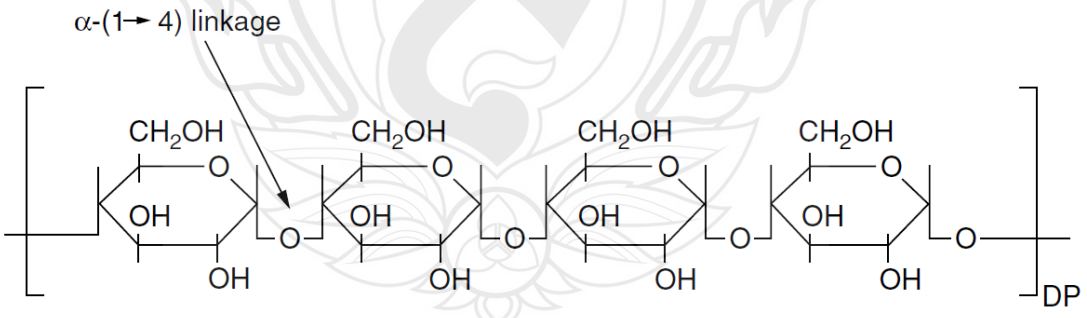
This thesis is composed of five chapters. The first chapter is an introduction on the research background, objectives and scope of research. The second chapter is a literature review which provides more details on starch, thermoplastic starch, cellulose, bacterial cellulose, cellulose whisker and cellulose whisker in nanocomposites. The third chapter reports the method of preparation of BCNWs, starch/BCNWs bionanocomposite films and characterization techniques of BCNWs, and starch/BCNWs bionanocomposite films. The results are discussed in detail in the fourth chapter. The final chapter summarizes the thesis.

CHAPTER 2

LITERATURE REVIEW

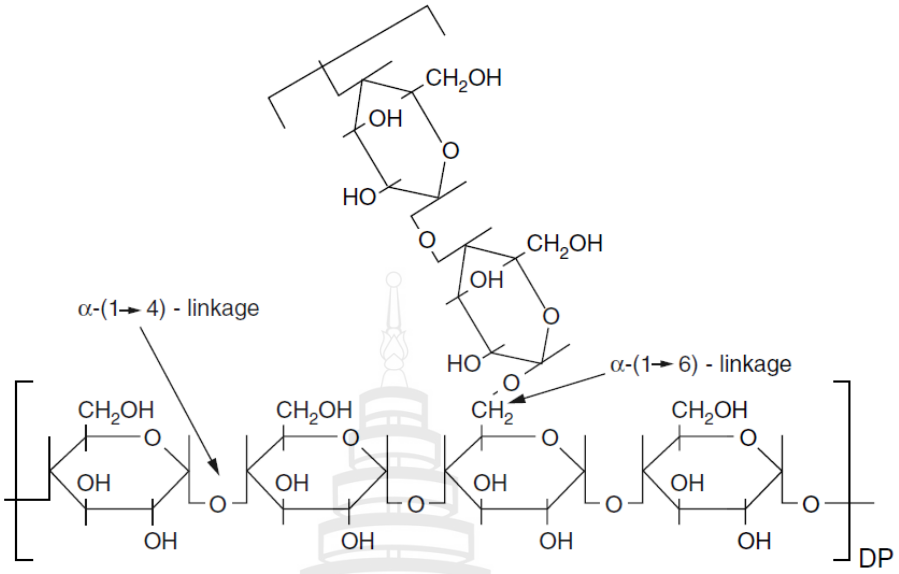
2.1 Starch and Thermoplastic Starch (TPS)

Starch is a linear or branched polysaccharide made up of repeating glucose groups with α-1,4 glycosidic linkages in giving rise to a chain length of 500-2,000 glucose units. Starch consists of two major compounds: amylase (Figure 2.1) and amylopectin (Figure 2.2). In the presence of hot water, the starch grains are hydrated in a process called gelatinization. Initially, the grains are swollen and subsequently disrupted and then the starch chains are released in the aqueous medium resulting in the formation of a gel. On cooling, the gel undergoes retrogradation, which results in the formation of a compact three dimensional network whose structure is based on the interaction between adjacent starch chains (Cheetham & Tao, 1998; Parker & Ring, 2001; Talja, Helen, Roos & Jouppila, 2007).



Source Cui (2005)

Figure 2.1 Structure of Amylose



Source Cui (2005)

Figure 2.2 Structure of Amylopectin

The amylose content estimated by all of the procedures based on iodine complex formation might be considered as apparent amylose content. Table 2.1 gives the apparent and absolute amylose content of starch from various sources.

Table 2.1 Amylose Content of Starch from Various Crops

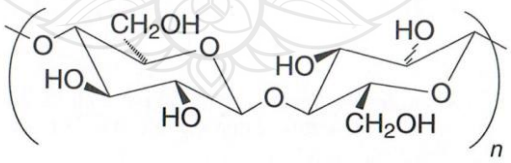
Starch	Amylose content (%)
Rice (Japonica)	17.5
Potato	21.4
Corn	30.2
Wheat	21.7
Tapioca	16.7
Maize (Normal)	21.5
Acorn	28.4

Source Hizukuri (1996), Hoover & Sosulski (1991)

Starch, in granular form, is not a thermoplastic polymer and hence exhibits poor melting processability. The granular structure of starch can be disrupted by adding plasticizers (Yu, Dean & Li, 2006; He et al., 2006). The plasticizers (e.g., water and glycols) interact with the polymer chains, and then reduce the interaction between adjacent chains (Bastida et al., 2005). thus making starch suitable for common processing such as extrusion or injection molding (Woehl et al., 2010; Dufresne, 2004). The above material thus obtained is termed "thermoplastic starch" (TPS) (Fakirov & Bhattacharayya, 2007; Woehl et al., 2010).

2.2 Cellulose

Cellulose is considered to be the most abundant renewable polymer in the world (Dufresne, 2004). Its structural material is naturally organized as microfibrils linked together to form cellulose fibers. Cellulose is biosynthesized by a number of living organisms ranging from higher to lower plants, some sea animals, bacteria and fungi (Heux, Dinand & Vignon, 1999)**.** Cellulose is a linear homopolymer (polysaccharide), in which β-D- glucopyranose rings are connected to each other with β-1-4 glycosidic linkages. The basic chemical structure of cellulose is presented in Figure 2.3. Each repeating unit bears three hydroxyl groups with the ability to form hydrogen bonds to adjacent chains. This plays a major role in directing the crystalline packing and also governing the physical properties of cellulose (Hull & Clyne, 1996).

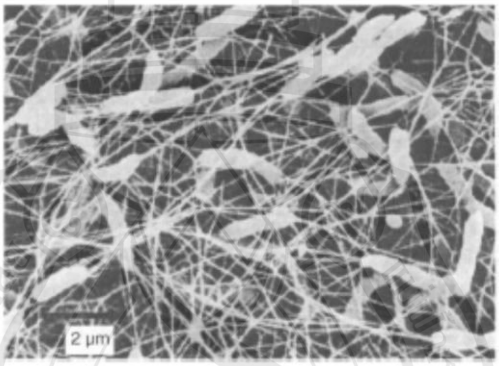


Source Baillie (2004)

Figure 2.3 Structure of Cellulose

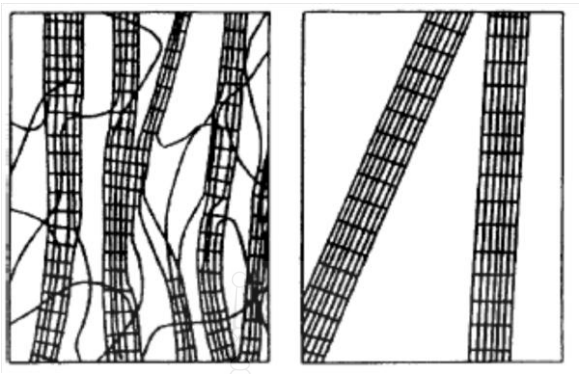
2.2.1 Bacterial Cellulose

Another variety of cellulose, which has caught the attention of biocomposite researchers, is the "bacterial cellulose" (BC). The BC is found on the surface of *Acetobacter* cultures, mainly *Acetobacter xylinum*, in a form of a gelatinous and translucent pellicle. A scanning electron micrograph of the pellicle (Figure 2.4) reveals a random network of cellulose microfibrils with a width of less than 100 nm (Iguchi, Yamanaka & Budhiono, 2000). BC is chemically identical to plant cellulose, but its macromolecular structure and properties differ from plant cellulose (Figure 2.5) (Bielecki, Krystynowicz, Turkiewicz & Kalinowska, 2005).



Source Iguchi et al. (2000)

Figure 2.4 Scanning Electron Micrograph of Bacterial Cellulose Nanofibrils and Bacteria (Acetobacter)

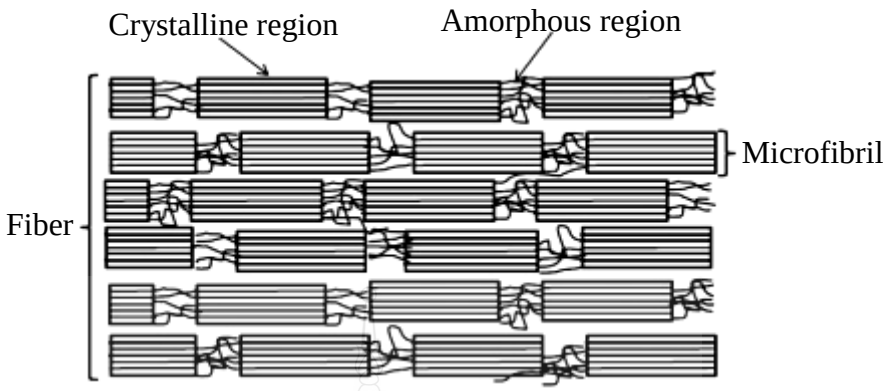


Source Iguchi et al. (2000)

Figure 2.5 Schematic Model of Plant Cellulose Fibrils (left); of BC Microfibrils (right) Drawn in Comparison with the `Fringed Micelles'

2.2.2 Cellulose Whiskers

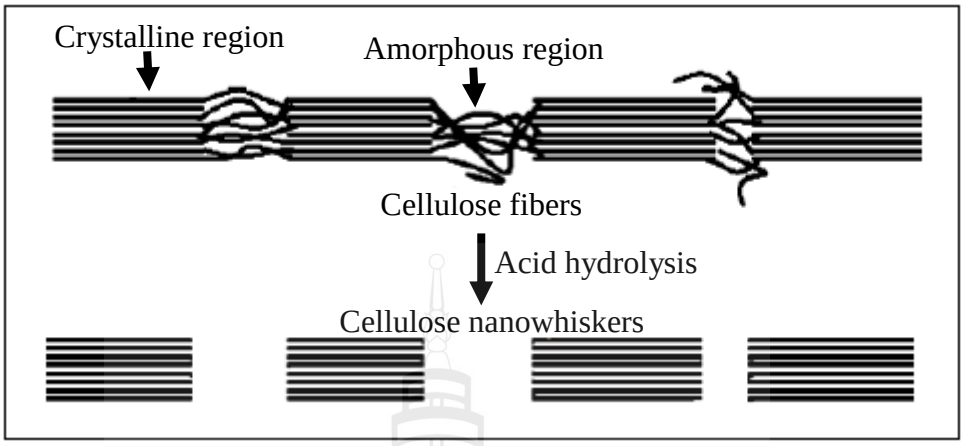
Cellulose chains are aligned parallel to each other in the so-called "crystalline" regions of the microfibrils. The cellulose microfibril constitutes the basic structural unit of the plant cell wall; each microfibril can be considered as a string of cellulose crystallites, linked along the chain axis by amorphous domains as depicted in Figure 2.6 (Whistler & Richards, 1970). The cellulose amorphous regions are randomly oriented in a spaghetti-like arrangement leading to a lower density compared to crystalline regions (Saxena & Brown, 2005; Lima & Borsali, 2004).



Source Whistler and Richards (1970)

Figure 2.6 Schematic Diagram of the Physical Structure of a Semicrystalline Cellulose Fiber

Cellulose whiskers represent the crystalline regions extracted from cellulose, mainly by acid hydrolysis. The amorphous regions of cellulose are more accessible to acid attack compared to crystalline regions and therefore, under controlled conditions, the amorphous regions are assumed to be removed whereas the crystalline regions remain, as illustrated in Figure 2.7.

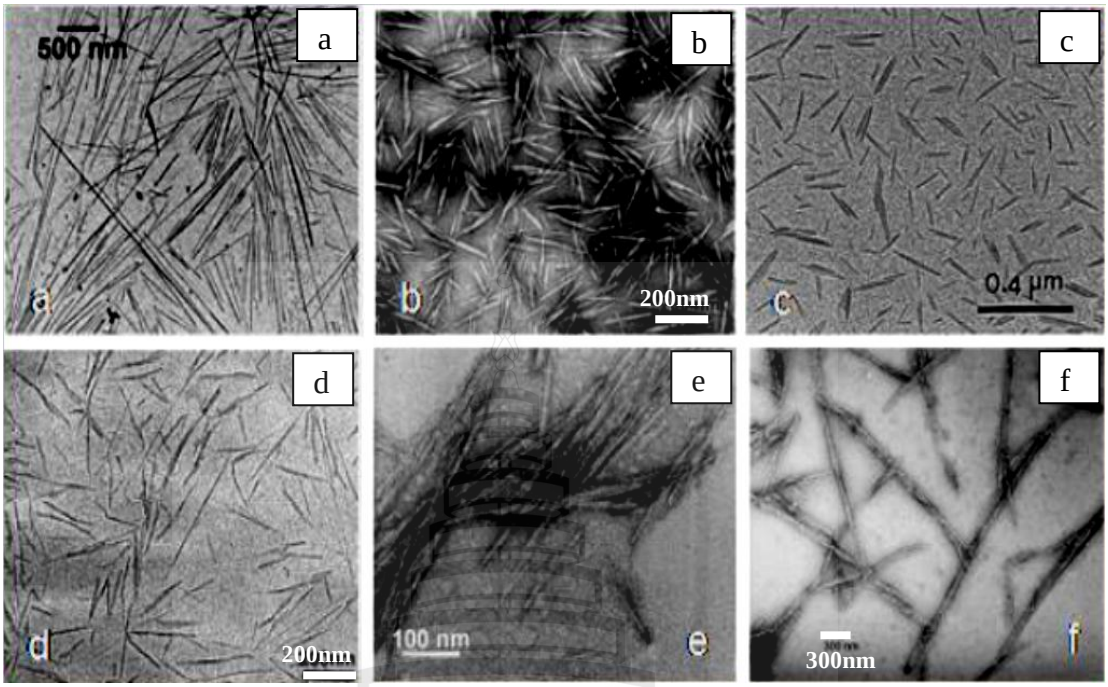


Source Chen, Lawton, Thompson and Liu (2012)

Figure 2.7 Illustration of Acid Hydrolysis of Semicrystalline Cellulose Fibers

Shape of cellulose microfibrils or nanocrystals depends on the nature of the cellulose source as well as the extraction conditions such as time, temperature, post treatment, and purity of materials (Dufresne, 2004; Bielecki et al., 2005; Whistler & Richards, 1970). Nevertheless, typical dimensions of the extracted nanocrystals or whiskers range from 5 to 10 nm in diameter and from 100 to 500 nm in length. Since the cellulose whiskers are devoid of chain folding, they contain only a small number of defects. The Young's modulus were determined by different authors and reported to be between 130 GPa (Li, Wang & Liu, 2011) and 250 GPa (Tang & Weder, 2010). There values are close to the modulus of the perfect crystal of native cellulose (Bielecki et al., 2005).

