# APPLICATION OF POLY (LACTIC ACID)/CELLULOSE NANOCRYSTAL NANOCOMPOSITE FILMS FOR THE PRESERVATION OF OXYGEN-SENSITIVE FOOD

By

Dangkamol Wongthanaroj

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# ABSTRACT

## APPLICATION OF POLY (LACTIC ACID)/CELLULOSE NANOCRYSTAL NANOCOMPOSITE FILMS FOR THE PRESERVATION OF OXYGEN-SENSITIVE FOOD

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The development of enzymatic browning is one of the main problems in the preservation of fresh-cut and oxygen sensitive fruits such as avocados. This study employed packaging solution based on PLA/cellulose nanocrystal (CNC) films with strong oxygen and UV barrier properties to prevent browning in avocado and prolong its shelf life. Specifically, the effects of package material types (neat PLA vs PLA/CNC nanocomposite) and storage conditions (temperature and time) on discoloration (browning), in-package humidity level and gaseous compositions, microbial growth, as well as shelf life were assessed. Enzymatic browning was visually evaluated and total discoloration ($\Delta E^*$) quantified as function of storage time and temperature to estimate the shelf life of the avocados using polynomial third degree regression equation. Nanocomposite package inhibited the browning of avocado when compared to neat PLA package due to the higher oxygen barrier of nanocomposite film, which effectively delayed oxidation of packaged avocados. Compared to neat PLA film, packaging with nanocomposite film significantly slowed down the avocados' respiration and maintained a high humidity atmosphere inside the package, which delayed the enzymatic browning, irrespective of storage conditions. PLA/CNC nanocomposite package extended the shelf life of fresh-cut avocados by additional 54 h and 98 h compared to neat PLA package, when stored at 23°C and 4°C, respectively. PLA-based films prevent the microbial growth. These results clearly demonstrate the potential of PLA/CNC film to extend the shelf-life of oxygen-sensitive food products.

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2021

Dedicated to my parents
Montol Wongthanaroj and Anchana Tatiyapanyalert

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# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Traditional petroleum-based plastics such as polyolefins, polyethylene terephthalate, nylons, etc. have been widely used in the packaging industry as materials for flexible or rigid formats to protect and preserve packaged foods, durable and non-durable goods, healthcare/pharmaceutical products, etc. due to their excellent mechanical properties, flexibility, transparency, barrier abilities, among others (Liu & Matuana, 2019; Natarajan et al., 2022; Rader et al., 2021; Vytejčková et al., 2017). Active food packaging that incorporates antioxidant, anti-ultraviolet, and other active ingredients into the plastics have also been developed in recent decades as a suitable method to inhibit microbial growth and extend the shelf life of foods (Miao et al., 2019; Shapi'i et al., 2020; Srisa & Harnkarnsujarit, 2020; Zhang et al., 2021). Unlike traditional materials that relies mainly on their barrier capacities to protect packaged products, active packaging materials in contrast continuously release antioxidants or remove undesirable compounds in the packages. They also decrease the photo-oxidation in the packaging; thus, keeping an internal environment appropriate for food storage (Vilela et al., 2018; Zhang et al., 2021).

Although conventional petroleum-based plastics have served the packaging industry in numerous applications, they are obtained from crude oil and their use is a major contributor to global greenhouse gas emissions since they are mostly either landfilled or incinerated at the end of their lives (Karkhanis & Matuana, 2019; Liu & Matuana, 2019; Natarajan et al., 2022; Shapi'i et al., 2020). The growing concerns about the environment coupled with legislations around the world banning specific materials derived from fossil fuels (e.g., single-use plastic packaging) are forcing the industry to look for alternative sustainable materials from renewable resources such as biobased and/or biodegradable plastics or toward a circular economy based on plastic recycling (Stark & Matuana, 2021).

Several bioplastics with a smaller carbon footprint and a better compostability compared to petroleum-based plastics have been developed in recent years. Some are commercially produced and tested for food packaging applications, including poly(lactic acid) (PLA), poly(butylene adipate-co-terephthalate) (PBAT), polyhydroxybutyrate (PHB), polybutylene succinate (PBS), polybutylene succinate-co-adipate (PBSA), starch, gelatin, etc.

The potential of active packaging based on bio-based and biodegradable plastics has been demonstrated by several investigators. For examples, extrusion cast films based on PLA/PBAT blends at different ratios (60/40 and 40/60) with incorporated trans-cinnamaldehyde (2-10%) as an antifungal agent showed a high antifungal efficacy by reducing bacterial and fungal growth in packaged breads, thus, extending shelf-life for 21 days at 30°C (Srisa & Harnkarnsujarit, 2020). Similarly, Miao and coworkers developed a biodegradable cast-extruded film based on PLA/PHB blends at 3-to-1 ratio that possesses antimicrobial ability by incorporating 6% (w/w) fennel oil into the blend. Not only the addition of fennel oil significantly improved the oxygen barrier performance of PLA/PHB film, but it also produced a film with a modified antioxidant ability and strong antibacterial ability that prolonged the shelf life of shucked oyster meats by 2-3 days (Miao et al., 2019). The potential application of solvent-cast starch films as antimicrobial food packaging has also been confirmed by incorporating chitosan nanoparticles synthesized via ionic gelation method into the starch matrix (Shapi'i et al., 2020). The results of *in vivo* study revealed that starch/nano chitosan film (15% w/w) was more efficient preventing the microbial growth in cherry tomatoes ($7\times10^{2}$ CFU/g) after 10 days period of storage at $10^{o}$C compared to neat starch film ($2.15\times10^{3}$ CFU/g). Recent study examined the bioactivities of solvent-cast chitosan/gelatin-based films loaded with tannic acid for the preservation of cold-stored fresh-cut apples (Zhang et al., 2021). The addition of tannic acid significantly improved the anti-ultraviolet and antioxidant capacities of chitosan/gelatin film. As a result, fresh-cut apples packaged by the film containing 1% tannic acid had a lower weight loss rate, reduced the degree of browning, inhibited lipid oxidase activity, and decreased malondialdehyde content during 10 days of storage at $4^{o}$C compared to the counterparts packaged in the film without tannic acid.

The use of biobased plastics with strong barrier capacities for food packaging applications has also received considerable attention in the scientific community. A systematic study assessed the suitability of PBS/PBSA co-extruded double layer films for vacuum packaging of raw chicken, turkey, and smoked turkey meat (breast) and compared the results with those obtained with the same foods packed with commonly used polyamide/low density polyethylene (PA/PE) double layer co-extruded films as the reference material (Vytejčková et al., 2017). Only minor changes of pH, water activity, microbiological quality, color, and profiles of volatile compounds were found during the storage throughout the shelf life of meats packaged in PBS/PBSA films compared to commonly used PA/PE film. Thus, confirming the practical application of PBS-based films for raw and smoked poultry meats. Recent investigation by Karkhanis and coworkers demonstrated the potential of extrusion-blown PLA films filled with 1% cellulose nanocrystals (CNCs) to extend the shelf-life of crackers, a moisture-sensitive food product compared to neat PLA films (Karkhanis et al., 2021). Crackers packaged within both PLA and PLA/CNC nanocomposite films were shelf-stable when stored below 50% RH at $25^{o}$C. However, the crackers packaged in the

PLA/CNC films had approximately 40% longer shelf-life compared to those packaged in neat PLA films when stored above 50% RH; clearly demonstrating the potential of CNC-based films to extend the shelf-life of dry-foods. This prolonged shelf life of crackers was attributed to the superior water barrier performance of the PLA/CNC films ($213.1 \times 10^{-16}$ $kg \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$) compared to the neat PLA counterpart films ($349.8 \times 10^{-16}$ $kg \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$) since the water vapor permeability of PLA film decreased in the range of 33-50% when only 1% CNC was added into the PLA matrix (Karkhanis et al., 2018a, 2018b; Matuana et al., 2016). Concerns on the toxicity and migration of CNCs due to their small dimensions have also been investigated. Numerous studies on the potential cytotoxicity, genotoxicity and ecotoxicity of CNCs through several *in vitro* and *in vivo* analysis showed no toxicity in food up to 4% (Fotie et al., 2020; Pradhan et al., 2020; Ede et al., 2020; Ong et al., 2020). Additionally, the migration levels of CNCs from the films in both polar and nonpolar food simulants were found well below the standard limits needed for food packaging materials (Fortunati et al., 2012; Silva et al., 2020), clearly showing the potential of CNC-based films for food contact applications.

