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DEVELOPMENT OF POLYLACTIC ACID (PLA)-BASED NANOCOMPOSITE FILMS FOR SMART FOOD PACKAGING APPLICATIONS

BY

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The thesis is approved as a creditable and independent investigation by candidate for the Master of Science degree and is acceptable for meeting the thesis requirement for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	:	Analysis of variance
CNC	:	Cellulose nanocrystals
CNF	:	Cellulose nanofiber
PLA	:	Polylactic acid
RH	:	Relative humidity
SEM	:	Scanning electron microscope
TEM	:	Transmission electron microscope
TS	:	Tensile strength
WVP	:	Water Vapor permeability
WVTR	:	Water vapor transmission rate

ABSTRACT

DEVELOPMENT OF POLYLACTIC ACID (PLA)-BASED NANOCOMPOSITE FILMS FOR SMART FOOD PACKAGING APPLICATIONS

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Most of current food packaging resources are non-renewable and non-biodegradable. The use of these materials has resulted in serious environmental issues. In recent years, the recognition of waste disposal problems and their impacts on the environment has risen the demand for packaging manufactured from renewable materials. However, there are some limitations of using biopolymers for food packaging applications. These limitations include unreliable mechanical and barrier properties. These issues may be addressed by combining biopolymers and nanotechnologies. Nanotechnologies are expected to improve the performance of biopolymer-based packaging materials for food packaging applications.

Cellulose nanocrystals (CNC) has shown a potential to be implemented as filler to reinforce synthetic polymer for food packaging applications, equating its unique attributes of biodegradability high surface area. In this work, the aim is to develop a friendly environmental nanocomposite film based on combination of polylactic acid (PLA) and CNC for food packaging applications using a solvent casting method. The main problem that restricts the utilization of nanocellulose as a reinforcing filler with hydrophobic polymers is their tendency to aggregate in the polymer matrix. Thus, a homogenization process was applied to the mixture in order to obtain an even dispersion of CNC in the PLA matrix. Three various internal mixtures at CNC content were used to prepare the nanocomposite films, which are 1,3,5 wt%. The prepared nanocomposite films were subjected to morphological analysis, tensile strength, water vapor, and moisture uptake tests. These tests were conducted to test CNC impact on the barrier and mechanical properties of the PLA matrix. The results of scanning electron microscope (SEM) images showed an appropriate dispersion of CNC at low concentration 1% and 3%, while aggregations of CNC were observed in the PLA matrix for the film containing 5%CNC. The tensile strength of the nanocomposite films significantly improved only with the film containing 3% of CNC by 40% compared to the control sample. The water vapor test conducted on the prepared films reviled an improvement of water vapor behavior of the film containing 3%CNC and 5%CNC by 20% and 42% respectively. The water uptake test reviled that the water sensitivity of the nanocomposite films increased with the presence of CNC at different contents (1%,3% 5%) in the PLA matrix. The obtained results from this study indicated that the prepared nanocomposite films presented relatively reliable tensile strength and water permeability compared to the pure PLA film. The improvement of the water permeability and the tensile strength showed a potential of the prepared nanocomposite films to be utilized in food packaging applications. However, further studies on the nanocomposite films properties required to confirm that.

CHAPTER 1: INTRODUCTION

1.1 Background

Plastics, glass, and metal are the conventional packaging resources used as food packaging materials. These materials are non-biodegradable and nonrenewable. However, an increase consciousness of waste disposal problems and their effects on the environment has risen the demand for packaging manufactured from renewable materials. Recently, this awareness has resulted in the development of biodegradable packaging materials, such as edible films and coatings covering certain disposable products (Tang et al., 2006; Tharanathan et al., 2003). Several biodegradable materials such as cellulose and starch are increasingly used in innovative packaging methods. These methods include using starches to make edible and biodegradable films for food packaging as well as implementing cellulose as filler to reinforce synthetic plastics (Tang et al., 2012).

According to Rhim's et al. (2013), Food packaging are accounted for 40% of the use of plastics. Almost 20% of them are used in disposable products for food packaging applications such as bottles, sheets, and cups. Food packaging has become an important topic with global effort to reduce disposal wastes. From 1988 to 2005, there was an increase of solid waste by 37%, and the packaging contributed to the total solid wastes at 31.2% (Imran et al., 2010).

Revising conventional packaging practices requires materials that not only are biodegradable but that also both provide protection and prolong shelf-life of foods. Biopolymers such as cellulose and starch have shown potential in food packaging applications due to useful characteristics such as acting as reliable mechanical and water barriers. Biopolymers are also ideal because they can be combined with other synthetic polymers or with additives such as antioxidants resulting in superior protection and greater overall functionality. However, in comparison with conventional materials, biopolymers’ performance is still inferior, and therefore biopolymers often do not fulfill the minimum requirements of food packaging. In order to extend biopolymers’ industrial applicability, multiple issues with biopolymer-based packaging must be addressed. These concerns include biopolymers’ degradation rates under different conditions, release of harmful compounds into packaged food, and potential for growth of unwanted microbes (Rhim et al., 2013).

In the efforts to improve the properties of biopolymers, various nanotechnologies have been used in food packaging. Biopolymers that incorporate nanoparticles, such as cellulose nanocrystals, have received considerable attention as promising packaging materials. These biopolymers called by bio-nanocomposites as they described in Rhim’s et al. (2013) review of smart packaging.

Nanocomposites exhibit many advantages. These advantages include acting as durable mechanical and gas or moisture barriers. They can also present more benefits such as sufficient biodegradability, low density, and adequate transparency. These properties are all desirable in food packaging (Dufresne, 2013). Such improved properties are attributed to the even dispersion of the nanoscale fillers incorporated into the polymer’s matrix (Rhim and Perry, 2007).

Among all the types of nanocomposites, cellulose nanocomposites are considered to be promising candidates for packaging applications. The characteristics of these cellulose nanocomposites rely on two factors: the origin of the nanocellulose, and the interaction between the nanocellulose and the matrix of the polymer (Azizi Samir et al., 2005).

Nanocellulose can be obtained by two methods: a mechanical method, and a chemical method. The nanocellulose obtained by the mechanical method is referred to as cellulose nanofibers (CNF), while the nanocellulose obtained through acid hydrolysis is called cellulose nanocrystals (CNC) (Azeredo et al., 2017).

Some authors have successfully prepared CNC through acid hydrolysis of cellulose microfibers, describing their morphology as rod-like nanocrystals. Their size and dimension mainly depend on the origin and the production process of the CNC. CNC presents a high aspect ratio varying from 10 (for cotton) to 67 (for tunicin) (Dufresne, 2013). The high aspect ratio of CNC is an important factor when they are used as reinforcing fillers (Dufresne, 2013). Recently, the use of CNC as filler in biopolymer matrices has increased. Much research has evaluated the effect of their reinforcement. The effects of CNC on the mechanical properties of different materials, especially tensile strength, are predominantly studied (Azeredo et al., 2017; Dufresne, 2013). CNC presents improved mechanical properties such as a tensile modulus of around 130 GPa and a tensile strength of 10 GPa (Dufresne, 2013)

1.2 Hypothesis

Blending and homogenizing cellulose nanocrystal in PLA matrix would be forming a new nanocomposite that would have properties of sufficient mechanical strength and thermal and moisture vapor permeability, and easy to be degraded and appropriate for use in food packaging.

1.3 Objective

The research goal is to develop a biodegradable environmental friendly nanocomposite film by blending and homogenizing CNC in PLA matrix using a solvent casting method. To achieve this goal, these objectives will be accomplished in this research.

Objective 1: fabricate biopolymer- based (e.g. PLA) nanocomposite films by blending and homogenizing CNC in PLA matrix using solvent casting method

Objective 2: investigate the impact of CNC content on the tensile strength of the prepared films and moisture barrier properties.

Objective 3: evaluate the possibility of using the nanocomposite films in smart food packaging application

1.4 Literature Review

1.4.1 Food Packaging Definition

Packaging is crucial to maintain the quality of food. It can maintain the benefits of food processing, enabling food products to travel long distances during distribution by providing protection for foods from external stimuli such as light, oxygen, and moisture (Marsh and Bugusu, 2007; Risch, 2009). Food packaging materials have three main functions: protecting and storing food and providing information about the product to consumers. Today, packaging materials that involve nanotechnology can provide better passive and active protection of foods (Risch, 2009). An example of passive protection is when the packaging provides an excellent barrier to protect food from deteriorating. On the other hand, active packaging involves ensuring a certain interaction between packaging materials and food to extend shelf life (Risch, 2009).

Conventional materials used in food packaging such as, glass, plastic, have been used to meet the goals of food packaging, but these materials have increased environmental issues and disposal management problem. Therefore, there have been increased effort to develop new packaging materials to enhance the food packaging and also provide choice and convenience to the consumers. Figure 1 shows the general requirement of food packaging (Rhim et al., 2013).

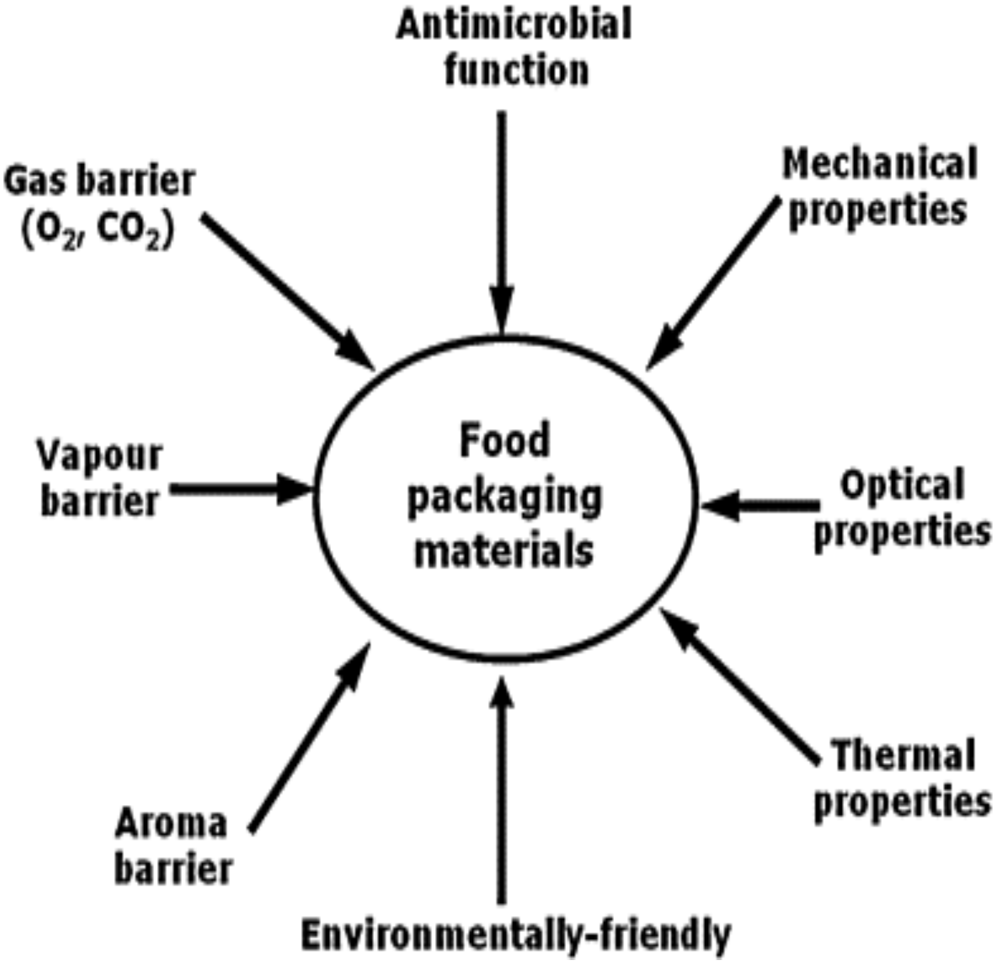

Figure 1.1 General requirement of food packaging material (Rhim et al., 2013).

1.4.2 Food Packaging Roles

Food packaging's main roles are to store food safely, to protect packaged food from external factors, and to reduce food waste. Moreover, the packaging should also provide the consumers with the necessary information about the food product such as nutritional and ingredients information, cooking instructions, and price. In addition to the main role, there are other (secondary) functions of the food packaging, which are traceability, convenience, and tamper indication (Marsh and Bugusu, 2007).

1.4.2.1 Protection

Proper food packaging can maintain the safety and quality of various food products. Packaging materials should protect packaged food from three types of external influences which are biological, chemical, and physical in order to prevent the deterioration of food, increase shelf- life, and enhance food quality (Marsh and Bugusu, 2007).