A variety of sources can be used to prepare cellulose whiskers, e.g. microcrystalline cellulose, bacterial cellulose, algal cellulose (valonia), hemp, tunicin, cotton, ramie, sisal, sugar beet, wood, and etc. Transmission electron micrographs of some cellulose nanocrystals are presented in Figure 2.8.



Source Siqueira, Bras and Dufresne (2010)

Figure 2.8 Transmission Electron Micrographs from Diluted Suspensions of Hydrolyzed (**a**) Tunicin (**b**) Ramie, (**c**) Cotton, (**d**) Sugar Beet, (**e**) Microcrystalline Cellulose (MCC) and (**f**) Bacterial Cellulose

Many different terms have been used in the literature to designate these rod-like nanoparticles. They are mainly referred to as “nanowhiskers” or cellulose nanocrystals. The terms microfibrils are also used, despite their nanoscale dimensions, leading to some misunderstanding and ambiguities (Paralikar, Simonsen & Lombardi, 2008). Some process, sources of raw cellulosic and extraction processes, are summarized in Table 2.2. Some sources for obtaining cellulose whiskers and their characteristics are listed in Table 2.3.

Table 2.2 The Different Process used to Describe Cellulose Nanowhiskers

Source	Process	References
Name Cellulose Nanowhiskers		
Ramie	H_2SO_4 hydrolysis	(Habibi et al., 2008)
Avicel	H_2SO_4 hydrolysis	(Petersson et al., 2007)
Cellulose Filter Paper	H_2SO_4 hydrolysis	(Rojas et al., 2009)
Grass Fiber	H_2SO_4 hydrolysis	(Pandey et al., 2009b)
Microcrystalline Cellulose (MCC)	LiCl:DMAc	(Oksman et al., 2006)
Name Cellulose Nanocrystals		
Cotton Whatman Filter Paper	H_2SO_4 hydrolysis	(Paralikar et al., 2008) (Mangalam et al., 2009)
Bacterial Cellulose	H_2SO_4 hydrolysis	(Grunert & Winter, 2002)
Cotton(cotton wool)	H_2SO_4 hydrolysis	(Morandi et al., 2009)
Microcrystalline Cellulose (MCC)	H_2SO_4 hydrolysis	(Bondeson et al., 2006)
Avicel, Pulp Fiber	Sonication	(Filson & Dawson-Andoh, 2009)

DMAc = N,N-Dimethyl Acetamide

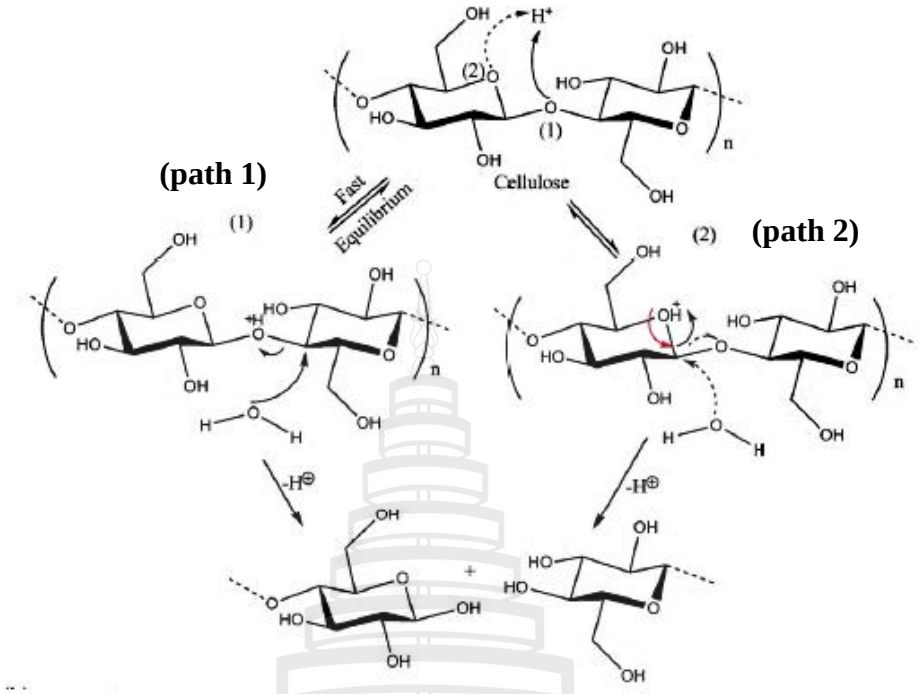
LiCl = Lithiumchloride

Table 2.3 Characteristics of Cellulose Nanowhiskers from Different Sources

Cellulose source	Length (nm)	Cross section (nm)
Tunicate	100-several microns	15
Bacterial	100-several microns	5-10, 30-50
Cotton	200-350	5-15
Wood	100-300	3-5
Sugar beet pulp	210	15

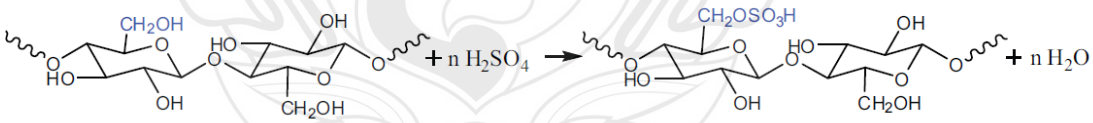
Source Gardner et al. (2008)

The extraction of crystalline cellulosic regions, in the form of nanowhiskers, is a simple process based on acid hydrolysis *(*Bielecki et al., 2005). Azizi Samir et al. (2005), described cellulose whiskers as nanofibers which have been extracted under controlled conditions that lead to the formation of high-purity single crystals.



Source Lu and Hsieh (2010)

Figure 2.9 Mechanism of Acid Hydrolysis of Cellulose



Source Li and Ragauskas (2011)

Figure 2.10 Esterification of Cellulose Hydroxyl Groups during Sulfuric Acid Hydrolysis

Sulfuric acid (H_2SO_4) hydrolysis of cellulose involves rapid protonation of glucosidic oxygen (Figure 2.9, path 1) or cyclic oxygen (path 2) by protons from the acid, followed by a separation of glucosidic bonds induced to water addition. Hydrolyzing cellulose with sulfuric acid also involves esterification of the hydroxyl groups. This esterification reaction generally proceeds to yield acid half-ester or the so-called 'cellulose sulfate' (Figure 2.10). The presence of sulfate groups on the cellulose nanocrystal surfaces results in negatively charged surfaces above acidic pH, leading to a stable colloid system of the nanocellulose suspension (Bondeson, Mathew & Oksman, 2006). This anionic stabilization via the repulsion forces was shown to be very efficient in preventing the aggregation of cellulose nanocrystals driven by hydrogen bonding (Lu & Hsieh, 2010).

Dong et al. (1998) studied the effect of hydrolysis conditions (time, temperature, and ultrasound treatment) on the properties of resulting cellulose nanocrystals. They reported that longer hydrolysis time leads to shorter monocrystals and also an increase in their surface charges. Characterization of cellulose whiskers were performed using different techniques such as Transmission Electron Microscopy (TEM), Nuclear Magnetic Resonance Spectroscopy (NMR), Atomic Force Microscopy *(*AFM) and X-ray diffraction (XRD) (Dong, Revol & Gray, 1998).

Wada et al. (1998) studied H_2SO_4 and HCl-prepared cellulose whiskers. They demonstrated that the charge of the surface is one of the main parameter which control the inter whisker interactions and the rheological behavior of their suspensions. The suspensions of charged whiskers showed no time dependence in viscosity. They explained that sulfuric acid provides more stable aqueous suspensions than hydrochloric acid because hydrochloric acid produced cellulose nanocrystals with minimum surface charge. On the contrary, sulfuric acid-prepared nanocrystals present a negatively charged surface, due to the esterification of surface hydroxyl groups to give charged sulfate groups (Wada, Kuga & Okano, 1998).

Later, Roman and Winter (2004) reported that the sulfate groups on the surface BCNWs induced the degradation of cellulose at lower temperatures and lower the BCNWs thermal stability (Roman & Winter, 2004).

Candanedo et al. (2005) studied the properties of cellulose nanocrystals by hydrolysis of softwood and hardwood pulps. They varied the hydrolysis time and

acid-to-pulp ratio in order to obtain cellulose nanocrystals. They explained that the reaction time is one of the most important parameters to be considered in the acid hydrolysis of wood pulp. Moreover, they reported that too long reaction times completely digest the cellulose to yield its component sugar molecules. On the contrary, lower reaction times will only yield large undispersable fibers and aggregates (Candanedo, Roman & Gray, 2005).

Wang, Ding and Cheng (2007) prepared cellulose nanocrystals by hydrolysis of microcrystalline cellulose with mixed acid composed of 30% (v/v) sulfuric acid and 10% (v/v) hydrochloric acid. The results showed the degradation of cellulose nanocrystals with sulfate groups started at lower temperature and two remarkable pyrolysis processes. When neutralized by NaOH solution, the degradation temperatures shifted to the higher temperature and occurred within a narrow temperature range characterized by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) at nitrogen current (Wang, Ding & Cheng, 2007).

Pandey et al. (2009a) prepared cellulose nanofibers from grass Zoysia (japonica and tenuifolia). It was found that cellulose whiskers have lower thermal stability than alkali treated fiber (Pandey et al., 2009a). In 2010, Rosa et al., prepared cellulose nanowhiskers by sulfuric acid hydrolysis from coconut husk fibers which had previously been submitted to a delignification process. Higher residual lignin content was found to induce a higher thermal stability to cellulose nanowhiskers (Rosa et al., 2010).

BCNWs were also prepared by acid hydrolysis of cotton cellulose, followed by freeze-drying. Lu and Hsieh (2010) confirmed that sulfuric acid removed amorphous cellulose to produce isolated cellulose nanocrystals with newly introduced sulfate groups on the nanocrystal surfaces. The results also showed that the introduced surface charges led to their effective separation (Lu & Hsieh, 2010).

Sanz et al. (2011) studied the effect of sulfuric acid hydrolysis time and further treatments such as neutralization and dialysis on the properties of the obtained nanoparticles. The calculated crystallinity indexes were deduced on the long hydrolysis times. The thermal stability of the material is significantly decreased because of the sulfate group. Neutralization produced a slight increase in the

crystallinity index and most importantly, it led to a remarkable increase on the BCNWs thermal stability (Sanz, Rubio & Lagaron, 2011).

2.2.3 Cellulose Whiskers in Nanocomposites

Since the first announcement of using cellulose whiskers as a reinforcing phase by Favier, Chanzy and Cavaille in 1995, new nanocomposite materials with original properties were obtained by physical incorporation of cellulose whiskers into a polymeric matrix. Until today, their research is considered as a very important work in the field of cellulose based polymer nanocomposites because it demonstrated the reinforcing potential of the high aspect ratio cellulose nanocrystals (Favier, Chanzy & Cavaille, 1995). Later in 2000, Angle`s and Dufresne prepared nanocomposite materials using glycerol plasticized starch as the matrix and a suspension of tunicin whiskers as a reinforcing phase. Tunicin whiskers consisted of slender parallelepiped rods with a high aspect ratio. After mixing the raw materials and gelatinization of starch, the resulting suspension was cast and evaporated under vacuum. The composites were conditioned at various moisture contents in order to evaluate the effect of this parameter on the composite structure. The resulting films were characterized using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), water absorption experiments, and wide-angle X-ray scattering. The unfilled matrix appears as a complex heterogeneous system composed of glycerol domains dispersed in an amylopectin continuous phase. Each phase exhibits its own glass-rubber transition, for which the temperature decreases as the moisture content increases owing to the plasticizing effect of water. The specific behavior of amylopectin chains located near the interface in the presence of cellulose probably led to a transcrystallization phenomenon of amylopectin on cellulose whiskers surface. This inherent restricted mobility of amylopectin chains most likely accounts for the lower water uptake of cellulose/starch composites for increasing filler content (Angle`s & Dufresne, 2000). Next, Angle`s and Dufresne (2001) studied the effects of whiskers content and water content on properties of the amylopectin/tunicin nanocomposite materials. The reinforcing effect of tunicin whiskers strongly depended on the ability of cellulose filler to form a rigid network, resulting from

strong interactions between whiskers such as hydrogen bonds. In addition, it was shown that increasing water content induced the crystallization of amylopectin chains and the accumulation of plasticizer in the cellulose/amylopectin interfacial zone (Angle`s & Dufresne, 2001).

Mathew and Dufresne (2002) prepared nanocomposites from sorbitol plasticized waxy maize starch matrix and tunicin whisker as the reinforcing phase. The composites were conditioned at different relative humidity levels. When increase tunicin whisker, the crystallinity of the system increases. The glass-rubber transition temperature (T_g) of the plasticized starch matrix increases up to whiskers content about 15 %wt loading. At higher whisker content, a decrease of T_g is observed. A significant increase in crystallinity was observed in the composites by increasing either moisture content or whiskers content (Mathew & Dufresne, 2002).

Garcia de Rodriguez, Thielemans and Dufresne (2006) prepared nanocomposites of sisal whisker reinforced poly vinyl acetate (PVAc). The resulting whiskers were rod-like particles with high aspect ratio. They studied water uptake of the sisal whisker PVAc composites. Water was not found to plasticize the composite significantly above the whisker percolation threshold. Below this threshold value, the composite was plasticized significantly at high water uptake (98% RH). (Garcia de Rodriguez, Thielemans & Dufresne, 2006).

Lu, Weng and Cao (2006) prepared plasticized starch (PS) biocomposites by casting with addition of ramie cellulose nanocrystalites (RN) of 0–40 wt% as fillers. The ramie cellulose nanocrystallites, having lengths of 538.5±125.3 nm and diameters of 85.4± 25.3 nm on average, were prepared from ramie fibers by acid hydrolysis. The scanning electron microscopy (SEM) showed a relatively good dispersion of the RN fillers in the PS matrix and good adhesion between the matrix and fillers of the PS/RN composites. Both tensile strength and Young's modulus increased from 2.8 MPa for PS film to 6.9 MPa and from 56 MPa for PS film to 480 MPa, respectively, with increasing RN content from 0 to 40 wt%. As the RN fillers increase in the PS matrix, the resulting composites also showed a higher water-resistance (Lu, Weng & Cao, 2006).