It should also be mentioned that the addition of 1% CNC into PLA, not only decreased its oxygen permeability by approximately 62-75%, but also reduced the light transmittance in the UV region of the spectra (250-380 nm) indicating that CNCs blocked light in this region, making the nanocomposite films attractive materials for packaging applications requiring UV light protection (Karkhanis et al., 2018a). Packages with strong UV-barrier capacity can protect packaged foods such as fresh-cut fruits by decreasing the photo-oxidation in the packaging, thus restraining the enzymatic browning on the product (Vilela et al., 2018; Zhang et al., 2021).

Despite the better oxygen barrier and strong UV-barrier properties of PLA/CNC nanocomposites films compared to neat PLA counterpart, as well as no demonstrated toxicity in food up to 4%, it is unknown how they will affect the shelf-life of oxygen-sensitive food products. Consequently, this study assessed the potential of PLA/CNC nanocomposite films in extending the shelf-life of fresh-cut avocados, which are oxygen-sensitive products. The study focused on the development of enzymatic browning catalyzed by polyphenol oxidase (PPO) on avocado flesh, which is one of the main problems in the preservation process of fresh-cut avocados (Soliva-Fortuny et al., 2002). Various protection techniques have been studied to control browning of avocados. Methods such as use of anti-browning agents (Soliva-Fortuny et al., 2002) and PPO inhibitors (Gómez-López 2002; Bustos et al., 2015), packaging with modified atmosphere (Gerdes and Parrino-Lowe, 1995), as well as treatments of avocados with thermal process (Zhou et al., 2016), high pressure processing (Woolf et al., 2013) and pulsed light processing (Aguiló-Aguayo et al., 2014; Ramos-Villarroel et al., 2011a, 2011b, 2014; Velderrain-Rodríguez et al., 2021) have been found to offer some protection against browning and prevent browning in avocados. However, the method that employs packaging solution based on PLA/CNC nanocomposite films with strong barrier capacities to prevent browning in avocado and prolong its shelf life has not been studied and was the main goal of this study. Visual appearances of both unpackaged (control) and packaged avocados were observed with digital photos taken periodically to qualitatively assess enzymatic browning, whereas their lightness, chroma, and discolorations were quantified as function of storage time and temperature to determine the shelf life of the products.

## 1.2 Objectives

The goal of this research was to evaluate the potential of PLA/CNC film to improve the shelf life of fresh-cut avocados, which are oxygen-sensitive food. To achieve this objective, the following specific objectives were proposed:

1. Investigate the effects of packaging material types (neat PLA versus PLA/CNC nanocomposite films) and storage conditions (room temperature versus refrigerated temperature) on the enzymatic browning on the flesh of unpackaged and packaged avocados through visual appearance observations;

2. Evaluate the effects of material types and storage conditions on the lightness and total discoloration of unpackaged and packaged avocados in order to gain an in-depth understanding of their enzymatic browning;

3. Assess the influence of the packaging material types on the headspace gas composition inside the package and percent moisture weight loss of packaged fresh-cut avocados; and

4. Estimate the shelf life of packaged avocados using discoloration data to determine the role of packaging material types and storage conditions on extending the shelf life of fresh-cut avocados.

## 1.3 Hypothesis

This research is intended to test the hypothesis that the addition of highly crystalline CNC into a PLA matrix will lead to nanocomposite film with improved oxygen barrier properties and extended the shelf-life of oxygen-sensitive packaged food.

## 1.4 Structure of thesis

The research rationale is introduced in the first chapter, followed by the background review on poly(lactic acid) and cellulose nanocrystals synthesis, film manufacture and property evaluation, and detail on packaging technology extending the shelf life of fresh-cut avocado in chapter 2. In chapter 3, the materials, experimental methods, and performance evaluation of packaged fresh-cut avocados are described. The results on the potential of PLA/CNC nanocomposite films in extending the shelf-life of fresh-cut avocados, which are oxygen-sensitive products is reported in chapter 4, along with their discussion. Finally, in chapter 5, the experimental data are summarized, and future work proposed.

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# CHAPTER 2

# BACKGROUND AND LITERATURE REVIEW

## 2.1 Introduction

This chapter covers the background and literature review related to the scope of research. It includes the synthesis of poly(lactic acid) (PLA) and its properties; the extraction of cellulose nanocrystals (CNCs) and their properties, as well PLA/CNC nanocomposite films manufacture and properties. Additionally, the literature on fresh-cut produce, the application of PLA/CNC nanocomposite films on food packaging, and related topics are included.

## 2.2 Poly(lactic acid)

PLA is a biopolymer derived from lactic acid, obtained from agricultural renewable source like corn, and it is compostable (Auras et al., 2004). Its processability and some of its physico-mechanical properties are comparable to petroleum-based polymers, making it a good candidate material for packaging applications.

### 2.2.1 Synthesis of PLA

Lactic acid (2-hydroxy propionic acid) is a basic constitutional unit of PLA manufactured from bacterial fermentation of carbohydrate resources or chemical synthesis (Lim et al., 2008). The chiral carbon atom gives a structure of lactic acid in two configurations: L-lactic acid (LLA) and D-lactic acid (DLA) (Figure 2.1) (Auras et al., 2004; Castro-Aguirre et al., 2016). Carbohydrates from renewable sources like corn, potato, or sugar cane are fermented through a homofermentative method. This method was chosen as a major fermentation process to produce lactic acid because it produces a greater yield than other methods (Auras et al., 2004; Castro-Aguirre et al., 2016; Garlotta, 2001; Lim et al., 2008).

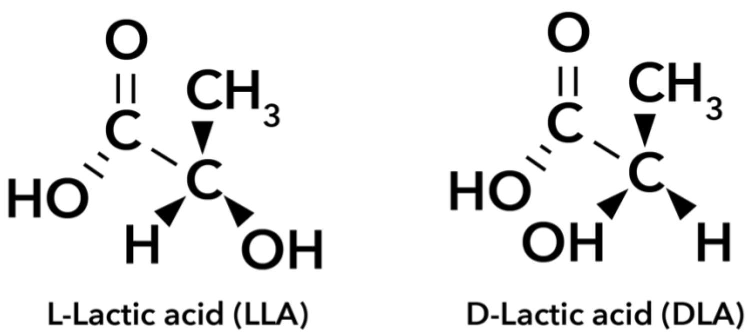


*Figure 2.1 Chemical structure of L and D-lactic, modified from (Auras et al., 2004).*

The high molecular mass of PLA provides good physical properties to materials (Auras et al., 2004). Two methods are used to obtain high molecular weight PLA, including the direct condensation polymerization and the ring-opening polymerization (ROP) as illustrated in Figure 2.2 (Masutani & Kimura, 2018). Direct condensation polymerization is a process that condenses hydroxyl and carboxylic acid functional groups under high vacuum and temperatures to remove water byproduct generated in the process (Castro-Aguirre et al., 2016; Gupta et al., 2007). This is the least expensive method; however, as lactic acid is converted into a higher molecular weight, the difficultly to eliminate a byproduct of water is increased. Therefore, this method is usually limited to a low molecular weight PLA. ROP is a method patented under Cargill Dow LLC. This method can produce a high molecular weight PLA using a cyclic dimer of lactic acid, mainly produced by depolymerization of low molecular weight PLA under low-pressure conditions (Avérous, 2008). To initiate the ring-opening polymerization, a transition metal compound is used as a catalyst to produce a high molecular weight PLA (Avérous, 2008; Masutani & Kimura, 2018).