Physical protection of packaged foods during distribution is necessary to ensure the quality of food since it encounters with many external mechanical damages such as shock and vibration. Paperboard and corrugated materials have good physical barrier properties, resisting the impacts and cursing damages, thereby they are used widely as packaging container and sometimes as packaging materials for instance fruit and eggs. using of the appropriate material in food packaging for physical protection not only protect the product but also the consumer. For example, the replacement of glass by plastic for soda products has decreased the danger from the broken glass container. (Marsh and Bugusu, 2007).

Food constituents can be affected when they exposure to environmental influences such as oxygen, moisture, and light. Therefore, a packaging materials with good barrier properties is important to protect food from compositional changes. Glass and metal provide good barrier properties against environmental influences, but since they used with other materials in packaging application, which might allow some permeability. For example, plastic lids used in metal cans to allow sealing and filling, which they might allow some level of permeability to some gases or vapor (Marsh and Bugusu, 2007).

1.4.2.2 Food Waste Reduction

Inappropriate food processing and packaging lead to food spoilage and as a result large amount of food is discarded, and consequently increasing the amount of food waste, which leads to environmental problems. Large amount of food waste has been reported in many countries where about 50% of the wastes are fruit. Increasing the shelf – life of foods by proper packaging can contribute to reduce food waste (Marsh and Bugusu, 2007).

1.4.3 Active Packaging.

One of the major reasons of deterioration of the packaged food is post- process contamination that caused by the use of inappropriate packaging materials or handling the food after processing. Recently, active packaging or smart packaging has applied in food packaging to reduce the deterioration of food that caused by microbial growth. Active packaging is a post- process protection system that requires certain interaction of food packaging and food. It can be in a form of films or coating, example the use of antimicrobial packaging films (Gandhi and Chikindas, 2007).

As it is mentioned above in food packaging role section, packaging materials can provide an active protection for packaged foods. One form of that, is smart or active packaging system. It is defined as a smart or intelligent packaging system that require an interaction between the packaged material and food or food surrounding atmosphere in order to maintain the quality and hence increase shelf –life of foods (Labuza and Breene, 1989).

Smart packaging is not just related to protecting food from the external environment by providing good barrier properties to the packaged food, but it also provides other important functions, maintaining food quality. Furthermore, Smart packaging can reduce the risk of the development of the pathogen, and provide the efficient control of surface contamination, which can be useful for foods with high water activity such as fish to control the microbial growth on the surface of these foods. (Scannell et al., 2000; Millette et al., 2007).

1.4.4 Active Packaging Concepts

1.4.4.1 Oxygen Scavengers

Potential microbial growth in food packages results from the occurrence of high levels of oxygen trapped within the packaging. These oxygen levels may also cause changes in color of the product, nutritional deterioration of packaged foods, and an overall decrease in the food's shelf-life (Hogan and Kerry 2008). Therefore, controlling and monitoring oxygen levels inside food packages can lower the rate of food spoilage. Including oxygen absorbing in food packaging is a viable technique for controlling oxygen levels in food packaging. Controlling oxygen levels in packaging often improves the quality and extends the shelf life of food products (Figure 1.2) (Ozdemir and Floros, 2004).

1.4.4.2 Moisture Control

Additionally, controlling for high levels of moisture within food packaging is important in reducing the water activity and thus limit microbial growth (Vermeiren et al., 1999). Moisture development in food packages can occur for different reasons such condensation and temperature fluctuation. Water accumulation inside food packaging leads to bacteria and mold growth, decreasing product quality and reducing the shelf life of foods (Ozdemir and Floros, 2004).

1.4.4.3 Antimicrobial Packaging

Active or smart packaging such as antimicrobial packaging interacts with the packaged food to discourage microbial growth (Appendini and Hotchkiss, 2002). Antimicrobial packaging material can be categorized into two categories: material that contains antimicrobial agents that migrate to the surface of the food, and material that contains antimicrobial agents bound to the surface of a film inside the package (Han 2003). Standard antimicrobial multi-layer active films consist of four layers: the outer layer, barrier layer, matrix layer, and control layer (Figure 1.3). Antimicrobial packaging, whether using multi-layer films or coating the inside of the packaging, can be used in order to control the growth of undesired microorganisms on foods (Labuza and Breene, 1989).

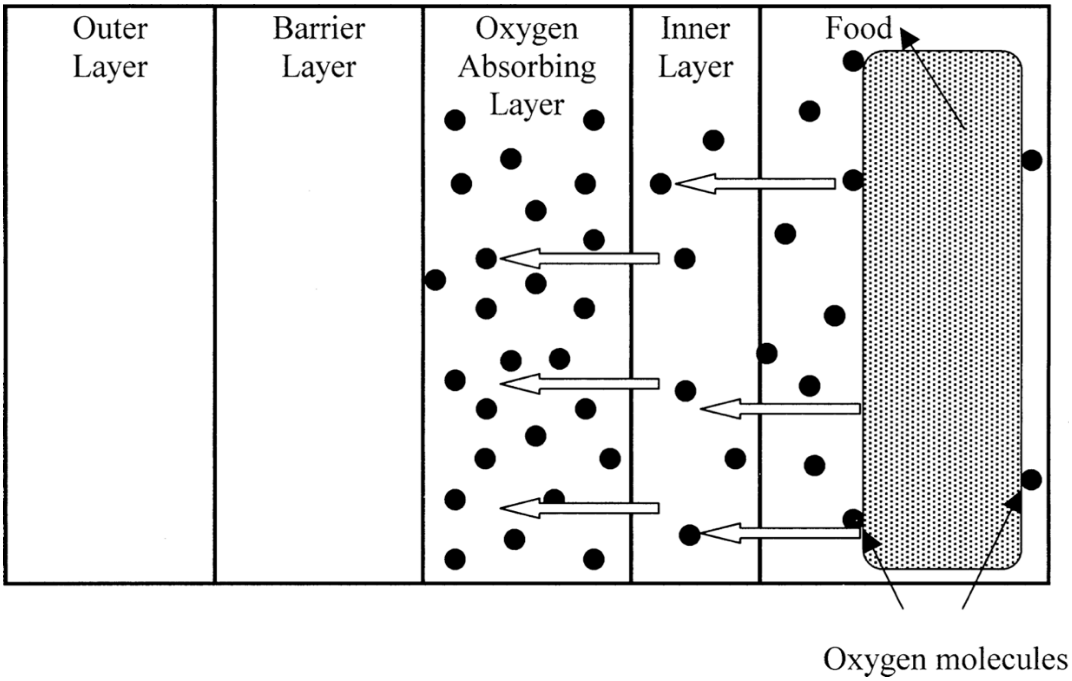

Figure 1.2 A structure of typical oxygen absorbing multi- layer film (Ozdemir and Floros, 2004).

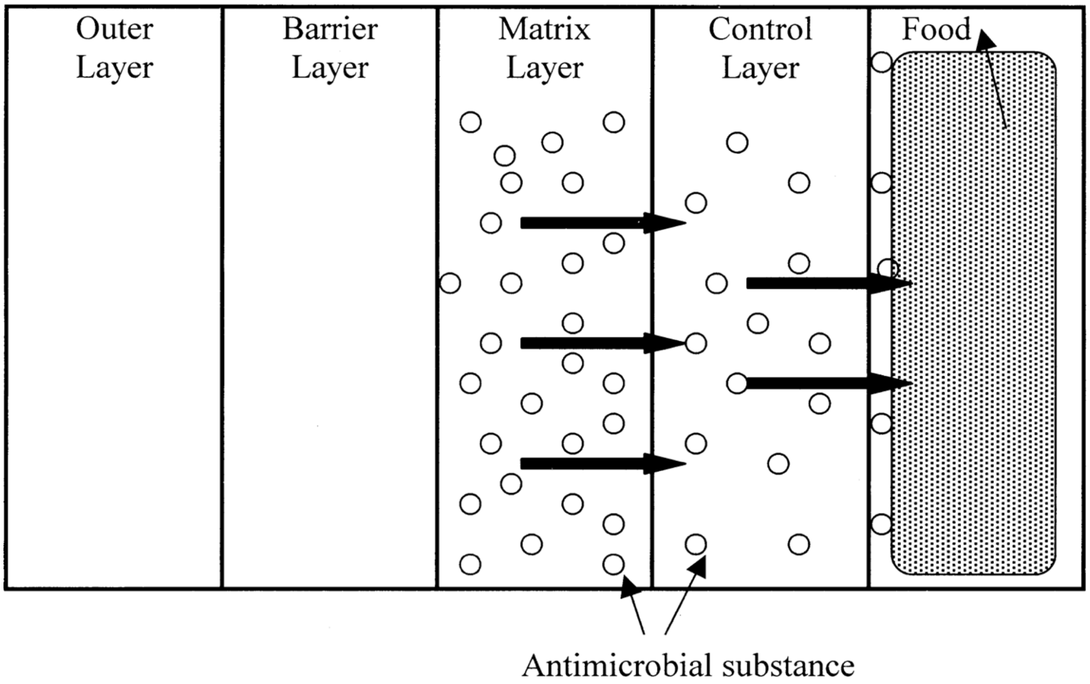

Figure 1.3 A structure of typical antimicrobial multi- layer active film (Ozdemir and Floros, 2004).

1.4.5 Bio-based Packaging Materials

Bio-based packaging materials are sourced from renewable and biodegradable materials are being utilized in food packaging applications. However, the two terms “bio-based” and “biodegradability” are distinct, not synonymous—biodegradable packaging materials can usually decompose naturally under certain conditions whereas bio-based packaging materials are not necessarily biodegradable despite initially being derived from biodegradable materials (Weber et al., 2002).

1.4.5.1 Biopolymers

Rhim’s et al. (2013) categorized biopolymers into three categories. These categories are based on the origin and the process condition as shown in Figure (1.4) (Rhim et al., 2013).

- Natural polymers—examples include cellulose, alginate, chitosan, and agar;
- Synthetic biodegradable polymers—examples include poly(l-lactide) (PLA), poly(glycolic acid) (PGA), and poly(vinyl alcohol) (PVA);
- Biopolymers produced by the fermentation of microbes—examples include pullulan and curdlan (Rhim et al., 2013).

1.4.5.1 Bio-nanocomposites

Bio-nanocomposites, a new type of material consisting of a polymer matrix incorporated with nanoparticles (ranging from 1–100 nm), are currently being considering as a promising innovation to improve the mechanical and barrier properties of biodegradable packaging. Rhim et al. (2013) expect that using nanocomposites will improve the mechanical, barrier, and thermal properties in food packaging (Rhim et al., 2013). For example, cellulose nanofibers (CNF) have been utilized as reinforcing filler to improve the mechanical properties of mango puree film such as glass transition temperature water vapor permeability (Azeredo et al., 2009).

