EFFECT OF NANO- FILLERS ON TENSILE PROPERTIES OF BIOPOLYMER FILMS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Agricultural and Biological Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2016

Urbana, Illinois

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ABSTRACT

There is a growing interest in biopolymers due to their biodegradability, biocompatibility, and edibility. Biopolymer applications include food packaging, biomedical applications, and as structural materials. However, their mechanical properties are limiting. Current research is focused on modifying the physical and chemical properties of biopolymers for better performance and broader applications. The addition of nanofillers has been proposed as a way to modify mechanical properties of biopolymers. This work is focused on biopolymers as edible materials, therefore biopolymers and fillers must meet this requirement. In this study, microcrystalline cellulose (MCC), nanocrystalline cellulose (CNC), $CaCO_3$ nanoparticles, hydrophilic nanoclay and hydrophobic nanoclay were separately added as fillers to the polymers, konjac glucomannan (KGM), hydroxypropyl methylcellulose (HPMC), and zein to investigate their effect on tensile properties of composite films. Tensile properties were measured by dynamic mechanical analysis (DMA) obtaining stress (σ), strain (γ), and Young's modulus (E). Results revealed significant differences between neat polymers. Fillers, at 2% w/w, affected film properties depending on their size, shape, and surface chemistry. Good dispersibility and the extent of polymer-filler interactions controlled the effect of nanofillers on film properties. Nano-size, high aspect ratio, and hydrophilicity showed significant effects on tensile properties over micro-size, low aspect ratio, and hydrophobic fillers. Hydrophilic nanoclay and CNC interacted better with biopolymer matrices than hydrophobic nanoclay, MCC, or $CaCO_3$. The load limit of CNC in KGM and HPMC to increase σ of neat polymers was 2% and 4%, respectively. Further addition resulted in γ and σ decrement. Load differences were attributed to differences in microstructure between KGM and HPMC. Addition of CNC up to 50% w/w resulted in a higher value of E, suggesting a volume fraction effect of nanocomposite components on films properties. The conclusions of this study were that the addition of nanofillers to biopolymers can lead to reinforced composites if there is good interaction between filler and matrix. Nanocomposite technology offers to broaden the application of biopolymers through the design of new materials with competitive mechanical, thermal, and barrier properties.

To my parents Idoya Goizueta Iraburu and Jose Resano Lazaro …

… and my sisters Lucia, Maria, and Paula.

ACKNOWLEDGMENTS

Foremost, I would like to express my most sincere gratitude to Dr. Graciela W. Padua for her guidance, trust, inspiration, and encouragement throughout this study. I greatly appreciate the generous research opportunity and knowledge that she shared with me during these years. I feel very grateful for having such a great advisor!

I would like to thank the members of the committee: Dr. Kent Rausch, Dr. Vijay Singh, for their time and suggestions. Especially to Dr. Kent Rausch for his guidance at the beginning of my MS.

I would also like to thank the technical help received from Lou Ann Miller, Kathy Walsh, and Tim Spila from the Frederick Seitz Materials Research Laboratory, University of Illinois. Thanks are extended to the Mechanical Science and Engineering Innovation Studio, University of Illinois, for the use of laser cutters.

I would like to give special thanks to my lab mates Lia Guardiola, Suzan Uzun, and Hemiao Cui for their help and friendship. Thanks are also extended to Lorena Rios-Acosta, Alejandra Botero, Nuria Castaneda, Xueqian Shi, Yimming Feng, Wan-Yuan Kuo, Ozan Khraman, Jingwen Cai, Luis Vargas, and Sindy Palma for the good times, inspiration, and pieces of advice during these years. You all are bright and passionate. I wish you the best!

I am grateful to my mother, Idoya Goizueta, for her support, trust, and love. She is an example of strength and courage. Thanks to my sisters Lucia, Maria, and Paula for being an example of perseverance and friendship. Especial thanks to my boyfriend Antonio for his patience, understanding, friendship, and love.

I am grateful to my friends back home Ana, Beatriz, Estela, Libe, Lorena, Silvia, Saioa, and Teresa for their understanding, patience, motivation, and love during many many years.

Last but not least, I would like to thank Rafael Garcia Santos and Maria Jose Beriain Apesteguia for their encouragement and help to persuade further education.

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INTRODUCTION

The food industry is becoming interested in the development of edible plastic materials for the design on novel foods. Edible plastics, formed out of biopolymers, are intended as moisture barriers, moist foods wrappers, and protective coatings. However, biopolymers are known to show limited mechanical properties, especially at high relative humidity values. A number of strategies are being tested to improve the mechanical performance of edible biopolymers.

The addition of fillers (composite polymers) is one methodology to approach stiffer and stronger materials. The advantages of composite materials have been quickly noticed and closely studied. Composite fillers evolved to nano- size. Researchers discovered new material properties when the added filler had at least one nano-size dimension, termed nanocomposites. Nano-sizing greatly increases the fillers surface area and allows for better matrix-filler interaction. Large surface area results in better integration, continuity, and homogeneity of the nanocomposite, leading to enhanced mechanical and thermal properties.

Following previous studies and hypothesis on reinforcement, this study is focused on the enhancement of tensile properties of biopolymers by the addition of nano-fillers. Konjac glucomannan (KGM), cellulose, and zein are examples of natural polymers with film-forming capacity that needs to be reinforced to enhance their tensile properties. Hydroxypropyl methylcellulose (HPMC) is an example of chemically modified cellulose. This semisynthetic polymer is a more water soluble version of cellulose with better gel and film forming abilities. The effect of edible fillers is evaluated: nanocrystal cellulose (CNC), microcrystal cellulose (MCC), and $CaCO_3$. Cellulose is widely used as filler because of its availability, versatility, and compatibility with many biopolymers. Calcium carbonate is the hardening component in egg shells and bones. It is abundant and inexpensive. The effect of hydrophilic and hydrophobic nanoclay fillers was also investigated. Nanoclays, although not edible, were selected as benchmarks because they are commonly incorporated for non-edible applications. They are reported as successfully enhance mechanical and gas barrier properties of polymer films because of their size and surface chemistry. Four of the fillers are nano-size, and one is micro-size, to study the effect of the size in the composite. Nanoclay plates have high aspect ratio, and nano $CaCO_3$ cubes have low aspect ratio to compare the effect of shape/aspect ratio in the composites. Hydrophilic and hydrophobic nanoclay have same size and aspect ratio but different surface chemistry to compare the matrix-filler interactions.

The objective of this study was to investigate the effect of filler type on tensile properties of KGM, HPMC, and zein polymer when 2% w/w CNC, MCC, $CaCO_3$, hydrophilic and hydrophobic nanoclay fillers were individually added to the matrices. The effect of different weight fractions of filler in the tensile properties of KGM and HPMC polymers was also studied by adding 2%, 4%, 8%, 16%, 32%, 50%, and 75% of CNC.

LITERATURE REVIEW

2.1 Composites

Composite materials consist of a discontinuous phase dispersed in a continuous one. Such phases are significantly different from each other in physical or chemical properties. The aim of mixing those components is tailoring a new complex material with upgraded properties. When these composites come from biological sources are consider bio-composites. When the discontinuous phase is particles with at least one dimension smaller than 100 nm the material is considered a nanocomposite (Dufresne et al. 2013). If both characteristics are occurring at the same time, the material is a bio-nanocomposite.

Nanocomposites have been investigated for more than 50 years and first referenced in 1950 (Okpala, 2013). Nanocomposites became more widely studied when Toyota's Central Research and Development laboratories began studies in polymer/layered silicate composites. The true start of nanocomposites began in 1990 when "Toyota first used clay/nylon-6 nanocomposites for Toyota car to produce timing belt covers" (Okpala, 2013). An example of other traditional nanocomposite is the addition of montmorillonite to polyethylene to improve tensile strength and modulus and decrease gas permeability.

Since then, nanocomposites have been widely used in commercial applications. For instance, nanocomposites can be found in medicine. Muzzarelli et al. (2007), prepared chitin nanofibril–chitosan glycolate composites for wound healing (Wilson and Omokanwaye, 2013). Nanocomposites are also present in the construction industry. Yao et al. (2012) showed how nanoclay improved physical and mechanical properties of asphalt mixtures. Viscosity and complex shear modulus of asphalt binder were remarkably increased by the addition of fillers.

Nanocomposites are also constituents of food and packaging materials. Polymer nanocomposites may act as antimicrobial agents, biosensors, reinforcement, and oxygen scavengers (Othman, 2014). The incorporation of nanoparticles into polymers can control microorganisms' levels. For instance, Tankhiwale and Bajpai (2009) incorporated silver nanoparticles into cellulose filter paper as a strategy to develop antibacterial food-packaging material. They reported how the toxicity of silver prevents *E. Coli* growth. Nanocomposites can also be present in packaging as active components to interact with the food or the environment and provide information. Hong and Park (1999) develop a color indicating package which informs the consumer about the quality of kimchi (a fermentable vegetable). They included into the inside face of the package lid a polypropylene resin film with $Ca(OH)_2$ (a CO_2 absorbent) with color indicator particles (bromocresol purple and methyl red). When kimchi ripens the pH falls, and the color indicator changes its initial color. Consequently, the consumer can visually detect the state of the product without opening the package. The addition of fillers can modify the properties of the matrix in several directions. Table 2.1 shows a list of functionalities that a composite can develop with the addition of specific filler.

Table 2.1. Fillers and their functions. Adapted from Thabet et al. (2011)

Function	**Fillers**
Enhancement of mechanical properties	High aspect ratio: Glass fibers Nanoclays
Reduced permeability	Plate-like: Nanoplates Glass flakes
Degradability	Organic fillers: Starch Cellulose
Biomaterials	Bone regeneration: Hydroxyapatite Tricalcium phosphate

2.2 Continuous phase: biopolymers

There is a growing interest in biopolymers due to their bio-related properties: biodegradable, biocompatible, and edible. They offer means to produce sustainable packing materials. Polylactic acid (PLA) and polycaprolactone (PCL) have been used in mulch films for agriculture (Hayes, 2012). Besides eco-friendly opportunities, biopolymers are studied for their biocompatibility to produce biomaterials. Materials that do not cause adverse effects when in contact with living organisms are considered biocompatible. They are implemented because they have functional properties to interface with biological systems (Tanaka, 2015). Therefore, they are used in tissue engineering for repairing or replacing hard or soft tissues in the human body (Kong and Xu, 2015). One example of the importance of biocompatibility and biodegradability of biopolymers is the design of a vascular prosthesis. The prosthesis has to degrade inside the body at a specific rate, while allowing cells to grow inside without harming them. Fast material degradation rate would result in a failure of shaping and function of the prosthesis. Slow material degradation rate would also result in failure because of lack of room for cells to grow inside the device. Kong and Xu (2015) studied the biodegradation rate and compatibility of the chitosan-heparin small-diameter artificial vascular prosthesis with subcutaneous tissues. They reported that chitosan-heparin degradation rate showed good biocompatibility with a small-diameter artificial vascular prosthesis. Another property of biopolymers is that they are edible. They make safe food contact surfaces although not necessarily meant to be eaten. Biopolymers are used as edible films or coatings on foodstuffs to improve quality and increase shelf-life (Fernandez-Pan and Caballero, 2011), change chemical-physical properties, or create new goods. For instance, cutting apples have been coated with chitosan/lauric acid to inhibit browning (Pennisi, 1992), with casein-lipid to reduce moisture loss (McHugh et al. 1994), and with alginic acudtasein-lipid to reduce both (Wong et al., 1994). Thobunluepop (2009) coated seeds with different chitosan-based polymers to protect them from rice seed borne fungi and to increase they shelf-life. Biopolymers are used to encapsulate food ingredients. Conjugated linoleic acid was encapsulated in whey protein concentrate (Jimenez et al. 2006). Parris et al. (2005) reported encapsulation of essential oils in zein nanospherical particles. Owed to their bio-related properties, biopolymers are a subject of study in several fields.

Biopolymers can be classified by their chemical composition. Polysaccharide biopolymers include cellulose, starch, konjac glucomannan, hydroxypropyl methylcellulose, pectin, among others. Protein-based biopolymers include zein, whey, soy wheat protein, collagen, gelatin, and casein. Finally, lipids or resins. There are also biopolymers based on synthetic renewable materials that are biodegradable.

2.2.1 Cellulose

Cellulose is a polysaccharide of 1,4-anhydroglucopyranose repeat units (Shanks, 2013). It is constitutive element of cell walls of plants and occurs naturally. Commercial celluloses are derived from trees and agricultural byproducts. Many cellulose forms, like wood, are directly included in building and furniture with little treatment (Shanks, 2013).Other types of cellulose products require separation, purification, and further processing. Applications of cellulose in new materials as either filler or matrix are continuously studied because of high performance and availability. Shanks (2013) described cellulose nanocomposites as polymers with exceptional mechanical properties, relatively low density, biodegradable, and able to replace many synthetic composites that have not been designed for specific purposes.

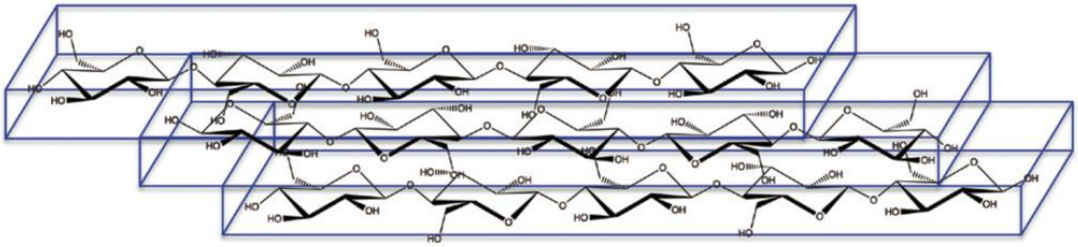

Figure 2.1. Rigid cellulose chain segments stack together in crystals (Shanks, 2013).

2.2.1.1 Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC), also called hypromellose, is a semisynthetic, hydrophilic, nonionic, cellulose derivative (Chen et al., 2015) obtained through the addition of methyl and hydroxypropyl ether groups (Ghosal et al., 2011)(Figure 2.2). Hydroxyl groups present in pure cellulose present strong inter- and intra- molecular interactions via hydrogen bonding leading to water-insolubility and crystallinity. Substitution of the -OH groups of the cellulose backbone by methyl and hydroxypropyl groups provides cellulose with water-solubility through the decrease in crystallinity of the molecule (Silva et al., 2008; Funami et al., 2007). Its molecular weight is 10-220kDa. HPMC molecules crosslink creating a 3D network. It is soluble in acids, organic solvents, and water. It is flexible, stable at heat and reasonable moisture levels,and tasteless and odorless. It is widely employed in the pharmaceutical industry. It is often used as drug delivery excipient because of its swelling capacity (Siempmann and Peppas, 2001). HPMC is also applied as a film or coating in capsules or tablets (Chang and Gray, 1978), glossing solutions, or component of tile adhesives and renders for water retention. Methylcellulose and HPMC can be used to reduce oil absorption through film formation (López et al., 2010). The use of 1% methylcellulose-sorbitol coating reduces the oil uptake of fried potatoes (García et al., 2002).