Svagan, Azizi Samir, Lars and Berglund (2007) prepared the nanocomposites of the highly plasticized amorphous amylopectin matrix of a 50/50 mixture of

amylopectin and glycerol with a microfibrillated cellulose (MFC) nanofiber (content in the range of 10-70 wt%). The starch matrix showed high compatibility with MFC and this facilitated the uniquely high MFC content achieved. SEM studies revealed a layered nanocomposite structure and good MFC dispersion. A modulus of 6.2 GPa, a tensile strength as high as 160 MPa, and a strain-to-failure of 8.1% were observed at 70 wt% MFC. The nanostructured characteristics of MFC and favorable MFC-matrix adhesion contributed to the delay material damage during deformation, ductility, and high toughness. It was suggested that nanostructured MFC network reinforcement has the potential to substantially improve the properties of commercial starch-based materials such as films and foams (Svagan, Azizi Samir, Lars & Berglund, 2007).

Glycerol-plasticized thermoplastic pea starch (TPS)/carboxymethyl cellulose (CMC) and TPS/microcrystalline cellulose (MC) composites were prepared using a screw extruder by Ma, Chang and Yu (2008). Composite films were investigated by dynamic mechanical thermal analysis (DMTA), mechanical properties, as well as water vapor permeability (WVP) and scanning electron microscope (SEM). SEM showed that there was good adhesion between starch and CMC or MC. MC increased the thermal stability, while CMC decreased the thermal stability, as well as the barrier of water vapor of the composites. DMTA revealed that the addition of CMC and MC improved the storage modulus and glass transition temperature (T_g) of the composites. Both CMC and MC increased the tensile stress and elongation at break at the low water content (13%). Water resistance of CMC and MC was better than TPS matrix. TPS/MC composites have better water vapor barrier than TPS/CMC composites because of the hydrophobic crystalline of MC led to reduction of permeability (Ma, Chang & Yu, 2008).

Takagi and Asano (2008) fabricated green composites from starch-based, dispersion-type biodegradable resin and cellulose nanofibers. The ingredients were blended together using a home-use mixer and a stirrer. Composites were prepared by hot pressing at 140 ^{o}C and 10–50 MPa. The flexural strength and flexural modulus of the composites increased with increasing molding pressure. Their mechanical properties showed good correlation with their density. It was suggested that the stirrer mixing treatment contributed to a uniform dispersion of nanofibers in the resin matrix (Takagi & Asano, 2008).

Cao, Chen, Chang, Muir and Falk (2008) prepared the cellulose crystals by acid hydrolysis of flax fiber. After mixing the suspension of flax cellulose nanocrystals (FCNs) and plasticized starch (PS), the nanocomposite films were obtained by the casting method. The effects of FCNs loading on the morphology, thermal behaviour, mechanical properties and water sensitivity of the films were investigated by means of X-ray diffraction (XRD), differential scanning calorimetry (DSC), tensile testing, and water absorption testing. SEM photographs of the failure surfaces clearly demonstrated a homogeneous dispersion of FCNs within the PS matrix and strong interfacial adherence between matrix and fillers. In particular, these nanocomposite films exhibited a significant increase in tensile strength and Young's modulus from 3.9 to 11.9 MPa and 31.9 to 498.2 MPa, respectively, with increasing FCNs content from 0 to 30 wt%. Also, with a loading of FCNs, the resulting nanocomposite films showed a higher water resistance (Cao, Chen, Chang, Muir & Falk, 2008).

Pea hull fibre nanowhiskers (PHFNW-t) were extracted from pea hull fibres (PHF) using sulfuric acid by Chen, Liu, Chang, Cao and Anderson (2009). The PHFNW-t was then blended with pea starch (PS) to prepare (PS/PHFNW-t) bionanocomposite films. Compared with the neat PS film and PS/PHF (hydrolysis time = 0 hours) film, the PS/PHFNW-t nanocomposite films exhibited higher ultraviolet absorption, transparency, tensile strength, elongation at break, and water-resistance. It was found that 8 hours was the most suitable time for hydrolysis of PHF by sulfuric acid in order to improve the elongation at break of the prepared bionanocomposite films (Chen, Liu, Chang, Cao & Anderson, 2009).

Liu, Zhong, Chang, Li and Wu (2010) prepared bamboo cellulose crystals (BCCs) using a combined HNO_3–$KClO_3$ treatment and sulfuric acid hydrolysis. The BCCs were then used to reinforce in the glycerol plasticized starch. The structure and morphology of BCCs were investigated using X-ray diffraction (XRD), scanning electron microscope (SEM), and nuclear magnetic resonance spectroscopy (NMR). At low concentration, crystals assembled into leaf nervations. At high concentration, crystals congregated into a micro-sized ‘‘flower” geometry. The different geometries of aggregation were due to high surface electrostatic energy and large surface area of BCCs. Tensile strength and Young's modulus of the starch/BCCs composite films

(SBC) were 12.8 and 210.3 MPa, respectively, which was much higher than those without bamboo crystals. By incorporation of the crystals, water uptake of the composites was decreased. The dispersion and polymorph of cellulose crystals were severely influenced by the different treatments and surrounding matrix, which ultimately affected the reinforcing effect on the plasticized starch-based biocomposites (Liu, Zhong, Chang, Li & Wu, 2010).

Kaushik, Singh and Verma (2010) studied properties of cellulose wheat straw nanofibril and TPS glycerol plasticized corn starch based nanocomposites. TEM, SEM and AFM confirmed the nano-size of the extracted cellulose wheat straw nanofibrils with diameter of 30–70 nm. XRD results of the nanocomposites revealed an improvement in crystallinity with addition of nanofibrils. TGA depicted an increasing in residue left with increase in cellulose nanofibrils content. Reduction in water adsorption was also observed. Mechanical and barrier properties increased with addition of nanofibers (Kaushik, Singh & Verma, 2010).

Bendahou, Kaddami and Dufresne (2010) prepared nanocomposite films of cellulose whiskers and microfibrillated cellulose (MFC) extracted from the rachis of date palm tree with natural rubber as matrix. These films were obtained by the casting/evaporation method. The properties of the nanocomposite films were investigated using differential scanning calorimetry (DSC), toluene and water uptake experiments, dynamic mechanical analysis (DMTA) and tensile tests. MFC was in a form of rod-like nanoparticles with an average length and diameter around 260 and 6.1 nm, respectively. Results showed that higher filler–matrix adhesion dominated the behavior of MFC-based composites such as a lower water uptake and higher mechanical properties in terms of stiffness. The reinforcing effect was shown to be higher for nanocomposites with MFC compared to cellulose whiskers (Bendahou, Kaddami & Dufresne, 2010).

Das et al. (2011) prepared jute micro/nanofibrils (JNF) by acid hydrolysis. JNF reinforced starch/PVA based biocomposite films (SPVA/JNF) prepared by solution casting method. JNF content was varied between 0, 10 and 15 wt%. These biocomposite films were characterized by mechanical characterization, thermal analysis, moisture uptake test, scanning electron microscopy (SEM) and atomic force microscopy (AFM). The AFM study revealed the most uniform dispersion of fillers in

SPVA/JNF 10 wt%. Moisture uptake decreased significantly in the biocomposites when exposed to 93 %RH condition. Moreover, the 10 wt% JNF loaded film (SPVA/JNF 10) resisted the dissolution in water indicating the stabilization of the matrix within a cellulose network. Thus, the SPVA/JNF 10 wt% films exhibited the best combination of properties (Das et al., 2011).

Trovatti et al. (2012) prepared nanocomposite films by casting water-based suspensions of pullulan and nanofibrillated cellulose. The materials were characterized in terms of morphology, thermal stability, crystalline structure and mechanical properties. All bionanocomposites were very homogeneous, translucent and showed considerable improvements in thermal stability and mechanical properties (up to 5500% and 8000% in the Young's modulus and tensile strength, respectively) when compared to the unfilled pullulan films (Trovatti et al., 2012).

Chen, Lawton, Thompson and Liu (2012) investigated the effectiveness of cellulose nanocrystals (CNC) derived from potato peel waste as a reinforcement. and vapor barrier additive. The nanocrystals were derived from cellulosic material in the potato peel by alkali treatment and subsequently acid hydrolysis. TEM images revealed the average fiber length of the nanocrystals was 410 nm with an aspect ratio of 41. Mechanical and barrier properties were improved by the incorporation of these CNC into a polymer matrix, even at low loadings of 1–2%, but only when the chemistry of the matrix was distinctly different from that of cellulose. Water vapor transmission measurements showed a marginal reduction of water permeability for the PVA composite, whereas no effect was observed for the thermoplastic starch composite (Chen, Lawton, Thompson & Liu, 2012).

Recently, Maiju, Mathew and Oksman (2013) to study the extrusion processing of cellulose nanocomposites and the properties of the prepared composites. Cellulose nanofibers (CNF) were used in wet state (12 wt% water suspension) in the processing of the composites together with a thermoplastic starch matrix. Nanocomposites with 0, 5, 10, 15 and 20 wt% cellulose nanofiber content were prepared. The characterization methods were tested by conventional tensile testing, scanning electron microscopy and moisture absorption. Mechanical testing of the nanocomposites showed that the tensile strength and modulus of the starch matrix were improved, and the moisture sensitivity was reduced with the addition of

cellulose nanofibers. The tensile modulus increased linearly with the increasing nanofiber content, but the strength properties were the highest for the material with 10 wt% of cellulose nanofibers. Scanning electron microscopy study revealed aggregates of cellulose nanofibers in the composites, especially in the case of composites with 15 and 20 wt% of CNF, which explains why there was no improvement in the strength properties of these composites (Maiju, Mathew & Oksman, 2013).

CHAPTER 3

METHODOLOGY

3.1 Experimental

3.1.1 Materials

The materials used in this work were bacterial cellulose (BC) sheet from cultivation of bacterial cellulose (*A. xylinum* TISTR 975) Deionised water was supplied by Mae Fah Luang University Labaratory (S2 building), Sulfuric acid (96% w/w) was purchased from Merck, NaOH AR grade was purchased from Qrec, New Zealand. Corn flour brand Super-Find was purchased from local supermarket. Guar gum and glycerol (99.5 % w/v) was purchased from Sigma Aldrich and Analar Normapur, respectively.

3.1.2 Preparation of Bacterial Cellulose Nanowhiskers (BCNWs)

Firstly, a bacterial cellulose (BC) sheet was prepared by compressing a bacterial cellulose pellicle which was sandwiched between woven metal sheets (325 mesh) at 115°C for 5 min using compression machine (Hydraulic hot press, Scientific LP-S-80, Labtech Engineering). The dried BC sheet was used as a raw material for preparation of bacterial cellulose nanowhiskers (BCNWs). The acid hydrolysis was performed using 50% (w/v) sulfuric acid, at a cellulose/acid ratio of approximately 8 g/L, shaking in water bath at 50 ^{o}C for a fixed period of time (24, 48 and 72h). The BCNWs were obtained as a precipitate collected from 15 centrifugation cycles (Ultrasonic Centifuge Avanti j-30I Centrifuge) at 12,500 rpm and 15 ^{o}C for 20 min. Flow chart of the BCNWs preparation is shown in Figure 3.1.

In order to determine yield (%) of BCNWs, the precipitate was dried in a hot air oven at 100 ^{o}C for 2 hours. The yield (%) of the BCNWs is calculated by the following equation 3.1:

$$\text{Yield (\%)} = \mathbf{C/G} \times 100 \qquad \text{(Equation 3.1)}$$

where **C** is weight of the dried BCNWs (gram) and **G** is weight of the dried BC sheet (gram).

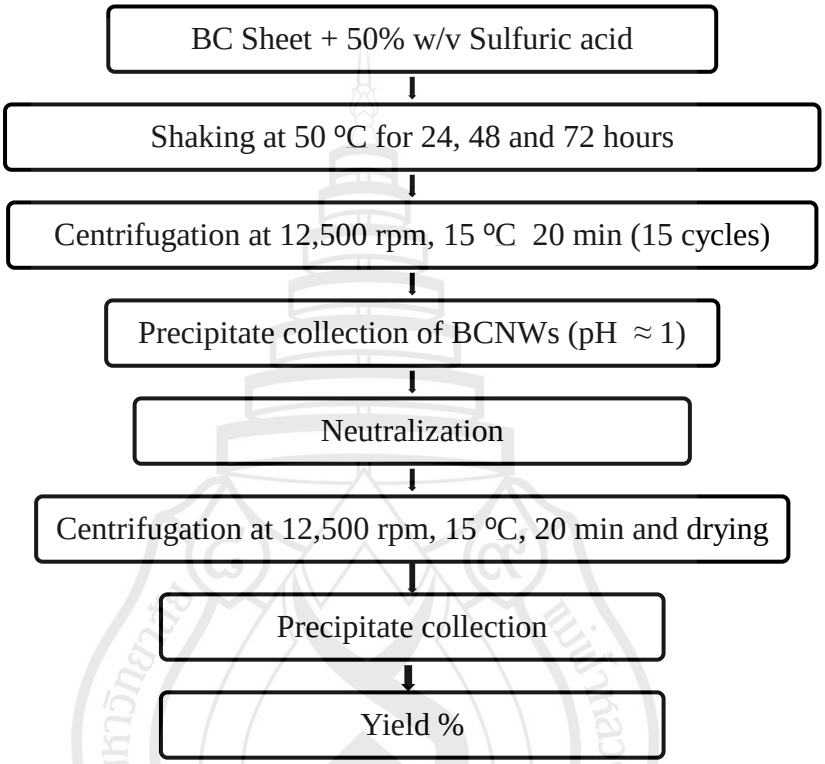


Figure 3.1 Flow Chart of Bacterial Cellulose Nanowhiskers (BCNWs) Preparation

3.1.3 Preparation of Bionanocomposite Films

3.1.3.1 Preparation of Standard Films

All ingredients in Table 3.1 was pre-mixed in a beaker until a homogeneous mixture was obtained. The mixtures were then heated at 80 °C using a hot plate for starch to gelatinize. It was continuously stirred for 20 minutes with heating. After that the mixture was degassed by sonification for 30 minutes. It was then poured onto a Petri dish and dried at 40 °C for approximately 2 days. The preparation steps are summarized as shown in Figure 3.2.

