UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

The incorporation of phenolic compounds into biopolymers as food packaging material can improve the functionality of packaging to maintain the quality of food during storage. This work aimed to improve the performance of corn starch/chitosan nanoparticles (CS/CNP) films on the extension of shelf life of cherry tomatoes by the addition of thymol at different concentrations (0, 1.5, 3.0, 4.5 w/w%). The resulting films were characterized in terms of optical, mechanical, and barrier properties using color spectrophotometer, texture analyzer (TA), and dry cup method, respectively. In terms of optical properties, only slight changes were observed on the total color changes and opacity of the CS/CNP films with the addition of thymol. Mechanical properties analysis revealed that the addition of thymol reduced the tensile strength (TS) of the films but the increment in the concentration of thymol did not significantly affected the TS of the films. The addition of thymol to CS/CNP films generally decreased slightly the elongation at break values but the decrement was pronounced for Young's modulus values. It was also found that the addition of 1.5 w/w% thymol increased the WVP of the films but the WVP reduced with the increase in thymol concentrations. Meanwhile, CS/CNP films incorporated with 3 w/w% thymol was found to be able to lengthen the shelf life of cherry tomatoes packed with the films whereby no significant changes for firmness and the lowest weight loss were found for cherry tomatoes packed with the films. Based on the observation, no mold growth occurred on the cherry tomatoes that were in direct contact with the 3 w/w% CS/CNP/Thy films during 7 days of storage period at ambient temperature. The CS/CNP/Thy films produced in this study were

demonstrated to have the potential to be used as active food packaging materials that can lengthen the shelf life of food products.

ABSTRAK

Penggabungan sebatian fenolik ke dalam biopolimer sebagai bahan pembungkusan makanan dapat meningkatkan fungsi pembungkusan untuk menjaga kualiti makanan semasa penyimpanan. Karya ini bertujuan untuk meningkatkan prestasi filem nanopartikel pati jagung / kitosan (CS / CNP) mengenai pemanjangan jangka hayat tomato ceri dengan penambahan timol pada kepekatan yang berbeza (0, 1.5, 3.0, 4.5 w/w%). Filem yang dihasilkan dicirikan daripada segi optik, mekanikal, dan sifat penghalang menggunakan spektrofotometer warna, penganalisis tekstur (TA), dan kaedah cawan kering (WVP). Dari segi sifat optik, hanya sedikit perubahan yang diperhatikan pada jumlah perubahan warna dan kelegapan filem CS / CNP dengan penambahan timol. Analisis sifat mekanikal menunjukkan bahawa penambahan timol mengurangkan kekuatan tegangan (TS) filem tetapi kenaikan kepekatan timol tidak mempengaruhi kekuatan tegangan filem secara signifikan. Penambahan timol ke filem CS / CNP secara amnya menurun sedikit pemanjangan pada nilai pecah tetapi penurunan tersebut diucapkan untuk nilai modulus Young (YM). Juga didapati bahawa penambahan 1.5 w/w% thymol meningkatkan kebolehtelapan wap air filem tetapi kebolehtelapan wap air berkurang dengan peningkatan kepekatan timol. Sementara itu, filem CS / CNP yang digabungkan dengan 3 w/w% timol didapati dapat memanjangkan jangka hayat tomato ceri yang dibungkus dengan filem di mana tidak ada perubahan ketara untuk kepejalan dan penurunan berat jisim paling rendah didapati untuk tomato ceri yang dibungkus dengan filem tersebut. Berdasarkan pemerhatian, tidak ada pertumbuhan kulat pada tomato ceri yang bersentuhan langsung dengan filem CS / CNP / Thy 3 w/w% selama 7 hari tempoh penyimpanan pada suhu persekitaran. Filem CS /

CNP / Thy yang dihasilkan dalam kajian ini terbukti berpotensi untuk digunakan sebagai bahan pembungkus makanan aktif yang dapat memanjangkan jangka hayat produk makanan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC	- Active packaging
AM	- Antimicrobial
AO	- Antioxidant
ASTM	- American standard testing material
CH	- Chitosan
CNP	- Chitosan nanoparticles
CTNP	- Chitosan thymol nanoparticles
CS	- Corn Starch
EAB	- Elongation at break
EOs	- Essential Oil
HEC	- Hydroxyethyl cellulose
PPI	- Peanut protein isolate
PBSA	- Poly(butylene-succinate-co-adipate)
PHA	- Polyhroxy-alkanoates
PE	- Polyethylene
PET	- Polyethylene terephthalate
PLA	- Polylactic acid
PP	- polypropylene
PS	- Polystyrene
PVC	- Polyvinylchloride
TG	- Tara gum
TM	- Tensile Modulus
TS	- Tensile strength
TA	- Texture Analyzer

TPP	- Sodium tripolyphosphate
ΔE	- Total color difference
WVP	- Water vapor permeability
WVTR	- Water vapor transmission rate
YM	- Young's modulus

CHAPTER 1

INTRODUCTION

1.1 Background

Food packaging plays a vital role in the food industries whereby it is an essential medium to protect food from contamination and to prolong the shelf life in order to provide high-quality food products for the consumers. According to Prasad and Kochhar (2014), food packaging plays a significant role as a barrier against oxygen, water vapor, ultraviolet light, and both chemical and microbiological contamination. Plastic material is used extensively for food packaging due to the reasons of low cost, lightweight, and high performance that link with good processability (Sangroniz et al., 2019). However, common conventional plastic packaging materials are synthetic polymers which usually derived from non-renewable petroleum resources that exhibit non-degradable properties (Othman et al., 2019; Zhao et al., 2008). The excessive amount of synthetic plastic waste generated municipal solid waste that is dumped into landfills which can negatively affect environmental sustainability (Singh and Sharma, 2016). This issue can be overcome by finding an alternative food packaging material. The substitution of synthetic polymers with biopolymers as food packaging materials is one of the promising alternatives. Renewable biopolymers such as polysaccharides (e.g., starch, alginate, pectin, carrageenan, agar, chitosan, etc.), proteins (e.g., gluten, gelatin, casein, whey protein, etc.), polyesters (e.g., polylactic acid), and their composites, derived from plant and animal resources are being widely investigated because these biopolymers can be used to replace non-degradable petrochemical-based plastics (Mangiacapra et al., 2006; Rhim and Ng, 2007).

Biopolymer based films are typically formulated with natural biopolymers originating from polysaccharides, proteins, or natural gums and these elements are capable of forming a cohesive and continuous matrix (Rhim et al., 2007). Bonilla et al. (2013) stated that biodegradable polymer based on natural polysaccharides specifically starch is reliable for food packaging due to environmentally friendly, flexibility, transparency, thermoplastic properties, and low cost. In particular, the corn plant is the primary source of native starches that are commercially available, and nearly more than 85% of starch production in the world is extracted from corn tree (Ibrahim et al., 2019).

However, starch-based films have limitations due to brittleness and exhibit low mechanical properties such as low tensile strength besides exhibiting poor moisture barrier properties (Bangyekan et al., 2006; Ren et al., 2017). An alternative to overcome the brittleness of the film is by adding plasticizers (Bonilla et al., 2015). Generally, plasticizers that are commonly used for the preparation of starch films are water, glycerol, sorbitol, and other low molecular weight polyhydroxy compounds (Bangyekan et al., 2006). Bangyekan et al. (2006) reported that plasticizers made from glycerol and sorbitol are being widely used because of their stability and edibility. The addition of the plasticizers contributes to a more flexible film, but the films become less strong.

Meanwhile, a further improvement can be done by blending starch with other natural polymers, hydrophobic substances, and/or antimicrobial (AM) compounds such as chitosan (CH) which can establish the functional properties of the starch-based film (Anker et al., 2001; Ayranci and Tunc, 2003; Flores et al., 2007; Garcia et al., 2000). CH can be found mainly from shellfish processing waste (Ren et al., 2017). Derivation of CH from deacetylated chitin can be attained from the exoskeletons of crustaceans and mollusks (Jasour et al., 2015). Recently, CH has attracted the researchers for the applications in food preservations due to non-toxic, biodegradable, biofunctional, biocompatible, and AM properties. Subsequently, a good adherence between starch and CH can be attained with the combination of hydrogen bonding; opposite charge attraction between CH cations and negatively charged starch film surface; and hydrophilicity (Julia et al., 2018).

An advancement of nanotechnology through the incorporation of nano-sized fillers or nanofillers in food packaging can be further applied to improve the properties of biopolymers whereby the nano-sized fillers will act as reinforcing function and the active ingredient in the bionanocomposite (Jamróz et al., 2019). According to Othman (2014), bulk CH can be synthesized into nanoparticles, namely chitosan nanoparticles (CNP) that have dimensions ranging from 1 to 100 nm. CNP is a type of organic nanofiller that exhibits low toxicity, biocompatibility, biodegradability, and AM activity. Besides, the incorporation of nanofillers will enhance the mechanical, thermal, and barrier properties of the biopolymers (Othman, 2014). CNP can be formed by ionic gelation (De Moura et al., 2008). This method is based on the principle of ionic bonding between positively charged amine groups of chitosan and negatively charged groups of polyanion-like tripolyphosphate (TPP) (Bodmeier et al. 1989; Pan et al. 2002). Significantly, TPP is used because it is non-toxic, multivalent, and able to form gels through ionic interactions.

Apart from that, the incorporation of CH and AM agent such as thymol into a biopolymer film is considered as a novel technique which leads to the enhancement of the shelf life and improves the sensorial properties of the food product (Lekjing, 2016). The drawbacks of directly adding the AM to the food products are that it may lead to the decrement of the action of active compounds and the alteration of the organoleptic properties of the foods due to the complexity of food components and the strong flavor of some agents (Rawdkuen, 2018). Hence, an alternative technique to overcome this limitation is by incorporating the AM compounds into the packaging matrix. In this context, active packaging (AP) applies the use of either synthetic or natural AM agents to protect food products from deterioration and the growth of microorganisms (Juneja and Sofos, 2005). Nowadays, consumers are concerned about the health and prefer natural-occurring substances rather than synthetic additive because some synthetic additives can be carcinogenic and toxic. The incorporation of phenolic compounds into the food packaging as a natural AM agent tends to be a great potential to improve the shelf life of perishable food products.

Phenolic compounds, which are the natural bioactive compounds and plant secondary metabolites are referred to extracts derived from herbs and essential oil (EOs). They contain many natural compounds such as thymol, linalool, and carvacrol.

Thymol (2-isopropyl-5-methylphenol), a volatile substance is the main phenolic monoterpene found in the essential oils extracted from plants such as thyme species and oregano belongs to the Lamiaceae family (Marchese et al., 2016). However, thymol which is a hydrophobic phenolic compound has intense flavor and odor. Thus, the incorporation of thymol into starch film formulation will overcome the sensorial impact on the food product (Nordin et al., 2020). The effects of adding different concentrations of thymol on the films were interference in terms of mechanical properties, barrier properties, and optical properties. The main reason was the different concentrations of hydrophobic constituents added to the film matrix that will affect the hydrophilic/hydrophobic balance of the film (Zhong et al., 2017).

Meanwhile, the typical problem related to the majority of perishable food such as milk, eggs, and most fresh fruits and vegetables is microbial growth that affecting food quality, alters textural property, and adversely affects the color and nutrition of a food product. In this study, cherry tomato is chosen as the food for shelf-life study. According to Mohan et al. (2016), tomato (*Solanum Lycopersicum* Mill.), is one of the widely grown vegetable crops, the second most important source of nourishment after potatoes for the world's population with a total production of around 160 million tons per year. Besides, tomato is one of the perishable fruits, and the ripeness changes continuously after harvesting. The study on the performance of the CS film incorporated with CNP and thymol to preserve the cherry tomatoes at ambient temperature storage condition may be one of the ways in managing this type of issue.

To the best of knowledge, most of the studies in the literature reported the effect of thymol on starch films without the additional incorporation of nano-sized fillers such as CNP. For instance, the studies on CS films incorporated with two EOs that are *Zataria multiflora* Boiss (ZEO) or *Mentha pulegium* (MEO) (Ghasemlou et al., 2013), CS films incorporated with glycerol and thymol (Nordin et al., 2020), and potato starch film incorporated with polysorbate-thymol (Davoodi et al., 2017). In the present study, CS films with the addition of thymol and CNP (CS/CNP/Thy films) were prepared via a solution casting method. The effects of thymol concentration on the optical, mechanical, and barrier properties of CS/CNP/Thy films were characterized. The application of the films was demonstrated on the shelf life of cherry tomatoes packed with the films in terms of firmness, weight loss, and observation of mold growth.

1.2 Problem Statement

Accumulation of conventional plastic waste from food packaging has led to a global environmental problem but this issue can be overcome by developing biopolymer-based food packaging material. Despite being environmentally friendly packaging, biopolymer-based packaging such as those made from starch has some drawbacks such as it exhibits poor mechanical and barrier properties due to its natural hydrophilic properties and porous structure of the starch matrix. This will limit the application of biopolymer-based food packaging. However, the incorporation of nano-sized fillers such as CNP can improve this limitation. CNP can act as the reinforcing agent and promote a good interfacial interaction with the biopolymer matrix. Besides, CNP exhibits good antimicrobial properties and has been used in food packaging and also coating.

Perishable foods such as tropical fruits, vegetables, meat, fish, and eggs have a short shelf-life which can affect the quality of the food. The use of AM agents has the potential to control this problem that usually occurs during storage. Despite that, there is a growing concern regarding the safety of synthetic additives. Alternatively, natural AM agents that have attracted much attention to food and packaging industries due to their potential action in food preservation besides being safe. Thymol is a natural phenolic compound derived from thyme oil and it is an effective antimicrobial agent against a wide variety of microorganisms. Thus, thymol can be used as food preservatives when added in small amounts which can delay microbiological contamination and food deterioration.

Nevertheless, thymol exhibits strong odor and volatility, so when it is added directly to the foods, their activity may be inhibited by many substances in the food itself, which will reduce its effectiveness. An efficient alternative is by using thymol as an AM agent into films than adding directly to the food because thymol can selectively and gradually migrate from the package onto the surface of the food. Hence, the incorporation of CNP and thymol into the starch films will exhibit AM properties and helps to control the deterioration of food products during the storage period. However, the properties of the films which are physical, mechanical, and AM are very much affected by the concentration of the thymol added into the films. Thus, it is important to investigate the effects of thymol concentrations on the properties of the CS/CNP/Thy films.

To the best of knowledge, no work has been done to produce CS/CNP/Thy films. Therefore, this study is directed towards developing and characterizing CS/CNP/Thy films, which is a novel formulation of film. Moreover, no work has been done to investigate the potential application of the CS/CNP/Thy films to be used as food packaging in the context of the shelf life of food packed with films and stored at ambient temperature. In this work, the real application of the CS/CNP/Thy films was demonstrated on the cherry tomatoes and the physical quality which includes firmness and weight loss, as well as mold growth on cherry tomatoes were evaluated during 6 days of storage period at ambient temperature.

1.3 Objectives

This thesis reports a comprehensive study of the preparation, characterization, and application of starch/CNP/Thy films. There are two primary objectives of the present work:

1. To produce and characterize optical (color, opacity), mechanical (tensile strength, elongation at break, Young's Modulus), and barrier properties (water vapor permeability) of CS/CNP films incorporated with different concentrations of thymol (0, 1.5, 3, and 4.5 w/w%).

2. To demonstrate the application of the CS/CNP/Thy films by investigating the shelf life (firmness, weight loss, and observation of mold growth) of cherry tomatoes packed with the films and stored at ambient temperature for 6 days.

1.4 Scope of the Work

The scope of work for the first objective was directed to produce CS/CNP films incorporated with different concentrations of thymol (0, 1.5, 3, and 4.5 w/w%) via a solvent casting method. The effects of different concentrations of thymol addition on the properties of the films were investigated in terms of color (color spectrophotometer), mechanical (texture analyzer (TA)), and barrier (water vapor permeability cup) properties.

The scope of work for the second objective was directed towards determining the shelf life of the cherry tomatoes packed with the films (CS, and CS/CNP/Thy films) stored at ambient temperature for 6 days. The quality of the cherry tomatoes was investigated through the analysis of firmness (TA) and weight loss of the cherry tomatoes. The mold growth of the cherry tomatoes was also observed throughout the storage period of 6 days.

1.5 Research Contributions

This work illustrates the continuing improvement on the biopolymer film as food packaging material whereby in this work, CS films were incorporated with CNP and thymol. The incorporation of CNP into the biopolymer matrix particularly starch will contribute towards the improvement of the mechanical, barrier, and antimicrobial properties of the films. A suitable concentration of thymol that is incorporated into the starch films with the addition of nanofillers of CNP resulted in starch films that exhibit high potential and efficient to be used for food packaging applications. The application of CS/CNP/Thy films as food packaging can improve the food quality during the storage period and may be applied in industry. The application may also reduce the usage of non-degradable food packaging material, thus sustainable and safe for the environment.

CHAPTER 2

LITERATURE REVIEW

2.1 Food Packaging

There are various types of basic packaging that have been applied for food according to the food requirements such as paper and paperboard, plastic, glass, metal, and a combination of the materials of various chemical natures and physical structures. Among all, plastics made from polymers provide several unique advantages when used as packaging such as lightweight, easy to handle, high efficiency for storage, and cost-effective (Narayanan et al., 2017). As a food packaging, the main functions are to control food quality and safety during storage and transportation, preventing gain or loss of moisture, prevent bacteria growth, and act as a barrier against oxygen, water vapor, carbon dioxide, and volatile compounds as illustrated in Figure 2.1 (Rhim et al., 2013; Shapi'i and Othman, 2015). In achieving the functional properties of food packaging, it must act as a protective barrier to provide physical protection and create proper physicochemical conditions for food products. Thus, food quality and safety can be maintained and the shelf life of the food can be prolonged.