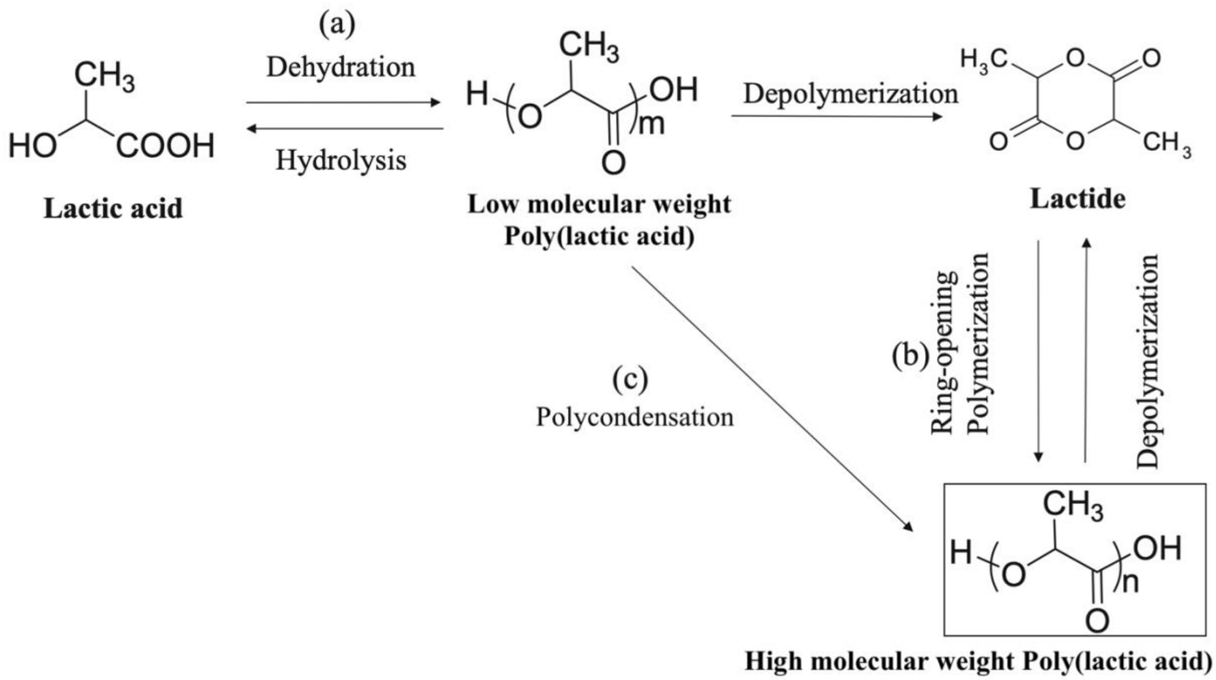


*Figure 2.2 PLA synthesis routes: (a) direct polycondensation of lactic acid, (b) ROP of lactide, and (c) polycondensation with additional method, modified from (Masutani & Kimura, 2018).*

### 2.2.2 Properties of PLA

The properties of PLA such as thermal, mechanical, and barrier vary with the polymer architecture and molecular mass. PLA can be manufactured from two configurations of lactic acid LLA and DLA resulting in different stereochemical structures of lactide, which affect the crystallinity of PLA (Auras et al., 2004; Lim et al., 2008). A composition of LLA greater than 93% yields a semi-crystalline PLA with a high degree of crystallinity, high melting point, and difficult to process (Auras et al., 2004; Castro-Aguirre et al., 2016). However, when the amount of DLA increases in a range that the amount of LLA is between 50-93%, PLA becomes amorphous with low melting point (Auras et al., 2004).

#### *2.2.2.1 Optical properties*

The light barrier properties expressed as opacity are related to the absorption of light in polymers and are crucial for packaging of light sensitive products. The chemical structures of PLA (L- or D- lactide) do not affect the light transmission since no significant differences in the light transmission between 300-1300 nm are reported for L- and D- based PLA (Hutchinson et al., 2006). In general, PLA almost has no transmission rate to UV-C, which is in the range of 190-230 nm, while nearly all UV-B and UV-A can pass through. UV-B and UV-C are the most damaging wavelengths for food (Auras et al., 2004). For the range of visible light (400-700 nm), the yellow and green colors in 540-560 nm appeared to be the most sensitive wavelengths to PLA because the average transmission is 95% within this range. This gives PLA a yellowness appearance making the film look old (Auras et al., 2004).

*2.2.2.2 Mechanical properties*

Mechanical properties describe the behavior of a material when a force is applied to it. Typically, PLA has good mechanical properties (Table 2.1) with the elastic modulus of 3,000-4,000 MPa and the tensile strength of 50-70 MPa. However, it is brittle with the elongation at break in the range of 2-10% and the glass transition temperature at 60-70°C (Perego & Cella, 2010). This brittleness constitutes a real drawback for its use in applications requiring materials with flexibility. Furthermore, the mechanical properties of PLA are affected by its chemical structures, with DLA and LLA structures having different mechanical properties due to the differences in their molecular weights (Table 2.1). The impact resistance of PLA increases with molecular weight (Grijpma et al., 2002). In contrast, the hardness is not affected by the molecular weight, but it is more influenced by the glass transition temperature of PLA (Perego et al., 1996). The effect of tensile strength is also mentioned by many investigators where the number is quite varied depending on the sample preparation process.

*Table 2.1 Mechanical properties of PLA with different molecular weights and crystallinities (Perego et al., 1996).*

| Mechanical Properties | Units | Crystalline PLLA | Amorphous PLLA |
|---|---|---|---|
| Molecular weight | Da | 71,000 | 67,000 |
| Crystallinity | % | 45 | 3 |
| Tensile strength | MPa | 66 | 59 |
| Elongation at break | % | 4.0 | 7.0 |
| Modulus of elasticity | MPa | 4,150 | 3,750 |
| Impact resistance, Izod, notched | kJ/m | 6.6 | 2.6 |
| Rockwell hardness | Scale H | 88 | 88 |

#### *2.2.2.3 Barrier properties*

Barrier properties are the ability of materials to obstruct the transfer of small molecules or permeants such as water vapor, oxygen ($O_2$), carbon dioxide ($CO_2$), ethylene, and some volatile compounds. They are crucial to the packaging industry, especially for food packaging since they determine the ability of a package to prolong the product's shelf life. For instance, the deterioration of oxygen-sensitive food is related to the oxidation process. Therefore, shelf life extension of a such food will require packaging materials with high $O_2$ barrier to prevent the occurrence of the oxidation reaction.

Generally, polymer morphology plays a fundamental role in barrier properties because the crystalline structure obstructs the diffusion of permeants, thus resulting to high barrier materials. In contrast, the amorphous structure has a lower capability to block the permeant (Almenar & Auras, 2010). Comparing the barrier properties of PLA to other polymers (Table 2.2), both $O_2$ and $CO_2$ permeability coefficients are comparable to polyethylene terephthalate (PET) but significantly lower than polystyrene (PS). Moreover, the water vapor permeability of PLA is also lower than that of PS (Auras et al., 2004).

*Table 2.2 Comparison of barrier properties of PLA and other polymers (Almenar & Auras, 2010; Auras et al., 2004).*

| **Barrier properties** | **Units** | **PLA** | **PET** | **PS** |
|---|---|---|---|---|
| Oxygen permeability at 25°C, 70% RH | $10^{-17}$ kg·m/m$^2$·s·Pa | 0.121 | 0.0188 | 27 |
| Carbon dioxide permeability at 25°C, 0% RH | $10^{-17}$ kg·m/m$^2$·s·Pa | 2.77 | 0.173 | 15.5 |
| Water vapor permeability at 23°C | $10^{-13}$ kg·m/m$^2$·s·Pa | 80-360 | 110 | 670 |

## 2.3 Cellulose nanocrystals

Cellulose nanocrystals (CNCs) are natural fibers derived from lignocellulosic plants broken down into nanoparticle sizes (Wang et al., 2019). They are biodegradable, biocompatible, and have low toxicity but a high surface area. Their use in packaging applications is increasing due to their transparency, low density combined with high stiffness and tensile strength (Stark, 2016).