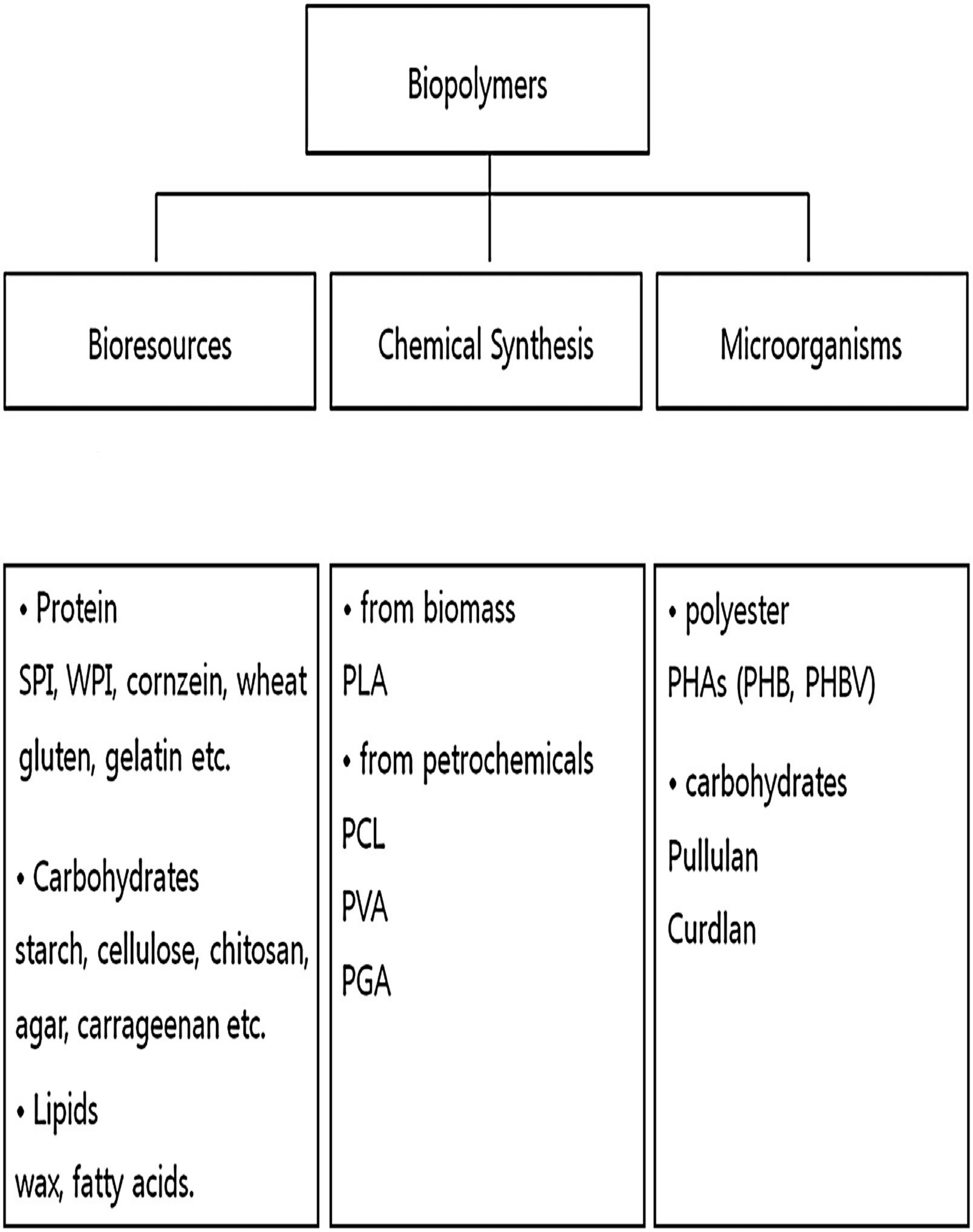

Figure 1.4 A Classification of biopolymers (Rhim et al., 2013).

1.4.6 Cellulose and Nanocellulose Structure

Despite its origin, cellulose is a linear homopolymer, consisting of repeating D-glucopyranose units linked by β (1,4) linkages, which form a cellobiose unit.This unit composes of two units of anhydroglucose, and every second anhydroglucose rotates 180 degrees with regard to adjoining units in order to form an oxygen covalent bond through dehydration as it is shown in Figure 1.5 (Li et al., 2015).

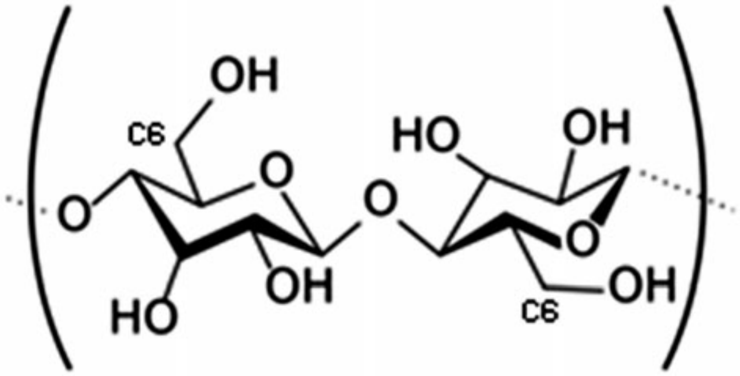

Figure 1.5 Cellobiose unit. (Li et al., 2015).

Hydroxyl group is the function group of the cellulose chains. Their interaction between each other stabilizes the cellulose chains. Three hydroxyl groups attach to each glucopyranose unit, and they contribute to form hydrogen bonds, which determine the physical characteristics such as strength and rigidity of the cellulose chain (Li et al., 2015).

The cellulose chains, shown in Figure 1.6, pack together to form highly ordered crystalline structures (microfibrils). However, disorder to these microfibrils occur to form two different regions: amorphous (disorder region) and crystalline (highly ordered region). As cellulose polymers aggregate in a liner structure, the interaction between hydroxyl groups form hydrogen bonds, stabilizing these polymers together. (Li et al., 2015).

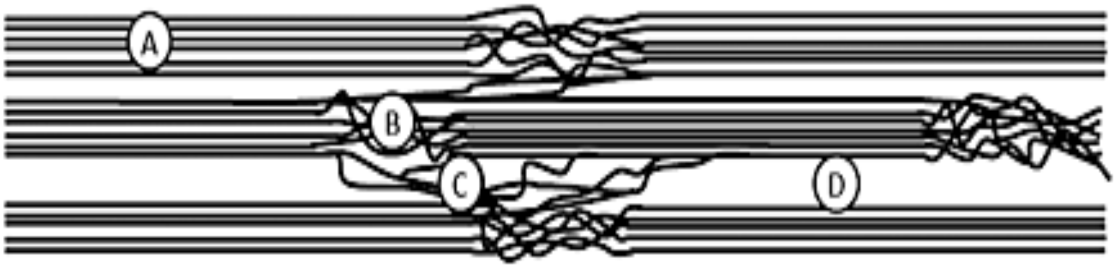

Figure 1.6. Idealized cellulose fiber structure. A where this alone is cellulose nanocrystals structure (CNCs); B is amorphous region (disorder regain); C is inter-fibrillar tie chains; D is voids (Li et al., 2015).

Nanocellulose is a term of cellulose fibers possess at least one dimension in nanometer. There are two methods to obtain nanocellulsoe. The first method is mechanical treatment, and the second is acid hydrolyses. In the acid hydrolyses, the separation of cellulose nanocrystals (nanowhiskers) is the product of the difference of solubility of the two regions (amorphous and ordered regions) in the cellulose chain. (Habibi et al., 2010).

1.4.7 Potential Application of Nano-cellulose in Food Packaging.

1.4.7.1 Reinforcement

As it is mentioned above that biopolymers present poor mechanical and barrier properties because of their hydrophilic nature. Recently, there has been an interest in using cellulose nanoscale materials such as CNC and CNF as a filler for reinforcing biopolymer films for food packaging applications. This interest comes from their beneficial characteristics such as biodegradability, low cost, and rather high aspect ratio. This high aspect ratio of cellulose material fillers is an important factor in enhancing the mechanical properties of nanocomposite films. Authors Cho and Park. (2011) and Khan et al. (2012) reported that the tensile strength of their cellulose nanocomposite films improved with the addition of CNC compared to the control samples (Rhim and Perry, 2007).

1.4.7.2 Coating and Edible films

Cellulose is a hydrophilic polymer, and thus usually shows poor compatibility with hydrophobic synthetic polymers and also poor thermoplastic properties. This limits the use of nanocellulose, especially cellulose nanocrystals, in nanocomposite fabrication by the extrusion method (Li et al., 2015). However, according to Li et al. (2015), CNC is easy to disperse in water, and thus it can be used as water-based coating for food packaging applications. This can be useful to improve the barrier properties of packaging materials.

1.4.7.3 Antimicrobial Nanocomposites Films

Antimicrobial nanocomposite films have received increased attention to be employed for food packaging. These films have antimicrobial activity, aiding to control the growth of unwanted spoilage microorganisms. Antimicrobial nanocomposite films possess desired characteristics for food packaging applications such as structural integrity, antimicrobial properties, and barrier properties, attributing to the incorporation of both nanoparticle fillers and natural antimicrobial agents in the film matrix (Rhim and Perry, 2007).

1.4.8 Preparation of Cellulose Nanocrystals suspensions

Under acid hydrolysis conditions, the amorphous regions of the cellulose chain are responsible for the separation of cellulose microfibers into monocrystals (Azizi Samir et al., 2005). In the cellulose chain, amorphous regions are surrounded and embedded within cellulose microfiber. The disruption of the amorphous region results in the release of cellulose microcrystalline because of the difference in kinetic energy between amorphous regions and crystalline domains. The generated substances are stable and physiological inert (Mazeau, K., 2003)

Azizi Samir et al. (2005) in his review of cellulosic whiskers, referred to cellulose nanocrystals by using the term cellulose microcrystalline and descried them as substances that occur naturally and have stiff rod-like or long straight crystals shape. The dimension of these substances can be vary depending on the origin of cellulose microfibers and process conditions (Azizi Samir et al.,2005). Many authors have reported the characteristics of cellulose nanocrystals suspensions from different natural sources such as beet pulp, cotton, and sugar. To study the characteristics of the cellulose nanocrystals suspensions, different techniques have been used such as transmission electron micrographs (TEM), small angle scatter (Azizi Samir et al., 2005). Terech et al. (1999) in their study of determining the dimensions of cellulose nanocrystals, prepared from marine animals, they used small angle scatter to determine the average of the cellulose nanocrystals dimensions (Azizi Samir et al., 2005).

1.4.8.1 Cellulose Nanocrystals Suspensions Stability

Microcrystalline cellulose or (CNC) suspensions stability, prepared by acid hydrolysis, depends on the solvent used in the hydrolysis procedure as well as resulted cellulose suspensions' surface charge, dimension, and size. (Azizi Samir et al., 2005). Dong et al. (1998) studied the effect of acid hydrolysis conditions and ultrasound treatment on the morphology of cellulose whiskers suspensions from cotton fibers. They found that there was an increased in the surface charge of the obtained cellulose whiskers suspensions (Azizi Samir et al., 2005).

According to Azizi Samir et al. (2005), the CNC suspensions prepared by sulfuric acid is more stable than those prepared by hydrochloric acid, and this is ascribed to the fact that sulfuric acid prepared CNC have negative surface charge, while hydrochloric acid prepared CNC do not present a charge (Azizi Samir et al., 2005).

CNC does not dissolve in common solvents, but it forms colloidal suspensions in polar solvents such as water (Azizi Samir et al., 2005). CNC suspensions are stable in high polarity solvents. However, surface chemical modification or coating of whiskers suspensions surface can generate stable suspensions in low polarity solvents (Azizi Samir et al., 2005). Goussé et al. (2002) prepared stable CNC suspensions by partial silylation of their surface. Moreover, Araki et al. (2001) successfully prepared stable CNC suspensions by using carboxylation-amidation procedure. Also Heux et al. (2000) observed how that the use of surfactant leads to stable cellulose whiskers suspensions (Samir et al., 2005).

De Souza Lima and Borsali. (2002) studied the dynamic, the static, and properties of CNC suspensions from tunicate, prepared by acid hydrolysis, using light scatter techniques. They observed several scattering peaks and explained them as electrostatic interactions between the suspension particles, which produces the structure order of these suspensions (Azizi Samir et al., 2005). However, these electrostatic interactions gradually disappeared after the addition of salt to suspensions. This indicated that CNC suspensions stability and order depend on the electrostatic interaction between CNC suspensions particles (Azizi Samir et al., 2005)

1.4.8.2 Nanocrystals Suspensions Birefringent

CNC aqueous suspensions, resulted from acid hydrolysis treatment, present the character of birefringent. This property caused by two origins in cellulose: The structural anisotropy produced from cellulose, and flow anisotropy as a result of cellulose alignment (Azizi Samir et al., 2005). This alignment of CNC has been studied by Ebeling et al. (1999). They found that it is shear- rate dependent alignment and reversible. At low shear rate, microcrystals align randomly in planer domains, but when the shear exceeded, they are broken to form cellulose whiskers suspensions alignment (Azizi Samir et al., 2005).

1.4.9 The Properties of Cellulose Nanocrystals (CNC) Composites

1.4.9.1 Mechanical Properties

Recently, the impact of CNC as a filler on the mechanical performance, tensile strength and modules properties of synthetic polymers has been studied more extensively. Favier et al. (1995) explained that the improvement of the mechanical properties of the composite film reinforces with CNC results from the percolation effect by CNC. CNC can create stiffness and strength effect in the polymer matrix by hydrogen bonds interactions (Favier et al., 1995). The increase of tensile and modules of nanocomposite films is restricted by the agglomeration of CNC. Additionally, they reported the optimum amount, which was 5 wt% (Huq et al., 2012; Khan et al., 2012).

Helbert et al. (1996), reported Young's modulus increased significantly of poly(S-co-BuA) film, reinforced with 30% of cellulose whiskers in comparison with the control sample. They explained that this improvement occurred because of the stiffness strength effect by CNC hydrogen bonds interactions.

Sung et al. (2017), They prepared successfully PLA/CNC composites for food packaging use by twin-screw extruder. Poly lactic acid (PLA) film reinforced with different contents of CNC (1%,3%, and5%). They reported that the nanocomposite films contain 1% and 3% of CNC had superior barrier and mechanical properties in comparison with the control sample.