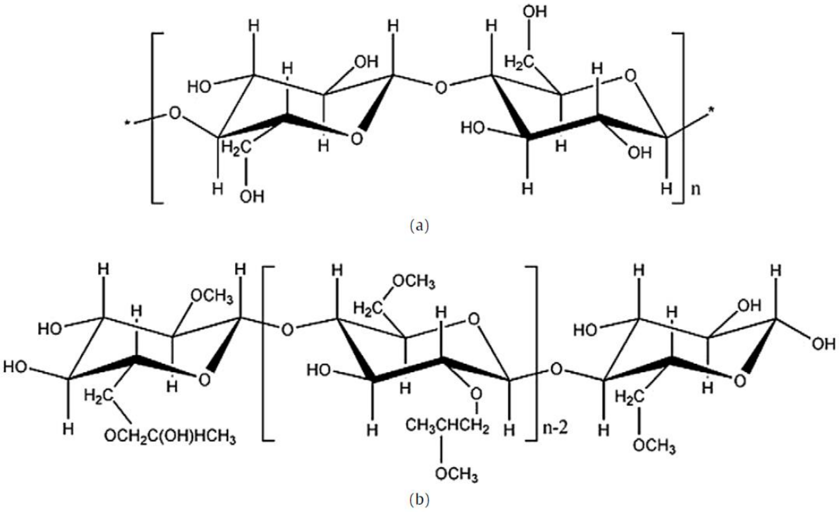

Figure 2.2. Structure of natural cellulose (a) and HPMC (b) (Silva et al., 2008).

2.2.2 Konjac glucomannan (KGM)

Konjac (Amorphophallus Konjac) is an Araceae perennial herb from South East Asia. The konjac glucomannan is extracted from the tuber, which contains around 49-60% (w/w) of glucomannan, 10-30% (w/w) starch, 2.6-7% (w/w) inorganic elements, 5-14% (w/w) crude protein, 3-5% (w/w) single sugars, 3.4-5.3% (w/w) ash and alkaloids (Li et al, 2005; Chuaa et al, 2010; Duke, 2004). The purified flour from the tuber is commonly known as KGM and was originally produced for food (Chuaa et al., 2010). KGM is a linear copolymer of glucose and mannose units linked by glycosidic β(1-4) bonds at a molar ratio of 1.0:1.6. It is naturally acetylated (Dave and McCarthy, 1997). Acetyl groups prevent the chains of glucomannan from approaching each other (Hui, 2005) and they are responsible for absorbing water. The deacetylation process results in formation of stable gels (Maekaji, 1974). Its molecular weight varies from 200 to 2,000 kDa (Keithley and Swanson, 2005). KGM can be used as dietary fiber. It has good rheological and gelling properties for emulsifying and stabilize food products (Chuaa et al., 2010). It is highly hydrophilic; KGM can absorb 100-times its volume in water. It is used as water binder in meat products in U.S. Clinical studies have demonstrated that supplementing the diet with KGM significantly lowers plasma cholesterol, improves carbohydrate metabolism, bowel movement and colonic ecology (Chuaa et al., 2010).

2.2.3 Zein

Corn has a paramount importance in U.S grain production. Corn kernels contain 75% starch, 9% fiber, 9% protein, 4% oil, 3% ash and simple sugars (Jeyakumar, 2015). These constitutes are extracted for food and industrial products, starch, sweeteners, corn oil, alcohol, resins, and fuel ethanol (Figure 2.3).

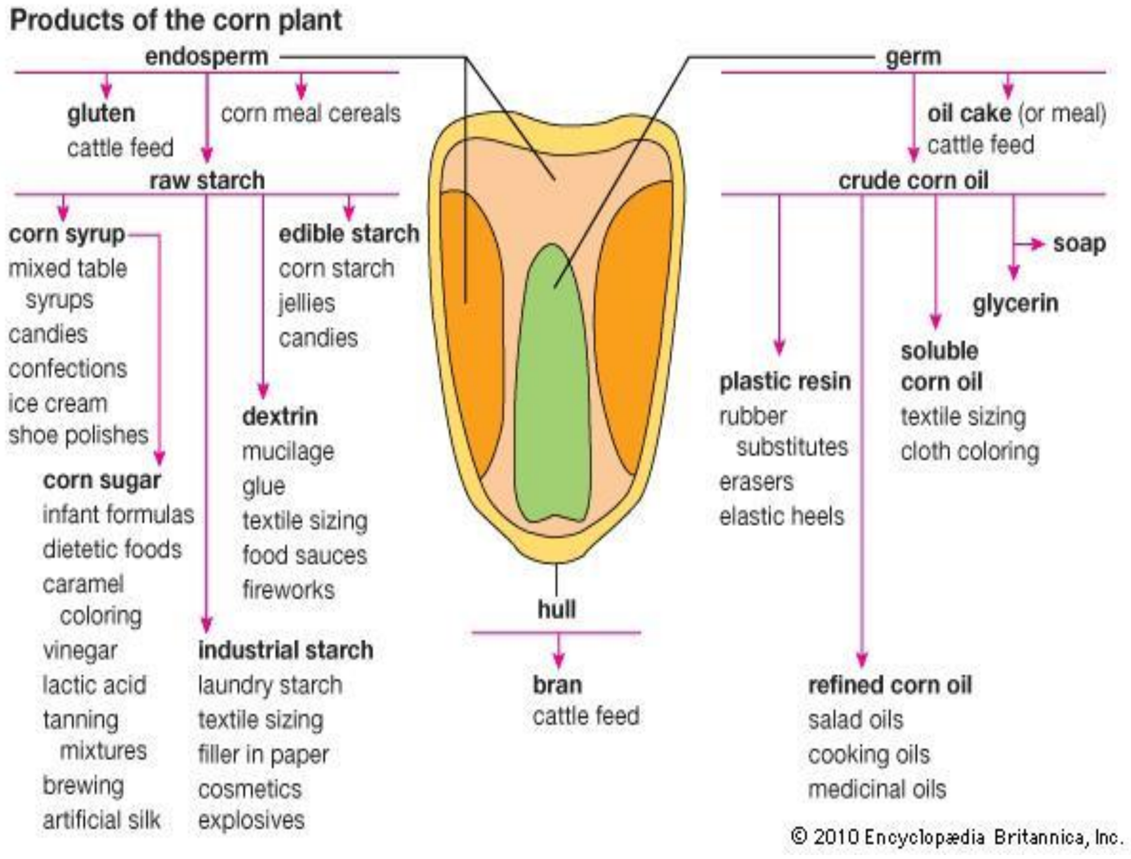

Figure 2.3. Scheme describing products derived from the corn plant. Retrieved on 8/31/2016 from https://www.britannica.com/plant/complant/images-videos/Products-derived-from-the-corn-plant/161954

Zein is a protein present in the endosperm of corn kernels. It constitutes 50-60% of the total endosperm protein. It is a prolamine, it is soluble in water-alcohol solutions, and is recognized for its film-formation ability. It is considered GRAS (Generally Recognized as Safe) by the FDA.

The most abundant amino acids in zein are glutamic acid, leucine, proline, and alanine. It lacks in acidic and essential amino acids lysine and tryptophan (Shukla and Cheryan, 2001). Zein comprises various peptides, α-, β-, γ-, and δ- zein of different charge, solubility, and molecular weight. (Esen, 1987). α- Zein has a molecular weight of 19 and 22 kDa, it amounts to 70-85% of total zein. γ-Zein has a molecular weight of 27 and 16 kDa and amounts to 10-20% of the total zein. δ- and β- Zein have a molecular weight of 10 and 17 kDa, respectively, forming 5-10% of the total zein (Figure 2.4).

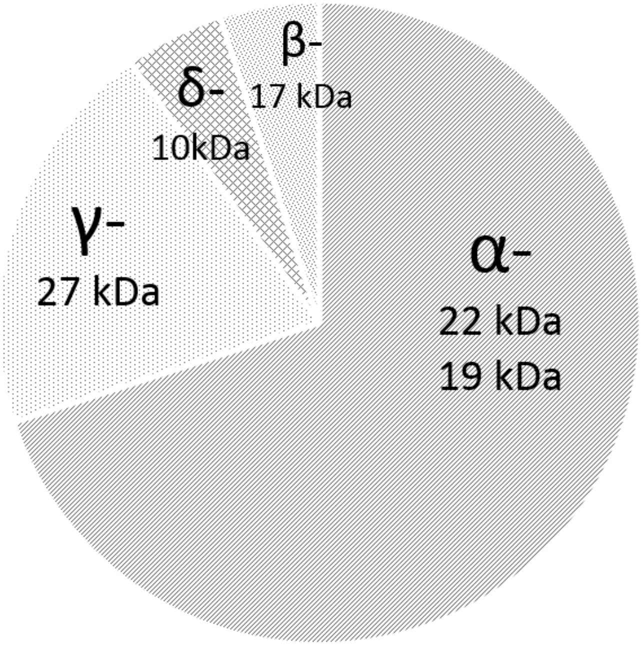

Figure 2.4. Molecular weight of zein peptides.

Its film-forming ability has been utilized to develop coatings, fibers, and films (Lawton, 2002). However, strong intermolecular forces in zein films make them too brittle for most practical applications (Xu et al., 2012). Zein is dense and brittle due to strong protein-protein hydrogen bonds. It can be plasticized by using water, glycerol and ethylene glycol to reduce such interactions (Vasile and Cazacu, 2013). For instance, Lai and Padua (1997) reported higher elongation of zein films when oleic acid was added to the composite. The tensile stress was also increased. Zein is used as coating for nuts, fruits, and drug tablets, for encapsulation, and adhesives (Lawton, 2002). Wu and Schawartzberg (1992) coated popcorn kernels with zein to increase the expansion ratio of popcorn. They reported that zein coating reduced moisture loss and expansion volume was insignificantly increased. Wong et al. (1996) reported zein as a good coating for improving strength and retarding interior quality deterioration of eggs.

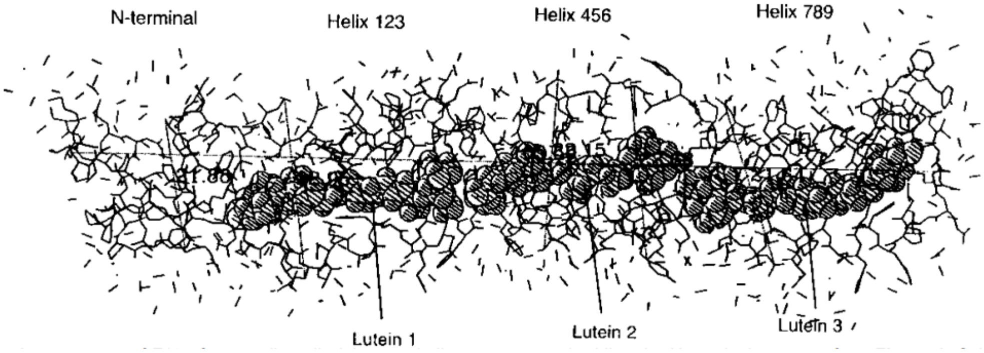

Figure 2.5. Complete structure of 19 kDa α zein. Ethanol is represented by fine lines (Momany et al. 2006).

2.2.4 Polylactic acid

Polylactic acid (PLA), also called polylactide, is synthetic biodegradable polyester composed of L and D lactic acid monomers. PLA is considered a renewable material because it can be produced by fermentation of carbohydrate from lactobacillus (Tang et al., 2012). Simple sugars like glucose, corn or potato maltose, cane or beet sucrose, and lactose are common sources for the fermentation. This process generates a small amount of byproducts. PLA is hydrophobic polymer due to methyl groups present in the backbone. Its molecular weight goes from 1 to 100 kDa. PLA is used for clothing, furniture manufacture, drug delivery, and tissue engineering. PLA is currently used as films, food service ware, and short shelf-life bottles and it is usually added to increase biodegradability and reduce costs (Gross and Kalra, 2002).

2.3 Discontinuous phase: nanofillers

The downside of biopolymers is that they present poor mechanical properties comparing to conventional non-biodegradable materials made from petroleum like polyethylene (PE) (Othman, 2014). One route to overcome that disadvantage is to develop nanocomposite structures by incorporating fillers into the matrix.

Nanofillers can significantly modify different properties of the materials into which they are incorporated (Marquis et al., 2011). For a successful modification of matrix properties, the filler has to be well integrating into the matrix (Angellier-Coussy et al., 2013). Good integration will lead to new physical-chemical properties of the composite. Usuki et al. (1993) and Okada et al. (1995) reported a polyamide-6 filled with nanoclays which they called “hybrid.” Later on, the term “nanocomposite” was defined (Marquis et al., 2011).

2.3.1 Properties

Ahmed and Jones (1990) reported that mechanical properties of composites can be affected by the characteristics of added fillers. Size, aspect ratio, and surface chemistry (Haafiz et al., 2015) of nanofillers, control their ability for dispersion and interaction with the polymer matrix; thus, affecting tensile properties of the composite (Figure 2.6).

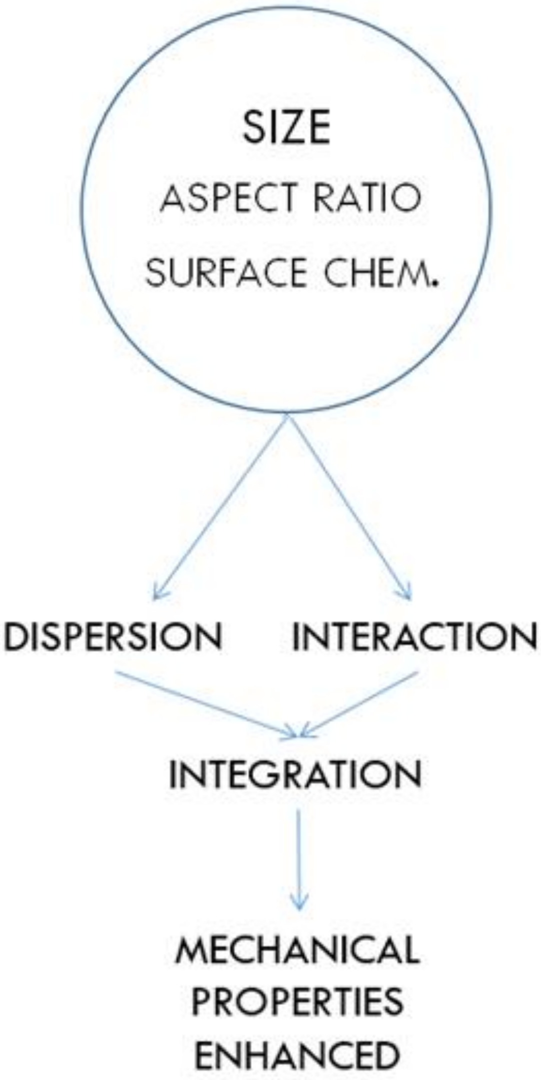

Figure 2.6.Important properties of fillers in composites.