### 2.3.1 Production of CNCs

Cellulose is the most abundant natural polymer mainly localized in plant cell walls. It consists of a repeating unit of cellobiose or a disaccharide of glucose (Figure 2.3) (Habibi et al., 2010; Phanthong et al., 2018). Generally, cellulose contains crystalline and amorphous regions in different portions depending on the cellulose source. Through the chemical process, the micro-crystallites can be extracted and isolated from cellulose, resulting in the formation of CNCs (George & Sabapathi, 2015).

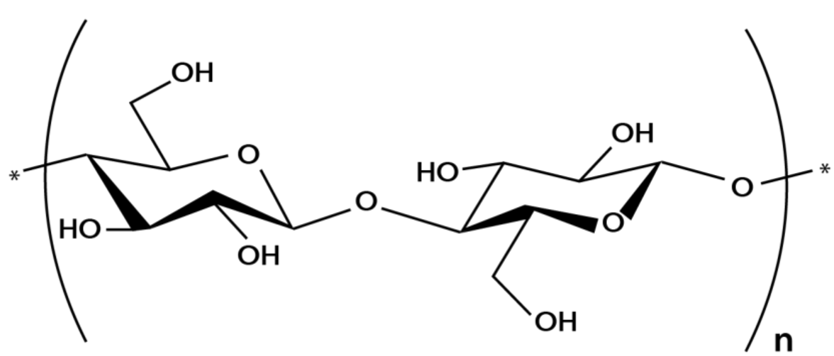

*Figure 2.3 Chemical structure of cellulose (Phanthong et al., 2018).*

The process of CNCs extraction can initially be done by a mechanical method using a shear force. However, this method requires higher energy consumption and produces CNCs with a lower crystalline fraction and yield than chemical methods (George & Sabapathi, 2015). The process of extracting CNCs is shown in Figure 2.4. At the beginning of the process, the original material goes through refining and lignin depolymerization, where the fiber and non-cellulose components are separated (Phanthong et al., 2018). After that, nanofibrillated cellulose is extracted. Subsequently, strong acid like sulfuric acid or hydrochloric acid is commonly used to extract the CNCs. This is the process of acid hydrolysis, where the crystalline region is isolated from the amorphous region, resulting in the production of cellulose nanocrystals (George & Sabapathi, 2015; Phanthong et al., 2018; Stark, 2016).

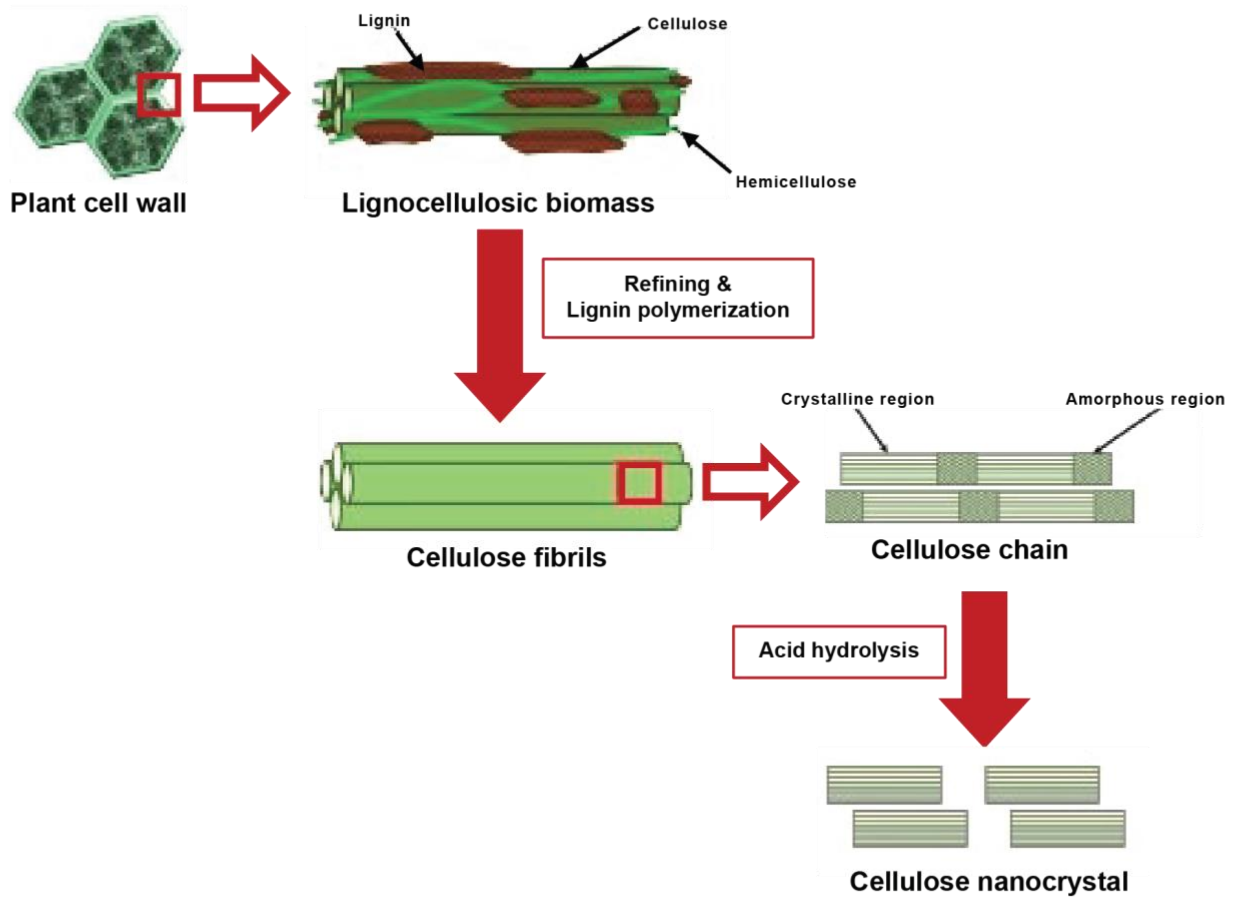


*Figure 2.4 Schematic of the production of CNCs, modified from (Phanthong et al., 2018).*

### 2.3.2 Properties of CNCs

Different sources of fiber and production processes influence the properties of CNCs, such as size, crystallinity, and the morphology. Usually, CNCs have diameters in the range of 4 nm to 25 nm and lengths of 100 nm to1000 nm. The tensile strength of CNCs is in the 7.5 - 7.7 GPa range. Their high crystallinity and biocompatibility make them excellent organic additives for improving the performance of various plastic matrices. Additionally, CNCs have excellent optical properties and are highly transparent (George & Sabapathi, 2015; Stark, 2016).

## 2.4 Processing technologies of composite films

Processing techniques play a vital role in determining the characteristics and properties of the film. Regarding the composite film, the processing method is a significant step in designating the dispersion of compound substances in a polymer matrix. Various methods can be used to process the composite films, including solvent casting and melt processing.

### 2.4.1 Solvent casting

Solvent casting or casting evaporation technique is one of the most common methods of producing nanocomposite films. This technique dissolves the polymer in a solvent such as water, alcohol, or an organic solvent. Later, the compound substances are added to the solution. The dispersion can be improved by stirring or sonication. After that, the solution is casted onto a mold, letting the solvent evaporates, and the composite films are formed (Oksman et al., 2016).