These examples show that implement of cellulose, especially CNC, as a filler can improve biopolymers performance however, the reinforcement effect depends on the properties of CNC.

Since the cellulose nanocrystals dimensions and properties depend on cellulose fibers origin as well as the processing conditions. Azeredo et al. (2012) in their study, they evaluated CNC effect, obtained from coconut and cotton, on the mechanical performance of alginate-acerola puree films. The results of their study suggested that both coconut cellulose whiskers and cotton cellulose whiskers have the same impact on the mechanical properties of the prepared films although coconut cellulose whiskers received to one or multi- stage belching. Also they highlighted the role of the chemical nature of the filler substances (hydrophilic or hydrophobic) in improving the mechanical properties. High compatibility between polymer matrix and CNC leads to better adhesion, and thus to maximize the mechanical properties of the nanocomposite (Azeredo et al., 2012).

Achieving an appropriate dispersion of CNC in polymer matrix mainly depends on the formulation of an adequate percolation network in the matrix, but the strong interactions between the CNC substances may restrict that because of their tendency of aggregations (Dufresne, 2010). Many methods and techniques have been used in order to overcome this problem. One of the possible techniques that can improve the dispersion of CNC in polymer matrix is the use of surfactants. For example, using surfactants increases the compatibility between a hydrophobic polymer matrix, such as PLA, and a hydrophilic filler such as CNC. The surfactant hydrophilic tail dissolves into the cellulose and the hydrophobic tail dissolves into the hydrophobic matrix, preventing aggregations of cellulose nanoparticles by steric stabilization (Hubbe et al., 2008).

Bondeson and Oksman. (2007) reported in their study that they used an anionic surfactant in order to enhance CNC dispersion in PLA matrix. CNC dispersion improved as the surfactant content increases. Accordingly, the prepared nanocomposite films presented superior mechanical performance compared to the pure PLA films though degradation on the PLA matrix was observed.

An alternative method to enhance the dispersion of CNC in polymer matrix is covalent linking. De Mesquita et al. (2012), successfully prepared CNC/chitosan bio nanocomposites through covalent linkage. CNC were functionalized with methyl adipoyl chloride. The results of the mechanical properties test showed an increase of the films tensile strength from 45 to 110 MPa, and also young’s modules from 1.35GPa to 3.75GPa with the addition of 60% of CNC.

1.4.9.2 Barrier Properties

Additionally, CNC potential to improve water barrier of biopolymers have been studied. Many authors have in their studies highlighted the improved barrier properties of variety of biopolymers with the addition of CNC at different contents. This improvement was ascribed to their small dimensions and large surface volume ratio, which leads to change the properties of the polymer matrix.

Water sensitivity and water vapor permeability are important properties for hydrophilic polymers when they are used in food packaging applications. Water sensitivity of a polymer is defined as the tendency of the polymer to form its own structure when it is affected by water. However, WVP refers to the move of water molecules throughout the polymer. CNC effect on these two properties was reported by many authors used different tests such as water solubility and water uptake (Azeredo et al. 2017).

Dhar et al. (2015) studied the effect of CNC obtained from bamboo (Bambusabalcooa) on the migration and barrier properties of poly (3-hydroxybutyrate) (PHB) polymer. They found that the PHB/ CNC films with the optimum CNC content, which was 2wt%, showed a significant decrease in the oxygen transmission rate by 65%. Also properties such as solubility and diffusivity of the film improved with the addition of CNC up to 2%. The improvement of the barrier properties resulted from the effect of tortuous of the CNC in the polymer matrix.

Azeredo et al. (2012), developed an edible film based on combination of fruit purees (acerola puree) and polysaccharides (alginate). The film incorporated with cellulose whiskers at different contents. Water vapor permeability of the film showed a significant improvement compared with unfilled films. This improvement was ascribed to the tortuosity effect of cellulose nanoparticles.

Additionally, the effect of CNC on the water resistance of different hydrophilic biopolymers has been reported by some authors Khan et al. (2012) and Dufresne et al. (2000).

1.4.10 Summery

Cellulose nanocomposites are promising candidates to revise non-renewable and non- biodegradable packaging materials due to their particular properties such as biodegradability and low cost. In addition to their basic properties, they are highly available and present reliable barrier and mechanical properties. Including active packaging is highly desirable in food packaging industry to maintain the quality and to extend shelf life of packaged foods. The use of biopolymers incorporated with nanocellulose as a packaging material could expend active packaging applications in food packaging industry.

CHAPTER 2: Study on Mechanical and Barrier Properties of Polylactic Acid Films Combined with Cellulose Nanocrystals

2.1 Introduction

Revising conventional packaging practices requires materials that not only are biodegradable but that also both provide protection and prolong the shelf-life of foods. Biopolymers such as cellulose and starch have shown potential in food packaging applications due to useful characteristics such as acting as reliable mechanical and water barriers. Biopolymers are also ideal because they can be blended with other synthetic polymers or with additives such as antioxidants or antimicrobial agents, resulting in superior protection and greater overall functionality. However, in comparison with conventional materials, biopolymers' performance is still inferior, and therefore biopolymers often do not meet the minimum requirements of food packaging. In order to extend biopolymers' industrial applicability, multiple issues with biopolymer-based packaging must be addressed. These concerns include biopolymers' degradation rates under different conditions, release of harmful compounds into packaged food, and potential for growth of unwanted microbes (Rhim et al., 2013).

Nanocomposites exhibit many advantages. These advantages include acting as durable mechanical and gas or moisture barriers. They can also present more benefits such as sufficient biodegradability, low density, and adequate transparency. These properties are all desirable in food packaging (Dufresne, 2013). Such improved properties are attributed to the even dispersion of the nanoscale fillers incorporated into the polymer's matrix (Rhim and Perry, 2007).

Among all the types of nanocomposites, cellulose nanocomposites are considered to be promising candidates for packaging applications. The properties of these cellulose nanocomposites depend on two factors: the origin of the nanocellulose, and the interaction between the nanocellulose and the polymer matrix (Azizi Samir et al., 2005).

Some authors have successfully prepared CNCs through acid hydrolysis of cellulose microfibers, describing their morphology as rod-like nanocrystals. Their size and dimension mainly depend on the origin and the production process of the CNC. CNC present a high aspect ratio varying from 10 (for cotton) to 67 (for tunicin) (Dufresne, 2013). The high aspect ratio of CNC is an important factor when they are used as reinforcing fillers (Dufresne, 2013). Recently, there has been an increased interest in using CNC as filler in biopolymer matrices. Much research has evaluated the effect of their reinforcement. The effect of CNC on the mechanical properties of different materials, especially tensile strength, are predominantly studied (Azeredo et al., 2017; Dufresne, 2013). CNC present improved mechanical properties such as a tensile modulus of around 130 GPa and a tensile strength of 10 GPa (Dufresne, 2013)

Poly lactic acid (PLA) is a linear thermo- plastic polyester, sourced from natural biopolymers such as starch. PLA exhibits a good biodegradability as well as it is easy to process. Thus, it has used in many applications and obtained the attention in number of applications and research (Sung et al., 2017).

This study aims to develop environmental friendly nanocomposite films based on combination of PLA and CNC for smart packaging applications via a solvent casting method. The objectives of this work are to fabricate biopolymer- based (e.g.PLA) nanocomposite films by blending and homogenizing CNC in PLA matrix via a solvent casting process. The properties of the nanocomposite films will be characterized and the effect of CNC contents on the properties of nanocomposite films will be also investigated as well in order to evaluate the potential of the produced nanocomposite films for smart packaging application.

2.2 Materials and Methods

2.2.1 Materials

Nanocellulose was purchased from the Process Development Center (PDC) at University of Maine, 5737 Jenness Hall, Orono, Maine, ME 04469. The properties of this CNC is shown in table 1. polylactic acid (PLA) was purchased from the Solutions of Consequences LLC, Grand Rapids, Michigan, MI 49505. The properties of this PLA is shown in table 2.

Table 2.1 The properties of CNC

Names	**Dimension**	**Density**	**CNC contents**
Cellulose nanocrystal	5-20 nm in diameter 150-20 nm in length	1.0 g/cm^3 aqueous gel	11.5-12.5 wt.% of solid in aqueous gel

Table 2.2 The properties of PLA

Names	Pellet size	Density
Poly lactic acid	3.6 mm in diameter	1.2 g/cm^3

The equipment used in this study include Omni Mixer Homogenizer, stainless steel dishes, desiccator, Dew point hygrometer, stable micro system texture analyser (model TA-HD plus).

2.2.2 Experimental Design

Based on our review of Khan et al. (2013) Work, the result of their study suggested that mechanical and barrier properties of thermoplastic biodegradable polymers improved with the addition of cellulose nanocrystals up to 5%. Accordingly, we decided to design our experiment based on a one-factor-at-a-time experiment design. Since in this work we want to evaluate the effect of different CNC contents on the mechanical and the barrier properties of PLA film matrix, we selected CNC contents as the factor with three levels (1%,3%, and 5%). Table 2.3 illustrates the experiment design and the conditions of films preparation:

Table 2.3 Synthesis conditions of CNC/PLA Films

CNC	**0 wt. %**	**1 wt. %**	**3% wt. %**	**5 wt. %**
PLA	4g	4g	4g	4g
Thickness	~ 0.16mm	~ 0.16 mm	~ 0.16mm	~ 0.16mm
Temperature	Room temperature	Room temperature	Room temperature	Room temperature
Mixing Time	45min	45min	45min	45min
Homogenization Time	15min	15min	15min	15min
Drying Time	24h	24h	24h	24h

As control, pure PLA and cellulose nanocomposite films will also be prepared respectively for comparison

2.2.3 Preparation of Polylactic Acid (PLA)/Cellulose Nanocrystals (CNC) Films

For the preparation of the films, we used the procedure reported by Hossain et al. (2012) with some modifications. Pure PLA films and PLA/CNC nanocomposite films were prepared using a solvent cast method. The procedure of this process is shown as Figure 2.1. 4g of PLA was dissolved in 100 ml of chloroform under continuous stirring until the PLA completely dissolved. The desired amount of CNC (1%, 3%,5%) was suspended in 10 ml of distilled water and then added to the PLA solution. After that, the two solutions were mixed for 45 minutes and then homogenized for 15 minutes. Afterwards, the homogenized solution was poured into stainless steel dishes and allowed to evaporated at room temperature for 24 h.

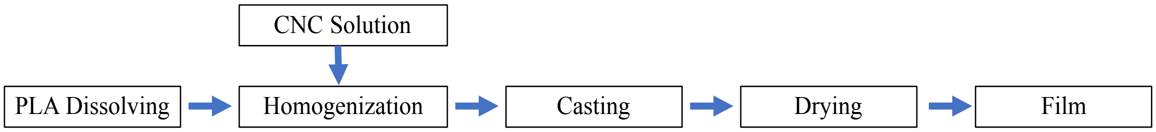

Figure 2.1. flowchart of casting process for fabrication of nanocomposite films

2.2.4 Examine Morphology of (PLA)/ (CNC) Films

In order to analyze prepared films morphology, scanning electron microscope (SEM) was utilized. Four samples of each film, pure PLA films, and nanocomposite films with 1%,3%,5% of CNC, prepared and dried at 100°C for 18. Then, they were stored at temperature of 22°C and relative humidity of 30% RH for three days before the test.