The size of fillers affects dispersion and interaction of the filler into the matrix. A reduction of filler size tends to enhance the strength of the composite (Lee et al., 2015). Lee et al. (2015) studied the effect of different sizes of $CaCO_3$ in glass fibers and polyester composite. They observe that smaller sizes of calcium carbonate showed higher strength compared to larger ones because they contributed to forming a densely packed microstructure. They attributed the enhancement to the larger surface area of the small fillers. Ozsoy et al. (2015) studied the influence of different loads of micro- and nano- filler Al_2O_3 and TiO_2 into epoxy composites. They concluded that tensile strength and elongation at break values of composites decreased while tensile modulus increased with the increased load of both micro- nano- fillers. However, they highlighted how small amounts of nano- size particles in epoxy have strong effect on tensile strength and ductility. Li et al. (2014) studied rice bran carbon/nitrile rubber composites. They concluded how smaller filler particles can disperse better in the matrix than larger fillers. Augmenting the size and the filler ratio caused difficulties in the homogeneous dispersion of fillers, leading to weak adhesion between matrix and fillers (Ozsoy et al., 2015).

Layered, plated, or irregular shape of fillers present better mechanical properties than spherical, cubical or acicular (Merinska et al., 2010; Idrus et al., 2010). Idrus et al. (2010) studied the effect of different shapes of silica fillers in natural rubber compounds. They tested cubes, elongated, and irregular shapes at four different loads from 10% to 40 %. They observed that elongated particles showed higher maximum torque compared to other shapes. Irregular shaped fillers presented the highest tensile strength, elongation at break, and hardness compared to cubical or elongated shapes. They attributed it to better filler-matrix interaction. Merinska et al. (2010) studied spherical silica and calcite, organosilicate Halloysite tubes, and modified clay plates as fillers in polystyrene. They concluded that layered fillers present high tensile and impact strength, and modulus than particles with another shape.

The aspect ratio is the particles length:thickness ratio (Idrus et al., 2010) (Figure 2.7). Higher aspect ratio of the filler contributes the strength of the composite whereas lower aspect ratio weakens the composite (Vallittu, 2015). Large aspect ratio results in large contact for filler-matrix interactions. When more contact area is provided to react with the polymer matrix, stronger bonding will occur. The enhancement of this interaction will improve the polymer-filler interaction and will, consequently, allow better stress transfer (Idrus et al., 2010). Consequently, fillers with large aspect ratio like platted clays tend to have a positive effect on the composite properties. Idrus et al. (2010) studied the effect of different shapes of ultrafine silica in rubber and concluded how the irregular silica shows the largest surface area, followed by elongated silica and cubical silica. Bras et al. (2010) showed the tensile modulus of cellulose nanocrystals films increased by increasing the aspect ratio (L/D) of the nanoparticles (Dufresne et al., 2013).

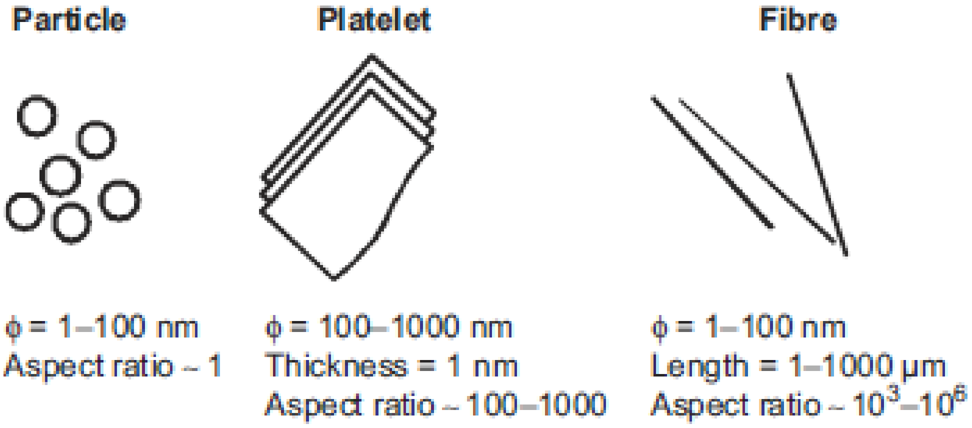

Figure 2.7. Various nanofiller shapes and typical aspect ratios (Pillai and Ray 2012).

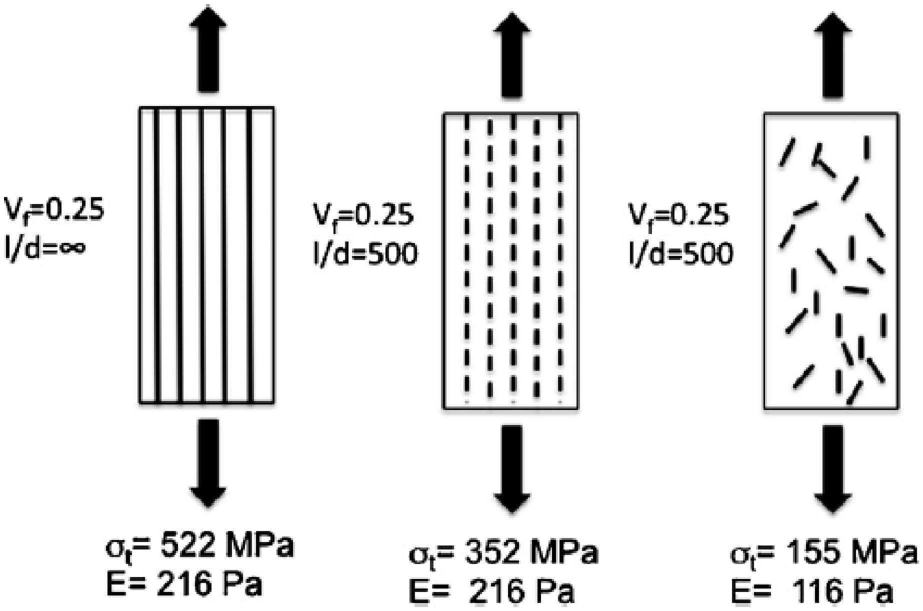

Figure 2.8. Influence of the aspect ratio (l/d) of fiber and their orientation to the tensile stress (σ), and modulus (E) of elasticity with the same volume fraction of fibers *(*Kardos, 1993*)*.

Surface chemistry refers to interaction between the continuous and the discontinuous phase of the composite. Surface chemistry is critical for the design of nanocomposites with desirable properties (Huang, 2014). Modifying the surface chemistry of the filler can lead to improved dispersion and filler-matrix interactions. Siqueira et al. (2009) chemically modified the surface of nanowhiskers and microfibrillated cellulose to improve their compatibility with polycaprolactone (PCL). They reported that the chemical treatment significant improved the mechanical properties of the nanocomposite whereas PCL-unmodified cellulose showed poor dispersibility. Eng et al. (2013) attributed the enhancement of mechanical properties of PLA/PCL/hydrophilic clay composite to hydrogen bonding between hydrophilic clays and PLA/PCL blend matrix. The surface chemistry of the clay promoted polymer-filler interactions that lead to desired mechanical properties. Haafiz et al. (2015) compared the effect of MCC and cellulose nanowhiskers on PLA. The performance of MCC was lower than cellulose nanowhiskers probably due to Van de Waal's attraction filler-filler forces instead of filler-matrix. However, the better performance of the nanowhiskers was attributed to hydrogen bonding between the filler and the matrix. Abundant filler-matrix interactions resulted in better interfacial adhesion and consequent improvement in tensile strength.

The fillers used in this study are two types of cellulose, calcium carbonate, and two types of clay. These five types of fillers were chosen to compare effects on size, surface chemistry, and aspect ratio on polymer matrices. Celluloses have the same surface chemistry but different sizes: microcrystalline cellulose (MCC) and nanocrystalline cellulose (CNC). Clays have different surface chemistries and same sizes: hydrophilic nanoclay and hydrophobic nanoclay. Calcium carbonate has different aspect ratio but same nano-size than clays and CNC.

2.3.2 Celluloses

Cellulose is the most used filler in the food industry. It is derived from wood sources, inert, and not digestible by humans. Cellulose is water insoluble but hydrophilic. Cellulose has been successfully added as reinforcement, water-retainer, preventive of phase separation, and tableting excipient (Ashori and Nourbakhsh, 2010; Vigo and Kinzing, 1992; Bai and Li, 2009; Haafiz, 2015; Habibi et al., 2010). The most common cellulose fillers are microcrystalline cellulose and nanocrystalline cellulose. Cellulose fillers can present the disadvantage of water absorption, poor wettability, incompatibility with many polymeric matrices, and temperature limitations (Siqueira et al., 2009).

MCC are environmentally friendly fillers that substitute mica, calcium carbonate, and clay (Shanks, 2013). It is generally extracted from cotton plants. MCC are crystalline flakes with 200:1 aspect ratio. MCC is water insoluble but hydrophilic at the same time, which allows it to swell and retain water. Even though MCC is not expected to be a reinforcing phase in the same way as are cellulose fibers, it can contribute via its binding properties (Shanks, 2013). MCC has been selected due to being successfully added as reinforcement, water-retainer, preventive of phase separation, and tableting excipient by many authors (Ashori and Nourbakhsh, 2010; Vigo and Kinzing, 1992; Bai and Li, 2009; Haafiz, 2015). For instance, Haafiz et al. (2015) added MCC and CNC to PLA to improve its thermal and mechanical properties. Ashori and Nourbakhsh (2010) reported enhanced tensile, flexural, and impact strength for polypropylene (PP)/MCC/wood flour composites when MCC was added. Bai and Li (2009) replaced silica with MCC in styrene butadiene rubber and reported an easier dispersion of the filler, while tensile strength, modulus and heat resistance were increased.

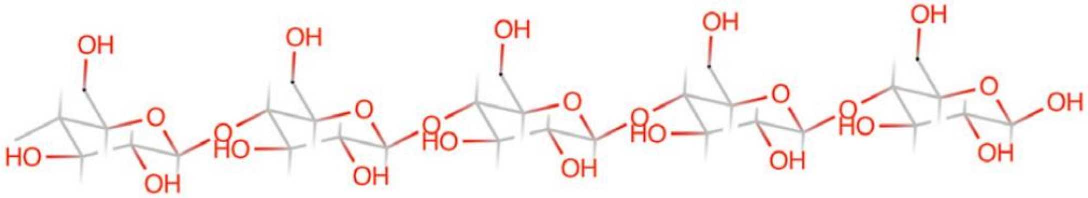

Figure 2.9. A representative segment of cellulose structure (Shanks, 2013).

CNCs are extracted from cellulose microfibrils via isolation of crystalline regions by acidic hydrolysis (Dufresne et al., 2013) (Figure 2.10). CNC are rod-like crystalline particles widely implemented as a nanocomposite because of their large surface area, excellent mechanical properties, and abundant –OH groups for surface functionality which help in dispersion (George and Sabapathi, 2015) and compatibility with hydrophobic polymers (Habibi, 2010). CNC aspect ratio varies from 5:1 to 20:1 depending on the source of extraction (Murphy, 1998; Vallittu, 2015). Their abundance, high strength, and stiffness, light weight and biodegradability serve as promising candidates for the preparation of bio-nanocomposites

(Dufresne et al., 2013). For instance, Qu et al. (2010) added CNC to PLA/Polyethylene glycol (PEG) to reinforce the neat polymer. They reported CNC as a good reinforcement, but it needs PEG to increased the compatibility between CNC-PLA. The addition of PEG to PLA/cellulose increased the tensile stress and elongation by 56.7% and 60% respectively.

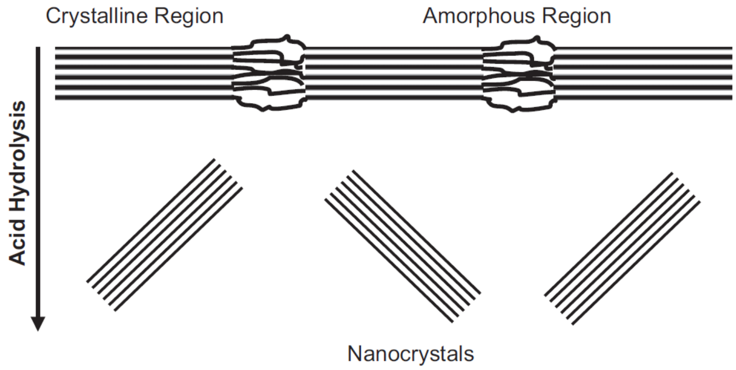

Figure 2.10. Acidic hydrolysis breaks down disordered (amorphous) regions and isolates nanocrystals (Dufresne et al., 2013).

2.3.3 Calcium carbonate

Calcium carbonate is commonly found in rocks, bones, and shells. It is one of the most abundant minerals, and it is relatively inexpensive (Lee et al., 2015). $CaCO_3$ is insoluble in water but unstable in acidic solutions. It is commonly used as filler for paints and coatings (Wu et al., 2015). Gao et al. (2009) et al. studied the tensile and compaction properties of polystyrene/nano $CaCO_3$ composite and reported that the strength and toughness of the polymer decreased when the filler was added. They attributed the poor mechanical properties to interfacial debonding and filler agglomerations. They also reported an increment in stiffness that resulted in improved tensile modulus and creep resistance when the content of filler increased.

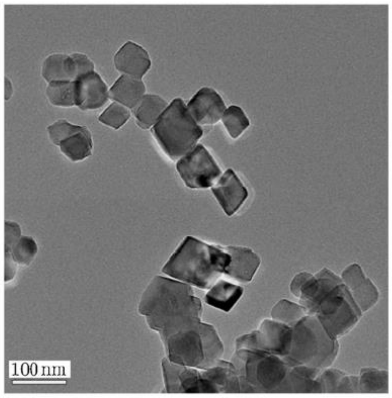

Figure 2.11. Transmission Electron Microscope (TEM) image of $CaCO_3$ nanoparticles (Gao et al., 2009).