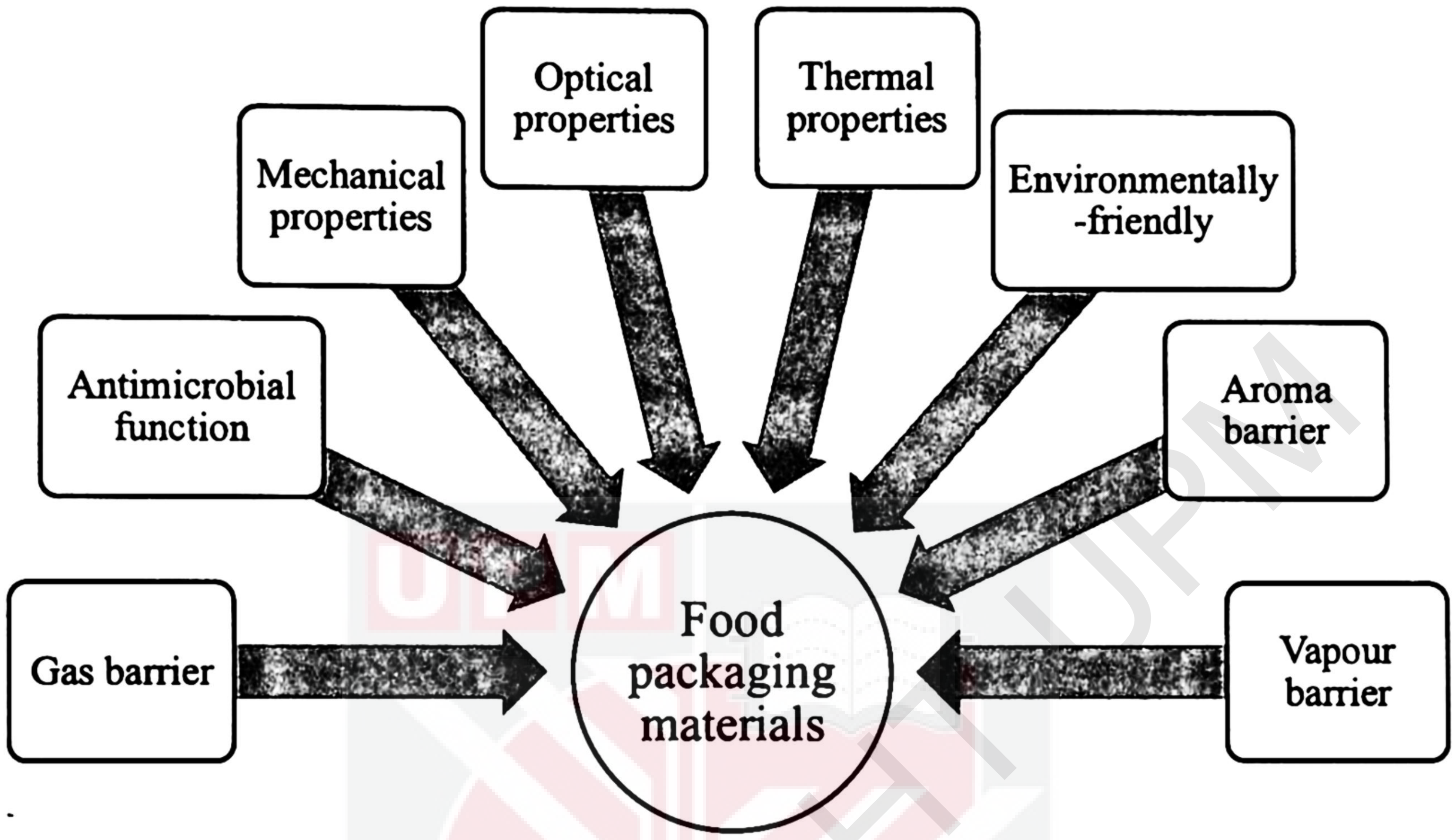


Figure 2.1 General properties required for food packaging materials (Source: Rhim et al., 2013)

However, conventional plastics that made from petroleum which is non-degradable, and some are difficult to recycle or reuse due to being complex composites that consist of varying levels of contamination (Song et al., 2009). The petrochemical-based plastics including polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyamide (PA) are widely used as packaging materials due to their large availability at relatively low cost; good mechanical properties like tensile and tear strength; good barrier to oxygen, carbon dioxide, anhydride and aroma compound; and good heat sealability (Siracusa and Dalla, 2008).

Though synthetic plastic packaging materials have been widely used for the packaging of various types of food, they have a negative impact on the environment since they cannot be easily degraded in the environment after use and they are non-totally recyclable and/or biodegradable (Siracusa and Dalla, 2008). In Asia, a report from World Wild Funds for Nature (WWF) in 2020 stated that the excessive plastics consumption from China, Indonesia, Malaysia, Philippines, Thailand, and Vietnam have contributed to 60% of the estimated 8 million tonnes of plastic that enter the world's oceans each year. Nowadays, the rise in single-use plastic and excessive discarding of the plastics has posed a major waste management challenge (Song et al., 2009) which causes many problems to ecological sustainability. On the contrary, only part of the plastics manufactured can be practically recovered, and not all plastics are suitable for recycling or re-use purposes (e.g., contamination of plastic by other materials, non-reshaped properties of thermosetting materials, and poor compatibility of co-blend) (Zhao et al., 2008). Hence, the amount of plastics disposed of has increased the volume of plastic wastes generated in the landfill. These problems can be overcome by finding alternatives to food packaging materials such as those made from biopolymers.

2.2 Biopolymers

Nowadays, consumers are concerned about the sustainability of the environment, and biopolymers are promising alternatives towards the usage of synthetic polymers. With regard to the environmental issue, excessive production of plastic made from non-degradable material can be reduced with the innovations of biopolymers as packaging materials. Biopolymers are polymers that contain monomeric units that are covalently bonded to form larger structures (Francis et al., 2013). Besides, biopolymers can be defined as 'biodegradable' by the enzymatic action of microbes, and it can break down naturally into organic components when exposed to the atmosphere, moisture, heat, and naturally occurring microorganisms (Mohan et al., 2017; Yadav et al., 2018). As illustrated in Figure 2.2, biopolymers-based packaging can be divided into four groups based on their origin and production. Bio-based polymers can be classified into two major categories which are natural bio-based polymers and synthetic bio-based polymers. The natural biopolymers are directly extracted or removed from biomass which constitutes of certain polysaccharides such as starch, cellulose, and proteins like whey protein and gluten (Yadav et al., 2018). On the other hand, synthetic biopolymers are derived from microbial production or fermentation (e.g. polyhydroxy-alkanoates (PHA) or through conventional and chemically synthesis either from biomass (e.g. polylactic acid (PLA) or petroleum (e.g. polycaprolactone (PCL)) (Othman, 2014).

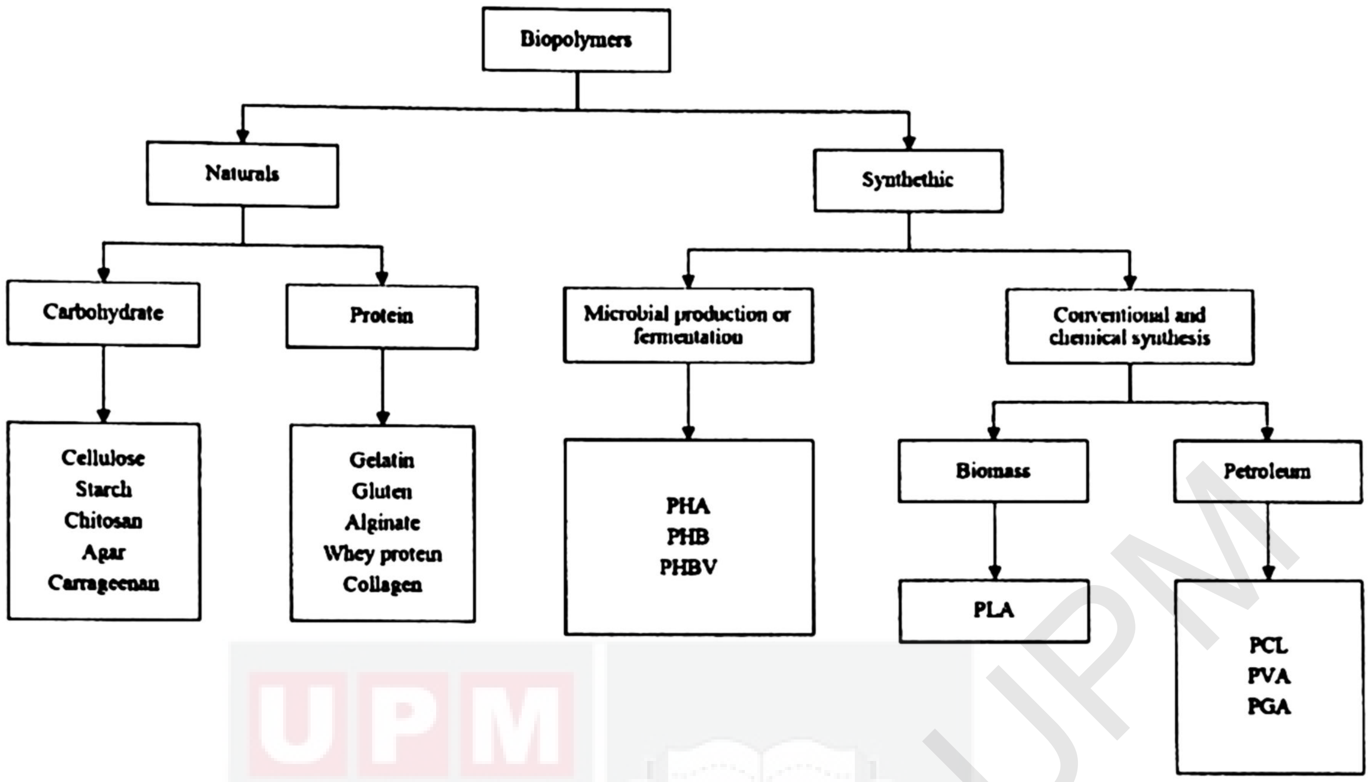


Figure 2.2 Classification of biopolymers

(Source: Othman, 2014)

Among the biopolymers, natural biopolymers are preferable due to environmentally friendly aspects and relatively abundant in nature. The most common type of natural biopolymer that has been studied to produce films for food packaging applications is starch and derivates (Othman, 2014). Starch can be extracted from potatoes, wheat, corn, or rice and is a well-known hydrocolloid biopolymer. Moreover, starch is easily available and is one of the cheapest groups of biodegradable polymers (Francis et al., 2013). Generally, starch consists of amylose (poly-α-1,4- d-glucopyranoside), a linear and crystalline polymer, and amylopectin (poly-α1,4-d-glucopyranoside and α-1,6-d-glucopyranoside), a branched and amorphous polymer. Figure 2.3 illustrates the structure of amylose and amylopectin which indicates linear and branched structure, respectively.

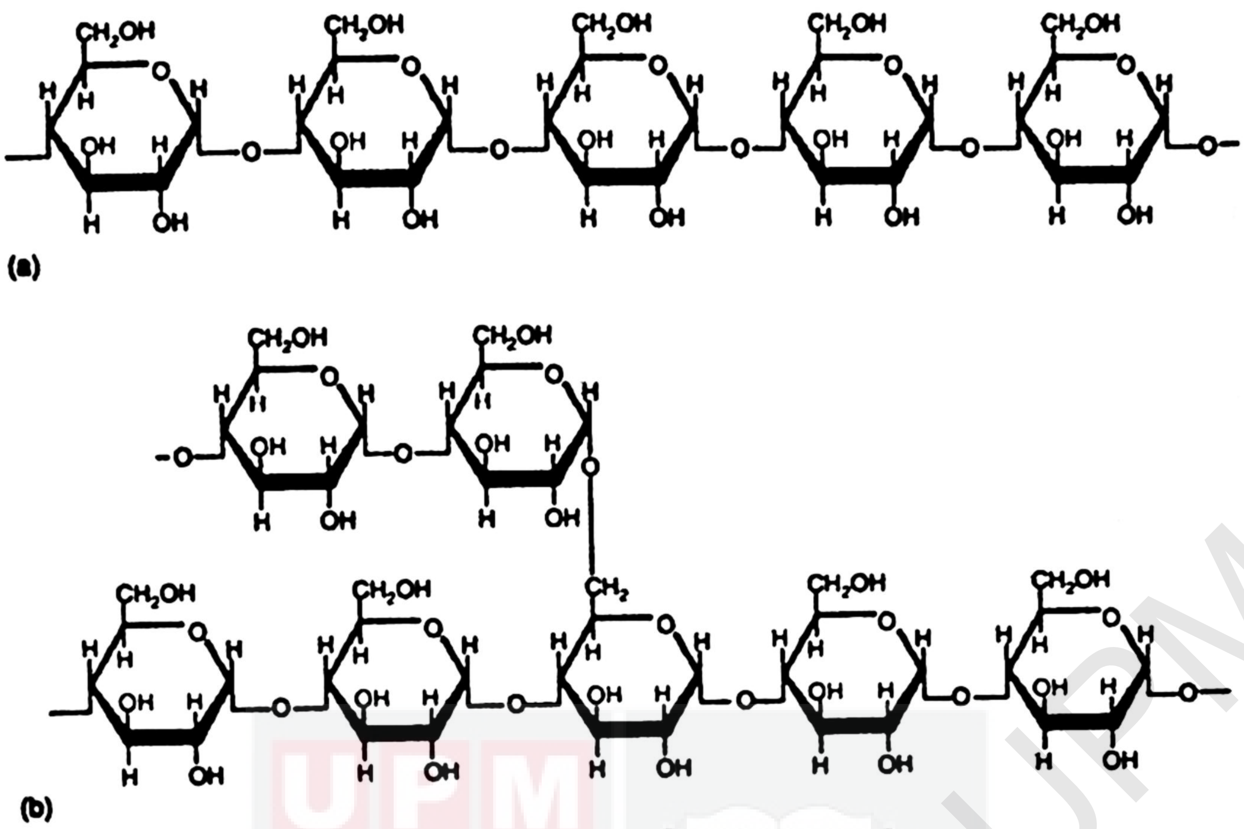


Figure 2.3 (a) Structure of amylose (b) Structure of amylopectin (Source: Francis et al., 2013)

Based on the previous study, the amylose content in relation to total starch content presented in CS is the highest which is 28–33% followed by wheat starch (30–32%), potato starch (18–20%), and cassava starch (16–19%) (Nordin et al., 2020). Besides, Domene-López et al. (2019) reported that the amylose content in CS was 24.8% which was higher compared to rice (16.9%), potato (20.5%), and wheat (24.5%). A high amylose corn starch contributed to the production of strong and flexible films due to amylose crystallization (Myllärinen et al., 2002). Thus, corn starch was utilized in this work due to high amylose content that exhibits excellent film-forming ability rendering strong, isotropic, odorless, tasteless, and colorless films (Campos et al., 2011).

However, the average chain lengths of CS are shorter than the single chain in linear amylose and the molecular size of amylose also varies between starches (Bertoft, 2017). By the addition of an adequate plasticizer, it is possible to obtain films with adequate mechanical properties, especially in films elaborated from high amylose starches (Campos et al., 2011). The main role of the plasticizers was to reduce the strong attraction of hydrogen bonds inside amylose and amylopectin molecules in the starch network and to facilitate the mobility rate of the polymer macromolecular chain. Hence, the glass transition temperature can be minimized and improvement in the flexibility and stiffness of starch-based plasticized materials can be achieved (Ibrahim et al., 2019). Besides, it was crucial to add plasticizer because native starches exhibit limited mechanical properties and are poor resistance to moisture (Yadav et al., 2018; Resano et al., 2018; Rhim et al., 2013). Since films formed from starch are brittle and difficult to handle; plasticizers are normally added to the film-forming solution before casting and drying procedures, which brings a way to overcome the brittleness of the films (Harunsyah et al., 2017).

2.3 Nanofiller

Nanofiller or nano-sized filler has gained interest among the researchers to overcome the limitations of the starch-based films that exhibit poor tensile properties and high water vapor permeability due to hydrophilic nature and sensitivity to moisture content (Sun et al., 2014). Improvement of the mechanical resistance of the starch-based films can be done using nanofiller as reinforcement in starch matrixes and few works have shown that the incorporation of nanofiller can increase tensile strength and elasticity modulus, and decrease the elongation capacity of the films (Harunsyah et al., 2017). According to

Jamróz et al. (2019), nanofillers can have different shapes and sizes, but their particle size is determined by the definition of the nanomaterials which is below 100 nm. The size of nanofillers is one of the keys which benefits the nanocomposite materials because they are based on a large surface area that leads to large interphase or boundary area between the biopolymer matrix and nanofiller. Generally, there are four types of nanofillers that are clays, organic, inorganic, and carbon nanostructure. The organic nanofillers which include natural biopolymers (e.g. chitosan (CH), cellulose) have gained enormous attention.

Chitosan ($C_6H_{11}NO_4$) is a linear polysaccharide and the second most abundant polysaccharide that can be found in nature after cellulose (Dutta et al., 2009). CH is a partially deacetylated polymer of N-acetyl glucosamine which is derived from alkaline deacetylation of chitin and it constitutes of β-(1,4)-linked-D-glucosamine residue with the amine groups randomly acetylated (Zhao et al., 2011). The source of CH is chitin which can be naturally found in the waste of crustacean shells such as crabs, shrimps, and lobsters (Goy et al., 2009). Moreover, CH has the benefits of biocompatibility, biodegradability, and low toxicity (Jang and Lee, 2008). Mohamed and Elmasry (2019) reported that CH has AM and antioxidant (AO) properties, and the ability to form protective films.