2.2.5 Sample Preparation of Tensile Strength Test

Strip-shape samples (the thickness is individually controlled) were prepared of the films (see figure 2.2). The samples were stored at 60% RH three days before the test. The dimensions of strips of film samples is shown in table 2.4

Table 2.4 The dimensions of strips of film samples

Film	**Length(cm)**	**Width(cm)**	**Thickness(mm)**
PLA	6	1.5	0.14
PLA/CNC 1%	6	1.5	0.14
PLA/CNC 3%	6	1.5	0.16
PLA/CNC 5%	6	1.5	0.18

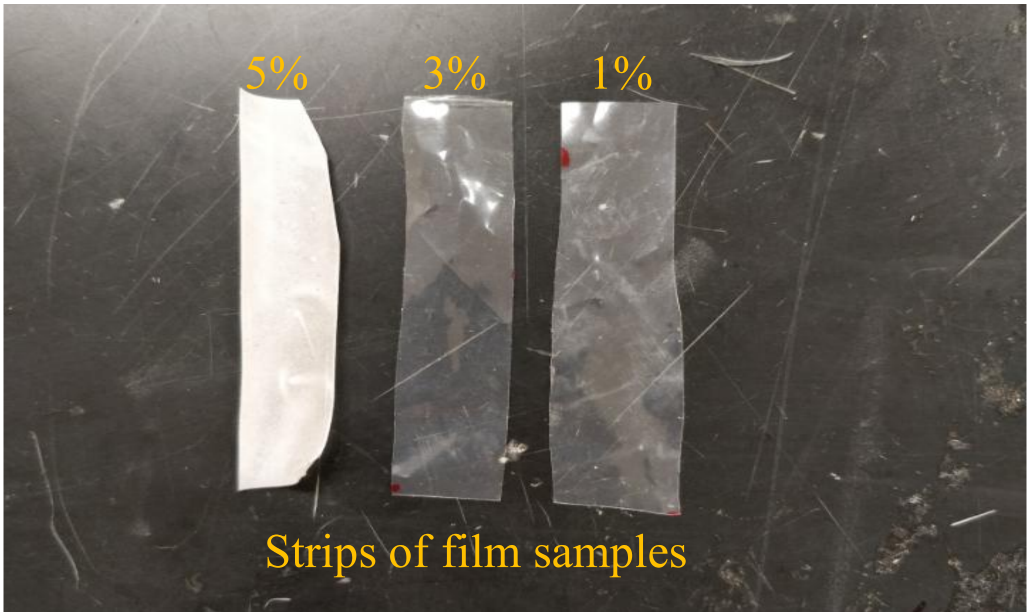

Figure 2.2 Strip-shape samples of, PLA/1%CNC, PLA/3 %CNC, and PLA/5%CNC nanocomposite films prepared for the tensile strength test

2.2.6 Determine Tensile Strength of the Nanocomposite Films

Tensile strength test was carried out in triplicate. The tensile strength of the films was measured at 25°C and 35% RH via a stable micro system texture analyser (model TA-HD plus). A load cell of 1 kN is applied. The length of the gauge was almost 20 mm. A crosshead speed of 2 mm/min was used (see figure 2.3).

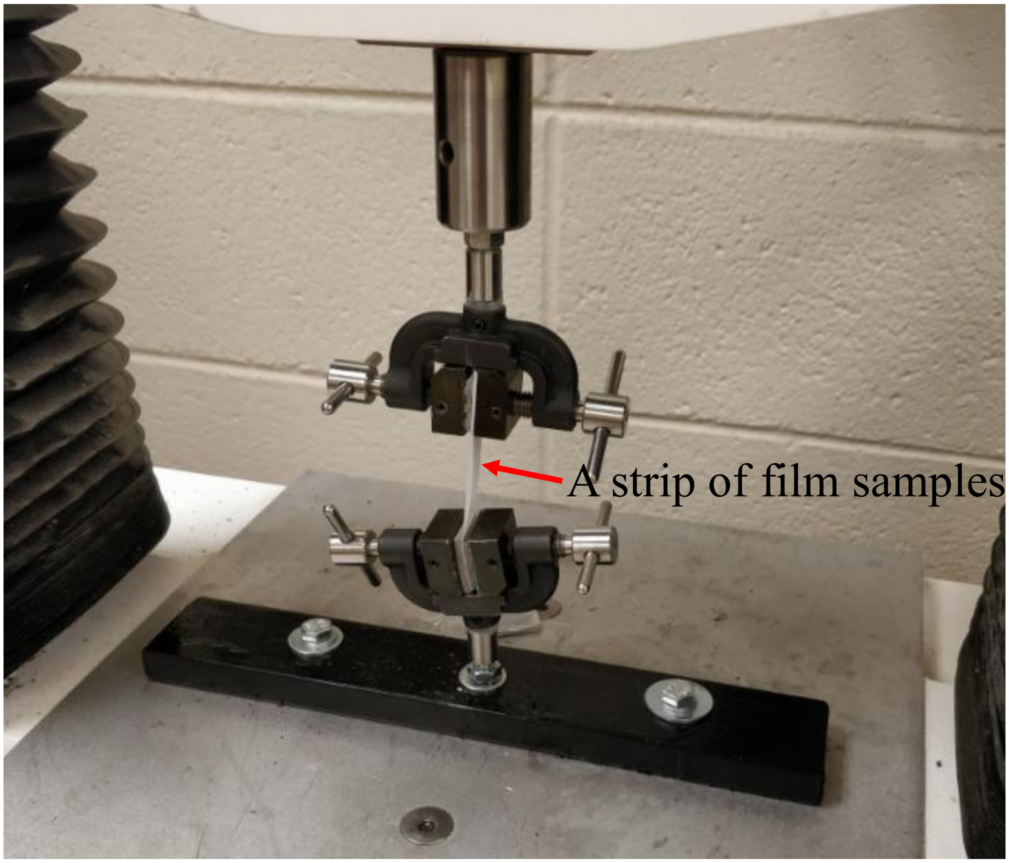

Figure 2.3 The equipment for tensile strength test

2.2.7 Sample Preparation of Water Uptake Test

Samples of 10mm in length and ~10 mm in width was prepared and dried at 100 °C for almost 18 h. Then they were weighted (M0) (see Figure 2.4).

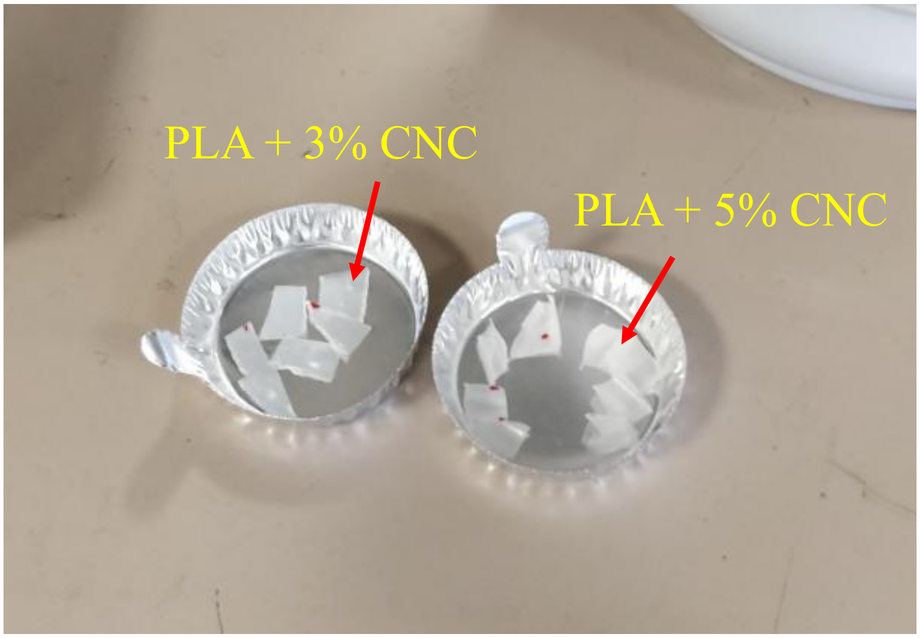

Figure 2.4 Samples of 10mm in length and ~10 mm in width of PLA/ 3% CNC and PLA/ 5% CNC nanocomposite films after dried at 100°C for 18 h**.**

2.2.8 Water Uptake Test

The moisture uptake test of films was evaluating through measuring the changes in the moisture uptake behavior of the films. The samples were placed inside a desiccator at room temperature and relative humidity of 95% (see figure 2.5). The samples are removed at different time intervals (t) and weighed (Mt) until reaching the equilibrium weight (M). The moisture uptake was calculated as follows in Eq. 1:

$$MU = 100\% *(Mt-Mo)/Mo \qquad (1)$$

Figure 2.5 Samples of pure PLA films and PLA/3% CNC films inside the desiccator at 95% RH and room temperature.

2.2.9 Sample Preparation of Water Vapor Permeability

The prepared films were cut into circle shapes (23 mm in diameter), and then they were glued on the top of a glass cup filled with 20 mL of distilled water. The headspace of the cup is 23 mm in diameter, and the samples are glued using hot silicon.

2.2.10 Water Vapor Permeability Test

Water vapor permeability (WVP) and water vapor transmission rate (WVTR) of the films were determined using a modification of the standard ASTM E 96. The whole ensemble was weighed, and then they were placed inside a desiccator at temperature of 22°C and relative humidity of 60%. The desiccator was attached to a pump to vacuum the air inside the desiccator (see figure 2.6). The samples were weighed at regular intervals during nine days and the WVTR is calculated according to the Eq. 2:

$$WVTR = (\Delta m/\Delta t)/A \quad (2)$$

Δm is the mass change (in grams), t is the time change (in days), and A is the area of the sample (in m^2). The WVP is calculated by the following equation:

$$WVP = WVTR*(L/\Delta P) \quad (3)$$

L is the sample thickness (in meters), and P is the difference of pressure across the two sides of the film (in kPa).

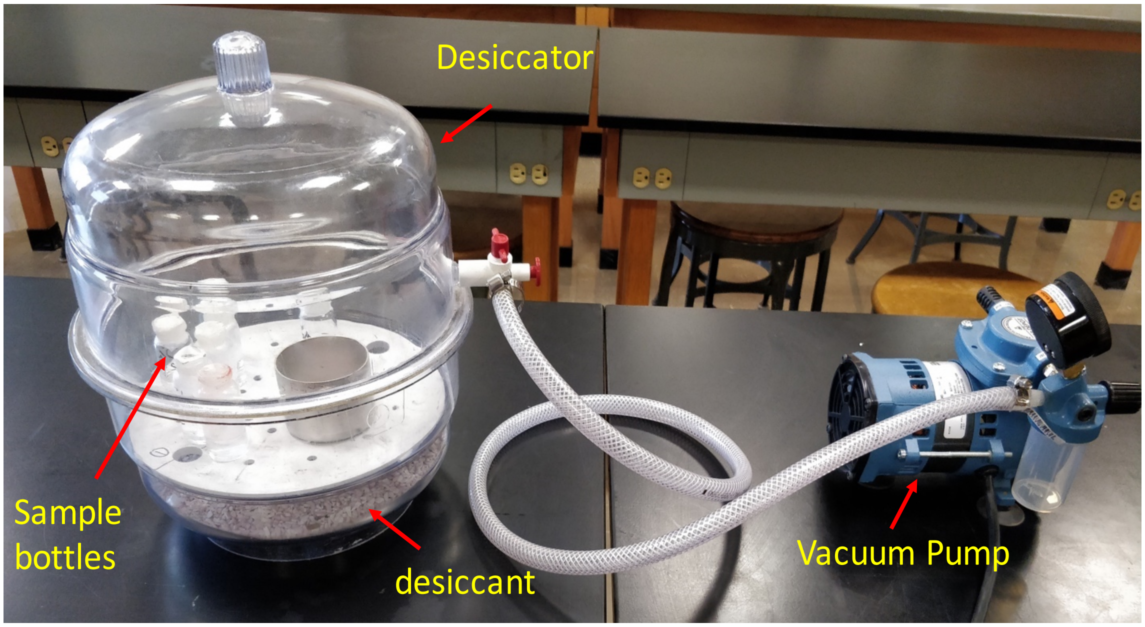

Figure 2.6 Water vapor permeability test system

2.2.11 Statistical Analysis

The impact of the different content of CNC (1,3,5 wt%) on mechanical and barrier properties of the PLA/CNC composite films were analyzed by Microsoft excel with the analysis of variance (ANOVA) and the significant differences in mean by RStudio (Version 0.99.491) with the Tukey's test at the interval of confidence of 95%.