Table 3.1 Ingredient for Preparation of Standard Film

Ingredient	Weight (g)
Corn starch	3.00
Guar gum	0.01
Glycerol (99.5%)	0.90
Deionised water	100.00

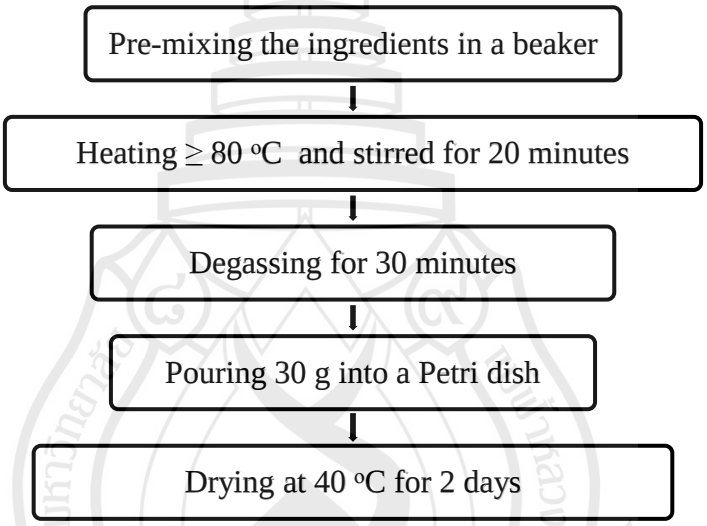


Figure 3.2 Flow Chart of Preparation of Standard Films

3.1.3.2 Preparation of Bionanocomposite Films

The pH of the prepared BCNWs measured after the centrifugation was being around pH 1. Then all the BCNWs were re-suspended in deionized water and adjusted pH to 3, 5, 7 by NaOH solutions of 0.5% and 5.0% (w/v) and subsequently centrifuged to obtain the pH adjusted BCNWs as a partially hydrated precipitate. To prepare the bionanocomposite fims, BCNWs were pre-mixed with deionized water (varied content 1, 5 and 10 wt% based on starch weight) in a beaker until a homogeneous mixture was obtained. Then other ingredients in Table 3.1 were added into the mixture. After that, all ingredients were heated at 80 ^{o}C using a hot plate for

starch to gelatinize. It was continuously stirred for 20 minutes with heating. After that the mixture was degassed by sonification for 30 minutes. Then, it was poured onto a Petri dish and dried at 40 ^{o}C for approximately 2 days. The preparation steps are summarized as shown in Figure 3.3.

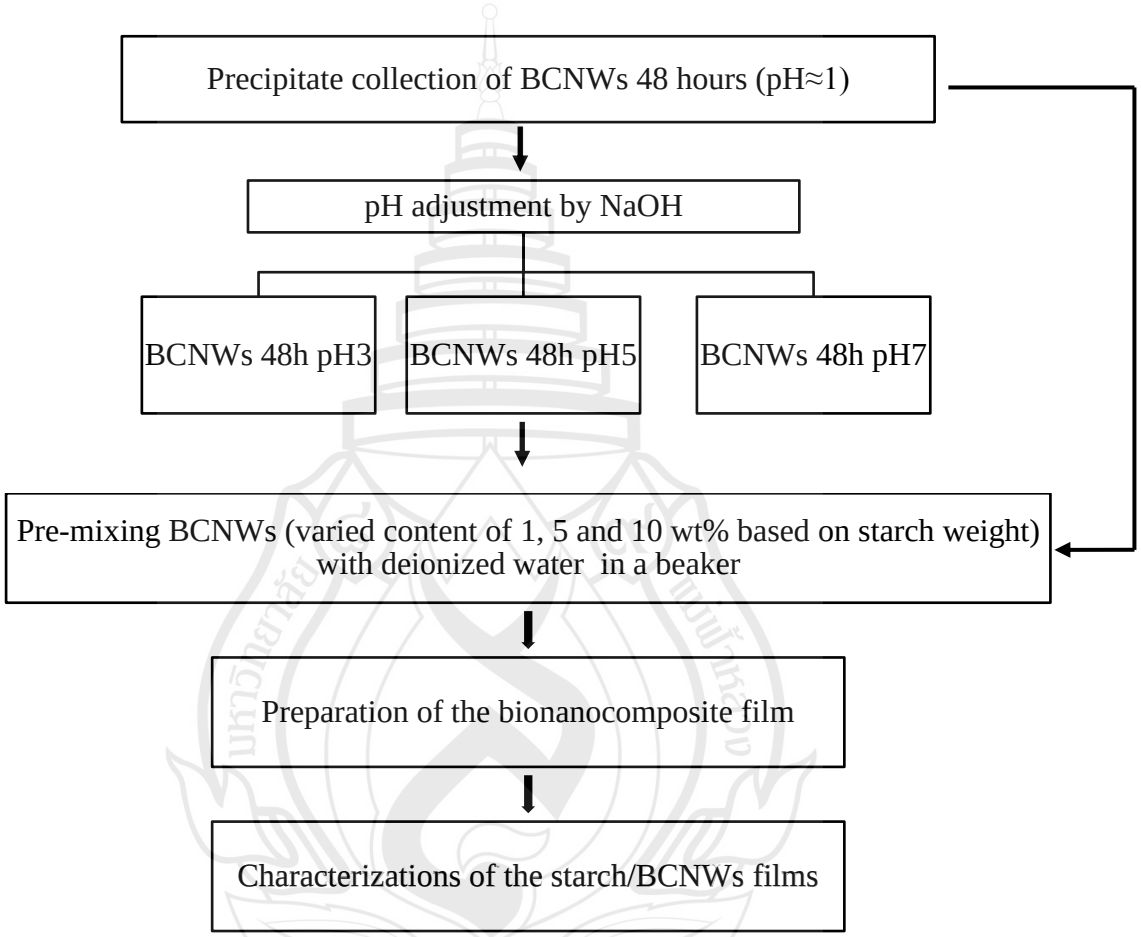


Figure 3.3 Flow Chart of Preparation of the Bionanocomposite Films

3.1.4 Characterizations

Prior to all characterization, samples were stored in condition of 50% relative humidity (RH) for 3 days (using a chamber of the saturated $Mg(NO_3)_2$ solution).

3.1.4.1 X-ray Diffraction (XRD)

X-ray diffraction patterns were detected using Cu Kα radiation, generated with X'pertPro MPD (Philips, Netherlands) at 40 kV, 20 mA. The X-ray beam was operated in reflection mode and the samples were examined over the angular range (2θ) of 10° to 45° with a step size of 0.02° and a count time of 4s per point.

3.1.4.2 Transmission Electron Microscopy (TEM)

One drop (8 μL) of 0.002% aqueous suspension of BCNWs was allowed to dry on a carbon coated grid (200 mesh). The nanocrystals were stained with uranyl acetate. Transmission Electron Microscopy (TEM) was performed using a JEOL, model JEM-2010, equipped with a digital Bioscan (Gatan) image acquisition system at 80 kV. Lengths and diameters of BCNWs were measured from several TEM micrographs. The reported dimension was averaged from measurements of 10 BCNWS.

3.1.4.3 Thermal Gravimetric Analysis (TGA)

Thermal analysis was carried out using a Mettler Toledo TGA/SDTA STAR 851e (Switzerland). Samples of approximately 5 mg were used. All the experiments were conducted using the constant heating rate of 5 °C/min, from 25 to 600 °C, under a nitrogen atmosphere (flow rate of 50 ml/min). The peak degradation temperatures of all samples were determined.

3.1.4.4 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) micrographs of the fractured surfaces of the pure starch film and starch/BCNWs bionanocomposite films were taken by a JEOL model jms-5410 LV. at an accelerating voltage of 10 kV. Prior to the examination, the surface of the specimen was sputter coated with a thin layer of gold.

3.1.4.5 Mechanical Test

Specimens with the dimension of 50 mm length and 7 mm width were cut from the films. The test was operated at a deformation rate of 3 mm/min using a load cell of 1 kN (Universal testing machine INSTRON Model 5566) with an initial grip separation of 30 mm. Average values of tensile strength, Young's modulus, and elongation at break were calculated from 5 specimens.

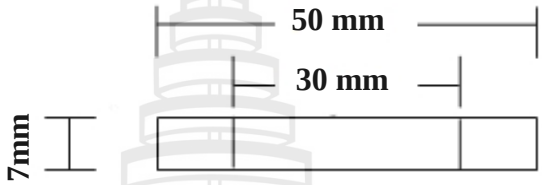


Figure 3.4 Illustration of Tensile Specimens

3.1.4.6 Moisture Absorption

Firstly, bionanocomposite specimens (dimension of 40 mm × 10 mm) were dried and then weighted (M_0). After that, the specimens were stored in condition of 75% RH (using a chamber of the saturated NaCl solution), and periodically removed and weighted (M_t). A minimum of four samples were tested for each film. Moisture absorption (M_a) at time *t* was calculated by the following equation 3.2:

$$M_a = \left(\frac{M_t - M_0}{M_0} \right) \times 100 \qquad \text{(Equation 3.2)}$$

where M_0 is the specimen initial weight and M_t is the weight after a time *t*.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of Hydrolysis Time on Properties of Bacterial Cellulose Nanowhiskers

In this research, bacterial cellulose nanowhiskers (BCNWs) were prepared by acid hydrolysis of the bacterial cellulose (BC) sheet using 50% (w/v) sulfuric acid at 50^{o}C. The effect of hydrolysis time of 24, 48 and 72 hours on the BCNWs' properties was studied. Figure 4.1 shows that yield (%) of the obtained BCNWs was decreased from 47.28±1.57% to 24.97±0.64% with increasing the hydrolysis time from 24 to 72 hours. The long the hydrolysis times, the more BC was hydrolyzed.

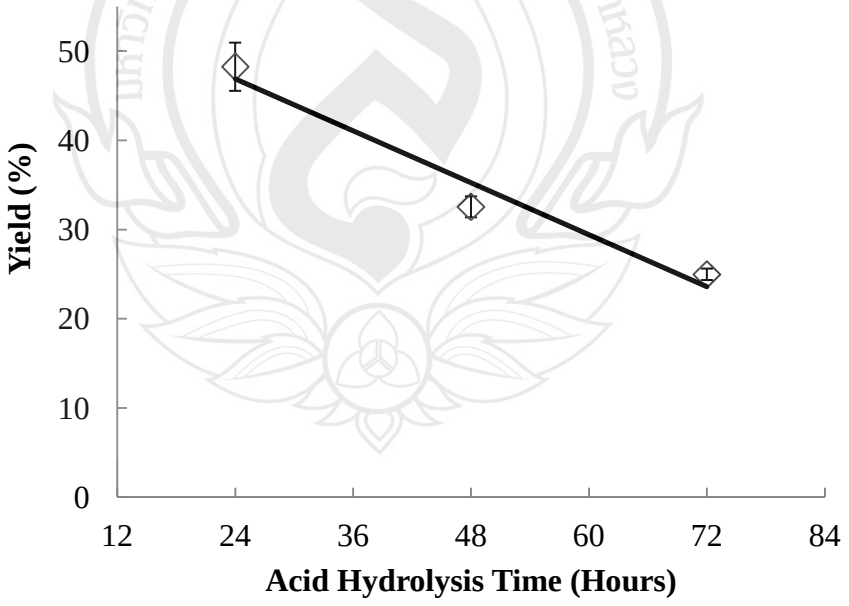


Figure 4.1 The Effect of Acid Hydrolysis Time on Yield (%) of the Bacterial Cellulose Nanowhiskers (BCNWs)

The effect of hydrolysis time on the crystallinity of the BCNWs is presented in Figure 4.2. The X-ray diffractions of the native BC and BCNWs after acid hydrolysis of 24, 48 and 72 hours (BCNWs 24h, BCNWs 48h and CNWs 72h, respectively) show three cellulose I characteristic peaks at 2θ = 14.7°, 16.4°, and 22.5° (corresponding to 101, $10\bar{1}$ and 002 crystal planes, respectively) (Lu & Hsieh, 2010). After short acid hydrolysis time of 24 hours, the peaks of BCNWs 24h diffraction patterns are sharper than that of the native BC because some of the amorphous regions which are more accessible than crystalline regions have been removed from the BC structure. crystalline regions. As time passes to 48 hours the peaks of BCNWs 48h become sharper than BCNWs 24h. This indicates that 24 hours are not is long enough for the acid to extract crystalline domains on BC structure. As time passes to 72 hours the peaks of BCNWs 72h tend to decline. With long hydrolysis times, amorphous regions have been largely eliminated then the acid attacked further into the crystalline regions. From the results of this study, therefore, the acid hydrolysis time of 48 hour was chosen for the preparation of BCNWs to use as a reinforcement in the next part.

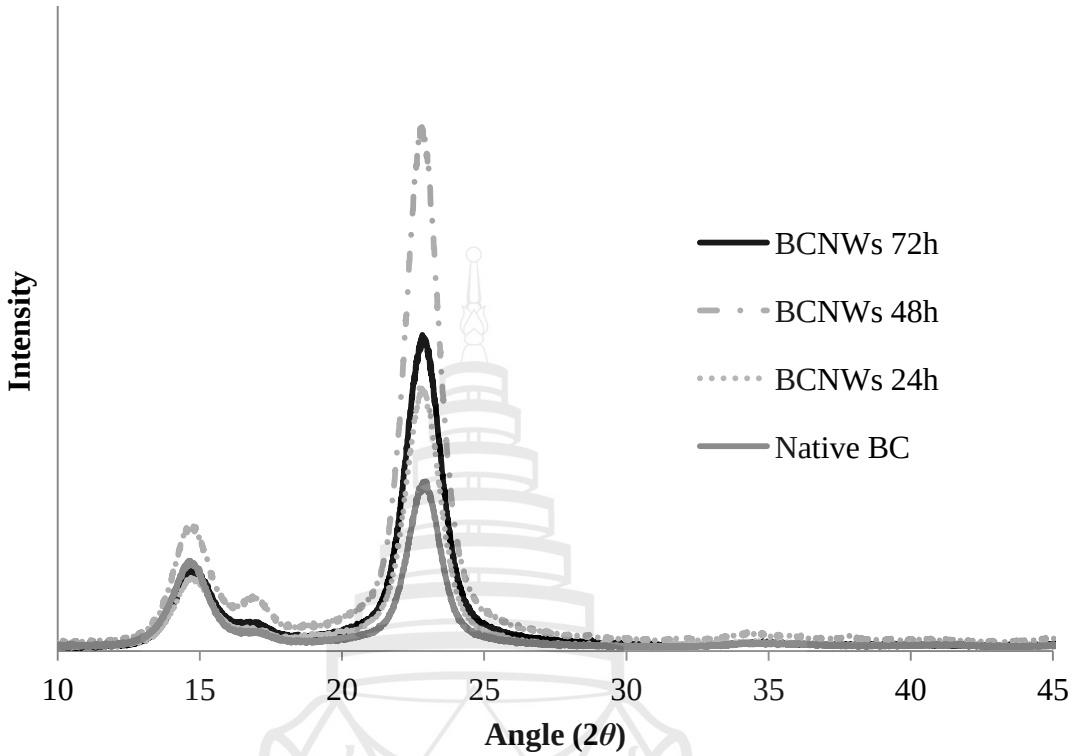


Figure 4.2 X-ray Diffraction Patterns of the Native Bacterial Cellulose (BC) and the Obtained Nanowhiskers (BCNWs) after Acid Hydrolysis Time of 24, 48 and 72 Hours (BCNWs 24h, BCNWs 48h and BCNWs 72h, respectively)