Several works have reported the utilization of CH as a filler in the biopolymer matrix such as the incorporation of CH in the CS film matrix which resulted in higher tensile strength (5.0 MPa) and elongation (108 %) compared to neat CS film (Julia et al., 2018). Shapi'i and Othman (2016) reported that there was 492% increment in TS value (21.23 MPa) of the starch film when CH was added up to 60% w/w compared to neat starch film (3.58 MPa). YM value of that film was also high which was 2842.5 MPa. Nonetheless, the EAB value was very low which was only 2.46%. The combination of hydrogen bonding, opposite charge attraction between OH^- of starch molecules and NH_3^+ of CH provides strong adherence between starch and CH molecules that resulted in the stability of two different compounds in the film (Bangyekan et al., 2006). Thus, CH can be a great option of filler to be incorporated into food packaging material. Chitosan nanoparticle or CNP has been widely considered as a potential nanofiller in the future development of bio-based packaging. A previous study by Shapi'i et al. (2019) has reported that the incorporation of CNP was more effective to improve the mechanical properties of the starch films compared to bulk CH (Shapi'i et al., 2019). CNP acts as the reinforcing agent that promotes good interfacial interaction with the biopolymer matrix, thus improving the structure and mechanical properties of the films compared to bulk chitosan (Shapi'i et al., 2017). Apart from that, Shapi'i et al. (2019) reported that starch films incorporated with CNP resulted in the significant improvement of the films against microbial growth when cherry tomatoes were wrapped in the films.

Ionic gelation is the most popular method used to produce CNP because the process is relatively simple and non-toxic (Rampino et al., 2013). It attributes to many advantages over other methods such as does not utilize toxic reagents, simple, mild process, and exhibits improvement in biocompatibility (Shapi'i and Othman, 2015). Tripolyphosphate (TPP) has often been used to prepare CNP via the ionic gelation method because TPP is nontoxic, multivalent, and able to form gels through ionic interactions. Through the process, the addition of TPP cations to CH solution generated intermolecular cross-linking reactions between the free amino groups of CH and the TPP cations, producing a bead gel of CH (Huang et al., 2009). In the process of ionic gelation, a positively charged amino group on CH reacts with negative TPP ions at room temperature and form molecular linkages, then simultaneously forming CNP with overall positive surface charge as shown in Figure 2.4 (Ibrahim et al., 2017). The formation of CNP is dependent on the concentration of TPP added into the CH. A study found that the optimum mass ratio of CH/TPP to form CNP was 5:2 (Huang et al., 2009).

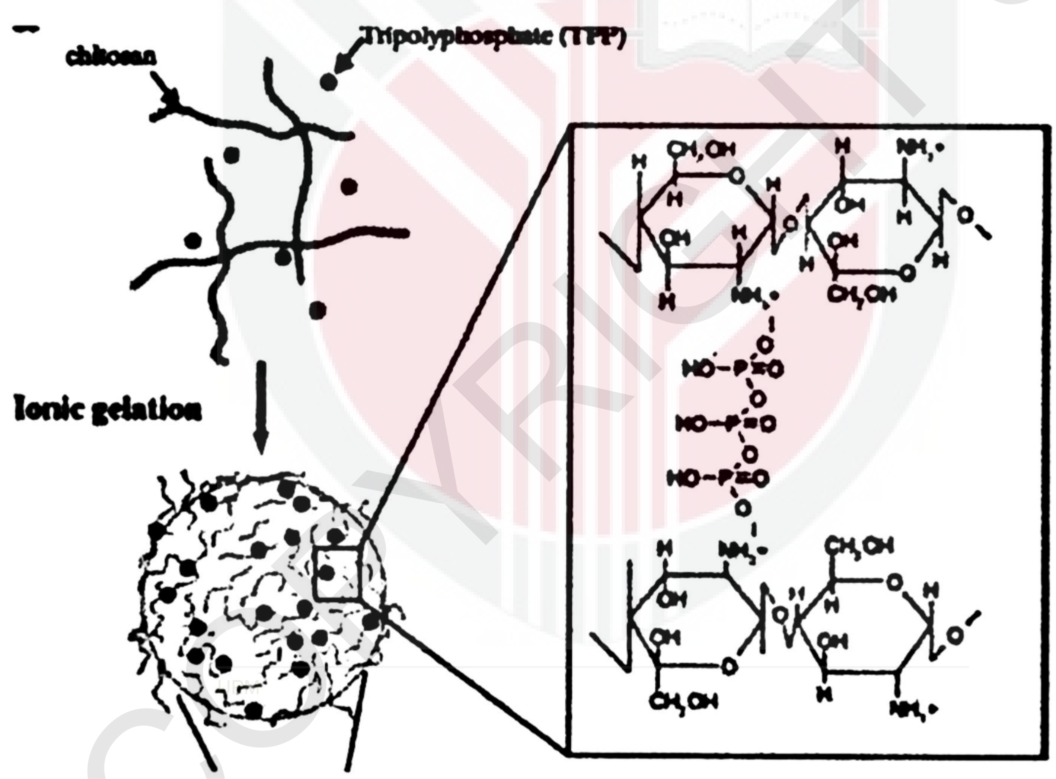


Figure 2.4 Ionic gelation of chitosan of chitosan with TPP

(Source: Ibrahim et al., 2017)

The main issue in food is the microbial contamination caused by bacteria, yeasts, and fungi which lead to food deterioration and short shelf life of food. Direct incorporation of phenolics compounds which are AM agents in food exhibits drawbacks such as undesirable modification of food flavor and alteration of food organoleptic.

Thus, a promising method could be the incorporation of phenolics compound in packaging material which resulted in the protection of food from microbial contamination, convenient, and easily applied to mass production (Ramos et al., 2013). These active ingredients cover many aspects such as factors that influence the shelf life of packaged foods, including physiological processes (eg, respiration of fresh fruit and vegetables), chemical processes (eg, lipid oxidation), physical processes (eg, staling of bread, dehydration), microbiological aspects (eg, spoilage by microorganisms), and infestation (eg, by insects) (Bodbodak and Rafiee, 2016). Thus, the incorporation of phenolics compounds was a great option to improve food safety, prolong shelf life, enhance sensory properties, and also maintain the quality of the food product (Bodbodak and Rafiee, 2016).

2.3.1 Thymol

Natural phenolic compounds such as thymol are one of the great options for the food packaging applications since they exhibit antimicrobial properties and have shown many different benefits on human health. Natural phenolic compounds are abundant bioactive compounds that can be easily obtained from different plant materials, agro-industrial wastes, and byproducts (Arcan & Yemenicio, 2011). According to Burt (2004), thymol which is a monoterpene phenol can be generally isolated from the aromatic plant thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*). Table 2.1 shows the approximate percentage (%) composition of thymol that mainly contributed up to 64% in both of the aromatic plants. Thymol is a phenolic and hydrophobic compound capable of binding bacterial proteins and giving rise to the disintegration and permeability of the cell membrane and, thus has a strong antimicrobial effect. These components show an additive effect that causes the inhibition of the growth of the microorganisms by damaging the integrity of the plasma membrane, affecting the pH and the balance of inorganic ions (Nieto, 2020). Besides, thymol exhibits lipophilic nature and these molecules could interact with bacterial membranes, altering their structure, and making them more permeable (Lambert et al., 2001). Thymol is generally regarded as safe (GRAS) food additive used in the USA, Europe, and China and preferable because originated naturally compared to the synthetic ones.

Table 2.1 Major components of Oregano and Thyme EOs

(Source: Burt, 2004)

Common name of EOs	Latin name of plant source	Major components	Approximate % composition
Oregano	*Origanum vulgare*	Carcavol	80%
		Thymol	64%
		γ-Terpinene	2-52%
		p-Cymene	52%
Thyme	*Thymus vulgaris*	Thymol	10-64%
		Carcavol	2-11%
		γ-Terpinene	2-31%
		p-Cymene	10-56%

Due to consumers' demands for food quality and safety, many researchers are now focusing on incorporating natural antimicrobial agents into packaging systems. The limitation of thymol is that it produces intense flavor and odor. Hence, the incorporation of thymol into the formulation of the starch film will reduce the sensorial impact on the food product. In the meantime, thymol has attracted the researchers' attention due to the broad spectrum of antimicrobial activity against food-borne pathogens such as *Salmonella typhimurium, Listeria monocytogenes, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus, Lactobacillus plantarum, Bacillus subtilis, Shigella sonnei,* and *Shigella flexneri* (Issa et al., 2017).

2.3.2 Effects of thymol in packaging film

Thymol was a potential phenolic compound as AM agent to be incorporated in food packaging material. A study has reported that PLA/poly(butylene-succinate-co-adipate) (PLA/PBSA) blend films containing thymol were very effective against fungal growth as compared to the neat PLA film and PLA/PBSA blend film (Suwanamornlert et al., 2020). The films have also been tested as bread packaging and resulted in the visible growth of yeast and mold delay by 7 days when packed in the films blended with thymol. However, all films blended with thymol exhibited a high reduction of TS and YM but higher EAB. The improved flexibility of the PLA film was due to the plasticizing effects of thymol that reduced the intermolecular interactions and increased the mobility of polymer chains.

Furthermore, thymol has been incorporated into other polymers such as PP. The incorporation of 8 wt% thymol in PP has been proven as an effective way to improve the quality of strawberries and bread samples during distribution and sale (Ramos et al., 2013). The strawberries were observed to undergo a physical deterioration due to the experimental storage conditions (25°C), but it was crucial to highlight that microbial growth was not observed until the end of the study (15 days). Meanwhile, PLA-based films incorporated with thymol (6 and 8 wt%) were proven to have a combination of antioxidant and antibacterial performance. Besides, most of the shelf-life study focused on the application of thymol as edible coatings. For instance, the effectiveness of the active coating loaded with different concentrations of thymol (500, 1000, and 1500 ppm) on the ready to use peeled shrimps was observed by Mastromatteo et al. (2010). They concluded that a slight AM effect was obtained when the coating was loaded with thymol. The effect of thymol in flexible PP films as AM packaging on the microbiological growth and sensory attributes of raw Atlantic salmon (Salmo salar) fillet stored in chiller was reported by Hurley et al. (2013). They reported that the application of active AM packaging reduced the microbial count of fillet salmon for least 18 days at 2°C and no modification of the sensorial properties were detected proving that the AM agent of thymol suitable for perishable foods packaging.

2.4 Properties of the films

2.4.1 Optical properties

The optical properties of the packaging materials include the color, opacity, transparency, and glossiness of the materials. A clear food packaging material is preferable among the consumers for the consumers to clearly see the food inside the packaging and observe through the eyes the food quality or any deterioration that happen to the food. Optical properties especially the color of packaging materials can be determined using CIELAB color parameters (L*, a* and b*) as the following:

1. L*: 0 (black) to 100 (white)

2. a*: -60 (green) to +60 (red)

3. b*: -60 (blue) to +60 (yellow).

Generally, non-composite food packaging films are transparent and colorless (Sonthornvit et al., 2010). However, the addition of other biopolymers inside the starch matrix will affect the film appearance. According to Abdollahi et al., (2012), all of the chitosan films incorporated with rosemary essential oil (REO) were transparent, with a slight yellowish tinge based on the values observed for b*. The changes in color were due to the natural color of the thymol and CH that was in the yellowish range.

A study by Zhong et al. (2017) investigated the effect of thymol concentration on the optical properties of peanut protein isolate (PPI)-based film. They found that the films incorporated with thymol were less transparent compared to control films (0% w/v thymol). The opacity of films increased when thymol concentration was increased from 0 to 2 % w/v due to the presence of polyphenols in the films that hindered the light transmission through the films. The color of the films also became yellowish and darker when incorporated with 2% w/v of thymol but no significant difference was observed for L*, a*, and b* values when the films were incorporated with low concentrations of thymol.

Meanwhile, another study observed the effects of thymol concentrations ranging from 0.5, 1, 1.5, 2, and 2.5% w/w in the hydroxyethyl cellulose (HEC)/wheatstarch-based films (Khairuddin et al., 2020). They found that all films produced were completely transparent, smooth, and glossy at the low concentrations of thymol (0.5, 1, and 1.5 % w/v) compared to the control film. The reason for the insignificant color change was due to homogenous surface morphologies for all films at low concentrations of thymol (Ramos et al., 2014). However, when the HEC/wheatstarch-based films were incorporated with higher concentrations of thymol (2 and 2.5% w/v), the films changed color to light creamy and exhibited a dull surface. This was due to higher lipid content from thymol which contributed to coarser microstructures of films due to deformation forces that act during the polymer chain aggregation.

2.4.2 Mechanical properties

Generally, the development of bio-based polymers is concerned about the mechanical properties and stabilities of the biopolymers to serve as the packaging material. The mechanical properties of materials are largely dependent on their behavior towards several vital conditions such as temperature, heating, and cooling rates, applied force, deformation, and the rate of deformation (Kumar et al., 2020). Kumar et al. (2020) also stated that there is a wide range of mechanical properties including YM; yield stress; Poisson's ratio; TS; storage and loss modulus; EAB; creep; and recoverable compliance. Among these, tensile property is one of the most studied mechanical characteristics of biopolymer film.

Mechanical properties especially TS and EAB of the films are related to their chemical structures. TS (MPa) is for strength measurement while the percentage of EAB (%) determines the elasticity of the films (Jamróz et al., 2019). TS indicates the maximum stress that the films can withstand during tensile testing whereas EAB means their potential to stretch (Choi et al., 2017). ASTM D882-09 is a standard test method for the tensile test of thin-film and is commonly used to characterize biopolymer thin film (Antoniou et al., 2015; Tang et al., 2008). TS, EAB, and tensile modulus (TM) are the main output results observed from a typical stress-strain plot. Physically, the tensile modulus predicts the stiffness of a material.

Shapi'i et al. (2019) found that the addition of CNP into the matrix of a starch biopolymer is more effective to improve the properties of the biopolymer compared to chitosan or CH. Table 2.2 shows that the addition of CH and CNP into neat starch films increased the TS and EAB of the films, thus proving the potential of chitosan to improve the mechanical properties of the neat starch film due to the compact and strong characteristics of the films. A more pronounced increment was observed when CNP was added into the films instead of CH. Good intermolecular interactions existed between the starch and CNP due to the tiny size of CNP that provided a large surface area of CNP to be exposed to the starch.

Table 2.2 Comparison of the mechanical properties of films based on the starch film with addition of CNP

(Source: Shapi'i et al., 2019)

Film	TS (MPa)	EAB (%)	YM (MPa)
Neat starch	1.12±0.03a	67.00±1.32a	5.96±0.58c
Starch/CH	3.16±0.07b	76.90±1.23b	4.43±0.16a
Starch/CNP	4.95±0.01c	90.77±1.40c	5.70±0.15b

Furthermore, a study by Antoniou et al. (2015) investigated the effect of CH and CNP on the mechanical properties of tara gum (TG) films. They found that the TS films improved while the elongation decreased by the addition of CNP. The incorporation of CNP increased the stiffness of the films due to the reinforcement effect of the nanoparticle in the film matrix. Figure 2.5 shows the mechanism when CNP was incorporated in the tara gum film. The CNP was more efficient to fill the free volume inside the film matrix due to the tiny size and regular spherical shape compared to bulk CH. Thus, more rigid and compact films structure produced when incorporated with CNP that led to the improvement of the mechanical properties of the films.

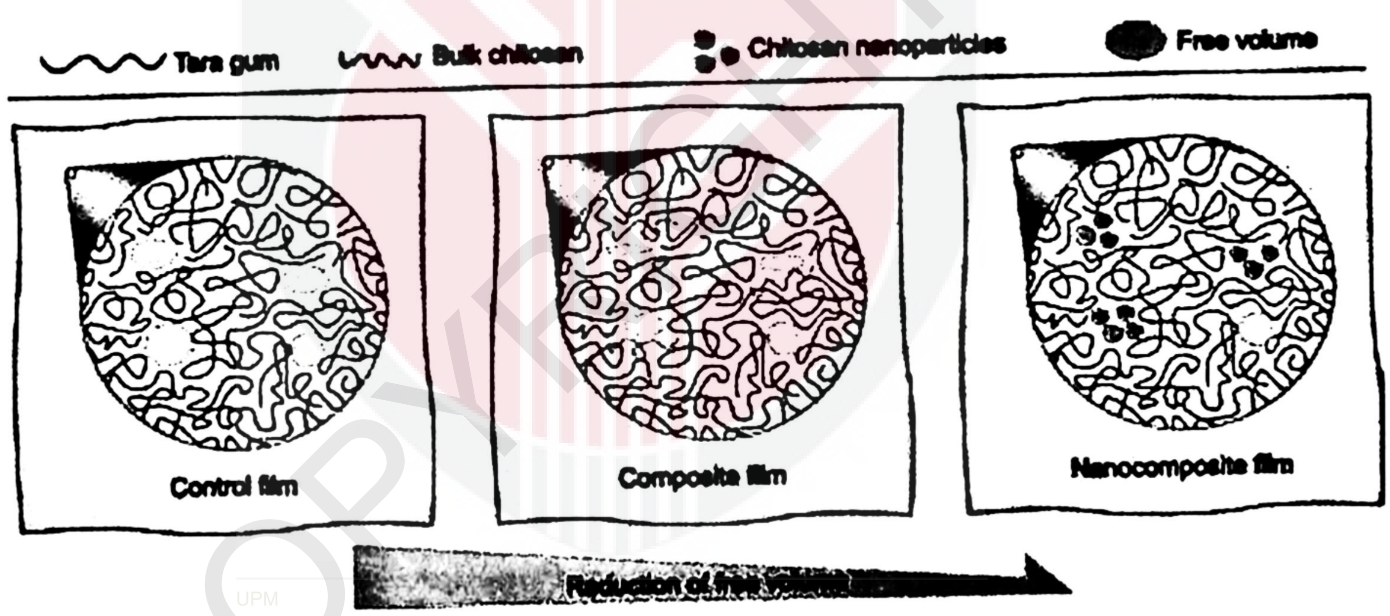


Figure 2.5 Effects of incorporation of CNP in tara gum film

(Source: Antoniou et al., 2015)

In addition, Davoodi et al. (2017) studied the dispersion of thymol with polysorbate in potato starch film-forming solution to produce polysorbate-thymol starch films. The presence of polysorbate-thymol in films resulted in a decrease in the TS of the films due to the formation of intermolecular interactions between hydroxyl groups of starch and polysorbate emulsifier. There was also an interaction that happened between the lipophilic and hydrophobic portions of polysorbate with thymol and starch, respectively. Besides, a study has reported the effects of incorporating different concentrations of thymol (0.5, 1, 1.5, 2, and 2.5% w/w) into the HEC/wheat-starch-based films (Khairuddin et al., 2020). They concluded that a slight increase in tensile properties of the films was achieved with the addition of thymol starting at 1.5% w/w accompanied by the decrement of YM values.