PLA/CNC nanocomposite films have been processed using this technique. This method provides a good dispersion of CNCs in the PLA matrix. However, there are some concerns for this method. For instance, the solvent residual can interfere with or reduce the mechanical and barrier properties of the film. Besides that, the notable limitation of this technique is that it is not scalable, and hence the process is difficult to implement at an industrial scale (Karkhanis et al., 2021).

### 2.4.2 Melt processing

Melt processing is the technique that is more suitable for high-volume production (Oksman et al., 2016). This approach requires heat and shear to soften the polymer and maximize the dispersion of nanomaterials (Mokhena et al., 2018). The dispersion can be improved by adjusting the design of melt compounders. Many strategies can be used to adjust the design such as the number of screws in an extruder, rotating direction, the diameter of screw, the distance of compounding. Recently, number of investigators have reported that the chemical functionalization, coating of nanocellulose, and grafting improve the dispersion of nanomaterials within the plastic matrix (Oksman et al., 2016). Since this process is subjected to heat, the processing temperature is very important. Not only that the temperature must be high enough to melt the polymer, but also it should not exceed the thermal decomposition temperature of CNCs to prevent thermal degradation. Melt processing can be accomplished through extrusion (blown and cast films), injection molding, compression molding, etc.

## 2.5 Barrier performance of PLA/CNC nanocomposite films

PLA alone does not have good barrier properties compared to PET as shown in table 2.2. Therefore, one of the challenging goals of developing PLA is to improve its barrier properties. The most widely used strategy reduces the mass transfer by increasing a tortuous pathway with the non-permeable barrier (Armentano et al., 2013; Matuana et al., 2016). One approach is adjusting material structures by forming multilayers or lamination with other high barrier plastics or materials, such as aluminum foil (Armentano et al., 2013). However, a multilayer material depletes the composability and recyclability of the materials. Another approach to increase the tortuosity is by increasing the crystallinity in the polymer matrix. This could be achieved through several methods, including: (i) manipulating the processing conditions such as adjustment of the cooling rate (Armentano et al., 2013; Liu & Matuana, 2019), and (ii) modifying the material formulations by adding inorganic additives like talc and clay (Liu & Matuana, 2019; Yu et al., 2012), or organic additives namely chitin/chitosan and natural fibers (Liu & Matuana, 2019; Suyatma et al., 2004).

The addition of CNCs into PLA matrix has been investigated by several researchers to improve the barrier performance of PLA (Karkhanis et al., 2018a, 2018b, 2021; Karkhanis & Matuana, 2019; Liu & Matuana, 2019; Stark, 2016; J. Wang et al., 2018). Indeed, results from different studies have shown significant improvement in water vapor and oxygen permeabilities of PLA by adding CNCs into the matrix. The improved barrier performance was attributed to the nucleating effect of CNCs, and the tortuosity effect induced by the crystals (Karkhanis et al., 2018a, 2018b, 2021; Liu & Matuana, 2019). CNCs are crystallites and acts as a nucleating agent in the PLA matrix; thus, increasing its crystallinity. This increased degree of crystallinity in PLA creates a tortuous pathway of gas diffusion, consequently improving the materials' barrier property (Karkhanis et al., 2018a, 2018b, 2021; Liu & Matuana, 2019; Stark, 2016; J. Wang et al., 2018).

A previous study of extrusion-cast PLA/CNC nanocomposites films indicated that the addition of CNC improves both the moisture and oxygen barrier properties of PLA films (Table 2.3) even with only 0.5 - 2 wt % of CNCs, due to the tortuosity effect (Liu & Matuana, 2019).

*Table 2.3 Barrier properties of extrusion-cast PLA and PLA/CNC nanocomposite films with various CNC contents (Liu & Matuana, 2019).*

| **% CNC** | **WVP at 38°C, 85%RH ($10^{-16}$ kg·m·m$^{-2}$·s$^{-1}$·Pa$^{-1}$)** | | **OP at 23°C, 0%RH ($10^{-20}$ kg·m·m$^{-2}$·s$^{-1}$·Pa$^{-1}$)** | |
|---|---|---|---|---|
| | **Average** | **% Change** | **Average** | **% Change** |
| 0 | 159.4 ± 9.7 | - | 353.6 ± 54.4 | - |
| 0.5 | 117.0 ± 4.2 | 26.6 | 267.4 ± 48.4 | 24.4 |
| 1 | 110.4 ± 6.6 | 30.8 | 212.1 ± 25.9 | 40.0 |
| 2 | 87.0 ± 8.0 | 45.4 | 178.8 ± 26.4 | 49.4 |

## 2.6 Potential of PLA/CNC nanocomposite films in food packaging

The poor barrier of PLA limits its packaging applications, especially for food packaging, where the barrier properties are crucial for extending the product's shelf life. As mentioned in previous section, the addition of CNCs improves the moisture and oxygen barrier properties of PLA films, making them suitable for food applications. However, there are many speculations on the toxicity of nano particles due to their small dimensions (< 100 nm) which have high potential to penetrate human tissue; thus, becoming harmful to human (Hannon et al., 2015; He et al., 2019).

In terms of food contact materials, conventional cellulose has been commercially used in the food industry for over 100 years and is certified as Generally Recognized as Safe (GRAS) in the USA. However, the use of fibrillated celluloses and nanocrystalline celluloses in contact with food still needs to be considered, as there are differences in characteristics, such as a nanoscale morphology and high surface area (Pradhan et al., 2020). Therefore, numerous studies have investigated the potential cytotoxicity, genotoxicity and ecotoxicity of CNCs (Ede et al., 2020; Fotie et al., 2020; Ong et al., 2020; Pradhan et al., 2020). The results showed no or very low cytotoxic, genotoxic and ecotoxic effects of CNCs through several *in vitro* and *in vivo* analyses. Interestingly, the fibrillated and nanocrystalline forms of cellulose were found as safe to use in food up to 4 wt% as conventional cellulose (Pradhan et al., 2020). The migration of CNCs in PLA/CNC nanocomposites has also been accessed and the overall migration levels in both polar and nonpolar food simulants were found well below the standard limits needed for food packaging materials (Fortunati et al., 2012; Silva et al., 2020). Karkanis and coworkers concluded that the low migration rates of CNCs from the material combined with no demonstrated toxicity in food up to 4% clearly show the potential of CNC-based PLA films for food contact applications (Karkhanis et al., 2021). Recently, the potential of PLA/CNC films to package crackers, a moisture-sensitive food product, as been demonstrated. The incorporation of 1 wt% of CNC into PLA significantly improved the moisture barrier properties of PLA/CNC nanocomposite film and extended the shelf life of cracker by 40% when stored at RH greater than 50% compared to the crackers packaged in neat PLA films as listed in Table 2.4 (Karkhanis et al., 2021).

*Table 2.4 Shelf life of cracker packaged with PLA and PLA/1%CNC nanocomposite films stored at different relative humidities at 25°C (Karkhanis et al., 2021).*

| **% RH** | **Shelf-life (hours) to reach 8% critical moisture content** | | |
|---|---|---|---|
| | **PLA** | **PLA/1%CNC** | **Delayed time (hours)** |
| 10 - 50 | Shelf-stable | Shelf-stable | - |
| 61 | 63.4 ± 0.6 | 86.1 ± 1.6 | 22.7 |
| 79 | 42.9 ± 0.7 | 59.9 ± 5.1 | 17.0 |
| 99 | 14.2 ± 0.2 | 20.1 ± 0.4 | 5.9 |

## 2.7 Fresh-cut produce

The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruit or vegetables that have been trimmed and/or peeled and/or cut into 100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience, and flavour while still maintaining its freshness (Rico et al., 2007; Watada & Qi, 1999). Recently, the fresh-cut product has been a value-added for fruit and vegetables where consumer's demand has increased due to the changes of consumers' lifestyle (Nicola & Fontana, 2014). However, as soon as the product is processed, packaging becomes essential to protect the fresh-cut produce and extend its shelf life. Therefore, the role of packaging for fresh-cut produce is to reach the consumer expectation where there should be no defect while keeping the product in an optimum maturity and fresh condition. Moreover, its appearance, sensory quality, texture, firmness, and taste, including the nutrient quality, should also maintain a good standard (Otto et al., 2021; Watada & Qi, 1999).