2.3.4 Clays

Clays are the most common filler used. Clays are small crystalline particles of different composition of alumina silicates. Their crystal lattice consists of a two tetrahedral silica sheets and one octahedral alumina or magnesium oxide sheet (Figure 2.12) (Tang et al. 2012). Montmorillonite is one of the most investigated clays for polymer nanocomposites. Its structure is one octahedral alumina sheet sandwiched between two tetrahedral silica sheets bound by Van der Waal's (Qutubuddin and Fu, 2002). Bentonite is an abundant type of clay containing high percentage of Montmorillonite, Illite or Kaolin clays. Nanoclays are sheet-type or plate structure with length and width measured in hundreds of nanometers, and thickness of one nanometer. Their aspect ratio is large, from 100-1000:1 (Pillai and Ray, 2012). A chemical modification of the clay surface is often achieved, with the aim to match the polymer polarity (Pillai and Ray, 2012; Alexandre and Dubois, 2000; and Ray et al. 2003). For instance, hydrophobic nanoclay is bentonite nanoclay coated with trimethyl stearyl ammonium in order to disperse it well in hydrophobic polymers. For successful reinforcement of polymer, these nanofillers have to be exfoliated. Tang et al. (2012) define an exfoliated nanocomposite as nanometer thick platelets distributed homogeneously throughout the matrix (Figure 2.13). Clays have been reported to improve mechanical properties without large loading required. They increase heat deflection temperature, and gas reduction and liquid permeability (Okpala, 2013). Incorporation of clay into polymers reduces costs (Okpala,2013). The addition of montmorillonite to chitosan matrix frequently exhibits higher modulus, higher strength and higher thermal stability, and reduced gas permeability than neat chitosan matrix due to strong matrix-filler interactions (Pillai and Ray, 2012).

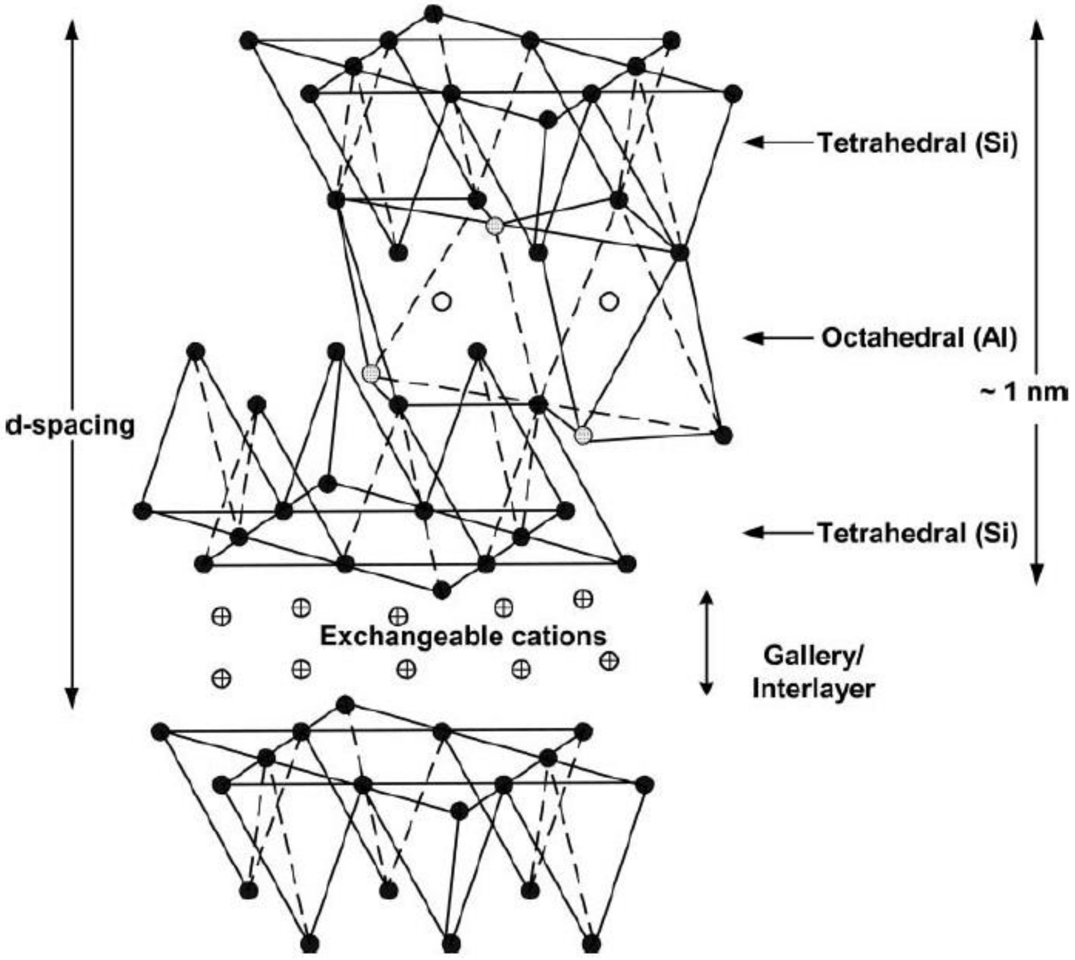

Figure 1 Structure of 2:1 layered silicates.

Figure 2.12. Structure of 2:1 layered silicates (Tang et al., 2012).

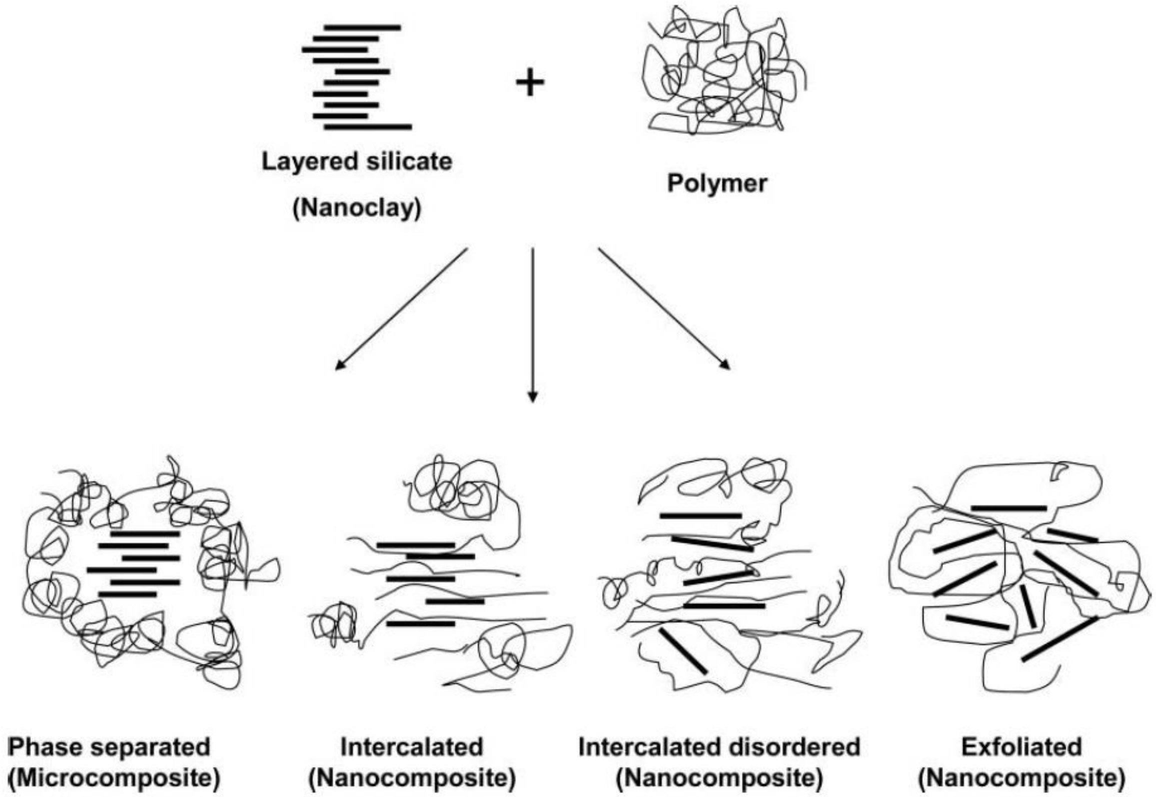

Figure 2.13.Possible structure of nanocomposites (Tang et al., 2012).

Nanocomposites have been proven to be a promising option to improve mechanical properties of biopolymeric films and potentially replace conventional packaging materials (Tang et al. 2012). The election of the matrix and filler has to be carefully chosen regarding the desired composite properties. The addition of fillers to coatings and edible films may enhance their mechanical properties.

2.4 Mechanical properties

Many authors have studied how to reinforce materials by the addition of different fillers. Increasing elasticity or stiffness, reducing the coefficient of thermal expansion and water permeability are only a few examples of those studies. Thus, mechanical properties have to be measured for proper characterization of the novel materials. This work is focused on tensile properties.

2.4.1 Measurement of tensile properties

Tensile tests measure the ability of a material to endure forces that pull the material apart and report on the ability of the material to elongate before breaking. Tensile test can be performed by dynamic mechanical analysis.

Dynamic Mechanical Analysis (DMA) describes the technique of applying an oscillatory or pulsing force to a sample and analyzing the response of the material to that force (Menard, 2008). When a force is applied to a material, in this case, tensile stress, a deformation is expected (Figure 2.14). This is explained by Hooke's law which relates the stress to the strain of a spring by a constant, k (Menard, 2008).

$$\sigma = k * \gamma$$

Where k is the spring constant, σ is the force applied, and γ is the strain. This behavior is defined by stress-strain curves. Increments in the spring constant result in stiffer materials. Thus, Young's modulus (E) would also increase (Menard, 2008). Then,

$$E = d\sigma/d\gamma$$

Where E is the Young's modulus.

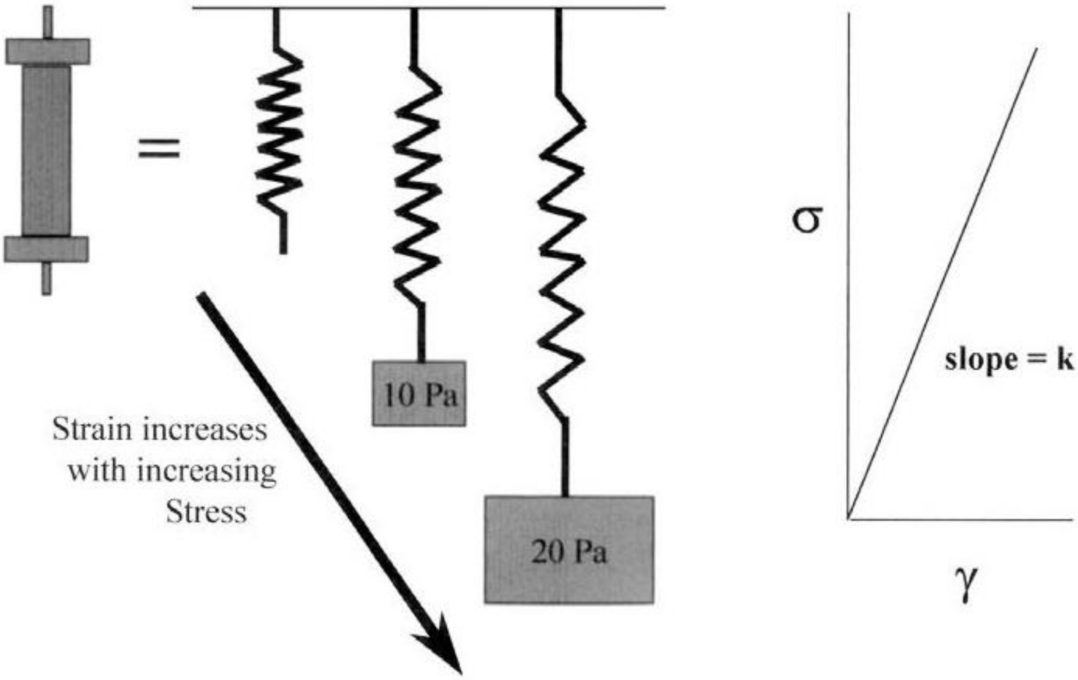

Figure 2.14. Representation of Hooke's Law and stress–strain curves. Elastic materials show a linear and reversible deformation on applying stress (within the linear region). The slope, k, is the modulus, a measure of stiffness, for the material. For a spring, k would be the spring constant (Menard, 2008).

2.4.2 Stress-strain curves

Stress-strain curves describe the typical behavior of materials under a force. They are graphic representation of the variation of strain with the tensile stress applied (Figure 2.15). Tensile behavior is affected by temperature, humidity, or manufacturing process.

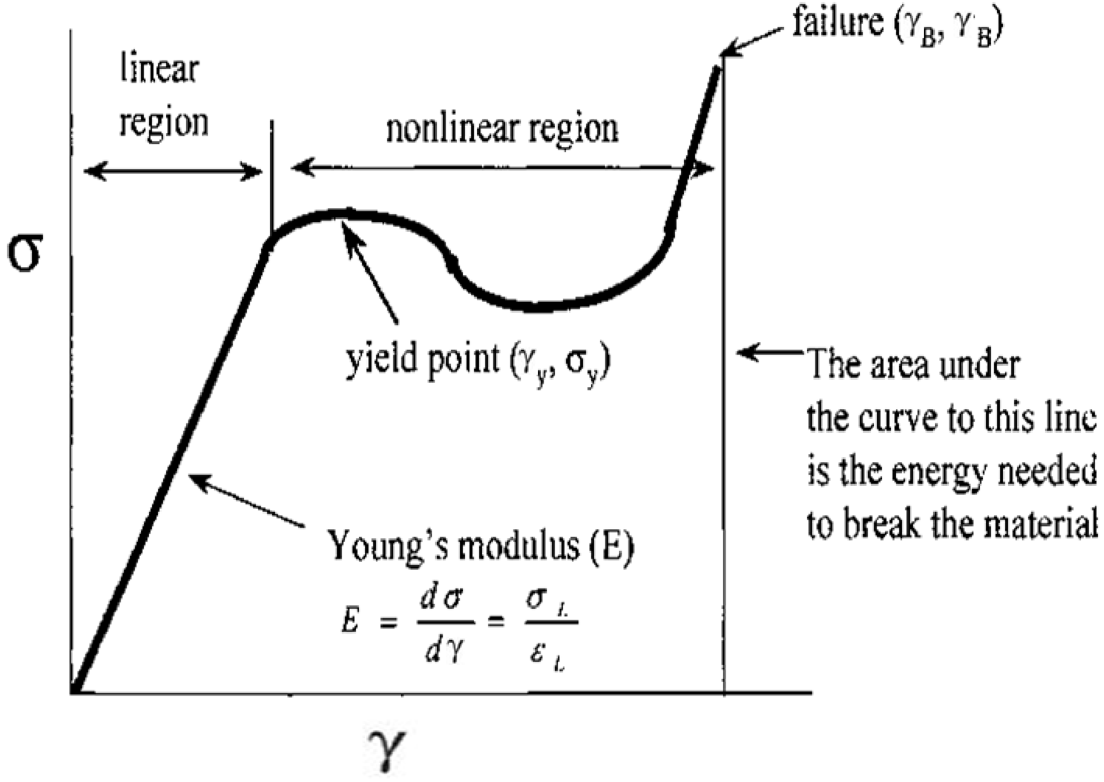

Figure 2.15. Representation of a general stress-strain curve and its components. Adapted from Menard (2008).

Where:

Tensile stress (σ): The tensile load applied per unit of original cross-sectional area at any given time. It can be expressed as σ = P / A, where P is load and A is cross-sectional area.