However, in another study carried out by Nordin et al. (2020), there was a reduction in mechanical performance when neat corn starch film was incorporated with thymol. According to Nordin et al. (2020), thymol caused major interruption within the starch network that subsequently reducing the intermolecular force between starch molecules. Moreover, the empty spaces within the starch matrix occurred due to the interruption by both glycerol and thymol which caused the matrix to lose its rigidity and strength (Nordin et al., 2020). Thus, it is significant to study the effects of thymol concentrations on CS films since previous research had stated that the mechanical properties could differ when there is the incorporation of thymol that exhibits hydrophobicity. However, no study has been done to investigate the effects of the mechanical properties of the films when there are both the reinforcement of CNP and thymol addition in the starch films matrix. In this work, the mechanical performance of starch/CNP films incorporated with different thymol concentrations was investigated via texture analyzer.

2.4.3 Barrier properties

The factors that influenced permeates diffusion through a film are the film structure, film permeability to specific gases or vapor, thickness, area, temperature, the difference in pressure, or concentration gradient across the films (Siracusa, 2012). Conceptually, permeability is generally related to the quantitative evaluation of the barrier properties of a material. The permeability is influenced by the size, shape, and, polarity of the penetrating molecule; the degree of cross-linking; and polymer chain segmental motion of the polymer matrix. Figure 2.6 illustrates the mechanism of water and gas permeate through the film which involved the process of adsorption, desorption, and diffusion. The water or molecules diffused through one side of the film matrix, move within the void space among polymer segments, and desorption of the water or gas molecules from the polymer surface to the other side of the film.

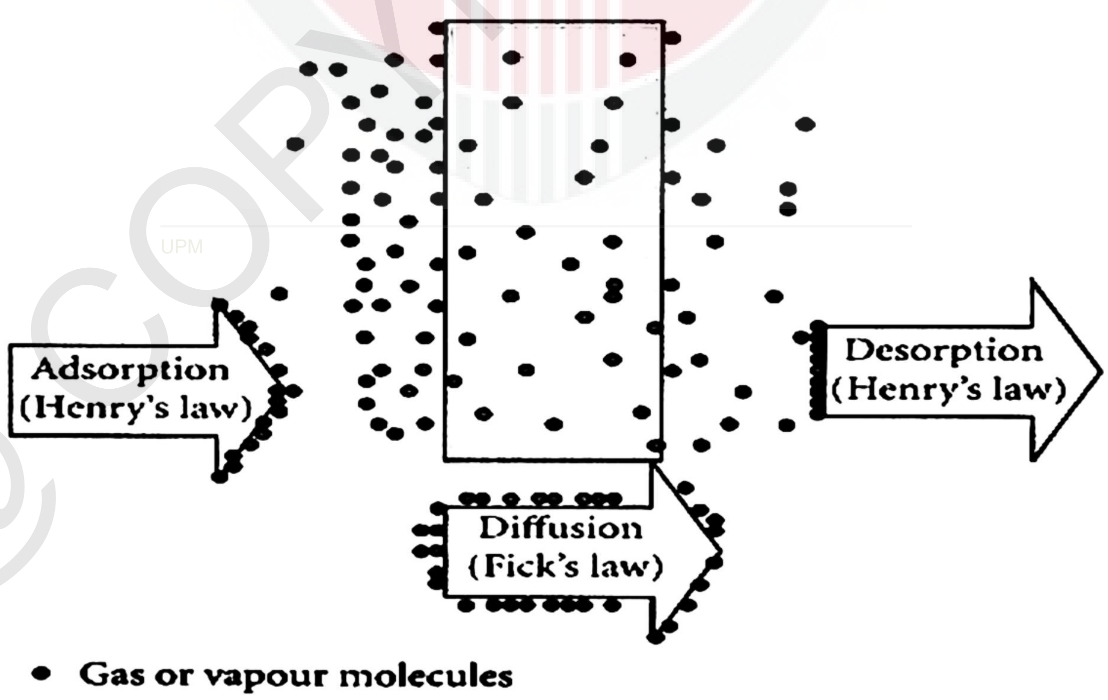


Figure 2.6 General mechanism of gas/vapor permeation through a plastic film

(Source: Siracusa, 2012)

The water vapor permeability (WVP) of the films is one of the important barrier properties to be investigated to packed fresh food products and products where dehydration and absorption of moisture should be at the lowest value (Jamróz et al., 2019). A major function of a packaging film is to retard moisture transfer between food and the surrounding atmosphere because excessive moisture in food packaged caused food deterioration to happen rapidly. The values of WVP of packaging systems should be at the lowest possible level in order to efficiently optimize the food package environment for extension of food shelf life. The factors that influenced WVP in biopolymer films are the degree of cross-linking whereby it enhanced intermolecular forces that reduce gas and water vapor permeability in food packaging materials (Liang et al., 2019). The next factor is the addition of plasticizer such as glycerol that primarily affects the water content of films at the stationary state of the permeation process which initiates greater plasticization and leads to the moisture transfer.

Neat starch-based films have limitations in terms of short shelf life caused by water absorption. Nevertheless, this limitation can be improved by the incorporation of reinforcing nanofiller to form the bionanocomposite films (Othman et al., 2019). Figure 2.7 shows the incorporation of CNP as nanofillers which contributed to large contact surface areas that provided the strong interaction between starch matrix and CNP; resulted in a compact and rigid film structure. The rigid structure can improve the mechanical and water vapor barrier properties compared to the neat starch film.

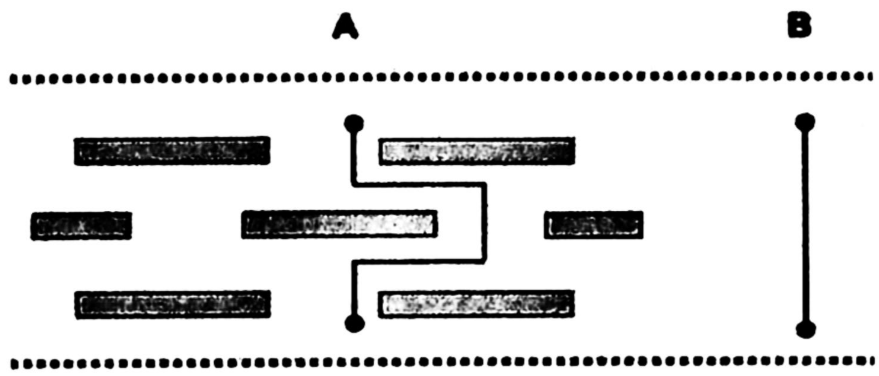


Figure 2.7 Schematic illustration of the tortuosity of (A) filled polymer (B) unfilled polymer

(Source: Shapi'i and Othman, 2015)

Besides, WVP was affected by the hydrophilic–hydrophobic ratio in the film matrix. A previous study had proved that the incorporation of polysorbate-thymol into potato starch film increased the WVP of the starch films (Davoodi et al., 2017). They stated that lipophilic compounds of thymol could act as water barriers in the films, while hydrophilic compounds could increase water transmission across the films. The increment of WVP of the films was caused by the polysorbate-thymol interactions that unstabilize the starch-starch polymer interactions which resulted in the reduction of the starch matrix network integrity. A study reported by Zhong et al. (2017) who incorporated thymol into modified PPI films found that the addition of thymol into the films lowered the WVP values than the control films. A decreasing trend of WVP was observed with the increment of thymol concentrations. They stated that this was due to the hydrophobic nature of thymol, which affects the hydrophilic/hydrophobic balance of the films. Besides, Medina et al. (2019) reported that the incorporation of chitosan thymol nanoparticles (CTNPs) into quinoa protein/chitosan (CQ) edible film resulted in a reduction of WVP values. In the CQ films incorporated with CTNPs, a homogenous distribution of CTNPs in the cross-sections of the films was observed. Thus, an interfacial interaction existed between CNPs with the biopolymeric matrix which indicated that CTNPs could act as filler.

In a study investigated by Li et al. (2020), WVP of gelatin films incorporated with thymol nanoemulsions did not significantly change due to the balance of hydrophobicity of thymol and film. Nonetheless, higher concentrations of thymol would generate higher WVP influenced by disruptive effects of thymol on the gelatin film network. In previous literature, hydrophobic components could also decrease the cohesiveness of the polymer network, causing an increase in WVP. Thus, it was important to study the effects of thymol addition in CS/CNP films on the barrier properties of the films because previous studies found the increase, decrease, or insignificant changes of barrier properties when thymol was added into the films.

2.4.4 Antimicrobial properties

Generally, several attempts have been made to develop food packaging in which AM agents such as CH and thymol are incorporated into polymeric materials. The antimicrobial activity of CH has been proven in a wide variety of microorganisms such as bacteria, yeasts, and fungi. AM agents carried by polymers can interact with the surface of the food to prevent foodborne microbial growth and control the diffusion rate of AM, thus ensuring the continuous and adequate presence of antimicrobials on food surface (Gharsallaoui et al., 2016). The antimicrobial mechanism of CH has been proposed to be mainly divided into three types. Initially, electrostatic attractions exist between positively charged chitosan chains and negatively charged bacterial cell walls that caused the absorption of chitosan onto the target bacteria. Subsequently, intracellular components will break down which leads to cell death. Next, the accumulations of CH surrounding the target bacteria isolate the bacteria from the environment and restrict nutrient intake and exchange (Huang et al., 2019).

A study reported by Shapi'i et al. (2020) stated that CNP showed better inhibition of gram-positive bacteria compared to gram-negative bacteria when the films were demonstrated on cherry tomatoes. This was due to CNP ($NH3^+$) tend to form a polymer membrane throughout the cell wall to shield gram-positive bacteria that are in close contact with CNP, thus blocking nutrient and oxygen supply from the metabolic activity of the bacteria. In the case of gram-negative bacteria, CNP diffuses bacteria across the cell wall and disrupts the cytoplasmic membrane resulting in bacteria leakage. This leakage destroyed the intracellular bacteria and killed the bacterial cells. Besides, a study by Medina et al. (2019) also reported similar findings whereby the inhibition of radial mycelia growth of *Botrytis (B.) cinerea* by the CNP was better than the controls treatment.

Apart from that, thymol is also a natural AM agent that exhibits antimicrobial properties that can prolong the shelf life of food. Thymol can exhibits the antimicrobial effect due to its lipophilic nature; its activity occurs at the lipid level, and the enzymatic complex of the membrane may modify the permeability and/or inhibit the cellular respiratory chain.

Robledo et al. (2018) investigated the incorporation of CTNPs into quinoa protein/chitosan (CQ) edible films. They found that the films were able to inhibit fungal growth especially *B. cinerea* or known as gray mold that commonly spoiled cherry tomatoes (Robledo et al., 2018). The most efficient inhibition was observed with the addition of 110 ppm thymol and they stated that hydrophobicity was an important factor that contributes to the presence of a free hydroxyl group to allow proton exchange. The greatest microbe inhibition was observed at relatively low thymol concentration which was related to the dispersion of thymol in nanoemulsion. This particular concentration allowed great dispersibility that increased antimicrobial effectiveness. Another study reported by Barrera-Ruiz et al. (2020) stated that CNP encapsulated with Thyme EOs showed high antimicrobial effect against foodborne pathogens such as *Enterococcus* species and *Klebsiella pneumonia.* The study also stated that thymol which is main components of Thyme EOs was capable to disintegrating the cell outer membrane. The inhibition of the pathogens was related to the interactions with the cell membrane of microorganisms and the hydrophobicity of the compounds

CHAPTER 3

METHODOLOGY

This chapter reports a detailed description of the materials and methods used to synthesize CNP and prepared CS/CNP/Thy films using the solvent casting method. This chapter also covers the materials and methods used to characterize the films which include optical, mechanical, and barrier properties. Apart from that, this chapter also covers the materials and methods used to investigate the shelf-life of cherry tomatoes packaged with the CS/CNP/Thy films in terms of firmness, weight loss, and observation throughout the 6 days of storage at ambient temperature. The overall methodology adopted in the present work is summarized in Figure 3.1.

Preparation of CNP via ionic gelation method

Fixed concentration of chitosan (CH) solution (15% w/w of solid starch)

↓

Investigation on the effect of different concentrations of thymol on the properties of the CS/CNP/thymol films

Vary concentration of thymol from 0, 1.5, 3.0, 4.5 w/w%

Characterization: Optical properties (ΔE, opacity), mechanical properties (TS, EAB, YM), barrier properties (WVP)

↓

Evaluation of CS/CNP/thymol films as food packaging films

Vary concentration of thymol from 0, 3 w/w% and neat CS film

Characterization: Firmness and weight loss of cherry tomatoes packaged with the films and stored for 6 days at ambient temperature

↓

Demonstration of CS/CNP/Thy films as food packaging films

Vary concentration of thymol from 0, 1.5, 3.0, 4.5 w/w%

Shelf-life of cherry tomatoes: Observation of mold growth on cherry tomatoes packaged with the films and stored for 6 days at ambient temperature

Figure 3.1 Summary of methodology

3.1 Materials

Chitosan (low molecular weight: 50 kDa, viscosity: 20-300 cP in 1% w/v of acetic acid, deacetylation: 75-85%) and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich, USA. Acetic acid, sodium hydroxide (NaOH), sodium bromide (NaBr), and Tween 80, corn starch (33% amylose, 67% amylopectin), glycerol, thymol, magnesium nitrate ($MgNO_3$), calcium chloride (CaCl), paraffin wax, and bee wax were purchased from R&M Marketing, UK. Cherry tomatoes were purchased from Kea Farm, Cameron Highlands, Pahang, Malaysia.

3.2 Synthesis of CNP

In this work, CNP was first synthesized via the ionic gelation process of CH and TPP before CNP was used as a filler in the starch films. All parameters were controlled based on the optimum parameters that produced the most stable and smallest size of CNP as reported by Shapi'i et al. (2019). Figure 3.2 shows the flow chart of the procedures involved in the synthesis CNP suspension. Firstly, chitosan (CH) solution (15% w/w of solid starch) was prepared by dispersing 0.45 g chitosan flakes into 50 mL aqueous acetic acid solution (1% v/v) using a magnetic stirrer (FAVORIT HS0707V2, Indonesia) for 30 minutes. Then, the pH of the solution was adjusted to pH 4.6 using 10% v/v NaOH.

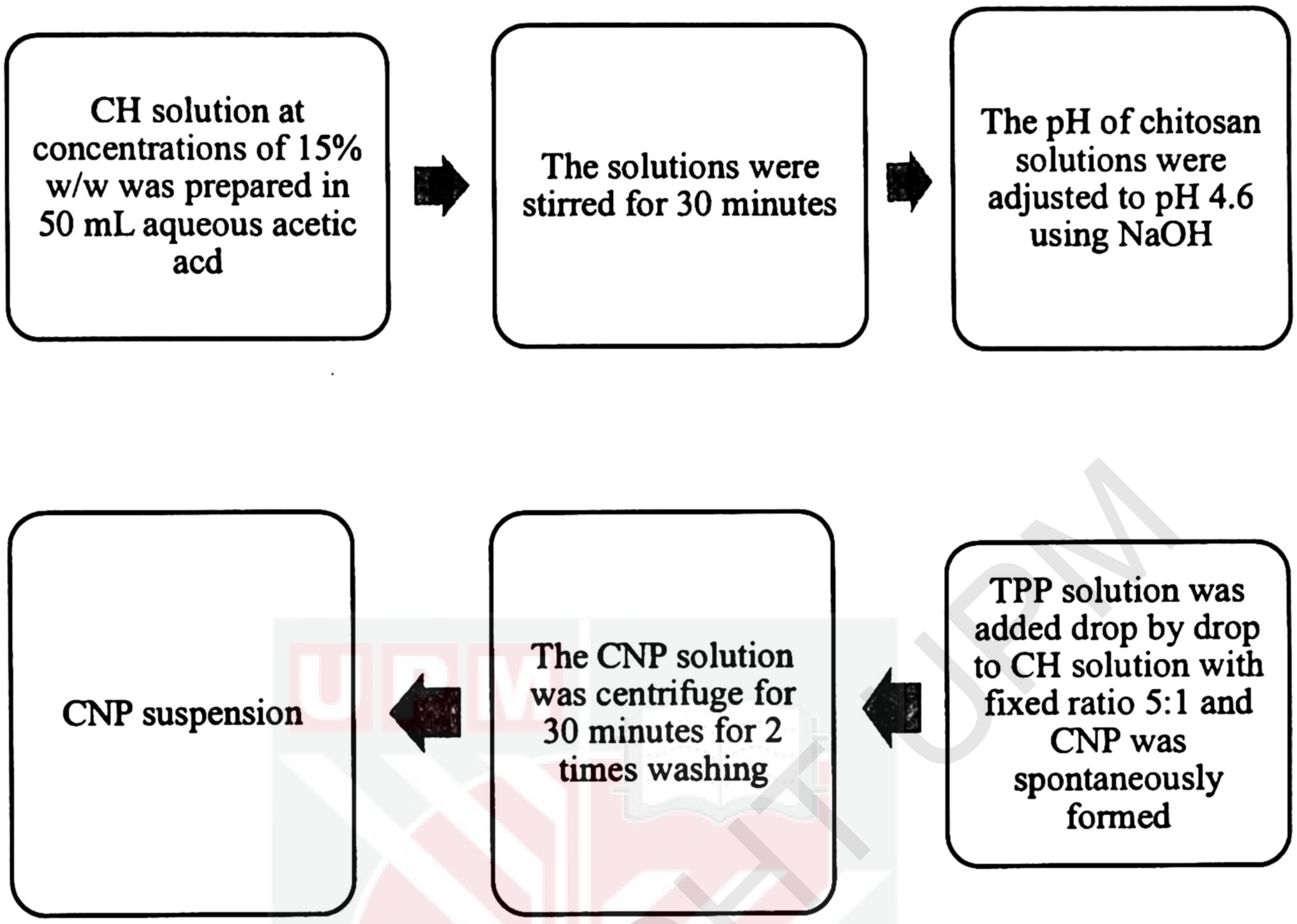


Figure 3.2 Steps for synthesizing CNP

TPP solution was prepared according to the ratio of chitosan to TPP (5:1) by dissolving 0.09 g of TPP powder in 50 mL distilled water. CNP was spontaneously obtained upon the addition of 50 mL of TPP solution drop by drop to the 50 mL CH solution under vigorous magnetic stirring at room temperature (25°C) for 30 min. Then, a centrifuge (Universal 320, Germany) was used to disperse the CNP emulsion at 9000 rpm for 30 minutes. The centrifugation process was done for two times washing and CNP suspension was produced.