### 2.7.1 Quality of fresh-cut produce

As the fresh-cut produce is subjected to minimally processed operations, the process usually removes the outer protective tissue, making the product easily wounded (Nicola & Fontana, 2014). In addition, it accelerates the product's degradation process. Therefore, the product becomes highly perishable and more susceptible to quality deterioration than the whole fruit and vegetables. Accordingly, from the aspect of the fresh-cut industry, the product's shelf-life depends on the time that the quality attributes, such as freshness, firmness, texture, color, aroma, and nutritional value, remain at a good standard and acceptable to the consumer (Francis et al., 2012; Nicola & Fontana, 2014; Rico et al., 2007).

#### *2.7.1.1 Appearance*

Appearance is the main factor affecting consumers' choice to purchase fresh-cut produce, as it is the first attribute that can lead consumers to decline the product. On the other hand, if the product appeared as freshly cut, its bright color surface can persuade consumers to purchase it (Francis et al., 2012; Koutsimanis et al., 2012; Nicola & Fontana, 2014). The product's appearance could be subjectively or objectively characterized. On a subjective measurement, it can be evaluated by human eyes using a rating scale. However, this method can be affected by human perception and the evaluation condition such as lightning during the evaluation process. In contrast, the appearance can be explained objectively, evaluated by measuring instruments, and reported as quantitative data (Francis et al., 2012).

Color evaluation using CIE L*a*b* color scale is the most frequently used and widely acceptable scale explaining the color that appeared on the product's surface (Francis et al., 2012). As shown in Figure 2.5, L* represents the lightness correlated with the development of whiteness as the L* increase. The color scale of a* signifies the greenness and redness from negative value to positive value, respectively. At the same time, b* expresses the color scale of blueness as a negative value and yellowness as a positive value (Konica Minolta Sensing Americas Inc., 2006). Moreover, their derivative measurement, Chroma (C*) and Hue (H*), can also be calculated from these measurements as follows:

$$C^* = \sqrt{{a^*}^2 + {b^*}^2} \quad (2.1)$$

$$H^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (2.2)$$

C* is also described as the intensity or the saturation of the color but it does not distinguish chroma color differences. Therefore, the scale of C* can be explained together with the H* value, distinguishing each color from another (Konica Minolta Sensing Americas Inc., 2006; X-Rite Inc., 2021).

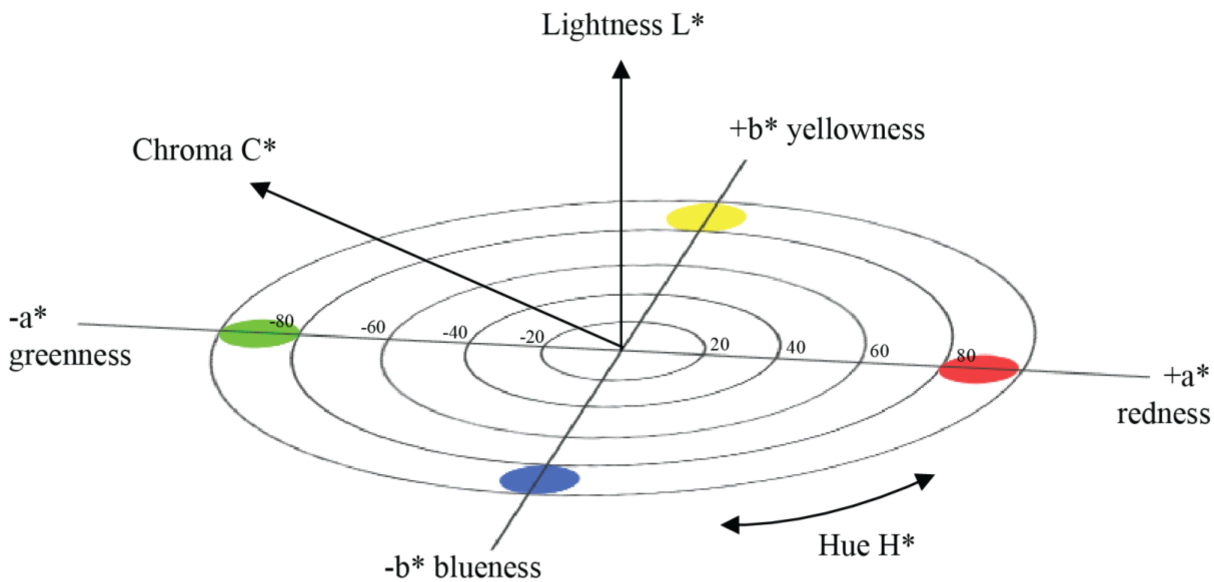


*Figure 2.5 Three-dimensional CIE L*a*b* and L*C*H* color spaces, modified from (Konica Minolta Sensing Americas Inc., 2006).*

Color changes in fresh-cut produce can occur from the degradation of color pigments like chlorophyll or carotene and the enzymatic browning reaction according to the oxidation reaction of phenolic compounds (Francis et al., 2012). These factors reflect the degradation process of fresh-cut produce. Therefore, the discoloration can systematically be recorded in the CIE L*a*b* color coordinates to monitor the degree of the product's degradation. For example, the discoloration of carrots can occur from the dehydration and lignin formation of the product, which later leads to the degradation of antioxidants and carotenes (Howard & Griffin, 1993; Rico et al., 2007). This phenomenon is correlated to the increasing value of L* due to the development of whiteness. Meanwhile, the browning reaction on the surface of fresh-cut apple resulted in the decreasing value of L*, which refers to the development of the brown pigment (Perez-Gago et al., 2005). However, in the measurement, the changes in the product's color do not change only one color parameter. Accordingly, the discoloration can be summarized as the value of ΔE* obtained from the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \tag{2.3}$$

where $\Delta L^* = L^*_{sample} - L^*_{standard}$; $\Delta a^* = a^*_{sample} - a^*_{standard}$; and $\Delta b^* = b^*_{sample} - b^*_{standard}$.

*2.7.1.2 Texture*

The texture is related to the firmness, crispness, juiciness, and hardness of fresh-cut produce, which is also highly expected from consumers when choosing to purchase the product (Kader, 2001). It is associated with tissue deterioration and can be used to index freshness and quality decline (Francis et al., 2012). Firmness losses in fruits and vegetables are related to both enzymatic and non-enzymatic processes. The losses of firmness in fruit are primarily caused by the enzymatic degradation of pectins, catalyzed by pectin methylesterase (PME) and polygalacturonase (PG). While in vegetables, tissue aging, senescence, water losses, and wounding effects are the major causes of textural changes (Francis et al., 2012; Li et al., 2010). Hence, softening of tissues in fresh produce is one of the most important factors limiting shelf-life, generally evaluated based on pressure (Li et al., 2010).

*2.7.1.3 Flavor*

Appearance might be the initial quality attribute of the product acceptance; however, the flavor has the most considerable impact on the consumer's desire and acceptability (Barrett et al., 2010). Flavor is defined as tastes and smells. Taste is a mouthfeel sensation perceived with the mouth, while smell is the aroma or odor received by the nose. Flavor can be challenging to quantify using instrumental analysis as it is based on the complexity of human perception, which comes from the interactions between many compounds where those measurements must relate to human acceptance (Kader, 2001; Francis et al., 2012). The quality of most fruit is influenced by sugar, organic acid, phenolic compounds, and volatile compounds. However, identifying and quantifying these contents are very time-consuming to evaluate. Moreover, the concentrations of these compounds must be in an optimum range to meet consumer perception (Kader, 2001). Ideally, the fresh-cut produce aims to stay in the ripeness stage; therefore, the flavor is usually described by the total soluble content and titratable acidity as it can indicate the maturity stage of produce (Barrett et al., 2010; Francis et al., 2012).