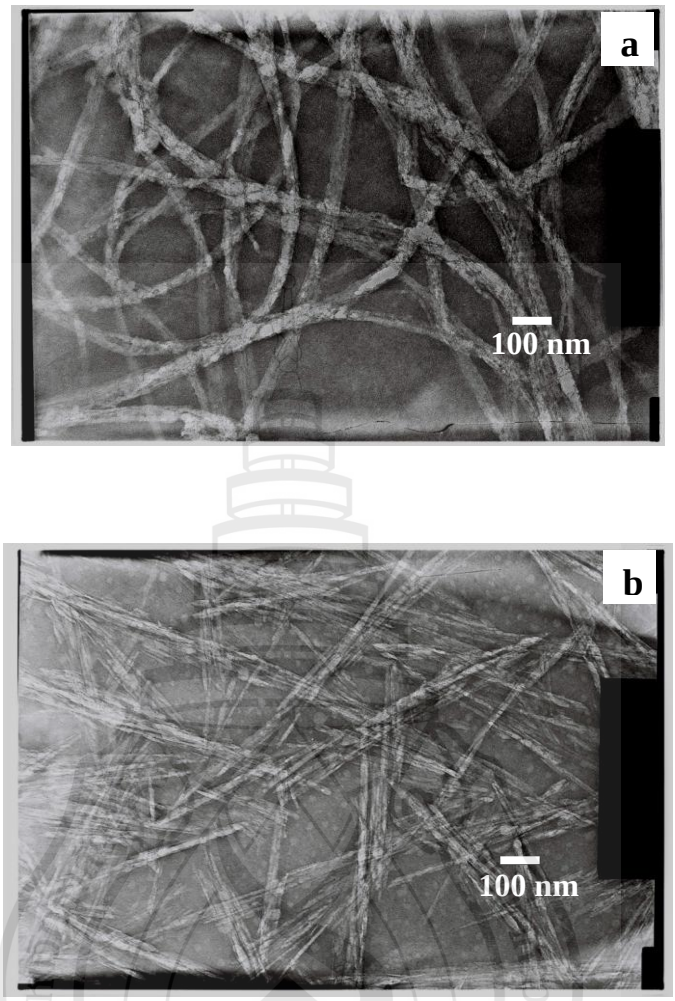


Figure 4.3 Transmission Electron Micrographs of (a) the Native BC and (b) Bacterial Cellulose Nanowhiskers after Acid Hydrolysis with 50% w/v Sulfuric Acid for 48 Hours (BCNWs 48h)

The morphology of the BC and BCNWs was studied by TEM. BC morphology showed continuous networks (Figure 4.3a). After acid treatments, morphology of BCNWs showed rod-like shapes as shown in Figure 4.3b. Diameters and lengths of BCNWs 48 h were estimated from several measurements on TEM micrographs. Averaged diameter and length of the BCNWs 48 h were approximately 28.18±1.99 nm and 637.61±147.10 nm, respectively.

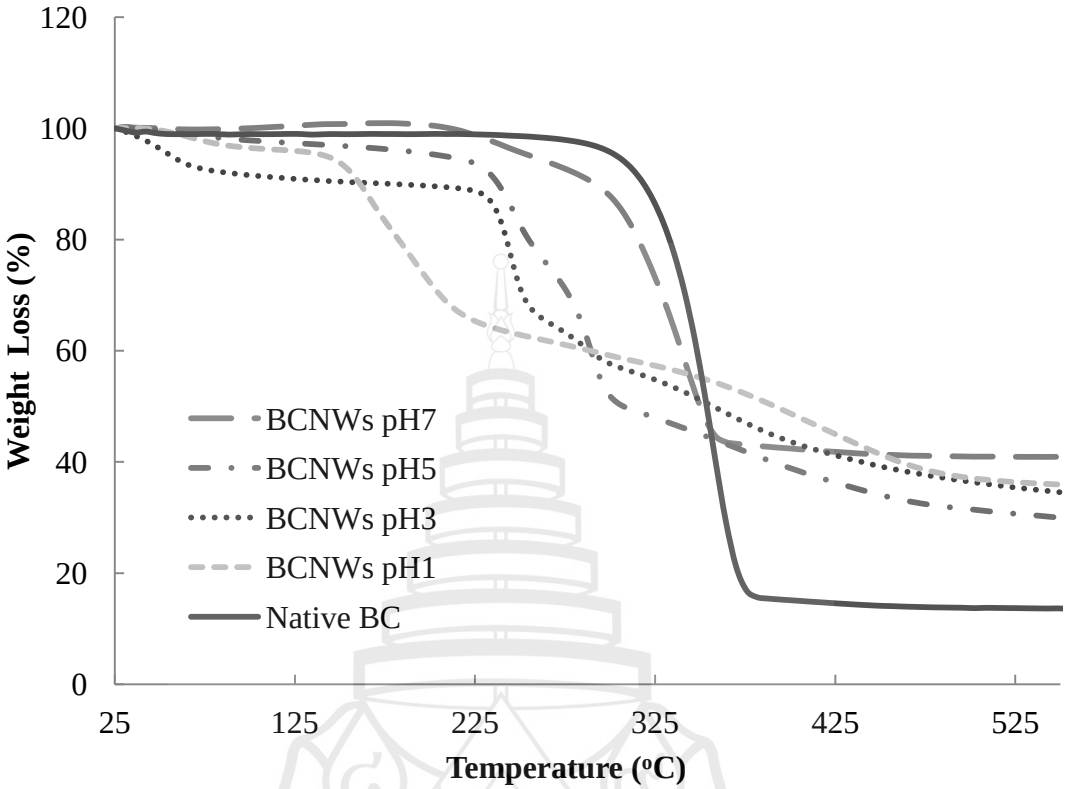


Figure 4.4 TGA Curves of the Native BC, BCNWs after Acid Hydrolysis Time of 48 Hours (BCNWs pH 1) and BCNWs 48 Hours with pH Adjusted to 3, 5 and 7 (BCNWs pH 3, BCNWs pH 5 and BCNWs pH 7, respectively)

Sulfuric acid introduced sulfate groups on the nanowhiskers surfaces due to the acid hydrolysis. The surface charges on BCNWs led to their effective separation for reinforcing in composites, however, it decreased thermal stability of BCNWs. Thermogravimetric analysis was carried out to investigate effect of BCNWs' pH on their thermal stability. Figure 4.4 shows TGA curves of the native BC, the BCNWs obtained after 48 hours of acid hydrolysis (BCNWs pH1) and BCNWs 48h with pH adjusted to 3, 5 and 7 (BCNWs pH3, BCNWS pH5 and BCNWS pH7, respectively). After acid hydrolysis treatment of 48 hours thermal stability of BCNWs pH1 was greatly decreased. Sulfate group is a well known decomposition catalyst that facilitates that formation of char residue (Kim, Nishiyama, Wada & Kuga, 2001). To improve thermal stability of BCNWs (Roman & Winter, 2004), pH of BCNWs was

adjusted by NaOH in order to remove the sulfated group on surface of the BCNWs (Favier et al., 1995). After adjusting pH of BCNWs to 3, 5 and 7, thermal stability of the BCNWs was gradually increased. Table 4.1 shows the peak degradation temperatures of the native BC and BCNWs with different pHs obtained from the DTG curves. It confirmed the increase in thermal stability of BCNWs with degree of pH adjustment.

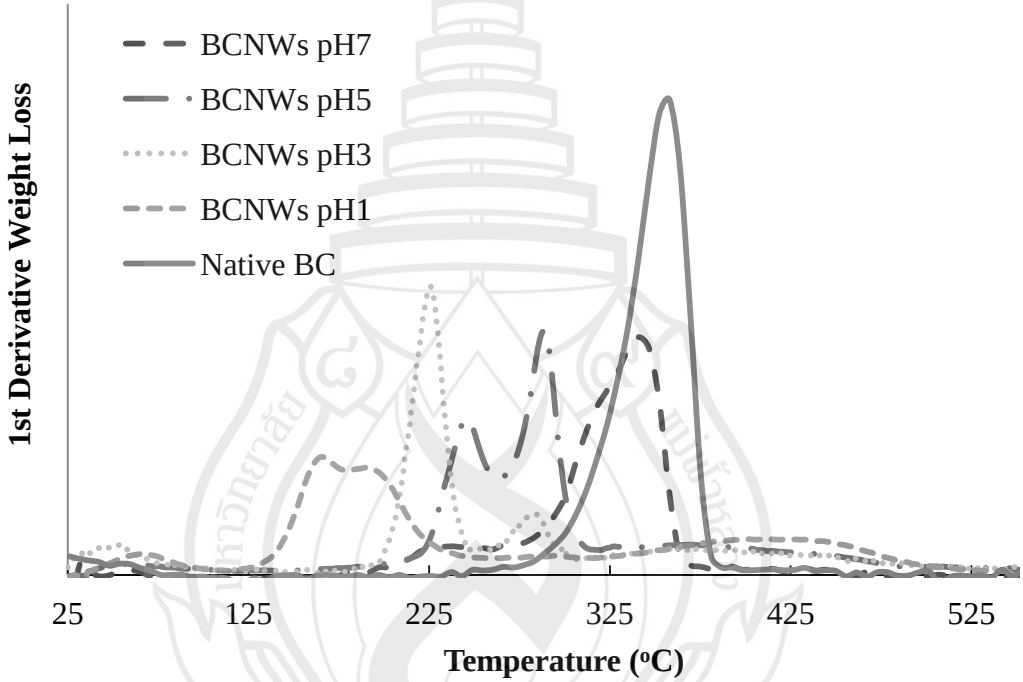


Figure 4.5 DTG Curves of the Native BC, BCNWs after Acid Hydrolysis Time of 48 Hours (BCNWs pH 1) and BCNWs 48 Hours with pH Adjusted to 3, 5 and 7 (BCNWs pH 3, BCNWs pH 5 and BCNWs pH 7, respectively)

From Figure 4.5, the native BC and BCNWs pH7 shows approximately one step of their degradations. However, the degradation steps of BCNWs pH1, pH3 and pH5 are obviously divided into two steps. It was explain that, the first step corresponds to the degradation of the more accessible regions (amorphous regions), which are highly sulfated, and the second step corresponds to the breakdown of the

crystalline fraction, which has been attacked by sulfuric acid (Julien, Chornet & Overend, 1993; Sanz et al., 2011).

4.2 Effects of Bacterial Cellulose Nanowhiskers' pH and Content on Properties of Bionanocomposite Films

The X-ray diffraction patterns of the corn starch granule, pure starch film and bionanocomposite films are shown in Figure 4.6. The diffraction peaks of the corn starch granular at 2θ = 15°, 17°, 18° and 23° are related to its A-type crystalline structure. The peaks of starch film diffractograms are lower than the native corn starch because after gelatinization, crystallinity was decreased. (Grande et al., 2009). For the bionanocomposite films, during preparation, it was found that the films with addition of BCNWs pH1 could not be obtained because all films was found to be cracked after drying. With addition of BCNWs pH3, BCNWs pH5 and BCNWs pH7, to varied contents of 1, 5, and 10 wt%, their diffraction peaks showed also three cellulose I characteristic peaks at 2θ = 14.5°, 16.4°, and 22.5°, revealing crystalline structure with preservation of the crystallinity of BCNWs in the bionanocomposite films. With increasing BCNWs contents the magnitude of the peaks are observed to increase, which corresponds to the typical crystal pattern of cellulose.

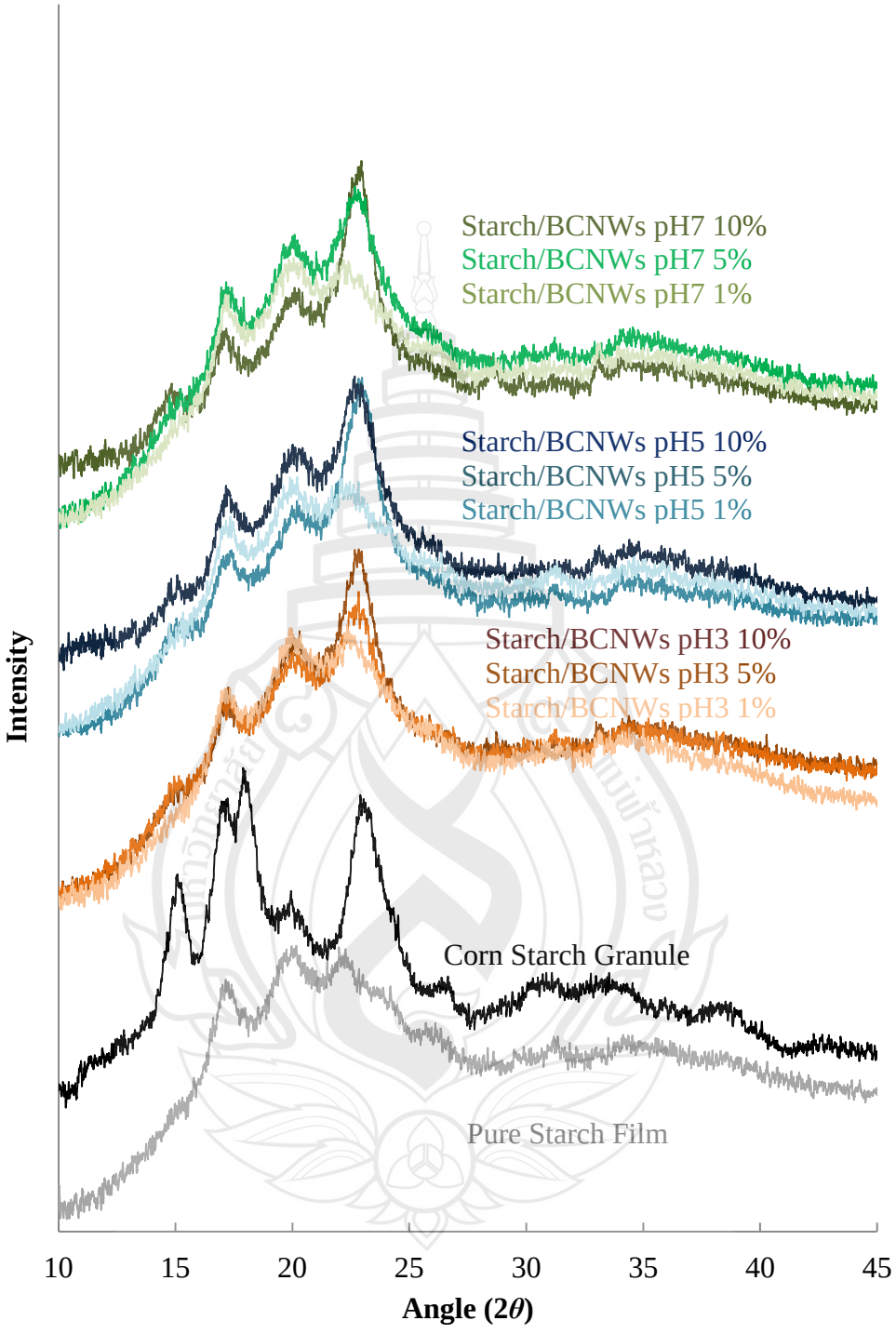


Figure 4.6 X-ray Diffraction Patterns of the Corn Starch Granule, Pure Starch Film and Bionanocomposite Films Reinforced with BCNWs of pH 3, 5 and 7 (with Varied Contents of 1, 5 and 10 wt%)