3.3 Preparation of CS/CNP/thymol Films

Table 3.1 shows the different amount of thymol added to the CS/CNP films to produce different concentrations of CS/CNP/Thy films. The weight of thymol was calculated based on the ratio of formulation as shown in Table 3.1 while the weight of CH, Tween 80, and TPP (g) were fixed.

Table 3.1 Formulation of starch-film forming solutions

Percentage (% per starch)	Formulation	Weight of chitosan (g)	Weight of thymol (g)	Weight of Tween 80 (g)	Weight of TPP (g)
0	1:0	0.45	0	-	0.09
1.5	1:0.1	0.45	0.045	0.3g/275 μL	0.09
3.0	1:0.2	0.45	0.09	0.3g/275 μL	0.09
4.5	1:0.3	0.45	0.135	0.3g/275 μL	0.09

The amount of 3 g of corn starch was dispersed in 50 mL distilled water–glycerol solutions and the composition of glycerol added into starch solution was fixed to 0.75 g Then, the solution was heated with continuous stirring using a magnetic stirrer until gelatinized completely at 85°C (Nordin et al., 2020). The CNP solution was prepared by dissolving 3 g CNP with 50 mL distilled water and underwent sonication (QSonica, 500W, 20kHz) for 15 minutes at 50% amp. The starch solution was then cooled down until 40 ± 2°C before mixed with CNP suspension.

The CS/CNP/Thy film solution was prepared by mixing 50 mL of CNP suspension and thymol that has been dissolved with Tween 80 solvent with a 50 mL gelatinized starch solution and stirred for 10 minutes using a magnetic stirrer. After mixing the CS/CNP/Thy solution, the solution was subjected to sonication for 5 minutes at 50% amplitude using an ultrasonic probe to produce a homogenous solution. An amount of 40 mL of the solution was poured into an acrylic petri dish (diameter: 14 cm) and left in the air-conditioned room (20°C) for 48 hours on the flat table. A starch film without the addition of CNP was also prepared as the film control. After drying, the film was peeled off from the petri dish and conditioned in a desiccator (RH: 51%, Temp: 30°C). Figure 3.3 shows a transparent and smooth film produced after peeled off from the petri dish. The thickness of the film was measured using a digital micrometer (Mitutoyo, Japan) at five random positions around the film. The average values of the thickness were used to calculate the TS, EAB, YM, opacity, and WVP.

Figure 3.3 Film after peeled off from petri dish

3.4 Characterization of the films

To study the effect of CNP and thymol addition on the starch films, all film samples produced in Section 3.3 were characterized in terms of optical, mechanical, and barrier properties.

3.4.1 Optical properties

The color of the films was determined using a color spectrophotometer (HunterLab, Ultrascan Pro, USA) by measuring the CIELAB coordinates (L*, a*, and b*). The color values were averaged based on at least five replicates. The total color difference (ΔE) was calculated using Equation (3.1):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad 3.1$$

where

ΔL*: the differential values of L* films

Δa*: the differential values of a* of films

Δb*: the differential values of b* of films

The differentials are between control film and film samples

The light transmittance of the film samples was measured using the same color spectrophotometer at a wavelength range of 200–700 nm. Then, the opacity values of the films were calculated using the following equation (3.2):

$$\text{Opacity} = \frac{A_{600}}{L} \quad 3.2$$

where

A_{600} : absorbance value at 600nm

L: film thickness (mm)

3.4.2 Mechanical properties

TS, EAB, and YM were determined by a tensile analysis performed using a texture analyzer (TA.XT2 Stable Micro Systems, UK) according to ASTM D882-09 (2009). Film strips (100 mm x 15 mm) were cut from each pre-conditioned sample film and placed between the grips. Initial grip separation and test speed were set to 60 mm and 0.5 mm s^{-1}, respectively. A minimum of five replications of each test sample was run.

The values of TS and EAB were determined from the software of the equipment. Meanwhile, the YM values were calculated based on the following equation (3.3):

$$\text{YM (MPa)} = \frac{\text{Stress(MPa)}}{\text{Strain(Dimensionless)}} \quad 3.3$$

3.4.3 Barrier Properties (Water Vapor Permeability)

The determination of WVP was carried out using a modified dry cup method according to ASTM E96 (ASTM, 2005). The films were cut into circular shapes using a cutter (diameter: 7 cm) and placed on the cup containing 10 g of CaCl (RH = 0%). The mixture of paraffin wax and bee wax (8:2) was heated using the magnetic stirring hotplate until the mixture melted. The mixture of waxes was then poured around the ring of the cup which acted as the sealant.

To maintain the surrounding humidity of the cups, each cup was stored in a desiccator containing saturated $MgNO_3$ to provide a constant RH of 51% at 30°C. A digital temperature humidity meter (Proskit NT-312) was used to monitor the relative humidity and temperature. Changes in the weight of the cup were recorded every 24 hours for 10 days and the weight was plotted against time to obtain the weight loss versus time graph.

The water vapor transmission rate (WVTR) was calculated as the following equation (Abdollahi et al., 2012):

$$WVTR = (W/t)/A \qquad 3.4$$

where

W/t: slope of weight changes versus time graph (g/h)

A: transmission area of the film ($28 cm^2$).

The water vapor permeability (WVP) was calculated as the following equation (Abdollahi et al., 2012):

$$WVP = (WVTR \times L) / (P_1 - P_2) \quad 3.5$$

where

L: average of film thickness (mm)

P_1: water vapor partial pressure in desiccator at RH = 51%, 21.64 x 10^5 Pa

P_2: water vapor partial pressure in the cup at RH = 0%, 0 Pa

3.5 Demonstration on the application of CS/CNP/Thy films as food packaging films

3.5.1 The shelf life of cherry tomatoes

The cherry tomatoes were washed under running tap water and dried to remove the excess moisture. Then, the tomatoes were packed in the films (dimension: 10 cm^2) and sealed (100-200°C, 5 s) using a sealer (SF-200, Malaysia). The quality of cherry tomatoes in terms of firmness and weight loss were analyzed on days 0, 3, and 6 of storage at ambient temperature (25 ± 3°C). Figure 3.4 shows the cherry tomato that has been packed using the films and sealed using a sealer.

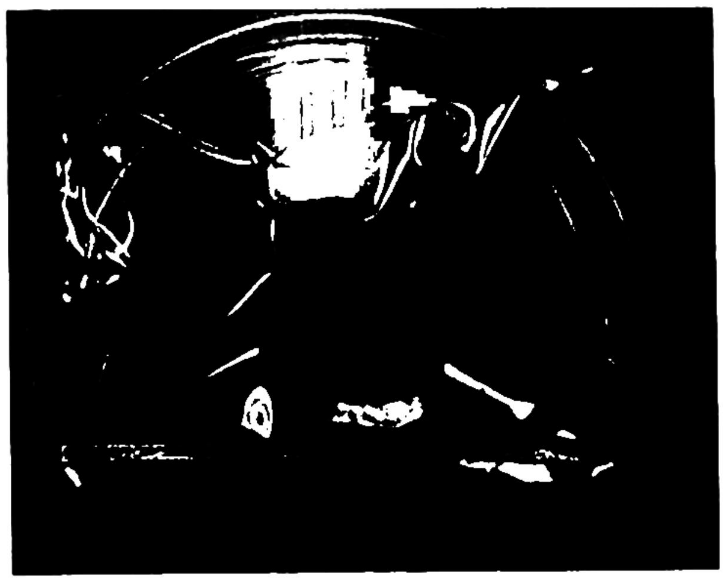


Figure 3.4 Cherry tomato that was packed using the produced films

3.5.1.1 Firmness

The firmness of the cherry tomatoes was determined using a texture analyzer (TA.XT2 Stable Micro Systems, UK) by measuring the force required to make a predetermined piercing using a standard probe. The tomatoes were compressed by the probe to a 2 mm penetration depth using a 2 mm cylindrical probe at a speed of 2mm/sec.

3.5.1.2 Weight loss

The weight loss of the cherry tomatoes was calculated using the following formula:

$$\text{Weight loss} = \frac{IW - FW}{IW} \times 100 \qquad 3.6$$

where

IW: the initial weight of fruits (g)

FW: the final weight of fruits at days 0, 3, and 6 (g)

3.5.2 The shelf life of cherry tomatoes: observation

In order to investigate the potential real application of the films on the food product, starch/CNP/Thy film, starch/CNP film, and neat starch film were put in direct contact with the tomato cherries. This was done to demonstrate whether the active compounds inside the films particularly thymol can migrate to the food to prevent the mold growth on the surface of cherry tomatoes.

The tomatoes were first washed under running tap water and dried to remove the excess moisture. The petri dish and knife were first sanitized using alcohol (isopropyl alcohol (IPA)) to avoid contamination. Then, the CS/CNP/Thy film was cut according to the size of the petri dish lid, and the films were placed inside the petri dish lid. Subsequently, cherry tomatoes were cut into half and placed into the petri dish. The lid that has previously placed with the film was closed. The cherry tomato was stored at ambient temperature (25 ± 3°C) and observed daily for 6 days. Figure 3.5 shows the cherry tomatoes that have been placed in the petri dish, in direct contact with the films.

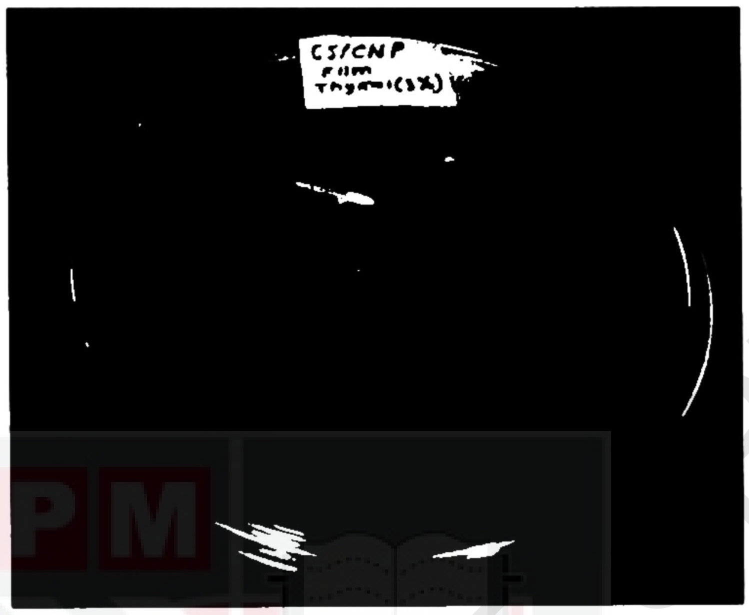


Figure 3.5 Shelf life study to observe mold growth on cherry tomatoes

3.6 Statistical Analysis

The statistical analyses of the obtained experimental results were performed by analysis of variance (ANOVA) using Minitab 17 software. Mean comparisons were conducted using Tukey's test at a 0.05 level of significance.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter presents results and discussion on the findings of the present work. Section 4.1 reports the results and discussion on the preparation and characterization of the CS/CNP/Thy films, including optical, mechanical, and barrier properties. Section 4.2 presents the demonstration on the application of the films as food packaging for cherry tomatoes whereby the shelf life of the cherry tomatoes was investigated in terms of firmness, weight loss, and observation of mold growth.

The results for mechanical properties were obtained for 0, 1.5, 3, and 4.5% of thymol concentrations. The study did not utilize higher concentrations of thymol because the films incorporated with a higher concentration of thymol were very brittle, difficult to peel off from the petri dish, and easily broken into pieces as can be seen from Figure 4.1. Furthermore, due to the properties of thymol that is volatile and heat-sensitive, the films were dried at room temperature for 48 hours instead of in an oven. The application of heat from the oven was found to disrupt the polymeric chain between starch matrix, CNP, and thymol, thus, the films dried using oven produced brittle structure as shown in Figure 4.2.

(a) (b)

Figure 4.1 Films with very brittle structure of (a) CS/CNP/Thy 6% and (b) CS/CNP Thy 7.5%

Figure 4.2 Films dried using the oven during casting for 12 hours at 50°C with 40% fan speed

4.1 Characterization of CS/CNP/Thy Films

In this work, starch/CNP/Thy films were produced with varying thymol concentrations as discussed in section 3.3. The effects of thymol concentration on the properties of the films were characterized in terms of optical properties (ΔE and opacity), mechanical (TS, EAB, and YM), and barrier (WVP) properties.

4.1.1 Optical Properties

Optical properties are important properties of food packaging materials as they can affect the protective function of the food package and influence the appearance and attractiveness of the food products (Berk, 2009). ΔE value which is formulated by L, a, and b values, represent black to white, green to red, and blue to yellow, respectively. The color (L*, a*, b*, and ΔE) and the opacity of the films added with different concentrations of thymol (0, 1.5, 3, and 4.5 w/w%) were determined and tabulated in Table 4.1.

Based on Table 4.1, all the films generally exhibited the same L*, a*, b* values. The addition of thymol reduced slightly the L* values and the reduction becomes pronounced at higher concentrations of thymol. This indicates a slight decrease in lightness due to the existence of thymol. Based on the work by Nordin et al. (2020), the L* values of CS films decreased with the addition of thymol due to the intrinsic color of thymol which is a white crystalline substance that caused the films to become slightly darker. Their work revealed that the L* value for CS films added with thymol was much lower than the findings in this study (33.0). The high L* value of all the films produced in this study was most probably due to the addition of CNP which increased the glossiness of films, hence, improved the appearance of the films (Shapi'i and Othman, 2016). A similar trend of L* values was also obtained when the different concentrations of thyme EO were added into starch/chitosan composite films (Mehdizadeh et al., 2012). Furthermore, Wang et al. (2019) reported that both neat CS film and CS film incorporated with orange-peel oil and zein nanocapsules exhibited high L* values, consistent with the findings in this work.

Optical properties are important properties of food packaging materials as they can affect the protective function of the food package and influence the appearance and attractiveness of the food products (Berk, 2009). ΔE value which is formulated by L, a and b values, represent black to white, green to red, and blue to yellow, respectively. The color (L*, a*, b*, and ΔE) and the opacity of the films added with different concentrations of thymol (0 to 4.5 w/w%) were determined and tabulated in Table 4.1.

Table 4.1 Optical properties of starch/CNP films. The data are reported a mean ±SD, n=3 and P < 0.05.

Concentration of thymol (% w/w)	L*	a*	b*	Total color difference (ΔE)	Opacity (A_{600}/mm)
0%	93.77 ± 0.14[a]	-1.10 ± 0.02[ab]	3.74 ± 0.19[b]	0	0.80 ± 0.04[b]
1.5%	92.99 ± 0.03[b]	-1.04 ± 0.05[a]	3.60 ± 0.21[b]	0.81 ± 0.03[b]	0.81 ± 0.02[b]
3.0%	92.95 ± 0.10[b]	-1.12 ± 0.07[ab]	3.62 ± 0.13[b]	0.84 ± 0.10[b]	0.87 ± 0.01[b]
4.5%	92.54 ± 0.05[c]	-1.19 ± 0.03[b]	4.24 ± 0.06[a]	1.33 ± 0.03[a]	1.06 ± 0.07[a]

In addition, Table 4.1 shows that there was no significant change in the a* values for all the films. The small negative a* values indicated that the films have a tinge of green color. Nonetheless, the a* values were very small and the tinge of green color was non-noticeable. Meanwhile, the CS/CNP films have a tinge of yellow color which can be observed from the small positive b* value due to the addition of CNP into the starch matrix. Maillard reaction between amino and hydroxyl groups of chitosan produced insoluble melanoidin compounds that lead to a tinge of yellow color films. The color was absorbed in wavelengths near the yellow region, due to the presence of CNP in the films (Gonçalves et al., 2020). When a higher concentration of thymol (4.5 w/w%) was added to the CS/CNP films, the b* value increased slightly because the high concentration of thymol (4.5 w/w%) incorporated in the starch matrix induced the light scattering on the surface of the films (Song et al., 2019). The light scattering played a role in the yellow pigment of the films due to wide-angle scattering, thus more yellow light is transmitted. However, the b* values were very small and the change in color was almost non-noticeable

Furthermore, ΔE was calculated to observe the color changes between CS/CNP and CS/CNP/Thy films, by which the color of CS/CNP films was used as the reference. There was a slight increment in ΔE values ($p<0.05$) when thymol concentrations were increased from 0 to 4.5 w/w% of thymol. This trend was expected due to the changes in L*, a*, and b* values as discussed earlier.

In addition, Berk (2009) stated that transparency is a vital property in packaging to allow the consumers to see the product inside the package and evaluate the quality of food products such as fresh meat, poultry, fruits, and vegetables based on physical appearance. In general, high opacity resulted in low transparency of the films. From Table 4.1, it can be seen that the addition of a high concentration of thymol (4.5 w/w%) increased slightly the opacity value of the films. Jessica et al. (2017) that incorporated

CH-starch films with natural extracts (oregano, blueberry, beetroot,) and Nordin et al. (2020) that produced CS films incorporated with thymol and glycerol reported that the opacity of their films increased slightly with the addition of thymol into the film matrix Zhong et al., (2017) reported that the opacity of PPI films increased slightly with the increase in thymol concentrations due to the presence of polyphenols in the films. The lipophilic compound inside thymol may hinder the light transmittance and caused light scattering, thus increased the opacity. However, the increment in opacity value found in this study was very small and when observed from Figure 4.3, the changes in opacity or transparency of the films were non-noticeable. In the meantime, the increment in the opacity values may provide benefits to prevent food decomposition via light, especially for light-sensitive food products.