2.3 Results and Discussion

2.3.1 Films Transparency

From a naked eye examination of the prepared films, the transparency of the pure PLA film and the nanocomposite films were not the same as shown in Figure 2.7. At low concentration of CNC (1% and 3%), the film containing 1%CNC was more transparent than the film containing 3% CNC. However, the two films' transparency were close to the pure PLA film. This indicated an even dispersion of CNC in the polymer matrix. However, at high concentration of CNC, the film had a granular structure, which might have been a result of the poor dispersion of CNC in the PLA matrix

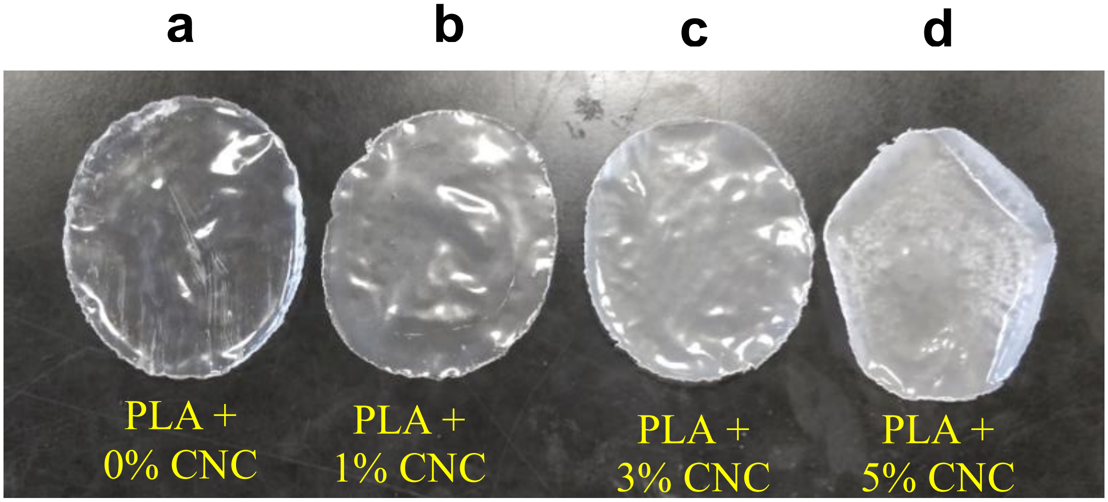

Figure 2.7 Transparency property of the prepared films (a) Pure PLA film, (b) PLA/1% CNC film (c) PLA/3%CNC film, (d) PLA/5% CNC film

2.3.2 Morphology of Polylactic Acid (PLA)/Cellulose Nanocrystals (CNC) Films

Since we want to understand changes in the films' mechanical and barrier performance, we examined the morphology of films via transmission electron microscopy (SEM). The results of SEM of the pure PLA films and the nanocomposites films with 1%, 3%, and 5% of CNC are presented in Figure 2.8. SEM images showed that the surface of nanocomposite films changed when the concentration of CNC increased. For the pure PLA film, the surface was rough and rigid, while the surface of films with CNC content up to 3% was relatively smoother than the pure PLA. For the films containing 1% and 3% of CNC, SEM images did not show any agglomerations of CNC in the surface of the polymer matrix, which indicated that filler substances were homogeneously dispersed. This might have occurred because of the processing route of films' preparation (Lee et al., 2013). However, for the nanocomposite films with high CNC content (5%), the presence of agglomerations of CNC in the surface of the PLA matrix was observed. These agglomerations in the surface of PLA matrix are due to the cellulose hydrophilic nature, that induces strong interactions between the cellulose nanoparticles by hydrogen bonds, leading to agglomerations in the polymer surface (Tserki et al., 2006).

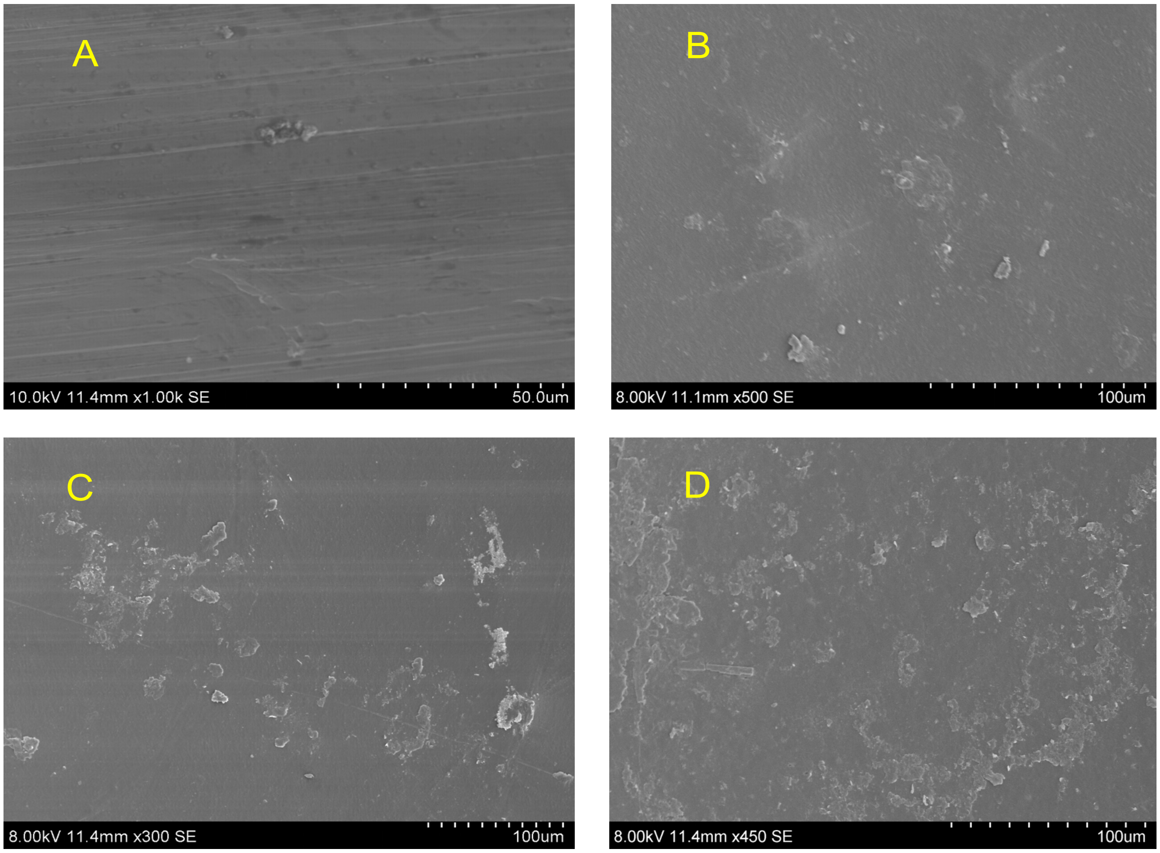

Figure 2.8 (a) SEM image of the surface of pure PLA film, (b) SEM image of the surface of PLA/CNC 1% film (c) SEM image of the surface of PLA/CNC 3% film, (d) SEM image of the surface of PLA/CNC 5% film

2.3.3 Tensile Strength Test

The obtained results of tensile strength test conducted on the pure PAL film and the nanocomposite films with different content of CNC (1%,3%,5%) are presented in table 2.5. Also the comparison of the ultimate strength point of the films are presented in Figure 2.6. For the pure PLA film, the yielded TS value (ultimate strength point) was 1.96 (g-force/cm^2). In the case of the film containing 3% of CNC, the TS increased significantly by 49% in comparison with the pure PLA film value.

However, in the case of the films which contains 1% and 5% of CNC, no significant increase in the TS was observed. This suggested that the film containing 3% of CNC was more ductile than the other films. Generally, the improvement of the mechanical performance of nanocomposite films results from different factors: compatibility between the polymer matrix and the filler, aspect ratios of the filler, and the orientations of the filler. However, in this work, we can hypothesize that the improvement of the tensile strength of the nanocomposite films caused by the stiffness effect of CNC that homogeneously dispersed in the PLA matrix (Favier et al., 1995). On the other hand, the decrease of the TS value of the film containing 5% CNC may have occurred because of the aggregation of CNC in PLA matrix. Authors Haafiz et al. (2013) and Pereira et al. (2014) reported similar results of the tensile strength of their cellulose nanocomposites. Haafiz et al. (2013) observed how that the poor compatibility between polymer matrix and CNC resulted in decrease in the tensile strength.

Table 2.5 Tensile strength test output

Film	Yield point (g-force/cm^2)	Ultimate strength point (g-force/cm^2)	Fracture (g-force/cm^2)
PLA	1529.33 ± 45	1961.36 ± 52	1763.53 ± 36
PLA/ 1% CNC	1624.26 ± 93	2118.2 ±	1914.03 ± 22
PLA/3% CNC	2318.06 ± 123	2936.266 ± 115	2690.23 ± 42
PLA/5% CNC	1451.9 ± 99	1761.033 ± 243	1547.46 ± 130

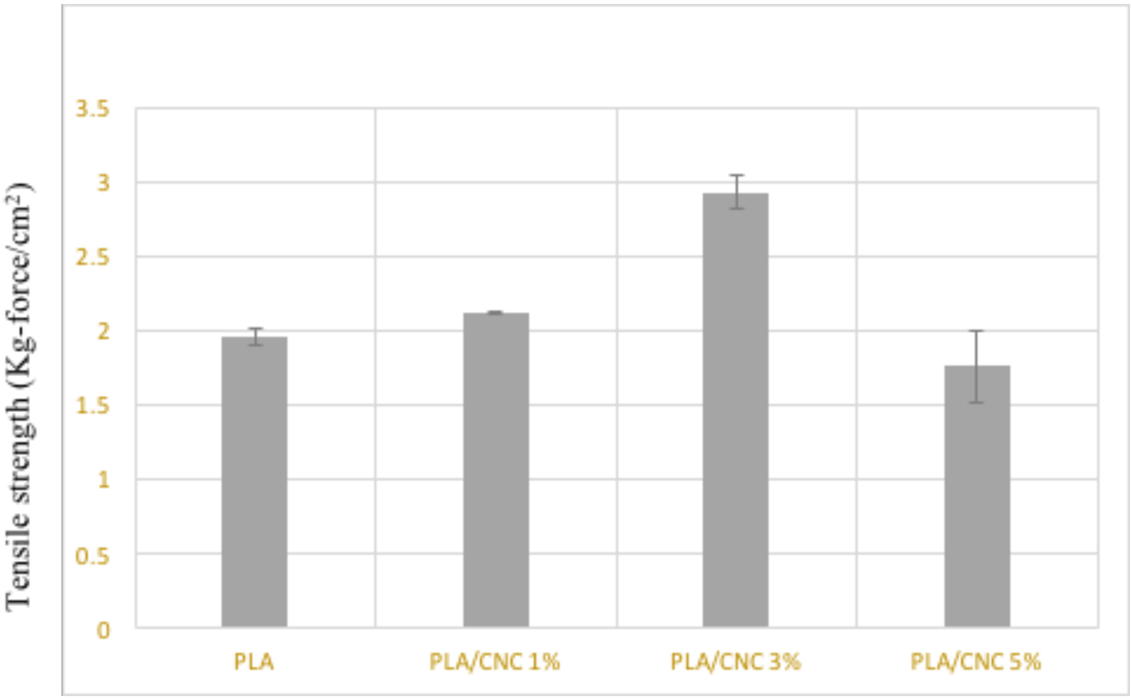

Figure 2.9. Tensile strength values of pure PLA and the nanocomposites films

2.3.4 Water Uptake Test

PLA is a recyclable biopolymer with water solubility resistance and hydrophilicity nature (Sung et al., 2017), ultimately making it a good candidate to be used in food packaging applications. The incorporation of reinforcing nanoparticles into PAL matrix might affect its moisture absorption behavior, and thus the moisture uptake test was tested in order to investigate CNC effect on the hydrophilicity nature of PLA, and also to determine the maximum absorbed amount of water by the pure PLA and the nanocomposites films.

The curves of water uptake percentage of the pure PLA and the nanocomposite films are presented in Figure 2.10. In the Figure, there are two zones; The first zone is where the water uptake rapidly increased, linear trend, and the second zone where the water uptake gradually decreased until it settled. For the pure PLA film, the water uptake percentage reached over 2.5%, and this value matches the values that reported by Liu et al. (2018). An increased of moisture uptake percentage was observed when CNC content increased from 1% to 5% in comparison with the pure PLA film value. The samples containing 1%CNC, 3%CNC, and 5%CNC yielded moisture uptake percentage of 5%, 7%, and 10% respectively. This increase is attributed to the CNC hydrophilic nature, incorporated into the PLA matrix (de Rodriguez et al., 2006).