*2.7.1.4 Nutritional value*

Nutritional value of fresh produce varies among commodities and cultivars, and a loss of nutrients begins after harvest (Kader, 2001). Regardless of this, cutting or wound stimulates ethylene production and increases the respiration rate, leading to a significant increase in the rate of nutrient loss (Barrett et al., 2010; Francis et al., 2012). After cutting, antioxidant and phytonutrients are exposed to oxygen and light; consequently, there are more susceptible to degradation (Francis et al., 2012; Rico et al., 2007). Vitamin C is one of the most sensitive vitamins which can be easily degraded when exposed to heat, light, and oxygen. For this reason, vitamin C is used as a freshness index (Barrett et al., 2010).

#### *2.7.1.5 Microbiological spoilage*

The growth of microorganisms on produce is often considered an attribute that limits the product's shelf life. Regarding fresh-cut produce, there is a higher risk of microbial spoilage according to the presence of cut surface that provides easy access to nutrients for microbes to grow and increased moisture content (Corradini, 2018; Rico et al., 2007). Among all factors of the quality of product, the potential of pathogen contamination and foodborne outbreaks have gained the most attention and are considered as essential factors related to the safety issues (Francis et al., 2012). The level of microbial growth is often correlated to sensory acceptability. In general, the shelf life of perishable products is associated with the microorganism counts above $10^6$ CFU/g or ml (Corradini, 2018). However, these values are product specific. There are significant differences in microbial counts between each product. This has been attributed to many factors from the process of cultivar; the soil and water used, the condition during harvest, the postharvest handling; and condition along the supply chain (Nicola & Fontana, 2014; Rico et al., 2007).

## 2.8 Fresh-cut avocado

Avocado is a highly perishable fruit because of its high metabolic rate, and it is challenging to commercialize as a fresh-cut product due to a rapid loss of quality after being processed (Maftoonazad & Ramaswamy, 2005; Velderrain-Rodríguez et al., 2021; Villa-Rodríguez et al., 2011). The quality loss is mainly attributed to the enzymatic browning and softening, as it is the first factor determining the product's acceptance or rejection (Velderrain-Rodríguez et al., 2021).

### 2.8.1 Quality of fresh-cut avocado

Once avocados are cut, the polyphenol oxidase (PPO) enzyme activity is the most challenging problem in the preservation process as it is the main factor of the mesocarp browning (Aguiló-Aguayo et al., 2014; Garcia, 2020; Ramos-Villarroel et al., 2011b; Soliva et al., 2000). The enzymatic darkening of the avocado pulp is a biochemical mechanism that happens during the catalysis reaction of an enzyme called polyphenol oxidase (PPO) (Dorantes-Alvarez et al., 1998; Pinheiro et al., 2009). With the presence of PPO in a pulp and oxygen in the air, the phenolic compound will oxidize and convert into quinones (Kahn, 1975). Quinones can polymerize and form a long chain of polyphenols; this then creates brown, red, or black pigments on avocado pulp, giving a browning appearance on the flesh (Kahn, 1975; Soliva-Fortuny et al., 2002).

In addition to the enzymatic browning, the product texture undergoes softening during storage due to the increasing rate of respiration and metabolism, which will also increase the susceptibility to microbial contamination (Soliva et al., 2000). Moreover, lipid oxidation may occur through reactive oxygen attacks on the unsaturated fatty acids. Subsequently, the process results in rancid odor giving an undesirable flavor and quality losses (Aguiló-Aguayo et al., 2014).

### 2.8.2 Techniques maintaining the quality of fresh-cut avocado

The process of maintaining the quality of fresh-cut avocado has been studied by many investigators (Aguiló-Aguayo et al., 2014; Dorantes-Alvarez et al., 1998; Gómez-López, 2002; Maftoonazad & Ramaswamy, 2005; Ramos-Villarroel et al., 2011b; Soliva-Fortuny et al., 2002; Velderrain-Rodríguez et al., 2021). Several factors influence this preservation process. Since the product needs proper storage conditions, factors such as temperature, relative humidity, atmospheric oxygen, and carbon dioxide should be optimized to preserve the fresh-cut fruit. Low-temperature storage can help decelerate the browning reaction as lower temperature slows down the enzymatic activity and the metabolism rate (Zauberman et al., 1985). However, the chilled storage can also induce chilling injury symptoms and expresses internal damage giving a browning pulp appearance (Hershkovitz et al., 2005).

While the cold storage aimed to slow down the reaction, anti-browning agents aimed to inhibit PPO activity can also be used to preserve the quality of fresh-cut avocado. Ascorbic acid, citric acid, and L-cysteine effectively retard the browning of avocado (Gómez-López, 2002). Recently, the intense light pulse (ILP) treatment was explored as a nonthermal technology to decontaminate food and food surfaces (Ramos-Villarroel et al., 2011b). Investigators have demonstrated that the application of very short ILP of broad spectrum light can inactivate microorganisms in foods (Aguiló-Aguayo et al., 2014; Ramos-Villarroel et al., 2011a, 2011b, 2014; Velderrain-Rodríguez et al., 2021). Still, this technique can also cause browning if the product is over-exposed to the treatments (Ramos-Villarroel et al., 2011a, 2011b, 2014). Treatments of avocados with thermal process (Zhou et al., 2016), high pressure processing (Woolf et al., 2013) have been found to offer some protection against browning and prevent browning in avocados. Other than that, packaging materials has also become a vital role in extending the shelf life of fresh-cut avocado. Various strategies have been investigated, such as modified atmosphere packaging (MAP) and active packaging.

### 2.8.3 Packaging technology extending the shelf life of fresh-cut avocado

Today fresh-cut produce is mainly packaged in flexible or rigid polymer containers such as bags, pouches, trays, or clamshell containers. These types of packaging are usually chosen based on the type of product or the technologies required to maintain the quality of the product with an ideal goal of providing physical protection, favorable atmosphere composition, and maintenance of flavor (Forney, 2007). For fresh-cut avocado, the factor that limits the shelf life is browning appearance because of oxidation reaction. Therefore, the strategy of choosing a packaging material for fresh-cut avocado is based on the permeation properties with a consideration of the atmosphere inside the package.

#### *2.8.3.1 Modified atmosphere packaging*

Modified atmosphere packaging (MAP) is a packaging technology that maintains the quality of product by using a modification of the gas composition in the package headspace. The atmosphere is modified to a favorable condition for product quality maintenance which has shown to increase the shelf life of many fresh-cut produce (Toivonen et al., 2009). However, the favorable atmosphere for each fresh produce is specific. The gas composition needs to be developed appropriately to fit with the characteristic of each produce. Gerdes & Parrino-Lowe (1995) used a nylon polyethylene laminated bag to package avocado halves at three atmospheric conditions including vacuum, air, and gas mixture (5%CO, 15%$O_2$, 80% $CO_2$). Interestingly, the vacuum showed the darkest flesh of avocado. The gas mixture did not reduce the occurrence of browning. Conversely, the texture was maintained and giving the best result under the anaerobic atmosphere (Gerdes & Parrino-Lowe, 1995). It is not surprising that the gas mixture did not give a better result maintaining the color. Rojas- Graü et al. (2009) reported that 0.25 -5% of $O_2$ combined with 10-20% of $CO_2$ successfully maintained the visual appearance of several fresh-cut fruits. However, with a high phenolic content fruit, the low $O_2$ and elevated $CO_2$ atmospheres cannot effectively inhibit the browning in fresh-cut produce. In fact, other treatments like inhibitor or antioxidant treatments are more effective than MAP technique (Rojas-Graü et al., 2009). Many investigators suggested that the MAP system in combination with antioxidant treatments delays the enzymatic browning (González-Aguilar et al., 2000; Soliva et al., 2000; Vilas-Boas & Kader, 2006).