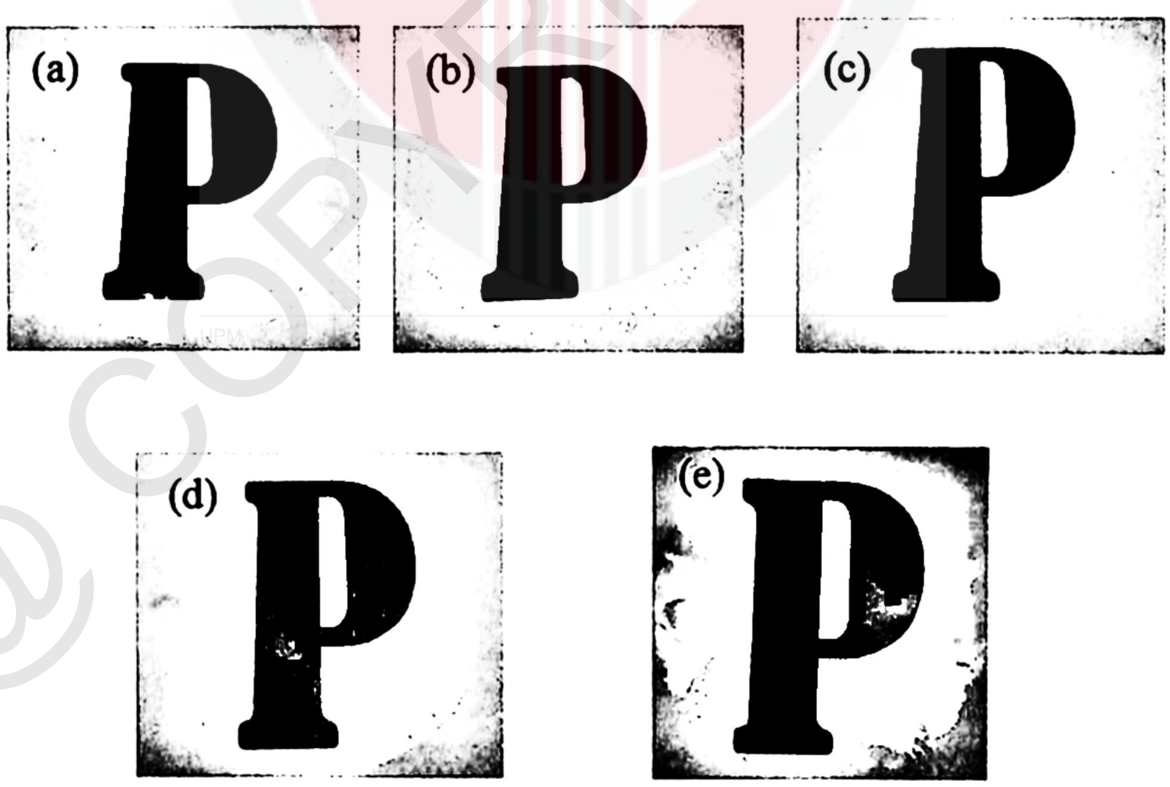


Figure 4.3 Appearance of films on alphabet of 'P'; (a) CS film (b) CS/CNP film (c) CS/CNP/Thy 1.5% (d) CS/CNP/Thy 3% (e) CS/CNP/Thy 4.5%

4.1.2 Mechanical Properties

Mechanical properties of food packaging material are crucial to evaluate the strength and durability of the material to package food products and withstand exert forces.

Figure 4.4 shows that neat CS film has poor TS but the incorporation of CNP into the neat CS film resulted in the increase in the TS. According to Shapi'i et al. (2019), the incorporation of chitosan especially in nanosized form or CNP, has the potential to improve the mechanical properties of starch films. The tiny size and regular shape of the CNP due to the crosslinking of chitosan and TPP during the ionic gelation process, facilitated the CNP to fill in the empty spaces between the matrix of the starch film (Lorevice et al., 2015). The large surface area of CNP exposed to the starch increased the intermolecular interaction between the starch and CNP. This brought the distances of adjacent starch chains closer (Ma et al., 2009), leading to an increase in the density of the starch film. The film became more compact, strong, and thus higher resistant to mechanical stress.

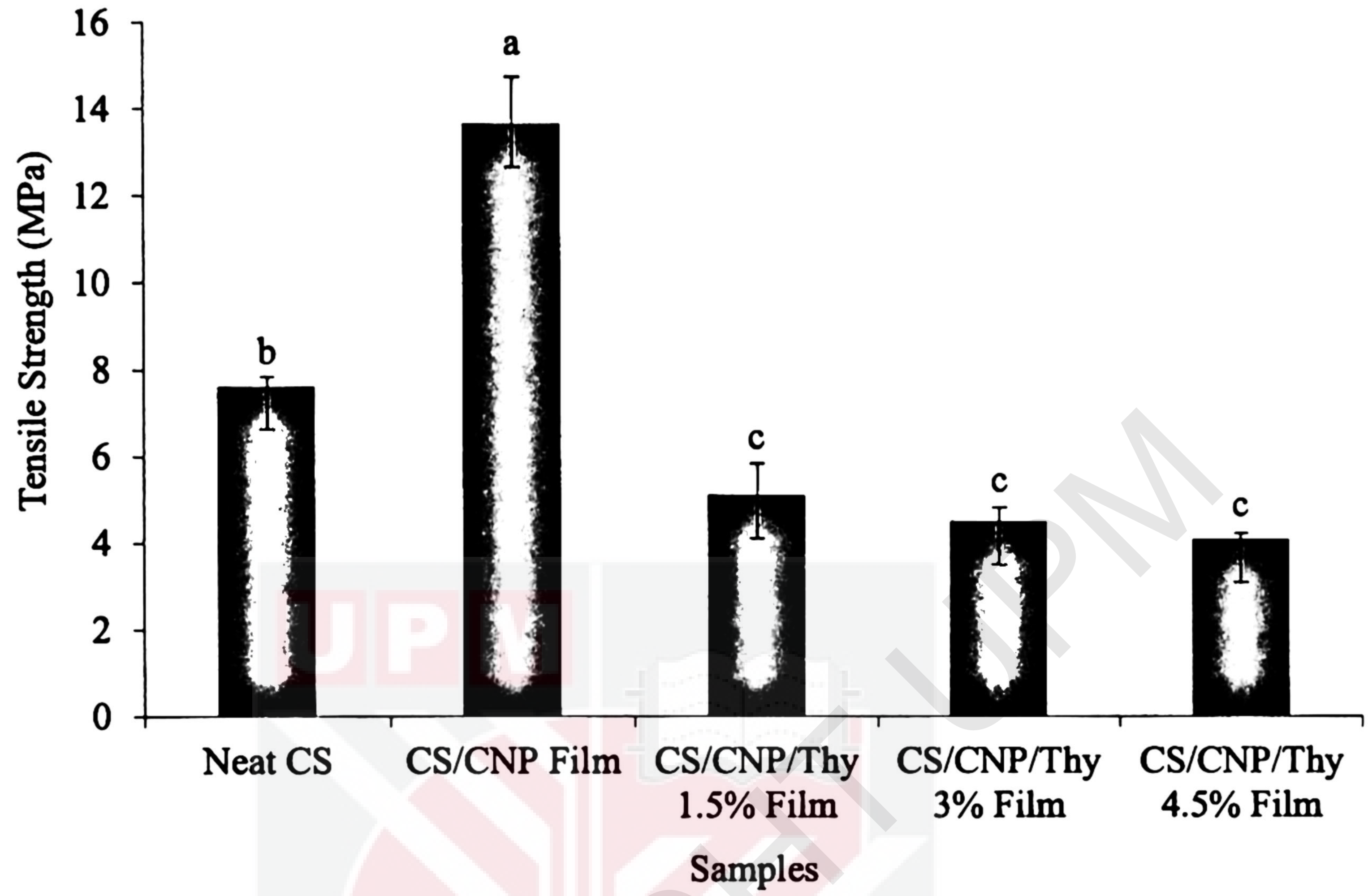


Figure 4.4 Effect of thymol concentration on TS of films. Different letters in the same graph indicate a statistically significant difference ($p<0.05$)

Figure 4.1 also shows the effect of thymol concentrations on the TS of CS/CNP/Thy films. The addition of 1.5 w/w% of thymol into the CS/CNP films caused a significant reduction in TS ($p<0.05$) from 13.7 MPa for CS/CNP films to 5.1 MPa for CS/CNP/thymol films which was a 63% reduction. However, when the concentration of thymol was increased from 1.5 to 4.5 w/w %, no significant change ($p > 0.05$) was observed for the TS of the films. The interaction between the starch biopolymer matrix and phenolic compounds resulting in the heterogeneity of the film structure, thus reducing the TS of the films. A similar trend of findings was revealed by Ramos et al. (2012) where they found a slight modification of TS when thymol was incorporated into the PP films. The reduction in TS was attributed to the complex structures formed between the lipids from the phenolic compounds extracted from EO and the starch polymers which reduce the cohesion of the starch network forces, thus subsequently decreasing the films' resistance to breakage (Jiménez et al., 2013). The addition of thymol possibly resulted in the lowered interaction between biopolymer monomers and may hinder polymer chain-to-chain interactions and reduce cross-linking. This finding is also consistent with the work of Nordin et al. (2020) who incorporated thymol and glycerol in CS film and found a decrease in TS by 96% compared to neat CS film. A similar trend of finding was also observed by Ghasemlou et al. (2013) who investigated the TS of starch film incorporated with ZEO or MEO. Tawakkal et al. (2016) reported that PLA composites incorporated with 30% w/w untreated kenaf (UK) or treated kenaf (TK) fibers with 0, 5, and 10% w/w thymol loadings experienced reduction in TS values. They reported that a localized plasticizing effect between the PLA and the thymol occurred which facilitated thymol molecules diffusion into the bulk of the matrix between the PLA chains. In this work, the addition of thymol interferes with the interaction between the polymer matrix and the CNP in the presence of the applied stress, and subsequently reduced the TS.

The percentage of elongation at break (EAB, %) determines the elasticity of the films and the potential of the films to stretch. Figure 4.5 shows that the EAB of neat CS films increased from 139 to 157% with the addition of CNP into the neat CS film. This was because most of the empty spaces within the starch matrix were filled up with CNP and formed strong hydrogen bonding which resulted in the increase of both TS and EAB. Moura et al. (2011) found that the addition of 40% w/w CNP increased the EAB of neat CMC films. They reported that the improvement of the films flexibility was attributed to the optimum particle size of CNP added into the films.

A similar trend of result was achieved when CS film and cassava film were added with CH (Julia et al., 2018). They found that the addition of CH in both starches films exhibited higher EAB values, 108% and 146% respectively compared to control films that presented higher deformation. This was due to the existence of synergic compatibility between starch and CH after film formation. When nanoparticles were presented, the biopolymer matrix reduces the number of interface defects and led the molecular movement of CNP molecules. Thus, the deformation capacity of polymeric matrix upon application of tension deformation was promoted, which allowed the films to have a higher elongation capacity until their rupture (Rodrigues et al., 2020)

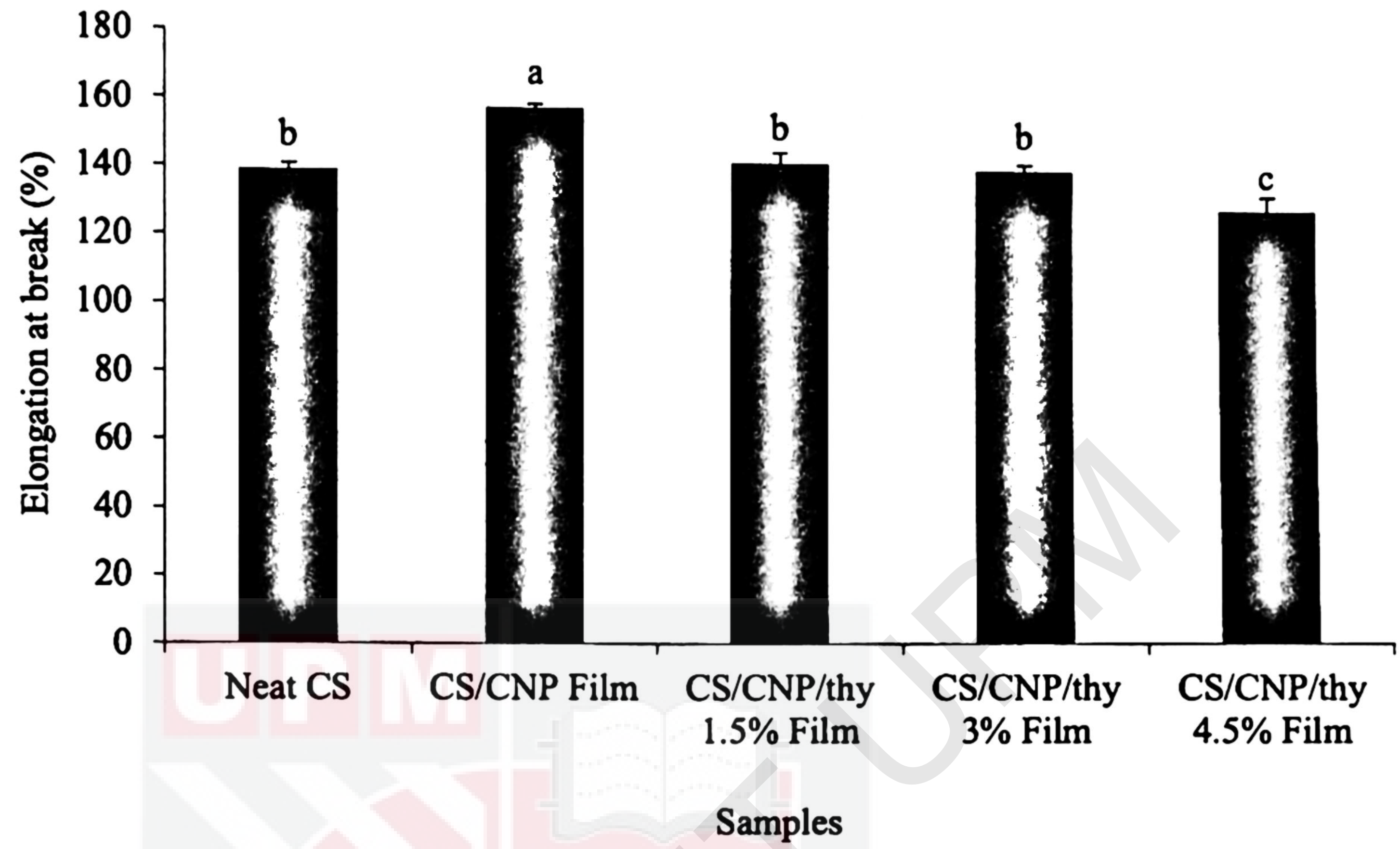


Figure 4.5 Effect of thymol concentration on EAB of films. Different letters in the same graph indicate a statistically significant difference (p<0.05)

Meanwhile, the incorporation of thymol (1.5 w/w% and 3 w/w%) did not significantly affect the EAB of the neat CS films. However, the addition of 4.5 w/w% thymol to the CS/CNP films caused a slight reduction in the EAB whereby EAB reduced from 140% to 129% for neat CS and CS/CNP/thymol incorporated with 4.5 w/w% thymol, respectively. As found by Robledo et al. (2018) and Nordin et al., (2020), the addition of thymol at a certain concentration formed a non-miscible phase in the starch matrix that caused segregation of the starch chains. Hence, the chain mobility reduces and this causes a reduction in the elasticity of the film. In this work, concentrations of 1.5 and 3 w/w% might be low to affect the EAB values of the films but 4.5 w/w% resulted in the decrease of the EAB of the CS/CNP/thymols films due to enough thymol to cause segregation of the starch chains. A similar finding was obtained by Altiok et al.

(2010) whereby the increment of thyme oil concentrations caused a reduction in EAB of CH film. They also stated that the decrement was caused by the increase in pore size and porosity of the films.