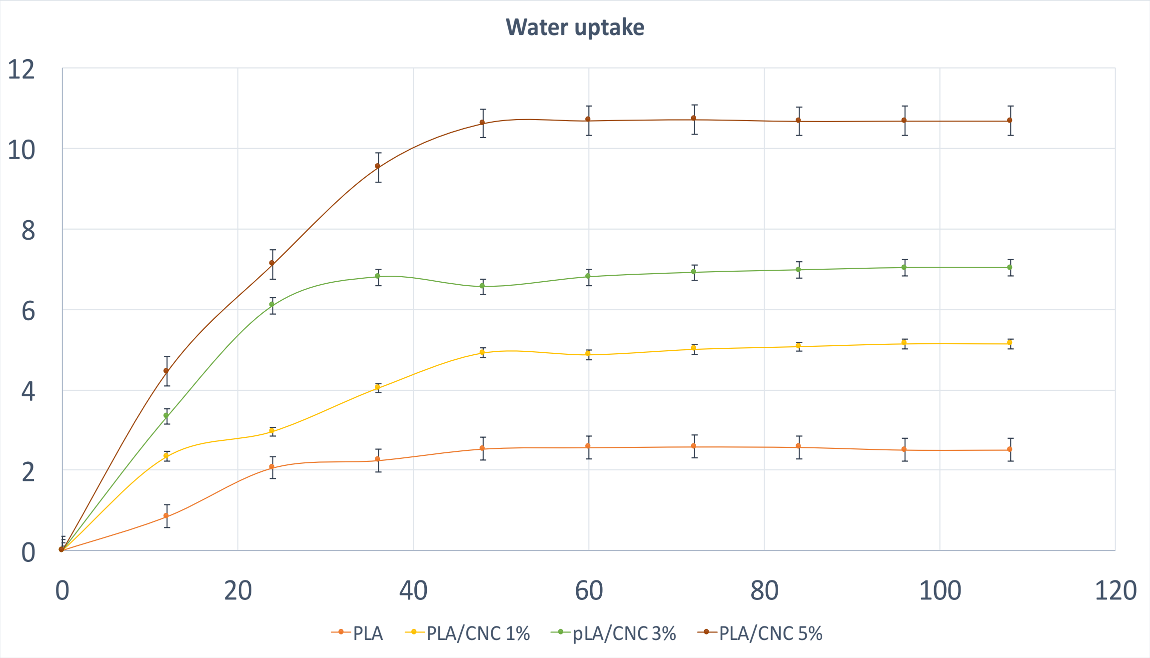

Figure 2.10 Water uptake percentages of pure PLA films and nanocomposite films for 5 days at 95% RH

2.3.5 Water Vapor Permeability Test

Water vapor permeability of the pure PLA film and the nanocomposite films contain different contents of CNC (1%, 3%, 5%) investigated with the assumptions that the thickness of sample films is the same, and the difference of water vapor pressure on both sides of the film is very small. Figure 2.15 shows the obtained values of WVP of the pure PLA film and the nanocomposite films with various content of CNC (1%,3%,5%). For the film containing 1%CNC, WVP significantly decreased by 20% compared to the control sample. In the case of the films containing 3% and 5% of CNC, WVP significantly decreased by 20% and 42% respectively. In the figure, we can see that there is a sharp decrease in WVP of the film containing 1% in day three, which might have been resulted from a systematic error or an error of the measurement at that day.

Sung et al. (2017) and Fortunati et al. (2012). reported Similar results of their cellulose nanocomposites. This reduction of WVP can be explained that the incorporation of CNC into the matrix of PLA films is thought to cause the increase of the tortuous path, leading water molecules to move in a low rate (Khan et al., 2013). On the other hand, for the films containing 5% of CNC, there was no significant decrease of the WVP observed in compression with the film containing 3% of CNC. This behavior of nanocomposite films contains high cellulose nanoparticles reported in a previous study by (Sanchez-Garcia et al., 2008). They reported that the WVP of the PLA/cellulose microfiber composite reduced with the samples containing 1% cellulose microfiber while no improvement in WVP observed for the sample containing 4%, and 5% of cellulose microfiber. This behavior indicated that the agglomerations of CNC have influence on their uniform dispersion. This behavior led to low tortuosity path and less efficient water barrier properties (Sanchez-Garcia et al., 2008). This conclusion is in accordance with the morphology images of the nanocomposite film with 5% CNC and other films in this study.

The yielded values of the WVP of the nanocomposite films and the pure PLA film in this study are bigger than the reported values in the mentioned studies above. This disagreement in values could be related to the difference in processing conditions. In our study, we measured the weight loss of distilled water with the assumptions that the thickness of all the films are the same, and the pressure difference across the film is negligible, while in their study they measured the weight gain of cells contain anhydrous calcium chloride at 25 C and RH of 50%. Generally, the WVP and (WVRT) mainly rely on the chemical nature (hydrophilic or hydrophobic) of the filler, process conditions, final arrangement of the nanocomposite with the added filler substances, and also the presence of voids and cracks on the films (Vásconez et al., 2009).

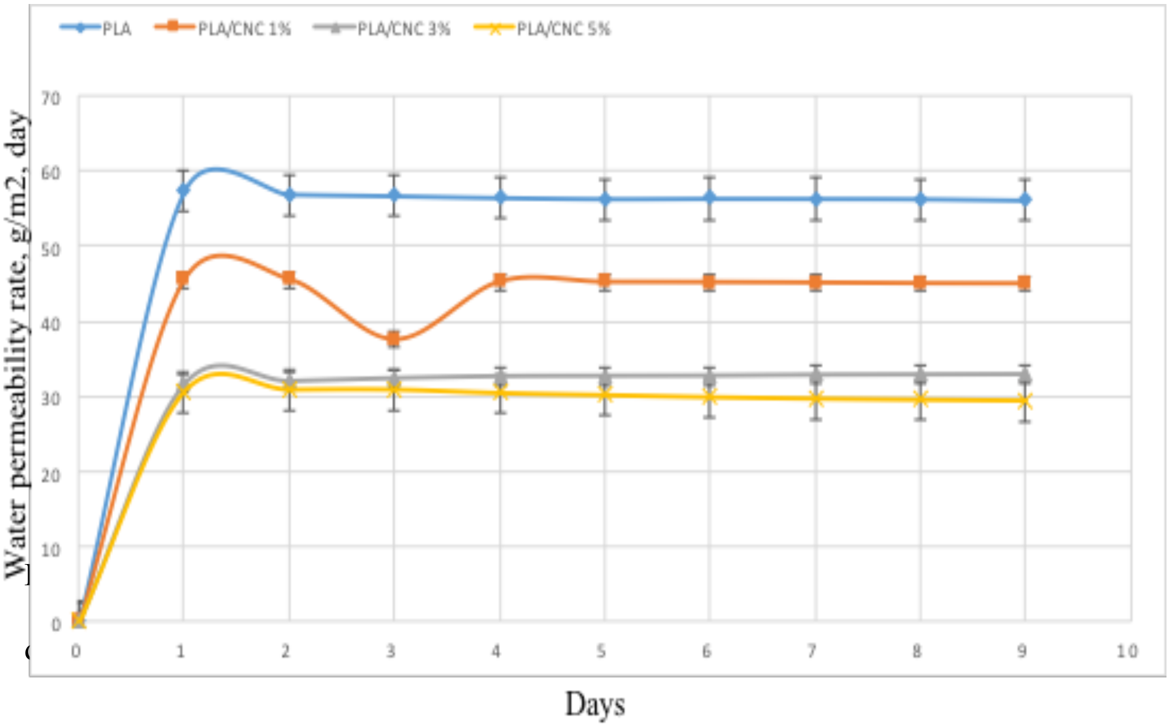

Figure 2.11 Water vapor permeability of pure PLA and the nanocomposite films

CHAPTER3: CONCLUSIONS AND FUTURE WORK

3.1 Conclusion

PLA/ CNC nanocomposite films were successfully prepared through the solvent casting method with various internal mixers at CNC content 1,3,5 wt%. The impact of CNC on barrier, optical, and mechanical properties were investigated in this work as well as the morphology of the prepared films. To evaluate the effect of CNC statistically on the tensile strength, water vapor permeability, and water uptake of the nanocomposite films, ANOVA procedure was used. To determine significant differences in mean, Tukey's test was used.

The morphology images of the nanocomposites films showed that CNC were evenly dispersed in the films containing 1% and 3%, while aggregations of CNC were observed in the PLA matrix for the film containing 5%. The tensile strength improved significantly only with the film contain 3% of CNC by 49%. A Significant decrease in the tensile strength of the film containing 5% of CNC compared to the PLA film, indicated that the aggregations of CNC in the PLA matrix disturbed the reinforcing effect of CNC on the films' mechanical properties. The result of the water permeability test conducted on the prepared films revealed that WVP of the films containing 3% and 5% of CNC improved significantly by 20% and 42% respectively. However, the presence of the CNC in PLA matrix at different contents 1,3,5 wt% had significant influence on films' water sensitivity. The water uptake test conducted on the prepared films showed that with the addition of CNC, the water uptake percentages increased. Generally, biopolymers show relatively inferior mechanical and barrier properties, which limits their use in food packaging applications. The obtained results in this study suggest that the PLA/CNC composite films have potential to be utilized in food packaging applications.

3.2 Future Work

In this study, the tensile strength, water uptake, and water vapor permeability properties of the PLA/CNC nanocomposite films significantly improved with the addition of CNC up to 3%, which indicated that the aggregations of the cellulose nanoparticles in PLA matrix disturbed the effect of CNC on the mechanical and the barrier properties. For future work, surface modification of cellulose nanocrystals (CNC) may improve their dispersion and consequently improving barrier and mechanical properties of the nanocomposites films.

In addition, the biodegradability of the nanocomposites films should be investigated at different temperatures to ensure packaged foods quality and safety when they are utilized in food packaging applications.

REFERENCES

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, *3*(2), 113-126.

Azeredo, H. M., Mattoso, L. H. C., Wood, D., Williams, T. G., Avena-Bustillos, R. J., & McHugh, T. H. (2009). Nanocomposite edible films from mango puree reinforced with cellulose nanofibers. *Journal of food science*, *74*(5), N31-N35.

Azeredo, H. M., Miranda, K. W., Rosa, M. F., Nascimento, D. M., & de Moura, M. R. (2012). Edible films from alginate-acerola puree reinforced with cellulose whiskers. *LWT-Food Science and Technology*, *46*(1), 294-297.

Azeredo, H. M., Rosa, M. F., & Mattoso, L. H. C. (2017). Nanocellulose in bio-based food packaging applications. *Industrial Crops and Products*, *97*, 664-671.

Azizi Samir, M. A. S., Alloin, F., & Dufresne, A. (2005). Review of recent research into cellulosic whiskers, their properties and their application in nanocomposite field. *Biomacromolecules*, *6*(2), 612-626.

Bondeson, D., & Oksman, K. (2007). Dispersion and characteristics of surfactant modified cellulose whiskers nanocomposites. *Composite Interfaces*, *14*(7-9), 617-630.

De Mesquita, J. P., Donnici, C. L., Teixeira, I. F., & Pereira, F. V. (2012). Bio-based nanocomposites obtained through covalent linkage between chitosan and cellulose nanocrystals. *Carbohydrate polymers*, *90*(1), 210-217.

De Rodriguez, N. L. G., Thielemans, W., & Dufresne, A. (2006). Sisal cellulose whiskers reinforced polyvinyl acetate nanocomposites. *Cellulose*, *13*(3), 261-270.

De Souza Lima, M. M., & Borsali, R. (2002). Static and dynamic light scattering from polyelectrolyte microcrystal cellulose. *Langmuir*, *18*(4), 992-996.

Dhar, P., Bhardwaj, U., Kumar, A., & Katiyar, V. (2015). Poly (3-hydroxybutyrate)/cellulose nanocrystal films for food packaging applications: Barrier and migration studies. *Polymer Engineering & Science*, *55*(10), 2388-2395.

Dong, X. M., Revol, J. F., & Gray, D. G. (1998). Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose. *Cellulose*, *5*(1), 19-32.

Dufresne, A. (2010). Processing of polymer nanocomposites reinforced with polysaccharide nanocrystals. *Molecules*, *15*(6), 4111-4128.

Dufresne, A. (2013). Nanocellulose: a new ageless bionanomaterial. *Materials Today*, *16*(6), 220-227.

Dufresne, A., Dupeyre, D., & Vignon, M. R. (2000). Cellulose microfibrils from potato tuber cells: processing and characterization of starch–cellulose microfibril composites. *Journal of Applied Polymer Science*, *76*(14), 2080-2092.

Ebeling, T., Paillet, M., Borsali, R., Diat, O., Dufresne, A., Cavaille, J. Y., & Chanzy, H. (1999). Shear-induced orientation phenomena in suspensions of cellulose microcrystals, revealed by small angle X-ray scattering. *Langmuir*, *15*(19), 6123-6126.

Favier, V., Canova, G. R., Cavaillé, J. Y., Chanzy, H., Dufresne, A., & Gauthier, C. (1995). Nanocomposite materials from latex and cellulose whiskers. *Polymers for Advanced Technologies*, *6*(5), 351-355.

Fortunati, E., Peltzer, M., Armentano, I., Torre, L., Jiménez, A., & Kenny, J. M. (2012). Effects of modified cellulose nanocrystals on the barrier and migration properties of PLA nano-biocomposites. *Carbohydrate polymers*, *90*(2), 948-956.