Strain (γ) (engineering strain or Cauchy strain): is the ratio of total deformation caused by a force over the initial dimension of the material. It is usually expressed in % basis: γ = (l/L)*100; where γ is strain, l is elongation, and L is gage length.

Elastic, or also call *Young's* or tensile, *modulus* (E): is the ratio of stress over strain, measured in in the linear region of the stress-strain curves in megaPascals (MPa). It is an indication of material stiffness (Baumgart, 2000).The larger the E, more stress is needed to stretch the material: therefore, it is stiffer. Flexibility is the opposite of stiffness (Santhosh et al., 2012).

After that linear region, the material could break without a plastic deformation (brittle materials) or it could deform longer until failure (ductile materials). The area under the stress-strain curve is proportional to the energy needed to break the sample (Menard, 2008). Figure 2.16 shows typical stress-strain curves for various materials:

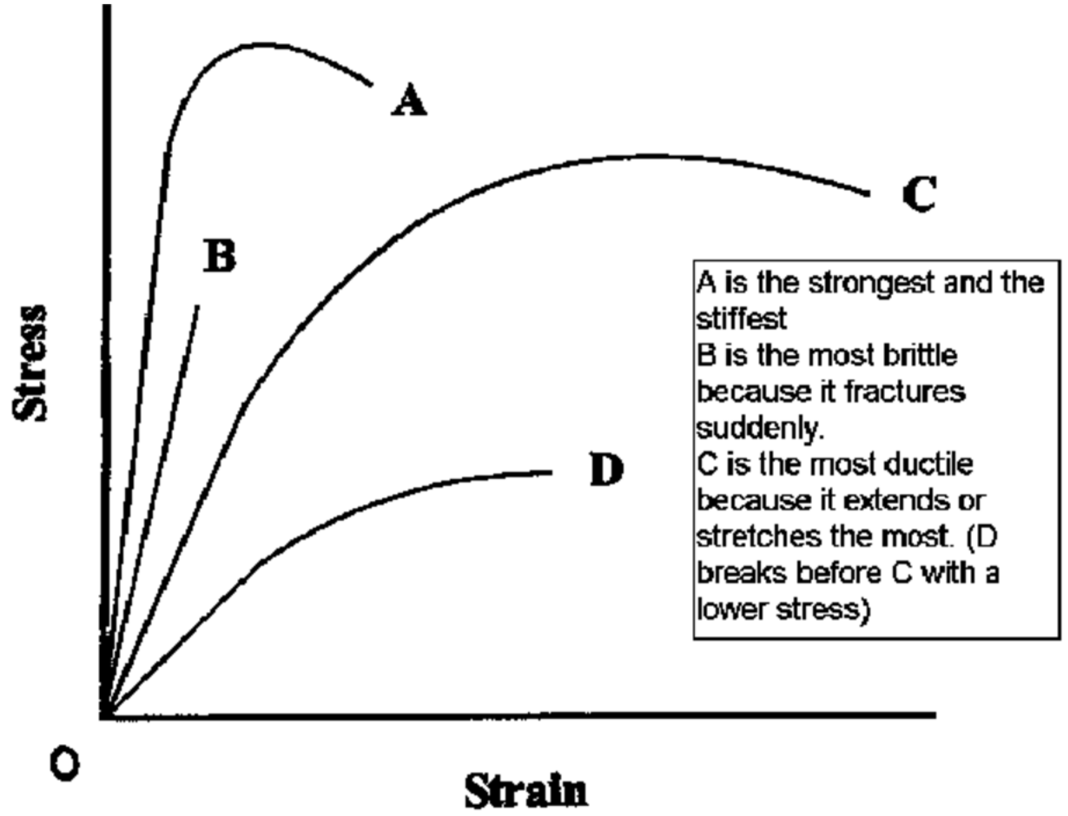

Figure 2.16. Different stress-strain curves for four materials *(*A Level Engineering, 2016*)*.

2.5 Morphology

Morphological properties are studied to evaluate dispersion of the filler into the matrix, compaction, and changes in the topographic profile of the polymer when the filler is added, and the tensile test is performed.

2.5.1 Scanning electron microscope (SEM)

Scanning electron microscope (SEM) is a microscope that produces images by scanning the sample with a focused beam of accelerated electrons as a source of illumination. A signal is produced when electrons from the beam source interact with the atoms present in the sample. These signals contain information about the surface topography and composition of the sample.

The most common detection of the signal is from secondary electrons. The components of this instrument are shown in figure 2.17.

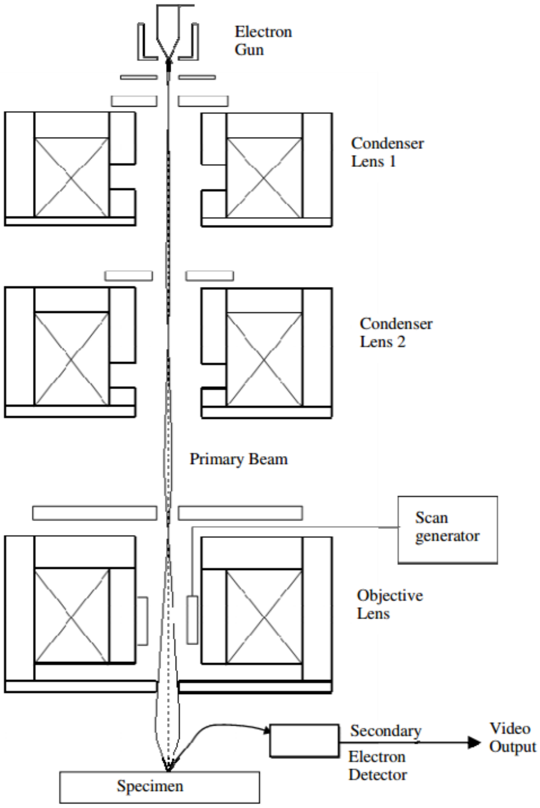

Figure 2.17. Schematic drawing of SEM *(*Khursheed, 2011*)*.

To obtain a signal, specimens must have electrically conductive surface and connection to ground to prevent electrostatic charge at the surface. Nonconductive specimens are frequently coated with an ultrathin conductive material to prevent them from charging by the source electrons. Commonly used coating techniques are low vacuum sputter coating or high vacuum evaporation.

Figure 2.18 shows an example of SEM imaging to analyze nanocomposites. Zeng et al. (2013) and Wu et al. (2011) investigated the effect of pure carbon nanotubes (MWCNTSs) in chitosan (CS). They studied SEM images of pure MWCNTs, pure CS, and MWCNTSs-CS nanocomposite.

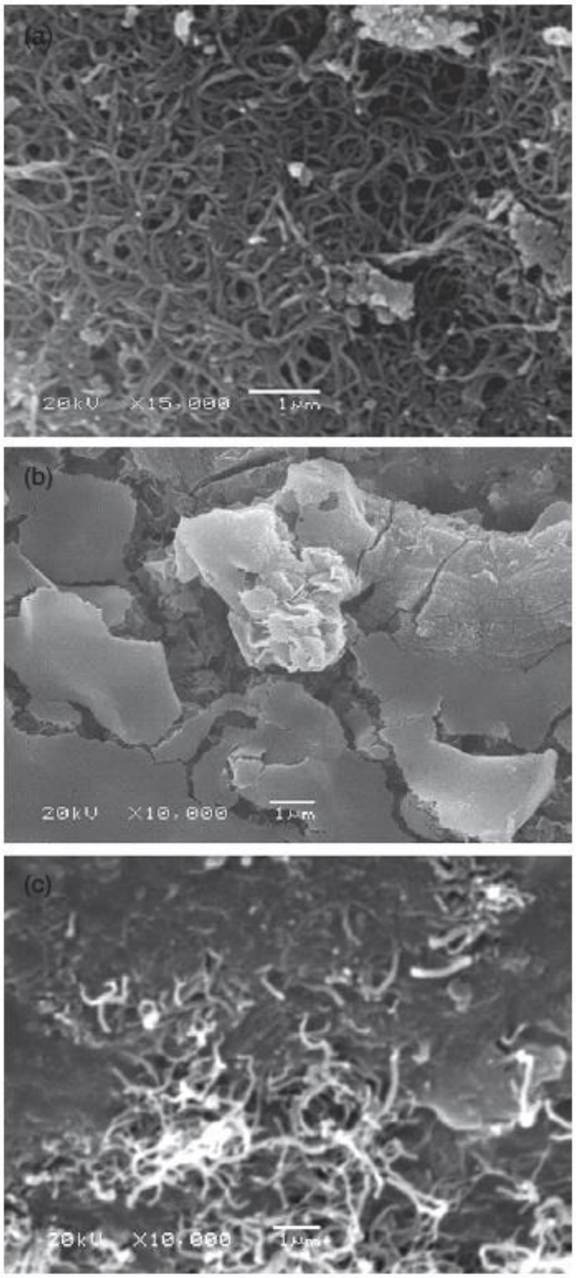

Figure 2.18. SEM images of pure carbon nanotubes (MWCNTSs) (a) (Zeng et al., 2013), pure chitosan (b) (Zeng et al., 2013), and the MWCNTs–CS nanocomposite (c) (Wu et al., 2011).

DYNAMIC MECHANICAL ANALYSIS OF KONJAC, HYDROXYPROPYL METHYLCELLULOSE, AND ZEIN

3.1 Introduction

The interest of the food industry in developing new types of foods and snacks requires the design of stiff and strong materials. Biopolymers are suitable materials for food applications. Biomaterials are already present in several packaging forms like injection molded bottles for carbonated drinks, cast films, and thermoformed trays (Tang et al., 2012). However, they show poor mechanical properties for other food applications (Tang et al., 2012). Researchers have considered nanocomposites to reinforce the polymer matrix (Othman, 2014; Terrazas-Hernandez et al., 2015) or decrease water vapor permeability (Souza et al., 2015) to meet package needs.

In this study, three biopolymers, konjac glucomannan (KGM), hydroxypropyl methylcellulose (HPMC), and zein were reinforced with five fillers, nanocrystalline cellulose, microcrystalline cellulose, $CaCO_3$ nanoparticles, hydrophilic nanoclay and hydrophobic nanoclay to study the effect of fillers on mechanical properties of biopolymer films.

The objective of this work was to investigate the effect of various nano-fillers selected to compare size (nano- and microcrystalline cellulose), shape (nanoclay plates, nanocrystalline cellulose needles, $CaCO_3$ cubes), and surface chemistry (hydrophilic and hydrophobic nanoclays) on tensile properties of biopolymers. According to several reports, the optimum filler load to improve matrix tensile properties is ≤5% (Rhim et al., 2013; Othman, 2014). For this study, a 2% w/w filler load was selected. A second objective was to investigate the effect of CNC filler weight fraction on tensile properties of KGM and HPMC.

3.2 Materials and methods

Zein (Showa Sangyo, Tokyo, Japan), hydroxypropyl methylcellulose (HPMC) (Methocel^{TM} E19, Dow Chemicals, Midland, MI), and Konjac glucomannan (KGM) (Ticagel® KGM High Viscosity, Tic Gums, Belcamp, MD) were used as film forming polymers. Microcrystalline cellulose, particle size 20µm, (Sigma-Aldrich, Milwaukee, WI), cellulose nanocrystals (CNC), 4–5 nm x 50–500 nm, (BioPlus^{TM}, American Process Inc., Atlanta, GA), calcium carbonate nanoparticles (cubic 90nm) (PlasmaChem, Berlin, Germany), hydrophilic bentonite nanoclay and hydrophobic nanoclay (25-30 wt. % trimethyl stearyl ammonium) (Sigma-Aldrich, Milwaukee, WI), were used as fillers. Ethanol 200 proof (Decon Laboratories, King of Prussia, PA), KOH (Fisher Laboratories, Fair Lawn, NJ), and magnesium nitrate (ChemCruz® Biotechnology, Dallas, TX) were used in the preparation of samples.

3.2.1 Film preparation

Zein films were prepared by dispersing powder zein (0.2 g/mL) and the corresponding filler (2%, w/w of zein) in 80% ethanol. All zein suspensions, except zein with CNC, were sonicated (VC 505, Sonics & Materials, Newtown, CT) at 200 W for 45 seconds. The zein sample containing CNC was not sonicated but stirred-mixed. HPMC films were prepared by dispersing HPMC (0.375 g/mL) in water and adding 2% (w/w of polymer) of the corresponding filler. HPMC suspensions were sonicated at 200 W for 2 min. A KGM mother solution was prepared by stirring 1.25% KGM (w/w) in water, followed by centrifugation (Model RC-5C, Sorvall, Newton, CT) at 12,000 rpm for 45 min. The pH was then adjusted to 7.5 with KOH (5%), and fillers were dispersed (2% w/w of polymer) in the KGM mother solution. All solutions were cast in 42 mL aluminum foil weighing dishes, dried at room temperature (22°C), and stored in desiccators at 50% RH for 48 h.

To study the effect of filler weight fraction on properties of biopolymer films, KGM and HPMC solutions were prepared as above and added to a series of CNC at weight fraction of 2%, 4%, 6%, 8%, 16%, 32%, 50%, and 75% w/w KGM. CNC neat polymer was prepared using 8 mL of CNC gel. All suspensions were sonicated at 200 W for 2 min., cast on 42 mL aluminum foil weighing dishes, dried at room temperature (22°C), and stored in desiccators at 50% RH for 48h.

3.2.2 Dynamic mechanical analysis (DMA)

DMA tests were performed using a Q800 DMA (TA Instruments, New Castle, DE). Tests were performed at room temperature using the film tension clamp. The force was ramped at 50 N/min. up to a maximum of 18 N or sample break. Stress/strain curves were analyzed using TA Universal Analysis software (TA Instruments, New Castle, DE) to obtain tensile strength (σ), strain (γ) at break, and Young's modulus (E) for each film strip.

Film specimens for DMA were cut in rectangular strips, 25 mm length and 2 mm width, with a laser cutter (Epilog Mini 24” Model 8000, Golden, CO) operated at 100 mm/s, 5000 Hz, varying the power from 3-12 W. Films were stored in a desiccator (50% RH) for 72 h before testing.

3.2.3 Morphology characterization (SEM)

SEM images of freeze-fractured film strips were collected to examine cross section morphology. Surfaces were sputter coated (Emitech K575, Ashford, U.K.) with gold to enhance surface conductivity. Samples were imaged by SEM (Model 6060LV, Jeol Ltd. Peabody, MA). Images were taken at an accelerating beam voltage of 5 kV, working distance of 10 mm, and spot size of 30 nm. These parameters were selected to obtain a clear image without damaging the film.

3.2.4 Statistical analysis

Analysis of the variance (ANOVA) and post-hoc Tukey-Kramer HSD test was conducted with R-Studio Statistics software (R Studio, Inc. Boston, MA). $p < 0.05$ was considered statistically significant. Three mechanical parameters were obtained from stress/strain curves. One way analysis of the variance (ANOVA) was conducted to compare the effect of the different fillers (2% w/w) among each other on each individual mechanical parameter (σ, γ, and E) for each of the polymers. The effect of different CNC loads in KGM and HPMC was investigated by one-way ANOVA comparing the different loads among each other. Data are presented as the mean ± standard deviation.