Young's modulus indicates the stiffness or rigidity of the film and a larger value correlates to a more rigid material (Pelissari et al., 2012) High YM indicates high stiffness of material whereas low YM value indicates the film has high elasticity. The YM values have been evaluated from the experimental stress-strain curves obtained for all the prepared films. Figure 4.6 shows the effect of thymol addition on the YM of CS/CNP films. Initially, the addition of CNP (15% w/w) into the neat CS film increased significantly the YM of the films from 33.7 to 63.6 Mpa. This finding is consistent with the TS value obtained earlier, whereby the increment in TS value indicates an improvement in the rigidity of the films, hence stiffer films, and thus increment in the YM value. A similar trend of results was achieved by Akter et al., (2014) whereby the CH added into starch films acted as a reinforcing agent in the films, thus increased YM values. In their study, YM values of the starch-based films improved significantly ($p < 0.05$) with the incorporation of CH. However, this finding was opposite to the finding obtained by Chylin et al. (2019) whereby YM values reduced when CH was added to the carrot cellulose nanofibre film. Pelissari et al. (2009) also found that YM values decreased when there were a the addition of 5 % w/w of CH to the cassava starch film. They explained that CH acted similarly to plasticizer and enhanced the ductility when incorporated in a starch network due to the direct interactions and the reduction of proximity between starch chains (Mali et al., 2006)

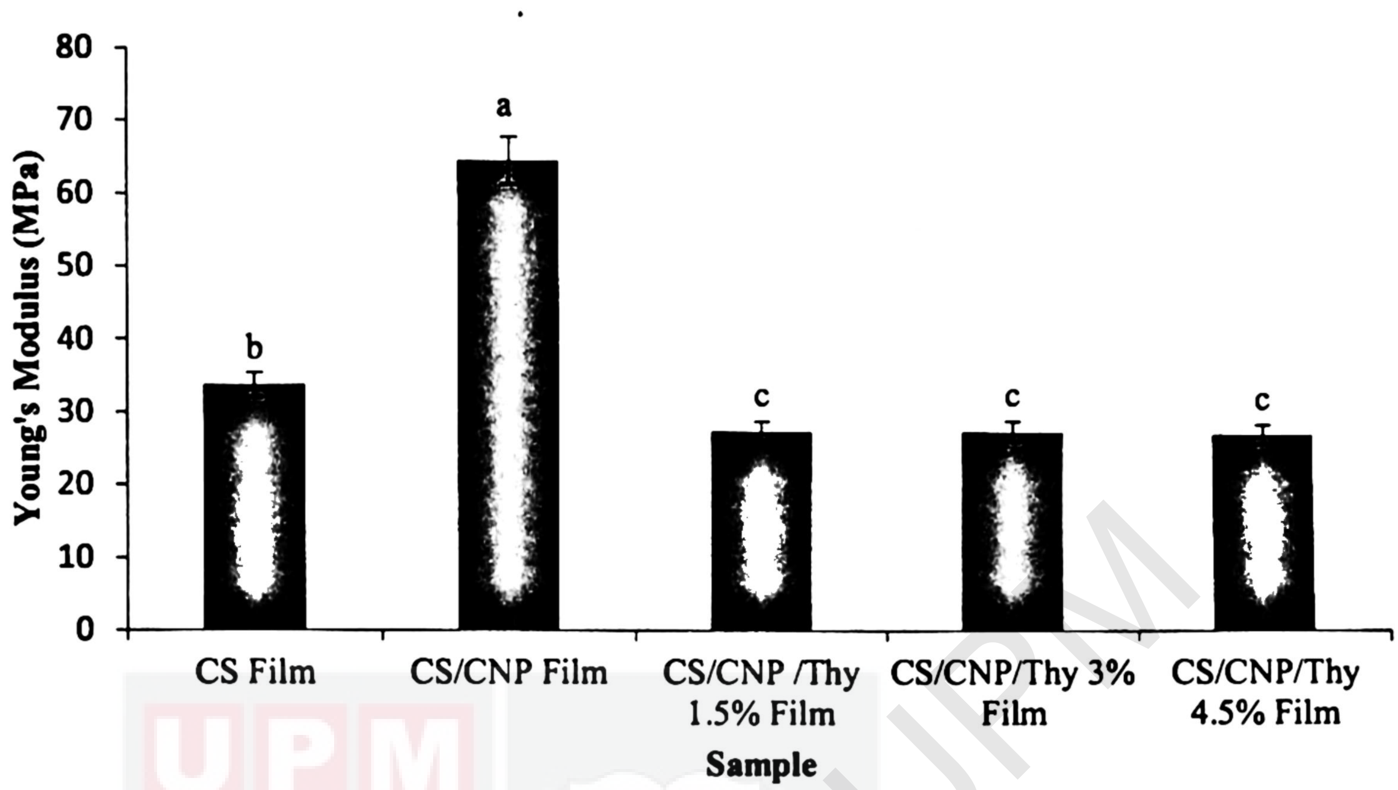


Figure 4.6 Effect of thymol concentration on YM of starch/CNP film. The data are reported a mean ±SD, n=3. Different letters indicate a significant difference at p<0.05.

Figure 4.6 also shows that the YM values decreased significantly ($p<0.05$) with the addition of thymol. Nonetheless, there were no significant change of YM when the thymol concentrations were varied from 1.5 to 4.5% w/w. Similar trend of results was reported by Villegas et al. (2019) whereby reduction of YM values were observed when thymol was added to PLA. This finding was related to the plasticizing effect of thymol that lead to the change of material stretchability which was proven through SEM observations of the films' cross-sections. Besides, Pelissari et al. (2009) found that YM of cassava starch-CH films increased with an increase in oregano EO concentration. This result was relevant to this study because oregano EO contained phenolic compounds that mainly comprised of thymol (Tavakoli et al., 2017). Besides, the addition of thymol may result in the lowered interaction between the starch monomers and may retard polymer chain-to-chain interactions and minimize cross-linking (Kavoosi et al., 2013). Hence, the decrease in rigidity of films was gained due to the increment of elasticity. Khairuddin et al. (2020) also found that the incorporation of 1.5% to 2.5% w/w thymol caused the YM decrement of hydroxyethyl cellulose (HEC)/wheat- starch-based films. They supported their findings by the plasticizing effect caused by the addition thymol to the polymer matrix. As a result, the ductile properties of the films increased, which was due to the changes of the materials' crystallinity. Furthermore, the addition of phenolic compounds extracted from EO resulted in a less dense film matrix that facilitated the movement of polymer chains, causing the films to be flexible and became less rigid (Pelissari et al., 2009), hence high YM value.

4.1.3 Barrier Properties (Water Vapor Permeability)

The main function of a food packaging is often to avoid or at least to decrease moisture transfer between the food and the surrounding atmosphere. Therefore, the WVP of food packaging material should be as low as possible. Evaluation of the WVP of polymer films is necessary if films incorporated with phenolic compounds are applied for foods at medium and high humidity. From Figure 4.7, it can be seen that the WVP of CS/CNP films increases from 0.66 x 10^{-7} to 1.96 x 10^{-7} g/Pa h m with the addition of 1.5 w/w% thymol. A similar trend of results was obtained by Wu et al. (2014) whereby the incorporation of 3 wt% thymol into neat PLA/PCL films resulted to the increment of WVP. The increment was due to the addition of thymol that increased the average pore size of the films. Wu et al. (2014) observed that many voids present in PLA/PCL/Thy films through scanning electron microscopy (SEM) images. Thus, the voids permitted more water vapor to transfer through the films. Similarly, Kavoosi et al. (2013) reported that the incorporation of thymol caused a significant increase ($p < 0.05$) in the WVP of gelatin films. Subsequently, the addition of thymol might hinder the polymer chain-to-chain interactions and minimized the cross-linking that previously existed within the biopolymer matrix. Thus, the WVP increased due to the disruption of the chain matrix.

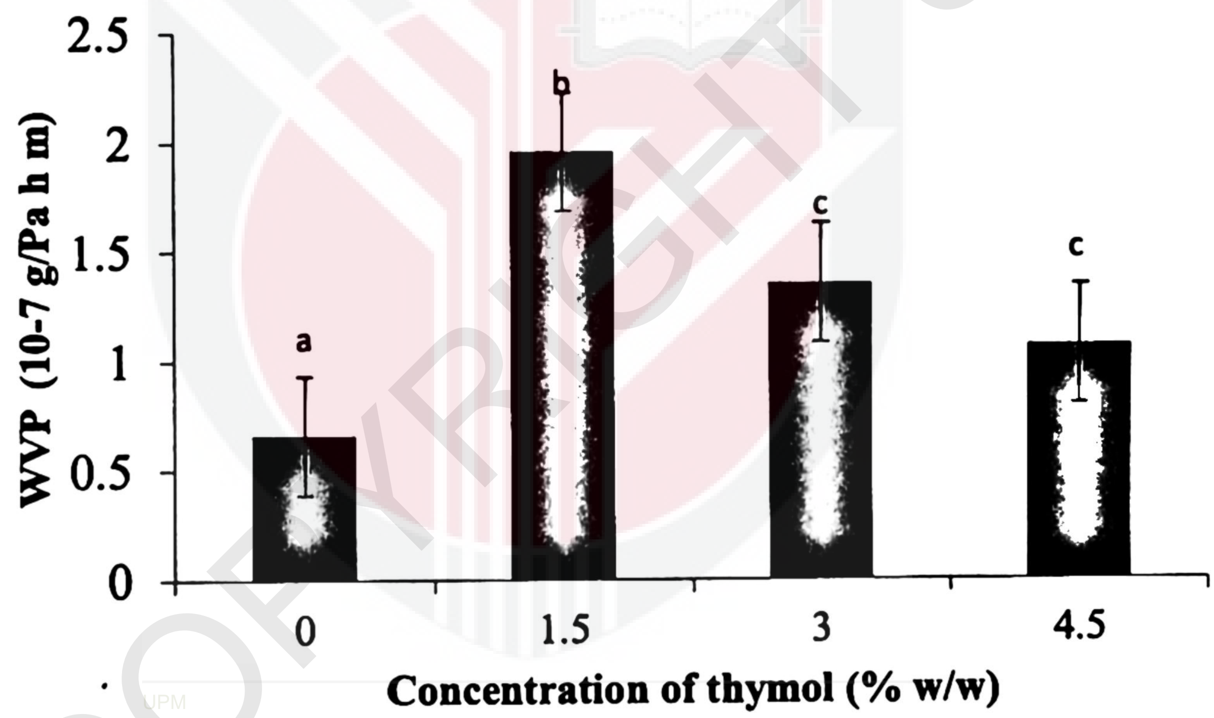


Figure 4.7 WVP of different concentration of thymol incorporated to CS/CNP films

The addition of higher concentrations of thymol (3 and 4.5 w/w%), reduced the WVP due to the hydrophobicity of the thymol (Kavoosi et al., 2013). The decrement in the WVP of the films may be due to the hydrogen and covalent interactions between the starch network and these polyphenolic compounds, whereby the interactions may limit the availability of hydrogen groups to form hydrophilic bonds with water and subsequently, lead to a decrease in the film's affinity for water (Shen et al. 2010). A similar trend of results was reported by Zhong (2017) that incorporated thymol into a modified peanut protein isolate (PPI) film. They found that the hydrophobic nature of thymol disrupted the hydrophilic/hydrophobic balance of the film, hence decreased the WVP. Thymol which is a major component of thyme EO that was hydrophobic, may act to enhance the hydrophobic nature of the film. Thus, this phenolic compound has the ability to limit water vapor penetration through the film (Moey et al., 2018). Besides, Pellisari et al. (2009) analyzed that the ratio of the hydrophilic-hydrophobic component determined the rate of water vapor transfer which mostly happens at the hydrophilic side. Therefore, increasing the ratio of hydrophobic could develop barrier properties. Another study carried out by Ghasemlou et al. (2013) proved that the addition of ZEO or MEO improved the barrier properties of starch film and gelatin films (Kavoosi et al., 2013).

4.2 Demonstration on the application of CS/CNP/Thy films as food packaging films

The shelf life of cherry tomatoes was determined in terms of firmness, weight loss, and observation of mold growth.

4.2.1 Firmness

Firmness is one of the important physical characteristics that determine the quality of cherry tomatoes. It is closely related to the ripening of the fruit that resulted in short postharvest life (Taye et al., 2019). According to Wang et al. (2009), the peak impact force decreases with the increase in ripeness. Peak impact force referred to the maximum force and the resistance to penetration of the cherry tomatoes. The ripening process leads to the softening of the tomato which requires only low force to penetrate the skin and the flesh of cherry tomatoes. Figure 4.8 shows the firmness of the cherry tomatoes packaged with different types of films over 6 days of storage at ambient temperature. In general, the firmness seems to decrease with the increase in the number of storage days for all the films during storage at ambient temperature because the cherry tomatoes underwent the ripening process during the storage. Othman (2008) explained that fruit firmness decreased due to the ripening process that occurs and the softening of vegetative tissues that are usually caused by catabolism of cell wall polysaccharides (hemicellulose). The hemicelluloses and pectin become more soluble, which caused disruption and loosening of the cell walls resulting in the softness of food during ripening (Abiso et al., 2015). Besides, change in the firmness of fresh products may occur due to loss of moisture through transpiration.

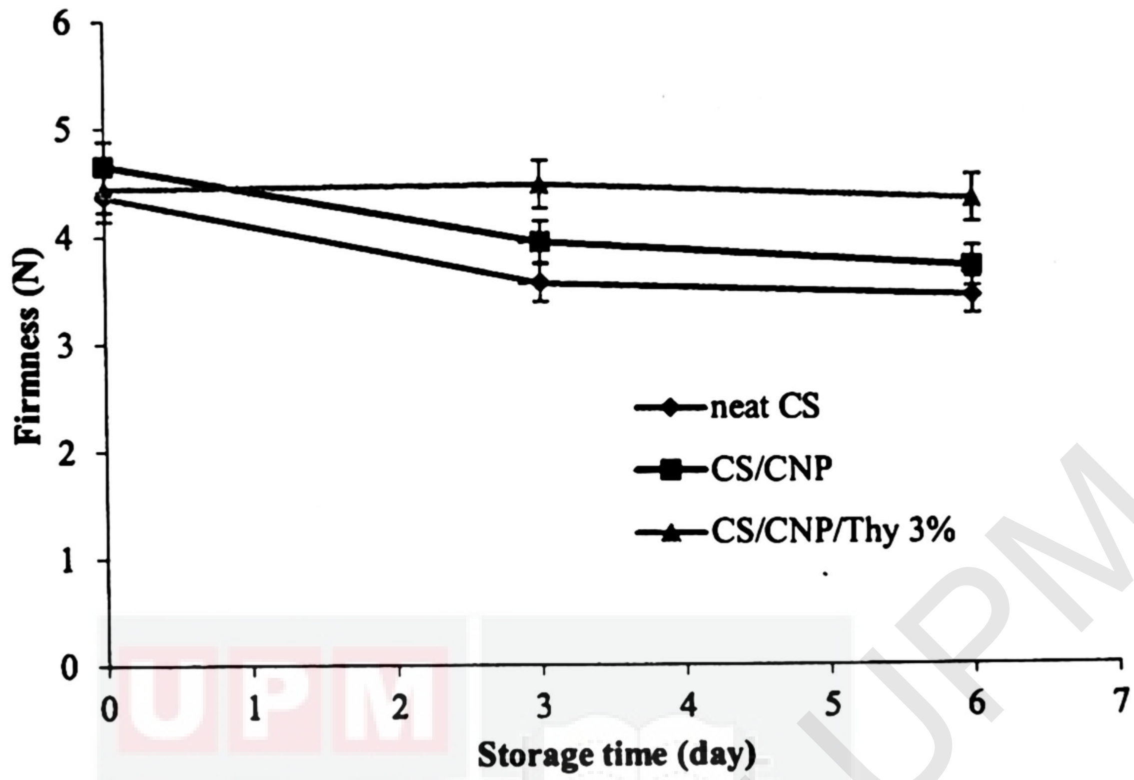


Figure 4.8 Firmness of cherry tomatoes packaged with (a) neat CS(b) CS/CNP (c) CS/CNP/Thy 3% films during 6 days storage at ambient condition

The cherry tomatoes stored in neat CS film which acted as control exhibited the maximum declination of firmness throughout the storage which was 20% of the initial firmness, followed by cherry tomatoes stored in CS/CNP films which was 19% reduction of the initial firmness, and 1.4 % reduction of the initial firmness for cherry tomatoes stored in CS/CNP/Thy 3% films.

The firmness of cherry tomatoes stored in CS/CNP/Thy 3% films was significantly maintained compared to CS and CS/CNP films. This was due to the presence of thymol that is an AM agent, released into the packaging which minimized tissue softening and helps in firmness retention during storage. Handling (2010) in his work found that the highest values of firmness (28 N) at the end of the storage period were achieved in strawberries coated with soy or gluten plus thymol. Correa-pacheco et al. (2015) also reported the same trend of results in which the avocado incorporated with chitosan-thyme essential oil nanoparticles (CSTEO-NPs) edible coating had higher fruit firmness compared to that of the untreated. Rahimi et al., (2019) found that chitosan combined with thymol positively affected the retention of firmness in peach fruits by minimizing water loss and fruit senescence, and reducing cell wall degradation by the action of inhibition of microbial propagation. In addition, Qin et al. (2016) stated that hot peppers stored in PLA/PLC/Thy could maintain the ripening process at a slow rate. The natural volatile compounds of thymol present in the headspace of food packaging can inhibit pathogen growth (Ramos et al., 2013), thus helps to retain fruit firmness.

Meanwhile, the firmness reduction of cherry tomatoes packed with CS/CNP films was slightly lower (19%) than that of neat CS films (20%) probably due to the existence of CNP that is also an AM agent. Nonetheless, the reduction was low because CNP requires the food to become in direct contact with it, before it can act as an AM agent.

4.2.2 Weight loss

The weight loss of fruits during storage is usually caused by water loss due to fruit transpiration (Medina et al., 2019). Figure 4.9 shows that weight loss (%) of cherry tomatoes is directly proportional to the storage time (day) for all the samples. Tomato cherries packaged in CS/CNP films exhibited a lower percentage weight loss compared to that packaged in neat CS film. This was due to the existence of CNP that had the ability to occupy the empty spaces of the neat starch film matrix and form a tortuous path within the starch matrix. Thus, it was harder for water molecules to permeate due to the relatively compact structure of the films (Antoniou et al., 2015). CNP was able to form a water barrier between the fruit and the external environment, thus minimizing the external transfer of water (Medina et al., 2019). The same trend of result was reported by Medina et al. (2019) whereby cherry tomatoes and blueberries coated with chitosan thymol nanoparticles that packed in clamshell exhibited a lower percentage of weight loss compared to control film.