Gandhi, M., & Chikindas, M. L. (2007). Listeria: a foodborne pathogen that knows how to survive. *International journal of food microbiology*, *113*(1), 1-15.

Goussé, C., Chanzy, H., Excoffier, G., Soubeyrand, L., & Fleury, E. (2002). Stable suspensions of partially silylated cellulose whiskers dispersed in organic solvents. *Polymer*, *43*(9), 2645-2651.

Haafiz, M. M., Hassan, A., Zakaria, Z., Inuwa, I. M., Islam, M. S., & Jawaid, M. (2013). Properties of polylactic acid composites reinforced with oil palm biomass microcrystalline cellulose. *Carbohydrate polymers*, *98*(1), 139-145.

Habibi, Y., Lucia, L. A., & Rojas, O. J. (2010). Cellulose nanocrystals: chemistry, self-assembly, and applications. *Chemical reviews*, *110*(6), 3479-3500.

Han, J. H. (2003). Antimicrobial food packaging. *Novel food packaging techniques*, *8*, 50-70.

Helbert, W., Cavaille, J. Y., & Dufresne, A. (1996). Thermoplastic nanocomposites filled with wheat straw cellulose whiskers. Part I: processing and mechanical behavior. *Polymer composites*, *17*(4), 604-611.

Heux, L., Chauve, G., & Bonini, C. (2000). Nonflocculating and chiral-nematic self-ordering of cellulose microcrystals suspensions in nonpolar solvents. *Langmuir*, *16*(21), 8210-8212.

Hogan, S. A., & Kerry, J. P. (2008). *Smart packaging of meat and poultry products* (pp. 33-54). John Wiley & Sons: West Sussex, UK.

Hossain, K. M. Z., Ahmed, I., Parsons, A. J., Scotchford, C. A., Walker, G. S., Thielemans, W., & Rudd, C. D. (2012). Physico-chemical and mechanical properties of nanocomposites prepared using cellulose nanowhiskers and poly (lactic acid). Journal of Materials Science, 47(6), 2675-2686.

Hubbe, M. A., Rojas, O. J., Lucia, L. A., & Sain, M. (2008). Cellulosic nanocomposites: a review. *BioResources*, *3*(3), 929-980.

Huq, T., Salmieri, S., Khan, A., Khan, R. A., Le Tien, C., Riedl, B., ... & Lacroix, M. (2012). Nanocrystalline cellulose (NCC) reinforced alginate based biodegradable nanocomposite film. *Carbohydrate polymers*, *90*(4), 1757-1763.

Imran, M., Revol-Junelles, A. M., Martyn, A., Tehrany, E. A., Jacquot, M., Linder, M., & Desobry, S. (2010). Active food packaging evolution: transformation from micro- to nanotechnology. *Critical reviews in food science and nutrition*, *50*(9), 799-821.

Khan, A., Khan, R. A., Salmieri, S., Le Tien, C., Riedl, B., Bouchard, J., ... & Lacroix, M. (2012). Mechanical and barrier properties of nanocrystalline cellulose reinforced chitosan based nanocomposite films. *Carbohydrate polymers*, *90*(4), 1601-1608.

Khan, R. A., Beck, S., Dussault, D., Salmieri, S., Bouchard, J., & Lacroix, M. (2013). Mechanical and barrier properties of nanocrystalline cellulose reinforced poly (caprolactone) composites: Effect of gamma radiation. *Journal of Applied Polymer Science*, *129*(5), 3038-3046.

Labuza, T. P., & Breene, W. M. (1989). Applications of “active packaging” for improvement of shelf-life and nutritional quality of fresh and extended shelf-life foods 1. *Journal of food processing and preservation*, *13*(1), 1-69.

Lee, J. H., Park, S. H., & Kim, S. H. (2013). Preparation of cellulose nanowhiskers and their reinforcing effect in polylactide. *Macromolecular Research*, *21*(11), 1218-1225.

Li, F., Mascheroni, E., & Piergiovanni, L. (2015). The potential of nanocellulose in the packaging field: a review. *Packaging Technology and Science*, *28*(6), 475-508.

Li, F., Mascheroni, E., & Piergiovanni, L. (2015). The potential of nanocellulose in the packaging field: a review. *Packaging Technology and Science*, *28*(6), 475-508.

Liu, W., Dong, Y., Liu, D., Bai, Y., & Lu, X. (2018). Polylactic Acid (PLA)/Cellulose Nanowhiskers (CNWs) Composite Nanofibers: Microstructural and Properties Analysis. *Journal of Composites Science*, *2*(1), 4.

Marsh, K., & Bugusu, B. (2007). Food packaging—roles, materials, and environmental issues. *Journal of food science*, *72*(3), R39-R55.

Mauriello, G. D. L. E., De Luca, E., La Storia, A., Villani, F., & Ercolini, D. (2005). Antimicrobial activity of a nisin-activated plastic film for food packaging. *Letters in applied microbiology*, *41*(6), 464-469.

Mazeau, K., & Heux, L. (2003). Molecular dynamics simulations of bulk native crystalline and amorphous structures of cellulose. *The Journal of Physical Chemistry B*, *107*(10), 2394-2403.

Millette, M. C. L. T., Le Tien, C., Smoragiewicz, W., & Lacroix, M. (2007). Inhibition of Staphylococcus aureus on beef by nisin-containing modified alginate films and beads. *Food Control*, *18*(7), 878-884.

Ozdemir, M., & Floros, J. D. (2004). Active food packaging technologies. *Critical reviews in food science and nutrition*, *44*(3), 185-193.

Pereira, A. L. S., do Nascimento, D. M., Morais, J. P. S., Vasconcelos, N. F., Feitosa, J. P., Brígida, A. I. S., & Rosa, M. D. F. (2014). Improvement of polyvinyl alcohol properties by adding nanocrystalline cellulose isolated from banana pseudostems. *Carbohydrate polymers*, *112*, 165-172.

Rhim, J. W., & Ng, P. K. (2007). Natural biopolymer-based nanocomposite films for packaging applications. *Critical reviews in food science and nutrition*, *47*(4), 411-433.

Rhim, J. W., Park, H. M., & Ha, C. S. (2013). Bio-nanocomposites for food packaging applications. *Progress in Polymer Science*, *38*(10-11), 1629-1652.

Risch, S. J. (2009). Food packaging history and innovations. *Journal of agricultural and food chemistry*, *57*(18), 8089-8092.

Sanchez-Garcia, M. D., Gimenez, E., & Lagaron, J. M. (2008). Morphology and barrier properties of solvent cast composites of thermoplastic biopolymers and purified cellulose fibers. *Carbohydrate Polymers*, *71*(2), 235-244.

Scannell, A. G., Hill, C., Ross, R. P., Marx, S., Hartmeier, W., & Arendt, E. K. (2000). Development of bioactive food packaging materials using immobilised bacteriocins Lacticin 3147 and Nisaplin®. *International journal of food microbiology*, *60*(2-3), 241-249.

Sung, S. H., Chang, Y., & Han, J. (2017). Development of polylactic acid nanocomposite films reinforced with cellulose nanocrystals derived from coffee silverskin. *Carbohydrate polymers*, *169*, 495-503.

Tang, X. Z., Kumar, P., Alavi, S., & Sandeep, K. P. (2012). Recent advances in biopolymers and biopolymer-based nanocomposites for food packaging materials. *Critical reviews in food science and nutrition*, *52*(5), 426-442

Terech, P., Chazeau, L., & Cavaille, J. Y. (1999). A small-angle scattering study of cellulose whiskers in aqueous suspensions. *Macromolecules*, *32*(6), 1872-1875.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technology*, *14*(3), 71-78.

Tserki, V., Matzinos, P., Zafeiropoulos, N. E., & Panayiotou, C. (2006). Development of biodegradable composites with treated and compatibilized lignocellulosic fibers. *Journal of applied polymer science*, *100*(6), 4703-4710.

Vásconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. *Food Research International*, *42*(7), 762-769.

Vermeiren, L., Devlieghere, F., Van Beest, M., De Kruijf, N., & Debevere, J. (1999). Developments in the active packaging of foods. *Trends in food science & technology*, *10*(3), 77-86.

Weber, C. J., Haugaard, V., Festersen, R., & Bertelsen, G. (2002). Production and applications of biobased packaging materials for the food industry. *Food Additives & Contaminants*, *19*(S1), 172-177.

APPENDIX A

ANOVA test results of the tensile strength test of pure PLA film and the nanocomposite films

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PLA	3	5884.1	1961.366667	2801.493333
PLA/CNC 1%	3	6354.6	2118.2	50.11
PLA/CNC 3%	3	8808.8	2936.266667	13306.90333
PLA/CNC 5%	3	5283.1	1761.033333	59290.41333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2394850.977	3	798283.6589	42.32180707	2.96494E-05	4.066180551
Within Groups	150897.84	8	18862.23			
Total	2545748.817	11				

APPENDIX A

Tukey's test results for the tensile strength test of pure PLA film and the nanocomposite films

```
$statistics

    Mean       CV  MSerror     HSD

  2194.217 6.259174 18862.23 359.1038

$parameters

  Df ntr StudentizedRange alpha  test    name.t

   8   4          4.52881  0.05 Tukey test$Film

$means

                    test$TS          std            r    Min      Max

PLA                1961.367     52.929135      3 1920.3    2021.1

PLA/CNC 1%    2118.200   7.078842        3   2113.2    2126.3

PLA/CNC 3%     2936.267   115.355552   3   2842.0    3064.9

PLA/CNC 5%     1761.033   243.496229  3   1553.1    2028.9

$comparison

NULL

$groups

        trt              means     M

1 PLA/CNC 3%   2936.26     a

2 PLA/CNC 1% 2118.20       b

3 PLA                 1961.36       b

4 PLA/CNC 5% 1761.03       b
```

APPENDIX B

ANOVA test result for the water uptake of pure PLA film and the nanocomposite films

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PLA	3	7.5813	2.5271	0.08356453
PLA/CNC 1%	3	15.444	5.148	0.020287
PLA/CNC 3%	3	21.109	7.036333333	0.039880333
PLA/CNC 5%	3	31.906	10.63533333	0.130377333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	104.6813856	3	34.89379519	509.1955412	1.80907E-09	4.066180551
Within Groups	0.548218393	8	0.068527299			
Total	105.2296039	11				

APPENDIX B

Tukey’s test result for the water uptake of pure PLA film and the nanocomposite films

```
$statistics
    Mean       CV   MSerror      HSD
  6.336692 4.131134 0.0685273 0.6844713
$parameters
  Df ntr StudentizedRange alpha  test     name.t
   8   4          4.52881  0.05 Tukey test3$Film
$means
  test3$Water       std    r    Min    Max
1    2.527100 0.2890753  3   2.257  2.832
2    5.148000 0.1424324  3   5.021  5.302
3    7.036333 0.1997006  3   6.842  7.241
4   10.635333 0.3610780  3  10.254 10.972
$comparison
NULL
$groups
  trt                  means        M
1   PLA/CNC 5%   10.6          a
2   PLA/CNC 3%   7.0           b
3   PLA/CNC 1%    5.1          c
4  PLA                  2.5          d
```

APPENDIX C

ANOVA test results for water vapor permeability test of pure PLA film and the nanocomposite films

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PLA	2	112.181	56.0905	7.51789088
PLA/CNC 1%	2	89.9973	44.99865	0.989683805
PLA/CNC 3%	2	65.9037	32.95185	1.395285125
PLA/CNC 5%	2	58.8901	29.44505	6.994174005

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	883.8718877	3	294.6239626	69.74572361	0.000655634	6.591382116
Within Groups	16.89703382	4	4.224258454			
Total	900.7689215	7				

APPENDIX C

Tukey’s test result for the water vapor permeability of pure PLA films and the nanocomposites films

```
$statistics

    Mean      CV  MSerror     HSD

  40.86779 5.067167 4.288374 8.430085

$parameters

  Df ntr StudentizedRange alpha  test    name.t

   4   4         5.757058  0.05 Tukey test$Film



$means

                     test$WVP      std     r     Min       Max

   PLA              56.09050 2.741877  2   54.1517  58.0293

PLA/CNC 1%  44.98375 1.116309  2   44.1944 45.7731

PLA/CNC 3%  32.95185 1.181222  2   32.1166 33.7871

PLA/CNC 5%  29.44505 2.644650  2   27.5750  31.3151

$comparison

NULL

$groups

        trt            means     M

1 PLA                   56.0        a

2 PLA/CNC 1%     44.9        b

3 PLA/CNC 3%     32.9        c

4    PLA/CNC 5%  29.4        c
```