The experimental design to test the effect of various fillers on tensile properties of biopolymers consisted of 3 polymers with 6 groups of fillers, each set had at least 8 specimens tested. A total of 229 strips were measured including controls (no filler). The experimental design to test the effect of filler weight fraction on tensile properties of biopolymers consisted of 9 different filler loads with at least 9 repetitions each. A total of 151 strips were measured. Table 3.1 and 3.2 describe the treatments for each objective.

Table 3.1. Number of experimental units for the study of the effect of 2% w/w filler in KGM, HPMC, and zein matrices (objective 1).

Objetive 1	Filler 2% w/w of polymer	KGM	HPMC	Zein
	Neat	25	11	11
	MCC	7	18	10
	CNC	26	17	12
	CaCO3	8	13	9
	Hydrophilic nanoclay	8	12	10
	Hydrophobic Nanoclay	10	12	10

Table 3.2. Number of experimental units for the study of the effect of different weight fractions of CNC in KGM and HPMC (objective 2).

Objetive 2	CNC % weight fraction load	0%	2%	4%	8%	16%	32%	50%	75%	100%	Total
	KGM	25	26	13	9	17	25	10	11	15	151
	HPMC	11	17	8	8	-	-	-	-	15	59

3.3 Results and discussion

3.3.1 Effect of polymer

Mechanical testing was done on the three neat polymer matrices to evaluate differences among them. Tensile properties, σ, γ, and E, were significantly different for KGM, HPMC and zein films (Figure 3.1 and Table 3.3). KGM films showed the highest values for σ (119.6±10.7 MPa) and E (4.6±0.7 GPa). HPMC films showed the highest values for γ (38.6±8.9 %) and the lowest E (1.7±0.3 GPa). Zein showed the lowest values for σ (49.7±9.7 MPa) and γ (4.2±1.6 %). Polylactic acid (PLA) values for σ, γ, and E (Jonoobi et al., 2010) are given for reference. Mechanical properties of PLA films were found close to those of zein.

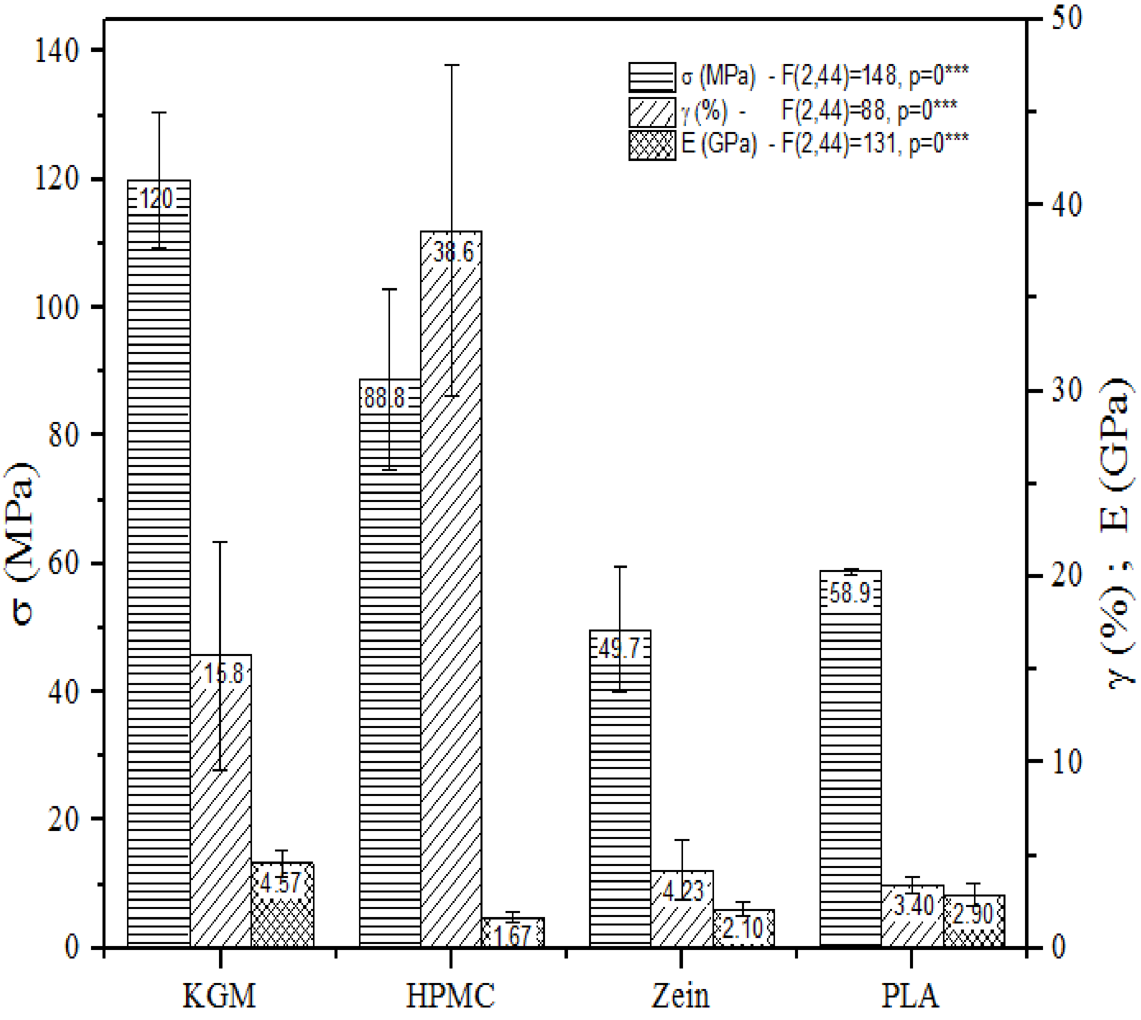

Figure 3.1. Effect of biopolymer on mechanical properties of films. KGM, HPMC, zein, and PLA are neat polymers that do not contain any filler added. Properties of PLA films are shown as reference values from Jonoobi et al. (2010).

Differences in tensile properties among the three polymers were attributed to their different molecular weight (MW) and chemical structure. The relation between mechanical properties and MW is affected by structural and external factors like chain orientation, crystallinity, and morphology. Therefore, the specific effects of MW can be determined only if all other variables are held constant (Nguyen and Kausch, 1999). Nguyen and Kausch (1999) compared mechanical properties of very low MW parafines with the mechanical properties of higher MW polyethylene (PE). They stated that in higher MW PE, a high number of Van de Waals interactions per chain could effectively immobilize the macromolecules in an entangled network; while paraffin molecules may easily slip past each other under stress. They also stated that E increased with MW in low MW regions and E tended to decrease in high MW regions due to misalignment of long polymer chains in the direction of the stress. KGM presents the highest E, and this could be attributed to its high MW. KGM has the highest MW (200 – 2000 kDa) of the group followed by zein which MW is 21-26 kDa and HPMC which MW is 17 kDa.

KGM presented the highest σ. The molecular structure of KGM polysaccharide chains can form bundles oriented alongside and create strong associations by inter-chain hydrogen bonds (Dave et al., 1998). Alkali treatment of KGM promoted deacetylation and increases polymer to polymer interaction (Dea et al., 1977; Brownsey et al., 1988). These interactions keep the polymer chains together while resisting higher σ. Zein films relatively low σ value was attributed to its morphology. This amphiphilic molecule is capable of forming layers by evaporation-induced self-assembly (Wang and Padua, 2012). These layers are probably not interconnected as KGM fibers are. HPMC presents a relative high σ due to its 3D network structure. When stress is applied, HPMC movable bonds are able to dissipate the energy and withstand higher σ. HPMC bonds change in conformation while stretching, so γ is the largest. KGM fibers are able to elongate before breaking because they are entangled. The observed brittleness of zein films was attributed to strong intermolecular forces in zein films (Xu et al., 2012). Their ductility is limited possibly due to the inability of the layered structure to dissipate energy.

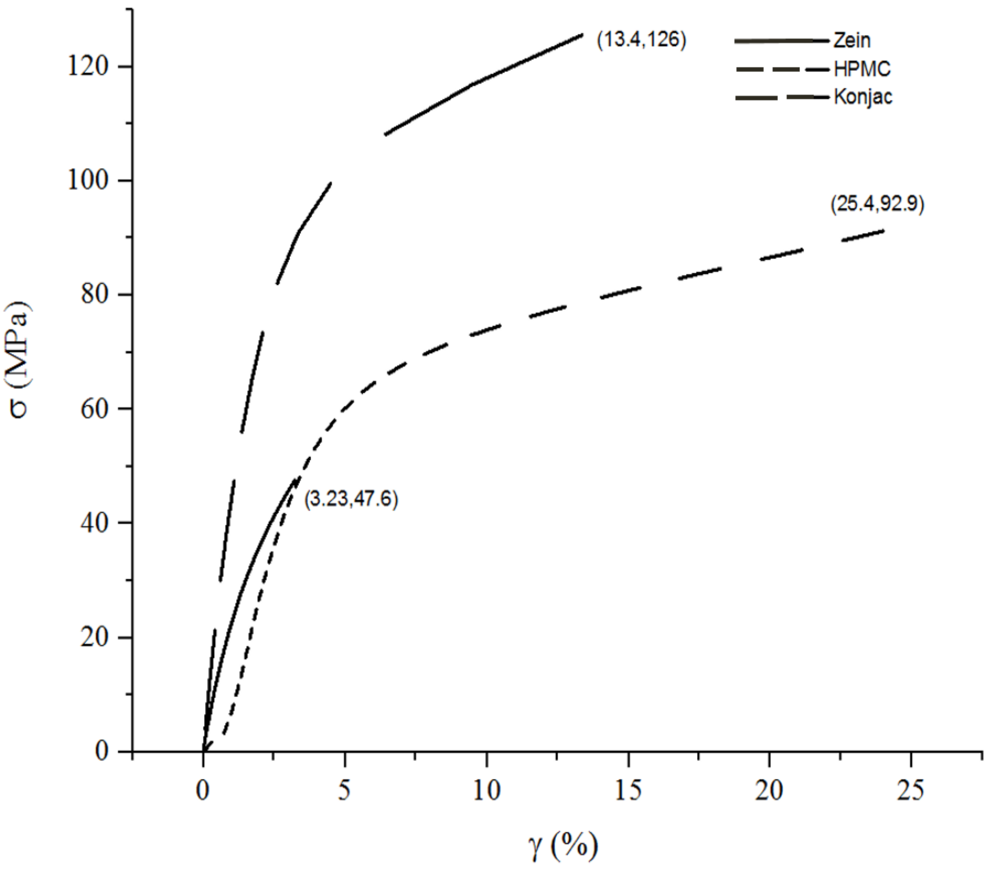

Figure 3.2. Stress-strain curves for a single strip of KGM, HPMC and zein films. These three curves represent the stiffness of KGM, the plasticity of HPMC, and the brittleness of zein.

The stress-strain curves shown in Figure 3.2 are consistent with the brittle behavior of zein films and the plastic deformation of HPMC films. KGM showed plastic deformation though not as pronounced as HPMC, which elongates considerably farther before failure. KGM was identified as the stiffest material, HPMC as plastic, and zein as brittle.

3.3.2 Effect of filler

The effect of added fillers, microcrystalline cellulose (MCC), cellulose nanocrystals (CNC), $CaCO_3$ nanoparticles, hydrophilic bentonite nanoclay, and hydrophobic nanoclays at 2% w/w to zein, HPMC and KGM films is shown in Table 3.3.

Size, aspect ratio, and surface chemistry are filler characteristics that are important for dispersion and interaction of filler with the matrix. Dispersion and interaction have a critical effect on composite characteristics.

Hydrophilic nanoclay significantly increased σ of KGM and HPMC films. Ahmed and Jones (1990) discussed that mechanical properties of composites could be affected by filler size, shape, and aspect ratio. Reducing filler size tends to enhance the strength of the composite (Ozsoy et al., 2015) (Lee et al., 2015). Layered, plated, or irregular geometries confer better mechanical properties than spherical, cubical or acicular ones (Merinska et al., 2010; Idrus et al., 2010). Mechanical properties are also affected by filler's degree of dispersion and interactions with the matrix (Haafiz et al., 2015). The increased σ of KGM and HPMC films by hydrophilic nanoclays was attributed to its small particle size, large aspect ratio and good compatibility with the polymers. Nanoclays plates of high aspect ratio and at least one nano-size dimension, maximized surface area to interact with the polymer matrix (Idrus et al., 2010). Eng et al. (2013) discussed the influence of hydrogen bonding between hydrophilic clays and PLA/PCL blends, which promoted polymer-filler interactions and enhanced mechanical properties in polymers. Hydrophilic nanoclay also increased σ of HPMC films, where good compatibility was also expected. The hydrophilic nanoclay also increased γ of KGM. Idrus et al. (2010) reported an increment in γ with the addition of micro-size silica to SMR L-grade natural rubber. They attributed it to high adhesion of filler to the matrix. Increased γ of KGM and HPMC films resulted in no net effect on E for either polymer. Hydrophilic nanoclay did not affect the properties of zein films, possibly due to the marked hydrophobic character of zein.

CNC increased σ of KGM and zein films, which was attributed to CNC nanoscale particle size and high aspect ratio (Murphy, 1998; Vallittu, 2015). Also, CNC's hydroxyl groups may have contributed to good dispersibility and polymer interaction (Haafiz, 2015). An indication of this interaction was the gelation observed during sonication of zein-CNC solutions. Hydrogen bonds between CNC and zein may have been formed during the sonication treatment strengthening zein microstructure to the point of making it solid-like. CNC increased E of zein films, which was also attributed to CNC nanoscale size, high aspect ratio, and surface hydroxyl groups. CNC did not affect the properties of HPMC nor γ of KGM and zein films. CNC has low aspect ratio compared with hydrophilic nanoclay, which may have led to lower polymer-matrix interactions with HPMC.

$CaCO_3$ is reported difficult to disperse in polymer matrices attributed to poor surface interactions resulting from its low aspect ratio (Gao et al., 2009). But in this work, $CaCO_3$ nanoparticles increased σ of KGM. This may be due to KGM's fibrous microstructure where $CaCO_3$ possibly filled the gaps between fibers leading to a stiffer and stronger matrix. $CaCO_3$ increased E of zein consistent with the observation that σ and γ decreased and E increased by the addition of $CaCO_3$ to PS (Gao et al., 2009). They explained that the rigid nanoparticles stiffened the matrix. $CaCO_3$ reduced γ of HPMC also possibly due to stiffening of the matrix. Vallittu (2015) discussed that cubic hydroxyapatite was effective at improving compression properties of materials. Its morphology performs better under compression than under tension forces.