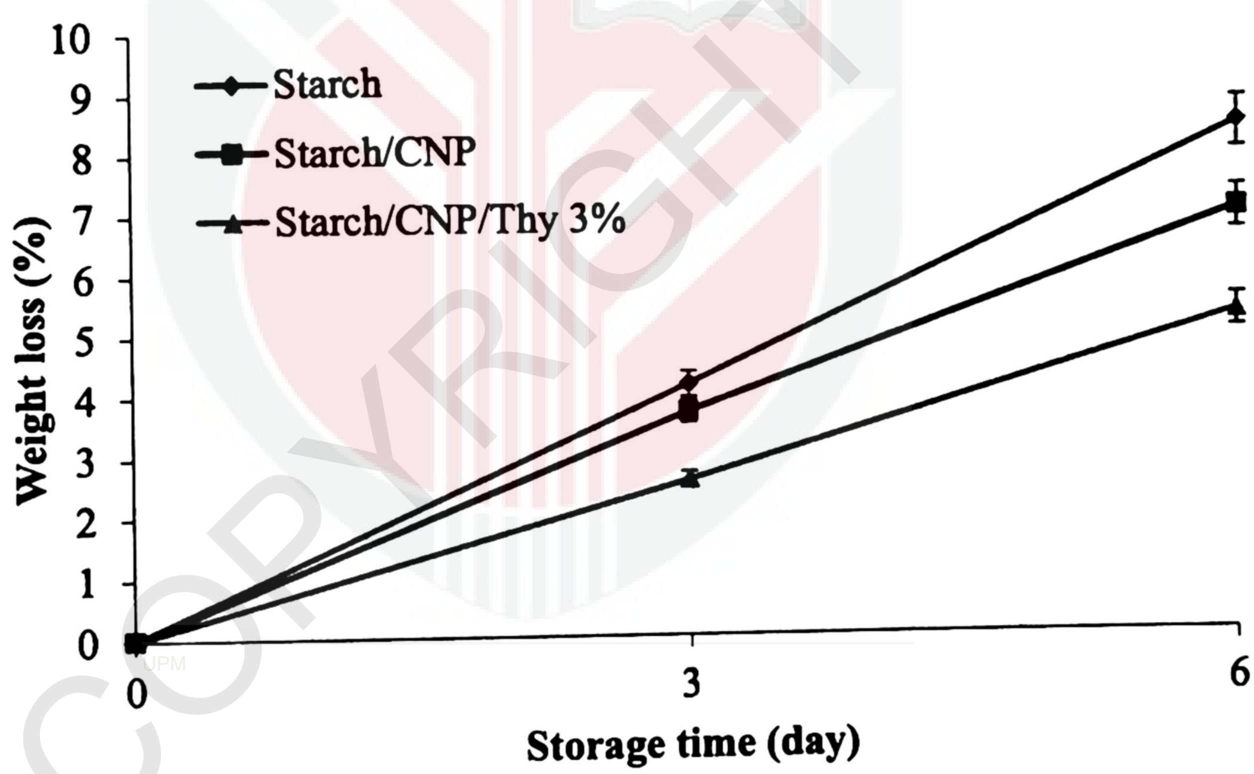


Figure 4.9 Percentage of weight loss of cherry tomatoes packaged in neat CS film (control), CS/CNP films and CS/CNP/thy 3%, stored at ambient temperature.

On the other hand, Cherry tomatoes that packed in CS/CNP/Thy 3 % films had the lowest weight loss whereby the weight loss was approximately 3.1 and 1.8% lower than that packed in neat CS and CS/CNP films on days 6, respectively. A study carried out by Sun et al. (2014) stated that the essential oil had hydrophobic traits which can reduce moisture loss of the food. A study carried out by Choi et al. (2016) suggested that hydrophobic essential oils such as oregano EO and bergamot EO in hydroxypropyl methylcellulose (HPMC) were potentially good water barriers to reduce moisture evaporation which directly causes the weight loss of fruit. Badawy et al. (2017) reported the same trend of result whereby the incorporation of thymol into the film-forming solution resulted in a slightly positive effect on the weight loss reduction of strawberries packaged with the gelatin/CH films. A treatment of strawberries with thymol also showed a similar effect whereby the strawberries exhibit the lowest weight loss after the storage for 15 days at 0°C due to the modifying internal atmospheres that slowed down the respiration rate of fruit (Handling, 2010). Hence, thymol was proven to be an effective phenolic compound to minimize the dehydration of fruit and caused delay in the reduction of weight loss percentage.

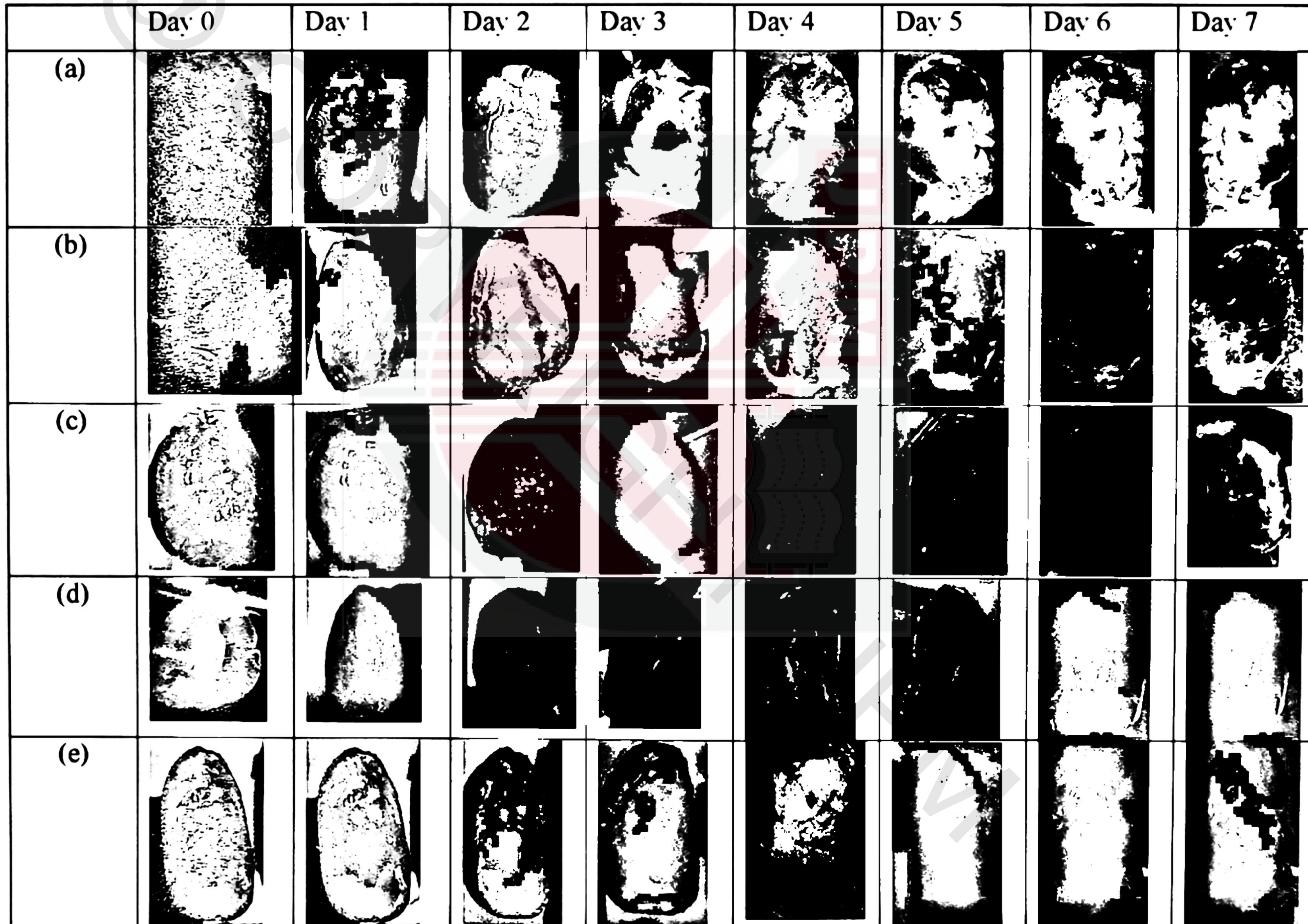
4.2.3 Cherry tomatoes: observation

The effectiveness of the developed films was evaluated by putting the films in direct contact with sliced cherry tomatoes and visually observed the occurrence of mold growth on the sliced cherry tomatoes with time. Table 4.2 shows the appearance of sliced cherry tomatoes samples from day 0 until day 7. It was found that mold growth on sliced cherry tomato samples that were in direct contact with the CS/CNP films was slower than that to neat CS film. The same observation was reported by Lustriane et al., (2018) whereby the incorporation of CH and CNP into the coating of banana was found to retard the decay and maintain the quality of the banana. Xing et al., (2016) stated that chitosan coating enriched with antimicrobials exhibited excellent inhibition on the growth of bacteria, yeast, and molds whereas it can retard the growth of microbes on the food. A study reported by Shapi'i et al. (2019) stated that starch/CNP films have potential as antimicrobial packaging. They found that cherry tomatoes packed with starch/CNP films exhibited highest the lowest mold growth due to the tiny size of CNP that can inhibit microbial growth.

Moreover, Table 4.2 shows that the CS/CNP/Thy 1.5% films exhibited satisfactory results in delaying the mold growth until day 6 of storage compared to CS/CNP films. The incorporation of thymol as AM agents could further slowdown the growth of mold on cherry tomatoes. A study by Medina et al. (2019) reported that chitosan thymol nanoparticles (CTNP) had better efficiency in inhibiting the mol growth compared to only CNP due to the additional of thymol which contributed to more AM contents. Table 4.2 also presented that cherry tomatoes in direct contact with CS/CNP/Thy 3% films have a consistent appearance with no microbial/mold growth until the end of the storage period. This result represented that 3 w/w% concentration of thymol incorporated in the films was the optimum concentration to exhibit the microbial growth and prolong the shelf life of the food product. The release of thymol from CS/CNP/Thy 3% film produced a suitable atmosphere that could reduce the respiration rate and fungal growth with minimal alteration of organoleptic properties and increased the shelf life of cherry tomatoes. The microbial mode of action of thymol has been postulated as disruption of cellular membrane functions and interference with active sites of enzymes and cellular metabolism (Marino et al., 2001) which may change the permeability of membranes of the microbes for cations and alter the ion gradients that lead the microbe cell to destruct and cell death.

Nonetheless, it was found in this study that cherry tomatoes in direct contact with the CS/CNP films incorporated with thymol concentration of 4.5 w/w% started to exhibit mold growth on the surface from day 3. The 4.5 w/w%% of thymol concentration might be too high, which interfered the gas diffusion through the films and the lack of gas diffusion might generate heat and anaerobic condition which led to mold growth (Lustriane et al., 2018). Besides, the problem may be due to the difficulty of uniform dispersion of the hydrophobic of thymol that caused non homogenize film-forming solutions. Moreover, thymol had limited water solubility, thus the undissolved thymol caused by non-homogenize solution applied at a concentration above the solubility limit impacts the visual quality and antimicrobial availability (Shah, et al., 2012). Thus, the incorporation of optimum concentration of thymol (3 w/w%) in the films exhibits the potential to increase the shelf-life of cherry tomatoes during storage as presented from the good results in the inhibition of the mold growth of cherry tomatoes.

Table 4.2 Appearance of cherry tomatoes exposed to the films (a) Neat corn starch film, (b) CS/CNP film, (C) CS/CNP/Thy 1.5% film (d) CS/CNP/Thy 3% film and (e) CS/CNP/Thy 4.5% film



CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

The primary aim of this work was to develop CS/CNP/Thy films as potential food packaging material. CS/CNP/Thy films have been successfully produced, characterized, and demonstrated as food packaging material. The main findings of this work have been summarized in Sections 5.1.1 and 5.1.2 according to the objectives of the present work.

5.1.1 Characterization of CS/CNP/Thy Films

Starch/CNP/Thy films that were produced at different concentrations of thymol were characterized in terms of physical, mechanical, and barrier properties. It was found that the addition of thymol at 4.5 w/w% increased slightly the opacity of the films due to the intrinsic color of thymol which is a white crystalline substance. The changes in the color properties of the films were also minimal and almost non-noticeable. Mechanical properties analysis revealed that the addition of thymol to the CS/CNP films reduced slightly the TS of the films but the increment of concentrations of thymol did not significantly affected the TS value. The addition of thymol to CS/CNP film decreased slightly the EAB and YM values ($p<0.05$). These were due to the plasticizing effects of thymol whereby lipids from the phenolic compounds reduced the cohesion of the starch network forces. It was also found that the addition of 1.5 w/w% to CS/CNP films increased the WVP but the WVP decreased with the increment of thymol concentrations due to the hydrophobic nature of thymol disrupted the hydrophilic/hydrophobic balance of the film and thymol has the ability to limit the water vapor penetration through the film.

5.1.2 Demonstration on the application of CS/CNP/Thy films as food packaging films

The shelf-life studies of cherry tomatoes packed using the produced films revealed that the CS/CNP/Thy 3% film was efficient to maintain firmness and the weight loss of cherry tomatoes compared to neat CS films and CS/CNP films after 6 days of storage. It was found that 3 w/w% thymol film was the optimum concentration to inhibit mold growth on cherry tomatoes after 6 days of storage. The results from this work confirmed the potential application of the CS/CNP/Thy films produced in this study as active food packaging materials that can enhance the shelf life of food products,

5.2 Recommendations

The present work found that the addition of thymol into CS/CNP film was able to maintain the quality of food during storage. Nonetheless, further study should be explored to enhance this research for better development of CS/CNP/Thy films.

1. It is recommended that for future studies to conduct the antimicrobial activity analysis of the films for food applications via microbial count rather than through visual observation of microbial growth.

2. It is suggested for future studies to encapsulate the thymol inside chitosan nanoparticles to reduce the volatility of the thymol and improve the performance of the films.

3. In order to obtain more reliable results, the film may be applied to other types of food such as fruits and meat and also extending the shelf life study storage period. The storage can also be conducted at different storage conditions such as different relative humidity and temperature.

4. Moisture sorption analysis is also important to determine the stability of the starch/CNP/Thy films toward moisture as hydrophilic properties of starch/CNP films might shorten the shelf life of the film.

5. In order to ensure the application of starch/CNP films is safe for the end-user, it is suggested to investigate the genotoxicity of starch/CNP films in the future. so that the films will be promising to be used as food packaging materials.

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APPENDIX

Appendix A. Linear regression analysis of moisture uptake over time for water vapor barrier analysis of films.

Linear regression was used to find the slope (W/t) of the moisture uptake (g) against time (day) graph. W/t then was used in the equation 3.4 to find the WVTR. R-squared (R^2) for each linear regression line was obtained from the graph.

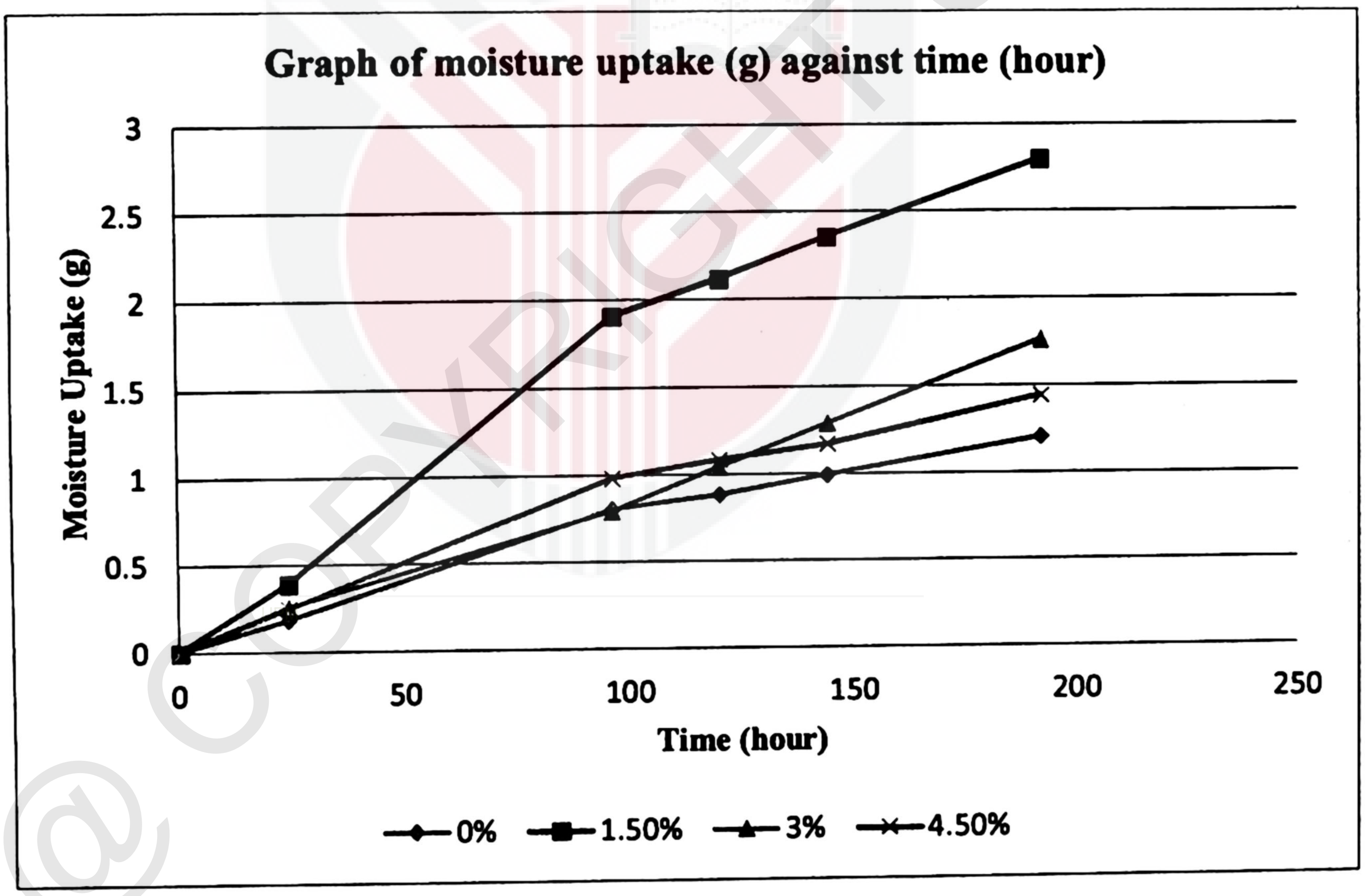

Figure G-1: Moisture uptake (g) against time (day) graph of films.

The equations of linear regression line and R^2 of each sample are tabulated in the Table G-1.

Table G-1: Equation of linear regression line and R^2 of CS/CNP films at different concentration of thymol.

Concentration of thymol (w/w%)	Equation Line	R-squared value (R^2)
0	y = 0.0064x + 0.0559	0.9726
1.5	y = 0.0153x + 0.1297	0.9655
3	y = 0.0089x - 0.0025	0.9955
4.5	y = 0.0076x + 0.087	0.9671

Note that the equations line are correspond to the y = mx + c where m is W/t.