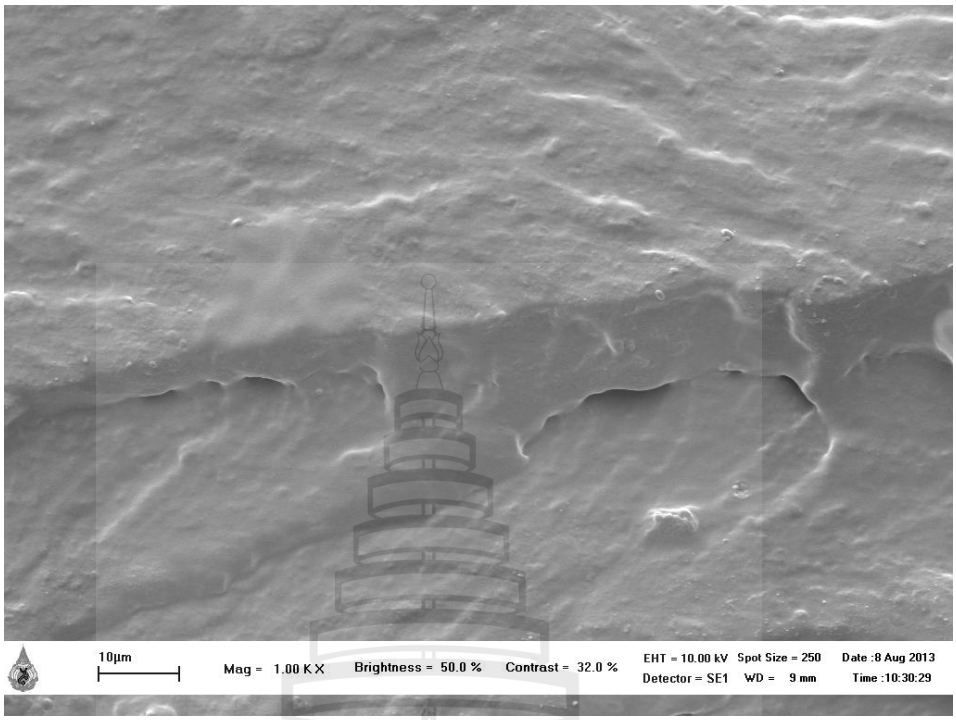


Figure 4.7 SEM Image of Fracture Surface of the Pure Starch Film

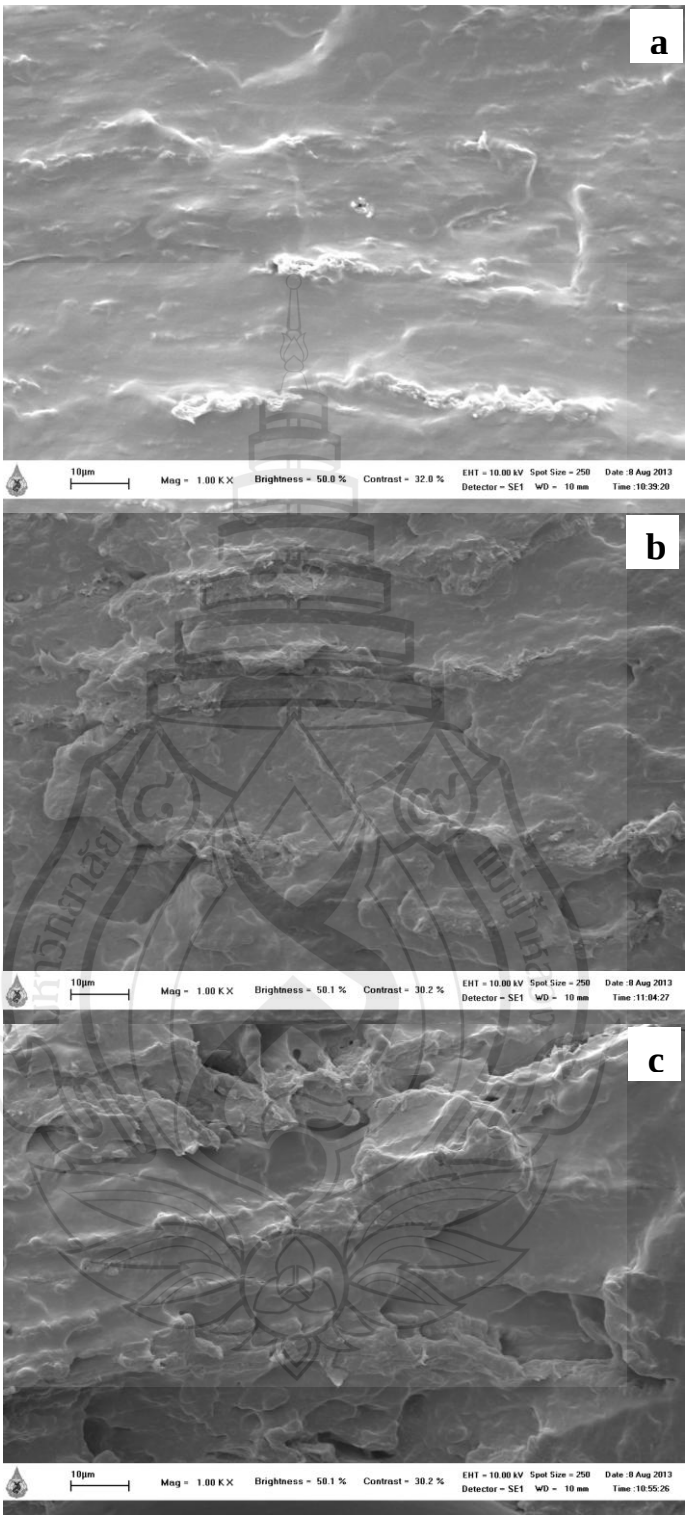


Figure 4.8 SEM Images of Fracture Surfaces of Bionanocomposite Films with Addition of 1 wt% (a), 5 wt% (b) and 10 wt% (c) of BCNWs pH 3

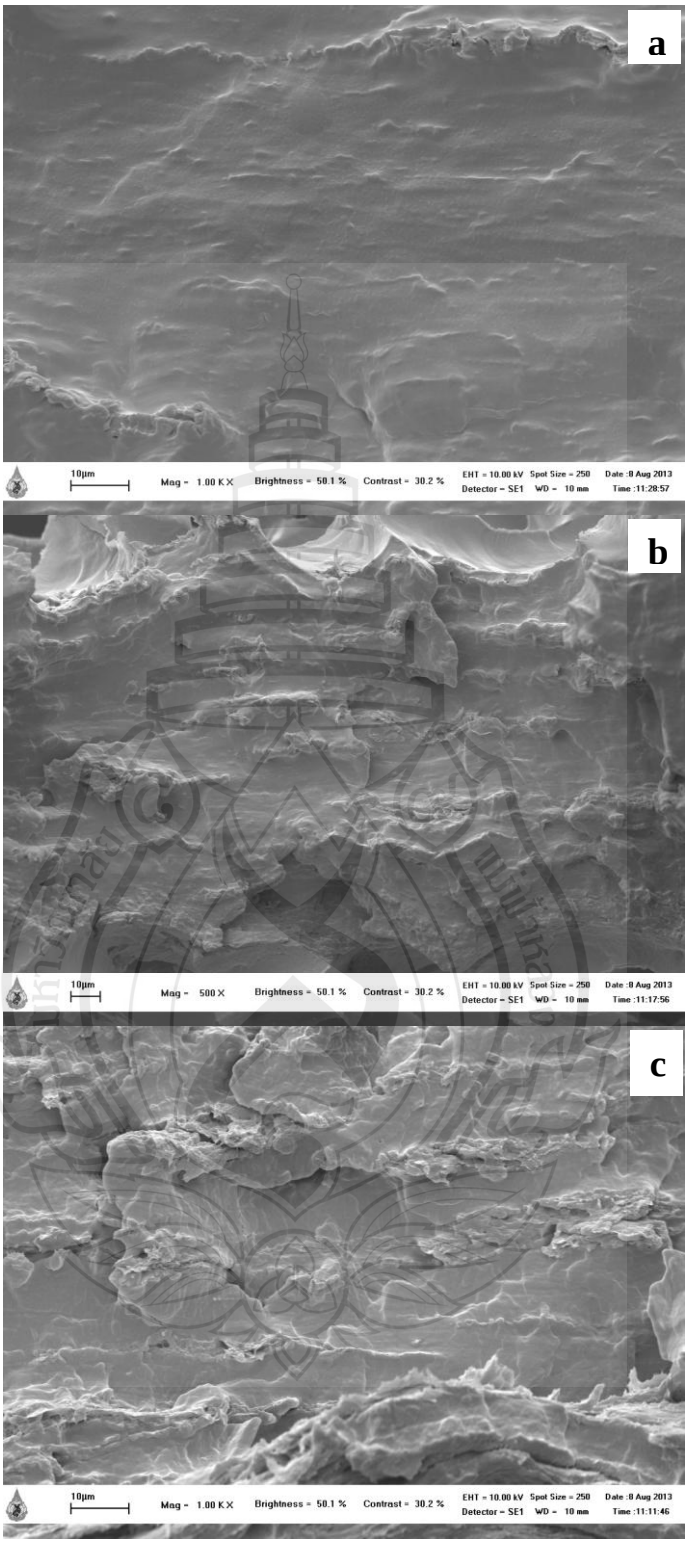


Figure 4.9 SEM Images of Fracture Surfaces of Bionanocomposite Films with Addition of 1 wt% (a), 5 wt% (b) and 10 wt% (c) of BCNWs pH 5

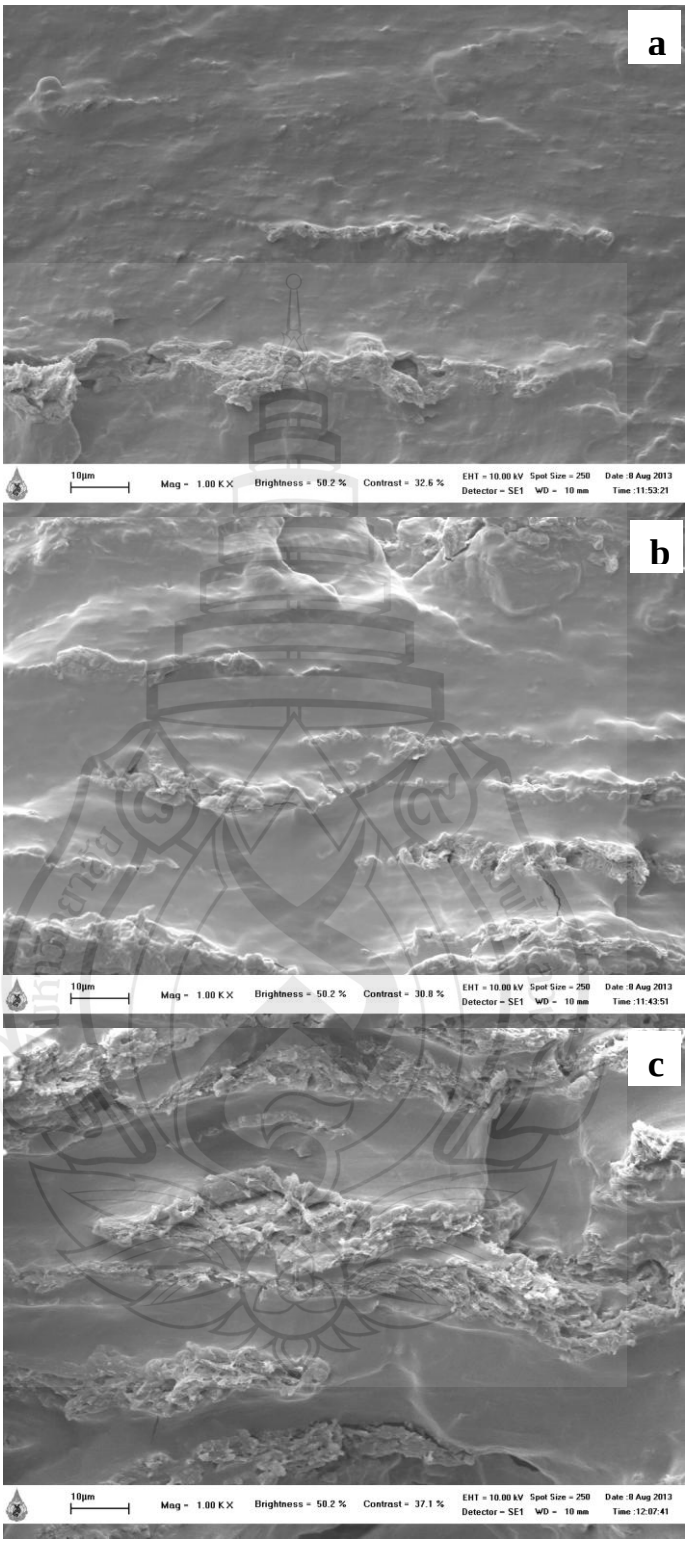


Figure 4.10 SEM Images of Fracture Surfaces of Bionanocomposite Films with Addition of 1 wt% (a), 5 wt% (b) and 10 wt% (c) of BCNWs pH 7

Figure 4.7 shows the smooth fractured surface of the pure starch film. For the starch/BCNWs pH3 films with varied contents of 1, 5 and 10 wt%, with increasing BCNWs content, the fractured surface become rougher. A good dispersion of BCNWs on fractured surface of these bionanocomposite films was observed (Figure 4.8a, b and c). The starch/BCNWs pH 5 films with varied BCNWs contents of 1, 5 and 10 wt%, show rougher surface than the starch/BCNWs pH 3 films. Some aggregates formation of BCNWs was found on their fracture surfaces. (Figure 4.9a, b and c). For the starch/BCNWs pH 7 films a poor dispersion and high aggregation of BCNWs was observed clearly at all BCNWs contents, particularly at 10 wt% content. (Figure 4.10a, b and c). The higher the pH, the lower degree of BCNWs dispersion in the bionanocomposite films was observed. This due to sulfate group on surface of BCNWs. The dispersion of BCNWs in the matrix and their compatibility are important for a reinforcing effect and improvement in the properties of composite materials (Trovatti et al., 2012).