Hydrophobic nanoclays are bentonite plates coated with a hydrophobic layer. Hydrophobic nanoclay significantly increased E of HPMC and decreased γ of zein and HPMC, but did not affect the properties of KGM. This may reflect a better interaction of the hydrophobic nanoclay surfaces with the least hydrophilic polymers.

MCC significantly decreased γ of HPMC films. Haafiz et al. (2015) attributed low γ values in polylactic acid composites to MCC aggregation by Van Der Waal's forces. Filler-filler interactions were stronger than filler-polymer ones. Poor interaction with the polymer lead to low σ (Qu et al., 2010).

Hydrophilic nanoclay and CNC were effective fillers. Hydrophilic nanoclay increased σ of KGM and HPMC. CNC increased σ for KGM and zein. Their performance was attributed to their hydrophilic surface, which facilitated dispersion. Nano-size, flat or elongated shape, and large aspect ratio enhanced filler-matrix interaction.

Table 3.3. Effect of filler on tensile properties of biopolymer films.

Mean		**Neat**	**MCC**	**CNC**	**$CaCO_3$**	**Hydrophilic clay**	**Hydrophobic clay**
KGM	σ (MPa)	119.96 ± 10.68^{b}	113.33 ± 7.87^{b}	130.88 ± 14.32^{a}	137.00 ± 6.96^{a}	140.00 ± 10.13^{a}	115.86 ± 17.76^{b}
	γ (%)	15.74 ± 6.09^{b}	12.83 ± 4.87^{b}	12.81 ± 4.55^{b}	18.67 ± 5.79^{ab}	25.50 ± 8.10^{a}	14.85 ± 7.01^{b}
	E (GPa)	4.57 ± 0.71^{a}	4.51 ± 0.30^{a}	5.01 ± 0.66^{a}	4.74 ± 0.53^{a}	4.10 ± 0.66^{a}	4.28 ± 0.46^{a}
HPMC	σ (MPa)	88.81 ± 14.26^{bc}	78.00 ± 5.87^{c}	99.82 ± 7.55^{ab}	82.53 ± 9.01^{c}	105.75 ± 8.84^{a}	80.75 ± 16.14^{c}
	γ (%)	38.64 ± 8.96^{a}	24.33 ± 8.90^{b}	42.65 ± 7.85^{a}	26.69 ± 9.28^{b}	43.67 ± 8.86^{a}	27.32 ± 7.38^{b}
	E (GPa)	1.67 ± 0.28^{b}	1.75 ± 0.33^{b}	1.86 ± 0.23^{ab}	1.86 ± 0.22^{ab}	1.95 ± 0.20^{ab}	2.08 ± 0.39^{a}
ZEIN	σ (MPa)	49.73 ± 9.72^{bc}	58.10 ± 8.53^{ab}	63.17 ± 9.39^{a}	51.56 ± 11.30^{abc}	59.60 ± 10.75^{ab}	40.20 ± 5.73^{c}
	Γ (%)	4.23 ± 1.57^{a}	4.21 ± 1.59^{a}	3.94 ± 0.98^{ab}	2.86 ± 0.77^{ab}	4.09 ± 1.4^{ab}	2.48 ± 0.79^{b}
	E (GPa)	2.10 ± 0.38^{b}	2.47 ± 0.27^{ab}	2.54 ± 0.33^{a}	2.64 ± 0.42^{a}	2.53 ± 0.35^{ab}	2.35 ± 0.38^{ab}

Different superscript letters (a, b, c, d) in the same row indicate significant differences ($P<0.05$).

Figure 3.3 shows the effect of filler on σ, γ, and E, over the three polymers studied. The plots suggested that each filler acts in a similar way across the polymers and that hydrophilic nanoclay and CNN were more effective than MCC $CaCO_3$, or hydrophobic nanoclay.

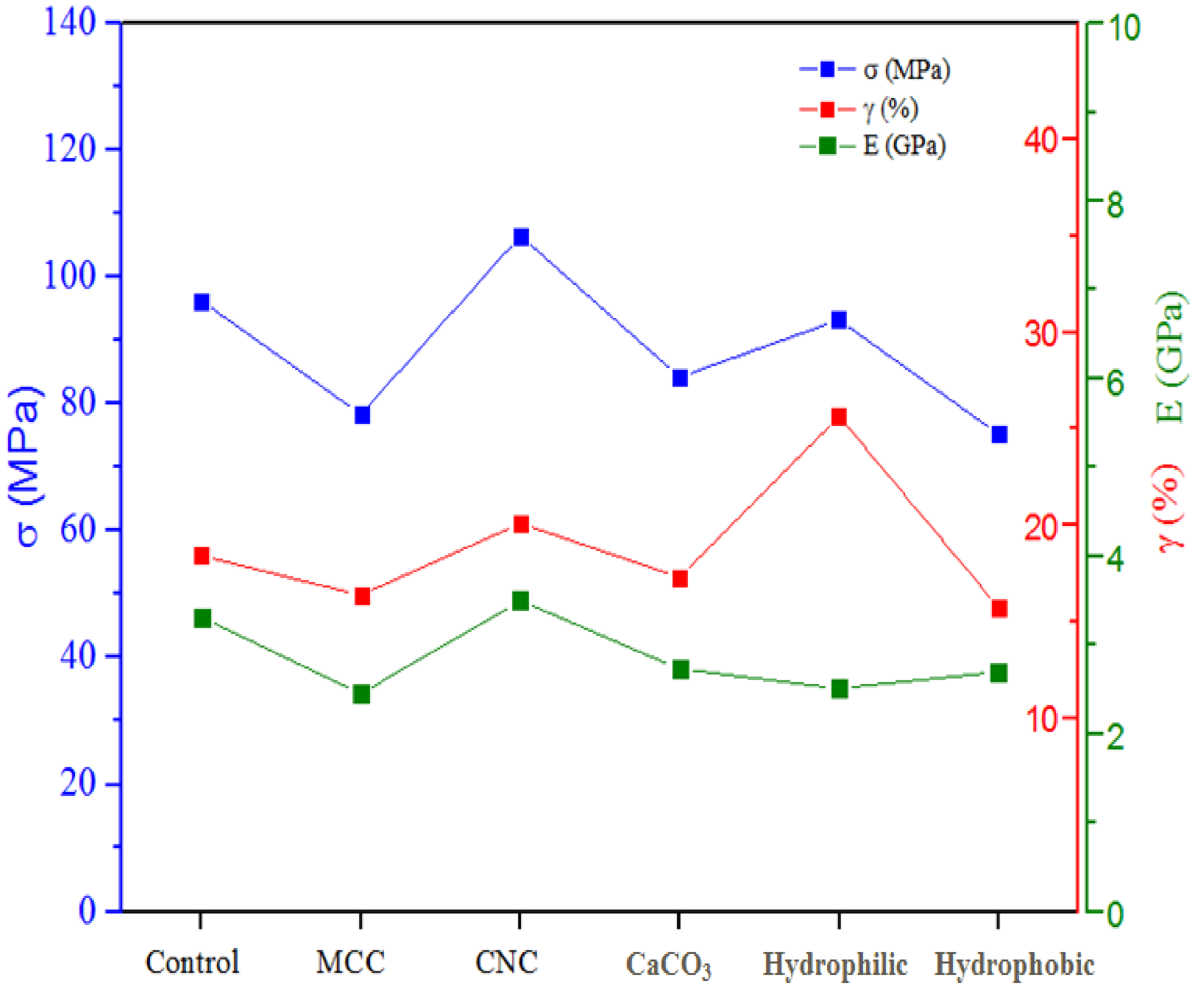

Figure 3.3. Effect of filler on tensile properties of KGM, HPMC, and zein grouped together.

3.3.3 Film morphology

SEM images of freeze-fractured cross sections of zein, HPMC, and KGM films are shown in Figure 3.4. SEM images of films containing 2% w/w fillers are also shown in Figure 3.4. KGM neat polymer film showed a structured lamellar cross section with gaps in between lamellae. The addition of CNC, $CaCO_3$, and hydrophilic nanoclay possibly contributed to fill in the gaps. In contrast, the HPMC neat polymer film showed a uniform surface. The addition of CNC and hydrophilic nanoclay to HPMC increased surface roughness. The addition of MCC did not modify the cross section of HPMC. Zein neat polymer film showed a smooth and homogeneous clean surface, consistent with brittle behavior. The addition of CNC and hydrophilic nanoclay resulted in visible stress lines from the freeze fracture. MCC did not to change the structure profile.

Nano-fillers changed the texture of neat polymer films, whereas the micro-filler did not show an effect. The high aspect ratio fillers, hydrophilic nanoclay and CNC, modified the surface of all three polymers, zein became glassier, HPMC became crumbly, and KGM became more compact thant the corresponding neat polymers. $CaCO_3$, MCC, and hydrophobic nanoclay showed limited effect on films cross- section surfaces, which was attributed to poor dispersion.

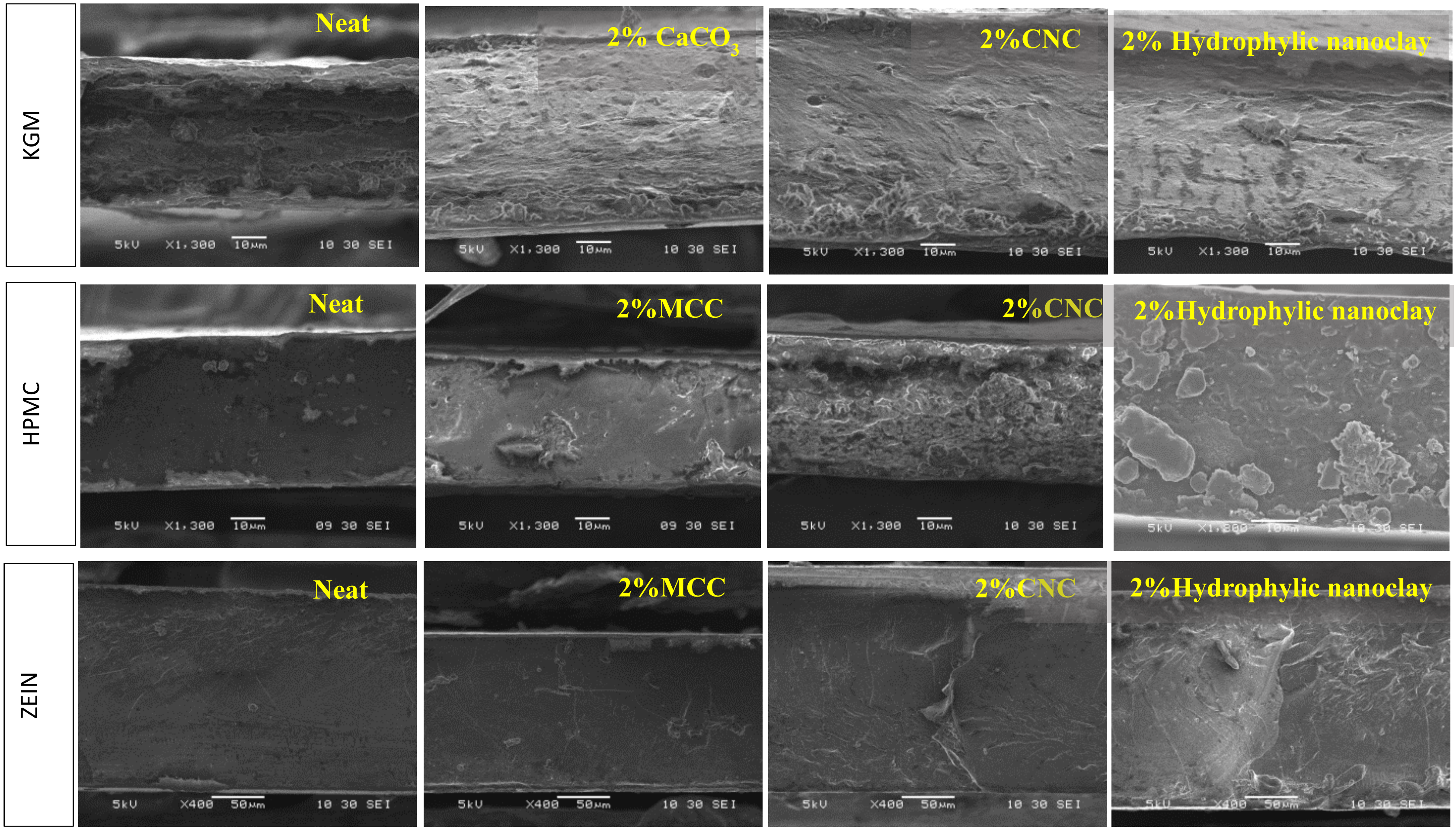

Figure 3.4. SEM images of freeze-fractured cross sections of KGM, HPMC, and neat zein polymers and SEM images of freeze-fractured cross sections of KGM, HPMC, and zein when 2% w/w of filler is added.

3.3.4 Effect of weight fraction

The effect of filler load on tensile properties of polymers has also been reported. The addition of fillers had a positive impact on mechanical properties of polymer films at weight fractions up to 5% (Rhim et al., 2013; Othman, 2014). Ozsoy et al. (2015) studied the influence of different loads of micro- and nano- fillers Al_2O_3 and TiO_2 on epoxy composites. They reported that σ and γ values of composites decreased while E increased with the increased load of both micro- and nano- fillers. Idrus et al. (2010) studied the effect of different shapes of silica as a filler in natural rubber compounds. They tested cubes, elongated, and irregular shapes from 10 to 40 parts per hundred of rubber. They concluded that elongated shapes had higher maximum torque compared to other shapes. Irregular shaped fillers presented the highest σ, γ, and hardness compared to cubical and elongated shapes. They attributed it to better filler-matrix interaction. Imoisili et al. (2013) studied cocoa pod-filled epoxy composites with 5 wt% to 30 wt% filler ratios. They reported decreasing mechanical strength values, while modulus and hardness increased when filler was added. They observed better filler dispersion at 5 wt% filler ratio (Ozsoy et al., 2015).

3.3.4.1 Effect of weight fraction in Konjac

The effect of weight fraction of 0%, 2%, 4%, 8%, 16%, 32%, 50%, 75%, and 100% of CNC filler on σ, γ, and E of KGM films is shown on Table 3.4. CNC added to KGM at 4% w/w increased film σ. However, σ began to decrease after that point. The γ decreased continuously through the weight fraction range, rapidly until 16% w/w filler load, slowing down afterward. E increased until 50% filler load, decreasing slowly afterward.

Table 3.4. Effect of weight fraction of CNC on tensile parameters of KGM films.

Tensile parameters	CNC weight fractions								
	0%	2%	4%	8%	16%	32%	50%	75%	100%
σ (MPa) F(8,142)=29.66; P=0 ***	120^{bc}	131^{ab}	144^{a}	120^{bcd}	111^{cde}	100^{e}	103^{de}	103^{de}	68^{f}
γ (%) F(8,142)=31.81 ; P=0 ***	16^{a}	12^{a}	12^{a}	6^{b}	6^{b}	4^{b}	3^{b}	3^{b}	2^{b}
E (GPa) F(8,142)=11.89; P=0 ***	4.6^{e}	5.0^{de}	6.1^{bcd}	6.0^{bcd}	5.9^{bcd}	6.3^{bc}	8.1^{a}	7.5^{ab}	6.7^{ab}

Gao et al. (2009) reported that E increased when increasing loads of $CaCO_3$ were added to PS. This was considered an effect of the filler. The behavior of E was described as a function of nano-filler volume fraction following $E_c = E_m \phi_m + E_f \phi_f$, where ϕ is volume fraction and the subscripts *c*, *m*, and *f* correspond to composite, matrix, and filler, respectively. Thus, homogeneously distributed nano-fillers lead to significant improvements in E. In this study, E maximum was observed at 50% filler, but decreasing afterward, suggesting that the high filler mass fraction controlled the film stiffness after 50% load. The shape of the functions σ, γ, and E vs. filler load is shown in Figure 3.5.

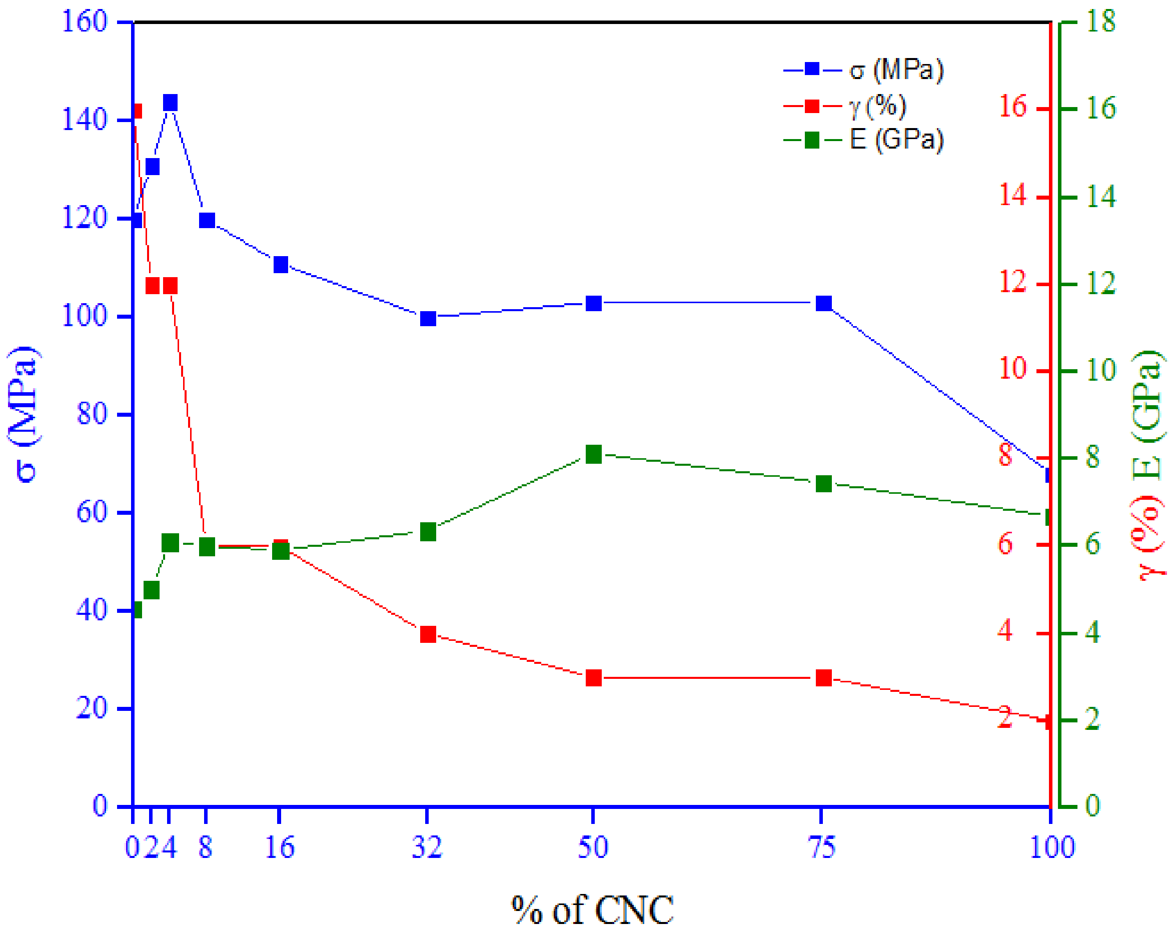

Figure 3.5. Effect of CNC weight fraction on tensile parameters of KGM films

SEM images of freeze-fractured cross sections of KGM films containing different weight fractions of CNC are shown in Figure 3.6. CNC addition to KGM matrix results in a change of KGM neat polymer.

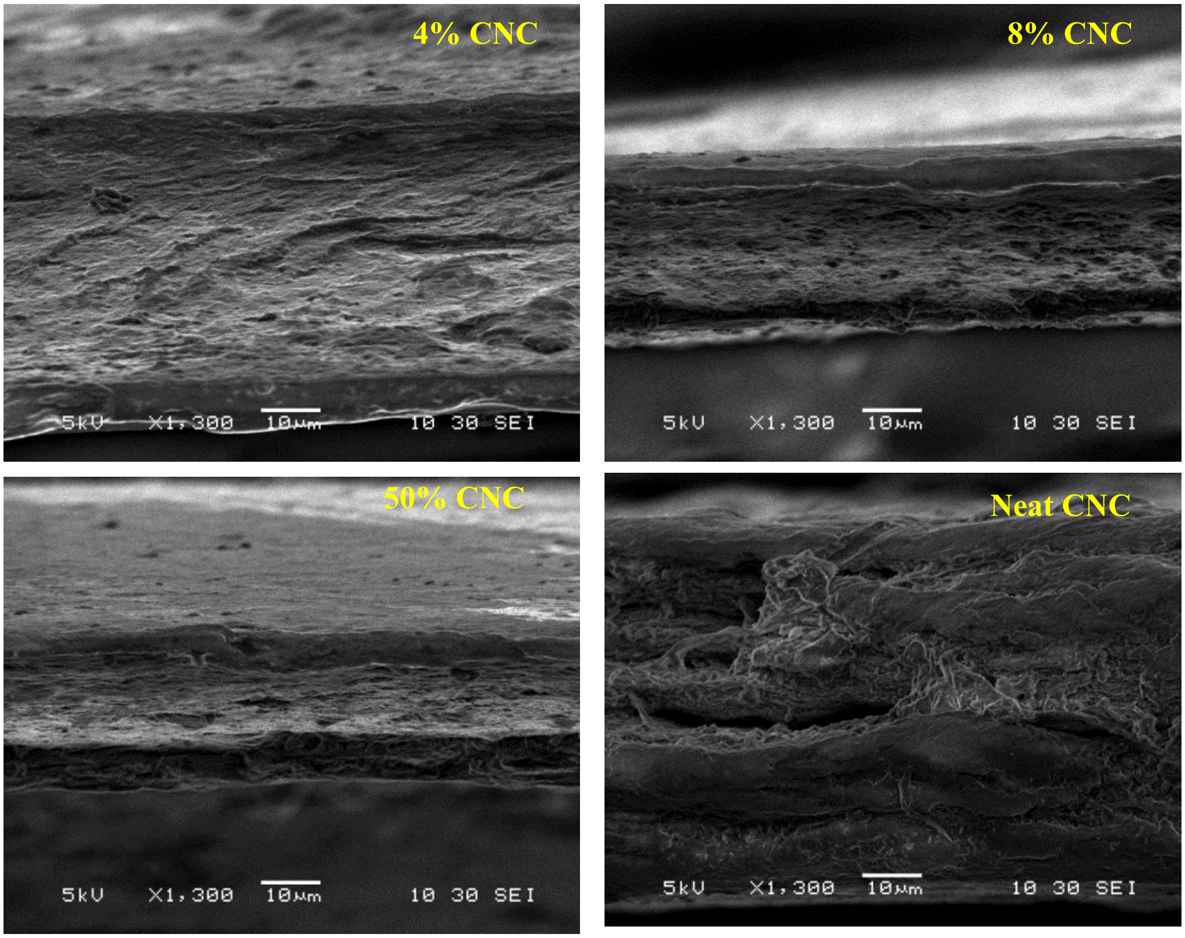

Figure 3.6. SEM images of weight fraction addition to KGM. SEM image of 100% CNC composite.

3.3.4.2 Effect of weight fraction in HPMC

In this study, CNC was added to HPMC at different weight fraction loads: 0%, 2%, 4%, 8%, 16%, 32%, 50%, 75%, and 100% (Table 3.5). After 8% load, films were not homogeneous enough to be measured due to the presence of multiple bubbles trapped in the film. These bubbles were generated during sonication and trapped because CNC thickens HPMC. Consequently, only data up to 8% was collected.

Table 3.5. Effect of weight fraction of CNC on tensile parameters of HPMC film. Additions above 8% were not homogenous; consequently, data was not collected.

Tensile parameters	CNC weight fractions								
	0%	2%	4%	8%	16%	32%	50%	75%	100%
σ (MPa)	89	100	71	52	-	-	-	-	68
γ (%)	39	43	28	13	-	-	-	-	2
E (GPa)	1.7	1.9	2.0	1.9	-	-	-	-	6.2

CNC added to HPMC at 2% w/w increased film σ and γ. However, both σ and γ began to decrease after that point. E smoothly increased with the filler load. HPMC is a 3D network where the integration of any filler in this polymeric structure is less accessible. The maximum σ point reached was at 2% of the load. The γ of the composite at 2% load was higher than that of the neat polymer, as discussed in Section 3.3.2. The behavior of E was expected to follow the same smooth increment as for KGM. Higher content of filler leads to higher stiffness due to the addition of a stiffer material. In this study, E slowly increased with load additions but was expected to decrease afterward. The shape of the functions σ, γ, and E vs. filler load is shown in Figure 3.7.

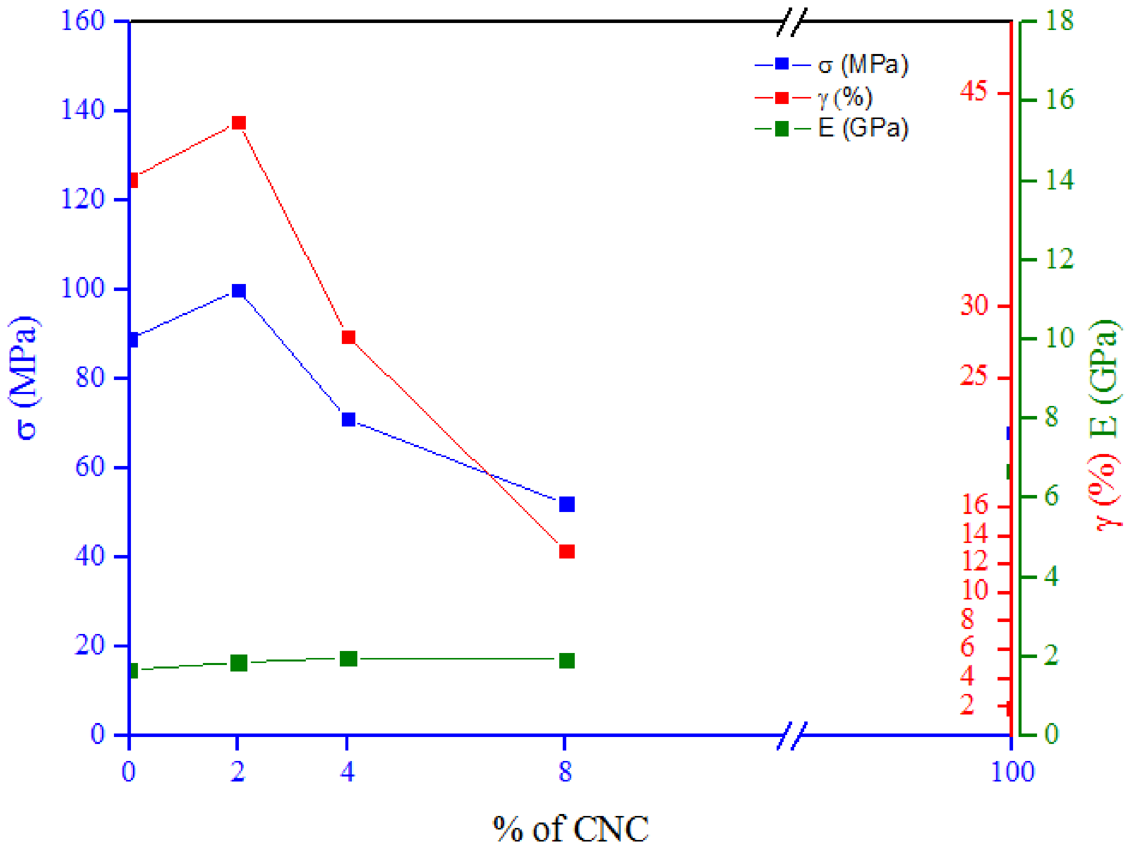

Figure 3.7. Effect of weight fraction of CNC on tensile parameters of HPMC film. Additions above 8% were not homogenous; consequently, data was not collected.

SEM image of freeze-fractured cross sections of HPMC film containing 8% CNC are shown in Figure 3.8. The addition of 8% w/w CNC also changes the profile of neat HPMC in a homogeneous way.

3.4 Conclusions

Tensile properties of composite films formed by the polymers KGM, HPMC, and zein and the fillers MCC, CNC, $CaCO_3$ nanoparticles, hydrophilic nanoclay, and hydrophobic nanoclay were studied by DMA and SEM. Differences in tensile properties were found between the three polymers, owed to differences in their chemical structure and MW. The fillers, which were different in size, shape, aspect ratio, and surface chemistry, showed different degrees of dispersion and interaction with the matrices, leading to specific results, case by case. On size, nano-sized CNC increased σ of KGM and HPMC neat polymers over microsized MCC. On surface chemistry, hydrophilic nanoclay increased σ of KGM, HPMC, and zein over hydrophobic nanoclay clay. Hydrophilic nanoclay clay also resulted in higher γ than hydrophobic nanoclay clay for KGM, HPMC, although not significantly for zein. On morphology, high aspect ratio hydrophilic nanoclay increased σ over low aspect ratio $CaCO_3$ for HPMC.

Load weight fraction of CNC filler affected mechanical properties of KGM and HPMC. Differences in load limits for KGM and HPMC were found. For HPMC, CNC addition rates up to 2% increased the σ. For KGM, CNC can be added up to 4% to increase the σ. These differences were attributed to differences in microstructure of these two polymers. KGM fibers were possibly able to accommodate larger amounts of filler. HPMC 3D network adsorbed a 2% of load of filler. E smoothly increased with the load regardless of the structure of the film. E was expected to decrease beyond 50% load in any film due to the high filler mass fraction controlled film stiffness.

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