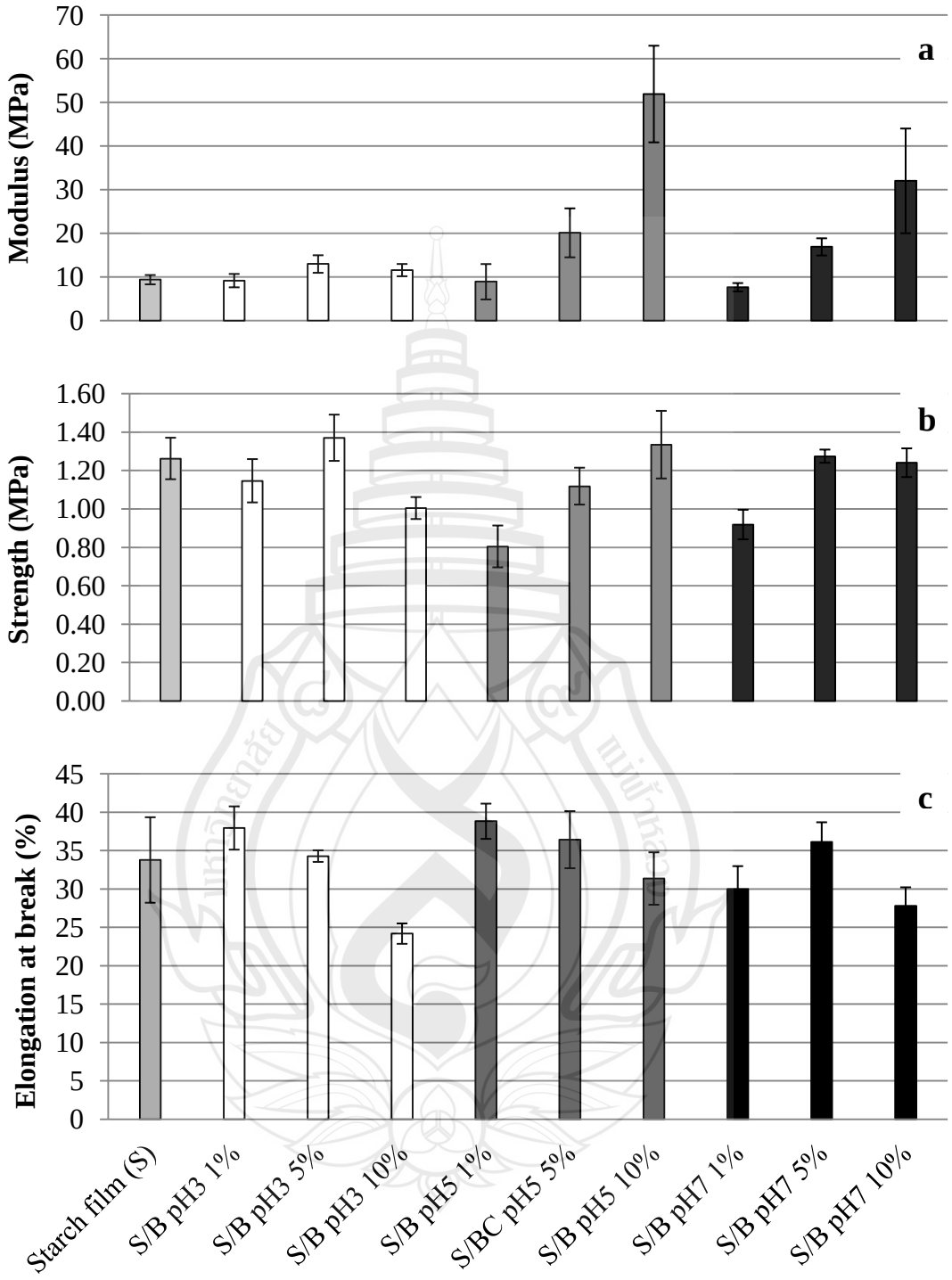


Figure 4.11 Young's Modulus (MPa) (a), Tensile Strength (MPa) (b) and Elongation at Break% (c) of the Pure Starch Film and Bionanocomposite Films Reinforced with BCNWs of pH 3, 5 and 7 (with Varied Contents of 1, 5 and 10 wt%)

Figure 4.11 shows the tensile properties of the pure starch film and bionanocomposite films reinforced with BCNWs of pH 3, 5 and 7 (with varied contents of 1, 5 and 10 wt%). It was found that the mechanical properties of the starch/BCNWs pH 3 which possessed a films good dispersion of BCNWs was not improved. because due to a poor interaction with BCNWs and the pure starch matrix. This likely caused by the sulfate groups on the surface of BCNWs. The mechanical properties of starch/BCNWs pH 7 films (with varied BCNWs contents of 1, 5 and 10 wt%) were also not much improved due to the large agglomerations and poor dispersion of BCNWs within the pure starch matrix. On the other hand, the starch/BCNWs pH 5 films show improved mechanical properties possibly because the optimum dispersion and sufficient interaction between BCNWs and the pure starch matrix in these composites. For mechanical properties improvement in composite systems both dispersion and interfacial adhesion are essential (Cao et al., 2008).

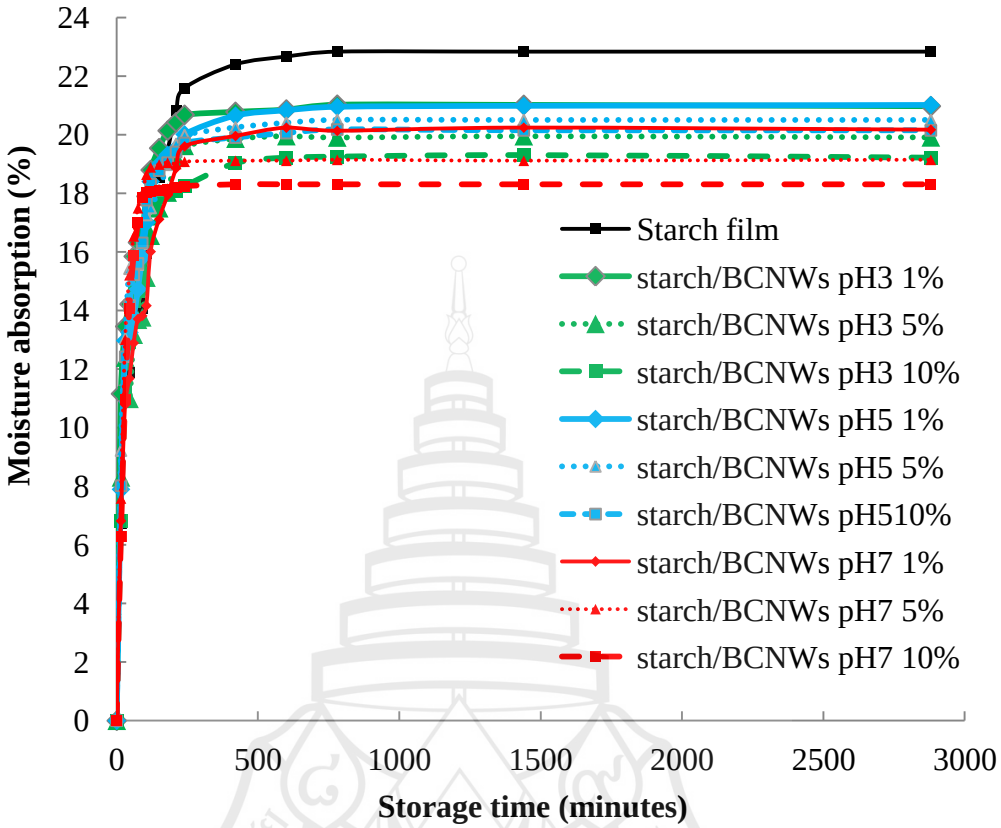


Figure 4.12 Moisture Absorption (at 75% RH) as a Function of Storage Time of the Pure Starch Film, and Bionanocomposite Films Reinforced with BCNWs of pH 3, 5 and 7 (with Varied Contents of 1, 5 and 10 wt%)

Though starch has been considered as one of the most promising materials for biodegradable plastics owing to its natural abundance and low cost, poor resistance to moisture absorption limits its wide applications. It is well known that addition of fillers is an effective way of decreasing its sensitivity to moisture and thus improving mechanical properties stability (Wan et al., 2009). Figure 4.12 shows the moisture absorption of the pure starch film and bionanocomposite films during conditioning in 75% RH as a function of time. The moisture absorption of pure starch film at equilibrium was 22.83%. With addition of BCNWs into the pure starch film, the moisture absorption at equilibrium was decreased as the BCNWs content increased from 1 to 10 wt%. The moisture absorption of the films of the starch/BCNWs pH 3 10

wt%, starch/BCNWs pH5 10 wt% and starch/BCNWs pH7 10 wt% were 19.22%, 20.14% and 18.05%, respectively. This suggest that a water resistance of all composite films greatly increased as compared to the pure starch film. The presence of BCNWs improved water barrier properties of the pure starch film because the higher crystallinity of BCNWs, their low moisture absorption and strong hydrogen bonding formed at the BCNWs-matrix interfaces. The water resistance of the starch/BCNWs pH7 films was higher than the starch/BCNWs pH 3 and starch/BCNWs pH 5 films because the sulfated group on the surfaces of the BCNWs of pH 3 and pH 7 result in the poor interactions with the starch matrix. So there is only poorly formed hydrogen bonding on their interfaces.

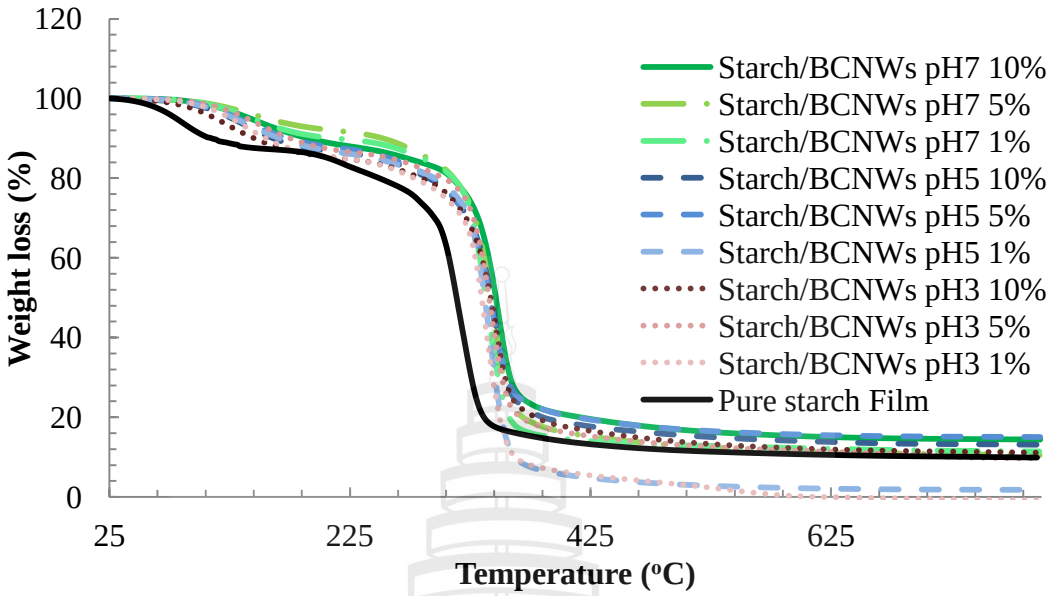


Figure 4.13 TGA Curves of the Pure Starch Film and Bionanocomposite Films Reinforced with BCNWs of pH 3, 5 and 7 (with Varied Contents of 1, 5 and 10 wt%)

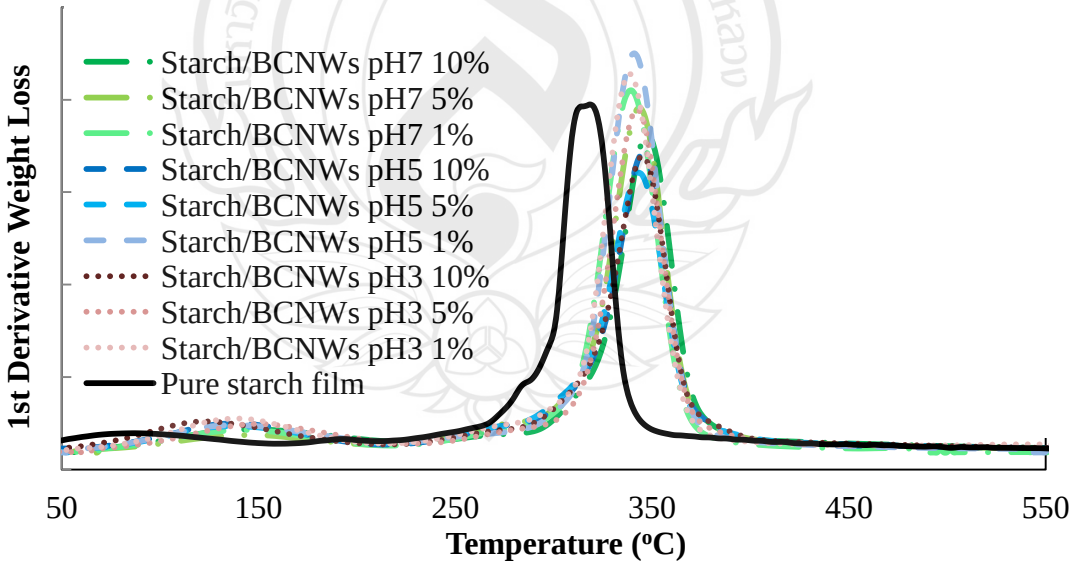


Figure 4.14 DTG Curves of the Pure Starch Film and Bionanocomposite Films Reinforced with BCNWs of pH 3, 5 and 7 (with Varied Contents of 1, 5 and 10 wt%)

Table 4.1 Peak Degradation Temperatures of the Native BC, BCNWs of pH 1, 3, 5 and 7, the Pure Starch Film and Bionanocomposite Films from Their DTG Curves

Sample	Peak Temperature (^{o}C)	
	1st Peak	2nd Peak
Native BC	358	-
BCNWs 48 h pH1	165	191
BCNWs 48 h pH3	226	280
BCNWs 48 h pH5	248	286
BCNWs 48 h pH7	340	-
Pure Starch Films	318	-
Starch/BCNWs pH3 1%	339	-
Starch/BCNWs pH3 5%	343	-
Starch/BCNWs pH3 10%	345	-
Starch/BCNWs pH5 1%	340	-
Starch/BCNWs pH5 5%	343	-
Starch/BCNWs pH5 10%	345	-
Starch/BCNWs pH7 1%	340	-
Starch/BCNWs pH7 5%	343	-
Starch/BCNWs pH7 10%	347	-

Thermogravimetric analysis (TGA) and differential thermogravimetry (DTG) curves of bionanocomposites films are shown in Figure 4.13 and 4.14, respectively. The peak degradation temperature of all films are listed in Table 4.1. Firstly, TGA curves shows an intitial drop about 100 - 150 ^{o}C which corresponds to a mass loss of water and glycerol (Averous & Boquillo, 2004). With addition of BCNWs, the thermal stability of the bionanocomposite films were significantly improved about 20 - 30 ^{o}C as compared to the pure starch film. The peak degradation temperatures of the bionanocomposite films systematically increase with increasing BCNWs content from 1 to 10 wt%. Regardless to pH of the BCNWs. The improvement in thermal stability of the bionanocomposite films with addition of BCNWs can be based on the fact that cellulose nanowhiskers have inherently good thermal stability and also due to the

intensive of hydrogen bonding between the pure starch matrix and BCNWs (Ahmad et al., 2012).

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

Bacterial cellulose nanowhiskers (BCNWs) was prepared by sulfuric acid hydrolysis (50%w/v) of BC sheet at 50°C to various treatment times (i.e. 24, 48 and 72 hours). BCNWs' yield (%) is decreased with longer acid hydrolysis time. It was found that acid hydrolysis of 48 hours was the optimum time to prepare BCNWs with the highest crystallinity. The acid hydrolysis transformed the continuous BC fiber network (native BC) into the isolated rod-like nanocrystals. The diameter and length of the BCNWs were approximately 28.18±1.99 nm and 637.61±147.10 nm, respectively. However, the sulfuric acid treatment leads to a decrease in the thermal stability of BCNWs which is due to the induced sulphate groups onto the BCNWs surfaces after acid hydrolysis. However, further pH adjustment can significantly improve the thermal stability of the BCNWs.

The bionanocomposite films of starch reinforced with BCNWs of pH 3, 5 and 7 at varied contents of 1, 5 and 10 wt% were prepared by film casting technique. With increasing BCNWs content, the bionanocomposites reveal the improvement in crystallinity, thermal stability and water resistance. Mechanical properties of the starch/BCNWs pH 3 and the starch/BCNWs pH 7 films were not improved because poor interaction between BCNWs pH 3 and pure starch matrix and large aggregation of BCNWs pH 7. The mechanical properties of the starch/BCNWs pH 5 film were improved possibly because the optimum dispersion and sufficient interaction between BCNWs and the pure starch matrix.

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APPENDICES

APPENDIX A

EFFECT OF ACID HYDROLYSIS TREATMENT ON YIELD % AND DIMENSIONS OF BCNWs

Table A1 Yield (%) of the Bacterial Cellulose Nanowhiskers (BCNWs) after Acid Hydrolysis with Different Treatment Times

Hydrolysis Time (hours)	Sample	Weight before acid hydrolysis (g)	Weight after acid hydrolysis (g)	%Yield
24	24-1	0.6412	0.3266	50.9357
	24-2	0.6449	0.2936	45.5264
	24-3	0.6478	0.3127	48.2711
			Average	**48.2442**
			SD	**2.7048**
48	48-1	0.6425	0.212	32.9961
	48-2	0.6452	0.2013	31.1996
	48-3	0.6483	0.2166	33.4105
			Average	**32.5354**
			SD	**1.1752**
72	72-1	0.6458	0.1632	25.271
	72-2	0.6471	0.1568	24.2312
	72-3	0.6432	0.1634	25.4042
			Average	**24.9688**
			SD	**0.6423**

SD= Standard Deviation

Table A2 Diameters of Bacterial Cellulose (BC). The Measurements were Obtained from Several TEM Micrographs

Sample	BC Diameters (nm)
1	47.04
2	41.48
3	38.09
4	39.43
5	42.31
6	39.32
7	43.85
8	46.01
9	49.62
10	49.62
Average	**43.68**
SD	**4.25**

Table A3 Dimensions of Bacterial Cellulose Nanowhiskers (BCNWs) after 48 hours of Acid Hydrolysis. The Measurements were Obtained from Several TEM Micrographs

BCNWs Samples	Length (nm)	Diameter (nm)
1	740.98	26.32
2	513.21	21.07
3	629.06	24.50
4	704.58	23.80
5	715.87	22.28
6	529.34	22.59
7	478.93	23.19
8	563.32	23.69
9	962.50	24.30
10	538.01	22.79
Average	**637.58**	**23.45**
SD	**147.10**	**1.43**

APPENDIX B

EFFECT OF BCNWs' pH AND CONTENT ON PROPERTIES OF BIONANOCOMPOSITE FILMS

Table B1 Tensile Properties of the Pure Starch Films

Starch films Specimens	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.25	36.89	8.22
2	1.12	24.25	10.76
3	1.21	35.30	8.77
4	1.33	38.37	8.91
5	1.40	34.07	10.21
Average	**1.26**	**33.78**	**9.37**
SD	**0.11**	**5.57**	**1.06**

Table B2 Tensile Properties of the Starch/BCNWs pH3 (with Varied Contents of 1, 5 and 10 wt%) Bionanocomposite Films

Starch/BCNWs pH3 1 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.00	33.20	8.96
2	1.10	38.50	9.31
3	1.17	38.00	10.30
4	1.15	40.50	8.62
5	1.31	39.50	11.93
Average	**1.15**	**37.94**	**9.82**
SD	**0.11**	**2.82**	**1.34**

Starch/BCNWs pH3 5 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.52	34.00	14.21
2	1.44	34.20	13.07
3	1.36	35.00	10.98
4	1.33	35.00	11.79
5	1.20	33.20	10.41
Average	**1.37**	**34.28**	**12.09**
SD	**0.12**	**0.76**	**1.55**

Starch/BCNWs pH3 10 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.04	23.00	10.06
2	0.94	25.00	8.93
3	1.00	22.90	11.17
4	0.96	24.00	9.86
5	1.08	26.00	11.70
Average	**1.00**	**24.18**	**10.34**
SD	**0.06**	**1.33**	**1.10**

Table B3 Tensile Properties of the Starch/BCNWs pH 5 (with Varied Contents of 1, 5 and 10 wt%) Bionanocomposite Films

Starch/BCNWs pH5 1 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	0.72	38.00	6.92
2	0.90	37.20	7.79
3	0.76	41.50	7.13
4	0.94	36.40	13.85
5	0.70	41.00	5.48
Average	**0.80**	**38.82**	**8.23**
SD	**0.11**	**2.30**	**3.25**

Starch/BCNWs pH5 5 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.08	33.00	19.57
2	1.24	37.00	15.91
3	1.08	33.00	26.95
4	1.00	42.00	13.55
5	1.19	37.10	13.94
Average	**1.12**	**36.42**	**17.99**
SD	**0.10**	**3.72**	**5.55**

Starch/BCNWs pH5 10 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.60	31.00	61.58
2	1.36	30.00	35.83
3	1.15	27.80	43.42
4	1.36	31.00	62.43
5	1.20	37.00	54.34
Average	**1.33**	**31.36**	**51.52**
SD	**0.18**	**3.41**	**11.62**

Table B4 Tensile Properties of the Starch/BCNWs pH 7 (with Varied Contents of 1, 5 and 10 wt%) Bionanocomposite Films

Starch/BCNWs pH7 1 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.01	33.00	8.84
2	0.96	25.00	9.27
3	0.94	31.00	7.94
4	0.82	30.00	6.78
5	0.86	30.90	7.19
Average	**0.92**	**29.98**	**8.00**
SD	**0.08**	**2.99**	**1.06**

Starch/BCNWs pH7 5 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.24	38.90	14.52
2	1.32	37.00	18.59
3	1.29	36.80	19.59
4	1.28	32.00	17.31
5	1.24	36.00	16.21
Average	**1.27**	**36.14**	**17.25**
SD	**0.03**	**2.55**	**1.99**

Starch/BCNWs pH7 10 wt%	Tensile Strength (MPa)	Elongation at Break (%)	Modulus (MPa)
1	1.32	25.00	51.31
2	1.32	27.50	37.37
3	1.16	26.00	29.58
4	1.20	30.50	25.78
5	1.20	30.00	25.78
Average	**1.24**	**27.80**	**21.32**
SD	**0.07**	**2.41**	**10.79**

Table B5 Moisture Absorption as a Function of Storage Time of the Starch Films Stored in Condition of 75% RH

Storage Time (minute)	Starch Films	
	Weight (g)	MA (%)
0	0.0742	0.00±0.00
15	0.0792	6.73±0.48
30	0.0831	11.99±0.71
45	0.0831	11.87±0.71
60	0.0847	14.10±0.53
75	0.0846	13.94±0.56
90	0.0847	14.07±0.57
105	0.0861	15.94±0.57
120	0.0877	18.12±0.20
150	0.0880	18.53±0.54
180	0.0887	19.47±0.98
210	0.0897	20.81±1.08
240	0.0903	21.59±1.32
420	0.0909	22.39±1.27
600	0.0911	22.66±1.24
780	0.0912	22.83±1.24
1440	0.0912	22.83±1.24
2880	0.0912	22.83±1.24

MA = Moisture Absorption

Table B6 Moisture Absorption as a Function of Storage Time of the Starch/BCNWs pH 3 (with Varied Content 1, 5 and 10 wt%) Bionanocomposite Films Stored in Condition of 75% RH

Storage Time (minute)	Sample					
	Starch/BCNWs pH3 1%		Starch/BCNWs pH3 5%		Starch/BCNWs pH3 10%	
	Weight (g)	MA (%)	Weight (g)	MA (%)	Weight (g)	MA (%)
0	0.0701	0.00±0.00	0.0603	0.00±0.00	0.0821	0.00±0.00
15	0.0778	11.16±1.89	0.0653	8.31±1.12	0.0877	6.81±0.84
30	0.0794	13.46±1.95	0.0678	12.39±0.44	0.0913	11.18±0.83
45	0.0800	14.22±1. 17	0.0669	10.99±0.45	0.0928	12.93±1.78
60	0.0811	15.85±1.55	0.0683	13.19±1.04	0.0939	14.32±1.37
75	0.0814	16.32±1.50	0.0686	13.69±0.83	0.0942	14.65±1.44
90	0.0816	16.59±1.50	0.0686	13.77±0.74	0.0945	14.95±1.58
105	0.0826	17.90±1.56	0.0694	15.12±0.76	0.0957	16.41±1.81
120	0.0832	18.80±1.67	0.0703	16.55±0.73	0.0966	17.57±1.94
150	0.0837	19.54±1.66	0.0709	17.50±0.78	0.0969	17.88±1.84
180	0.0841	20.14±1.84	0.0712	18.03±0.81	0.0970	18.00±1.83
210	0.0843	20.42±1.90	0.0719	19.21±1.13	0.0970	18.09±1.76
240	0.0845	20.68±1.88	0.0721	19.62±1.44	0.0972	18.24±1.72
420	0.0845	20.78±1.89	0.0723	19.87±1.45	0.0978	19.03±1.95
600	0.0846	20.86±1.90	0.0723	19.95±1.44	0.0980	19.22±1.89
780	0.0847	21.02±1.76	0.0723	19.91±1.44	0.0980	19.25±1.87
1440	0.0847	21.02±1.71	0.0723	19.95±153	0.0980	19.31±1.90
2880	0.0847	20.97±1.70	0.0723	19.91±1.51	0.0980	19.22±1.89

Table B7 Moisture Absorption as a Function of Storage Time of the Starch/BCNWs pH 5 (with Varied Content 1, 5 and 10 wt%) Bionanocomposite Films Stored in Condition of 75% RH

Storage Time (minute)	Sample					
	Starch/BCNWs pH5 1%		Starch/BCNWs pH5 5%		Starch/BCNWs pH5 10%	
	Weight (g)	MA (%)	Weight (g)	MA (%)	Weight (g)	MA (%)
0	0.0817	0.00±0.00	0.0780	0.00±0.00	0.0822	0.00±0.00
15	0.0882	7.90±0.44	0.0852	9.22±0.26	0.0888	8.02±0.86
30	0.0923	12.97±1.29	0.0882	13.03±0.40	0.0924	12.42±0.65
45	0.0926	13.25±1.11	0.0901	15.43±0.33	0.0938	14.17±0.30
60	0.0930	13.77±1.03	0.0901	15.47±0.51	0.0947	15.22±0.31
75	0.0938	14.72±0.59	0.0902	15.57±0.60	0.0950	15.61±0.28
90	0.0947	15.82±0.14	0.0908	16.28±0.56	0.0956	16.31±0.40
105	0.0956	17.00±0.54	0.0915	17.26±0.74	0.0968	17.77±0.34
120	0.0968	18.40±0.71	0.0917	17.50±0.50	0.0972	18.31±0.43
150	0.0972	18.97±0.69	0.0922	18.11±0.66	0.0976	18.77±0.40
180	0.0975	19.31±0.76	0.0929	18.97±0.52	0.0978	19.04±0.36
210	0.0977	19.52±0.76	0.0934	19.69±0.79	0.0981	19.41±0.44
240	0.0981	20.01±0.58	0.0937	19.98±0.65	0.0984	19.72±0.47
420	0.0986	20.65±0.37	0.0939	20.24±0.67	0.0985	19.90±0.51
600	0.0988	20.83±0.27	0.0940	20.40±0.75	0.0987	20.05±0.46
780	0.0989	20.95±0.23	0.0941	20.50±0.74	0.0988	20.18±0.46
1440	0.0989	20.98±0.13	0.0941	20.50±0.83	0.0987	20.15±0.46
2880	0.0989	21.01±0.19	0.0941	20.50±0.83	0.0987	20.15±0.46

Table B8 Moisture Absorption as a Function of Storage Time of the Starch/BCNWs pH 7 (with Varied Content 1, 5 and 10 wt%) Bionanocomposite Films Stored in Condition of 75% RH

Storage Time (minute)	Sample					
	Starch/BCNWs pH7 1%		Starch/BCNWs pH7 5%		Starch/BCNWs pH7 10%	
	Weight (g)	MA (%)	Weight (g)	MA (%)	Weight (g)	MA (%)
0	0.0705	0.00±0.00	0.0778	0.00±0.00	0.0832	0.00±0.00
15	0.0753	6.82±0.89	0.0834	7.59±2.57	0.0884	6.30±1.24
30	0.0781	10.78±0.44	0.0875	13.00±2.83	0.0923	10.97±0.60
45	0.0787	11.68±0.66	0.0894	15.20±1.70	0.0949	14.08±0.61
60	0.0796	12.89±1.06	0.0905	16.53±1.05	0.0964	15.87±0.76
75	0.0802	13.71±1.10	0.0913	17.49±0.87	0.0974	17.00±0.75
90	0.0802	13.82±1.23	0.0918	17.97±0.97	0.0981	17.87±0.74
105	0.0805	14.16±0.97	0.0923	18.64±0.67	0.0983	18.03±0.93
120	0.0818	16.01±0.85	0.0925	18.89±0.79	0.0983	18.05±1.06
150	0.0826	17.12±1.09	0.0926	18.98±0.93	0.0983	18.11±1.01
180	0.0831	17.94±1.31	0.0926	19.05±1.03	0.0984	18.14±1.04
210	0.0838	18.86±1.14	0.0926	19.00±1.14	0.0984	18.21±0.90
240	0.0843	19.60±1.07	0.0926	19.08±1.08	0.0984	18.25±0.83
420	0.0846	19.95±1.02	0.0927	19.11±1.14	0.0985	18.31±0.84
600	0.0848	20.24±1.06	0.0927	19.11±1.14	0.0985	18.31±0.84
780	0.0847	20.13±1.03	0.0927	19.14±1.19	0.0985	18.31±0.84
1440	0.0848	20.24±1.09	0.0927	19.11±1.14	0.0985	18.31±0.84
2880	0.0847	20.17±1.09	0.0927	19.14±1.19	0.0985	18.31±0.84

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