### *2.8.3.2 Edible coating*

Edible coating is an environmentally friendly technology that provides an additional protective coating which can give the same effect as MAP. This coating is a semipermeable barrier that can be adjusted to control moisture transfer, gas exchange, or oxidation process (Dhall, 2013; Oms-Oliu et al., 2008). One major advantage of edible coating is that the active ingredients, such as antioxidants and antimicrobial agents, can be incorporated in the coating matrix to increase the ability of packaging to prolong the shelf life of the product (Dhall, 2013; Rojas-Graü et al., 2009). Edible coating has been successfully used with several fresh-cut produce like apple (Perez-Gago et al., 2005) and pear (Olivas et al., 2003; Oms-Oliu et al., 2008). For avocado, like other fresh produce, an edible coating is predominantly used to preserve whole fruits. However, it has recently been explored for fresh-cut avocado as well. Mendoza-Gómez et al. (2017) has demonstrated the effect of gellan coating carrying antioxidants on the visual quality and color of avocado slices. Remarkably, the gellan coating with the addition of L-glutathione, D-isoascorbate, polysorbate 80, and glycerol, as a mixture of anti-browning agents, increased the shelf life up to 5 hours compared to the control (Mendoza-Gómez et al., 2017). The results have shown that this is an effective technique that can help reducing the discoloration by inhibiting the PPO activity and, therefore, increasing the shelf life of fresh-cut avocado.

*2.8.3.3 Combination of techniques*

Several treatments and techniques for extending food shelf life such as MAP and edible coatings have been explored for fresh-cut produce. Since each method has its limitations, the ability of using a single preservation method might not be appropriate to meet the expectation. Therefore, many studies have explored the potential of combined methods on food preservation. Usually, these combining methods are aimed to decrease the disadvantages of one method, enhance the advantages of one another, thus improving the ability of package to prolong the shelf life. For instance, the edible coating with anti-browning agents (Mendoza-Gómez et al., 2017) and the MAP with the addition of antioxidant treatment (González-Aguilar et al., 2000) is counted as the combination preservation technique. In this combined approach, the antioxidant or anti-browning agent helps control the enzymatic reaction. On the other hand, edible coating alone creates a protective barrier for the product, where the atmosphere can be adjusted to control respiration and retard enzymatic browning. Similarly, the MAP technique gives a favorable atmosphere to the product; thus, slowing down the respiration rate as well as the discoloration.

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# CHAPTER 3

# EXPERIMENTAL

## 3.1 Materials

As an oxygen-sensitive product, Mexico 'Hass' avocado was purchased from a local supermarket in Lansing, Michigan, distributed by Mission Produce (Oxnard, CA). The fruits were selected as a sizing grade of 40, extra-large size with a total weight of 9.5 - 11.5 oz. PLA, Ingeo biopolymer 4044D formulated for a reactive extrusion process was obtained from NatureWorks LLC (Minnetonka, MN) in a pellet form with less than 4% D-lactide content (Liu & Matuana, 2019). Freeze-dried CNCs (2015 - FPL - CNC - 071) were produced from the dissolution of wood pulp using the sulfuric acid process, acquired from the U.S. Forest Service (Madison, WI) (Moon et al., 2013). The prepared CNCs were varied in length with a range of around 100 - 300 nm and had a diameter around 5 nm.

## 3.2 Preparation of PLA/CNC composite films

PLA pellets and CNC powder were dried in the oven at 60°C for at least 24 hours before processing. Then PLA pellets and 2 wt% of CNC were mixed and blended in a high-intensity mixer at a speed of 22,000 rpm for approximately 1 minute. As previously described (Liu & Matuana, 2019), the cast film processing was carried out using a 32 mm conical counter-rotating twin-screw extruder with a 13:1 L:D ratio at 30 rpm. Films were produced through a slit die with a die gap of 3.4 mm. The processing temperature profile from the hopper to the slit die was set as 180-180-175-155°C. The chill roller was installed at a distance of ¾ inch apart from the slit die, was used to stretch the film; a winder then collected the film. The neat PLA film was manufactured using the same process and conditions. This film was characterized in a previous work reported that the oxygen permeability ($10^{-20}$ kg m $m^{-2}$ $s^{-1}$ $Pa^{-1}$) of PLA is 353.6 ± 54.4 and PLA/2%CNC is 178.8 ± 26.4 (Liu & Matuana, 2019).

### 3.3 Skin color and ripeness stage of avocado

The ripeness stage affects the discoloration of a fresh-cut avocado in a way that when the unripe avocado was cut, it appeared that the discoloration on the flesh would occur slower than a fresh-cut ripe avocado (Cox et al., 2004; Villa-Rodríguez et al., 2011). Thus, the ripeness stage needs to be monitored prior to the experiment. The parameters that can identify the ripeness stage of the avocado fruits are firmness and color parameters, which, as the avocado ripens, there is a correlation between them. The firmness will decrease along with the color parameters (Villa-Rodríguez et al., 2011). This study is focusing on the ripeness stage that the fruits are ready to consume. Therefore, according to Villa-Rodríguez study, the fruit skin color will have to remain below stage 4, as shown in Table 3.1. The Chroma meter (CR-400, Konica Minolta Tokyo, Japan) was used to quantify the color parameters in the CIE LAB color scale; L* (Lightness), a* (green/red chromaticity), and b* (yellow/blue chromaticity), recorded as an average of five measurements taken around the fruit equator. By following the value of color parameters clarified in the Villa- Rodríguez study, the L* (Lightness), H* (Hue), and C* (Chroma) were used. Hence, H* and C* can be calculated by using the data collected from a chromameter (L*, a* and b*) using the following equations (López-Malo et al., 1998; Soliva et al., 2000):

$$H^* = \arctan\left(\frac{b^*}{a^*}\right) \tag{3.1}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{3.2}$$

*Table 3.1 The ripeness stage based on avocado skin color.*

| **Ripeness stage based on avocado skin color** | **Color parameters**[1] | | |
|---|---|---|---|
| | **L*** | **C*** | **H*** |
| Stage 4 from Literature (Villa-Rodríguez et al., 2011) | 24.4 ± 0.4 | 6.8 ± 0.4 | 77.7 ± 1.8 |
| Colors at purchase | 25.2 ± 0.5 | 5.3 ± 0.9 | 96.5 ± 5.4 |
| Colors after reaching experimental stage 4 | 23.9 ± 0.5 | 4.1 ± 0.6 | 82.7 ± 11.7 |
| Colors at experimental stage | 22.1 ± 0.5 | 2.5 ± 0.8 | 54.4 ± 8.7 |

[1]Fifteen replicates were tested.

## 3.4 Preparation of PLA/CNC composite films

Table 3.1 shows the tracking of avocado skin color collected before it is ready for an experiment. The avocado fruits were purchased with similar skin color and stored at room temperature (23 ± 1°C) until they are ready to be cut. A chroma meter was used to track the skin color of the fruit. After the avocado skin color reached stage 4, the fruits were stored for 5 additional days before they were used in this experiment.

Before cutting, the fruits and knife were disinfected with 91% propyl alcohol. Then the fruits were cut in half, the pits removed, and each half of the avocado was immediately packed in prepared bags made of PLA and PLA/2% CNC. The unpacked avocado was also observed as a control sample.

## 3.5 Packaging and storage of fresh-cut avocado

Packaging bags were manufacture by cutting the films into 12 by 7 inches and folded them in half. Then two sides of the films were sealed using an impulse sealer (Linsnfield, Jiangxi, CN) with the 2 mm seal width. Level 4 of the impulse level was applied with a 330 W of impulse power. One side of the bag was left open to insert the fresh-cut avocado into the bags. Once inserted, the opened side of the bag was sealed the same way as the previous two sides. The same method was applied to both PLA and PLA/CNC films. Fresh-cut avocados packaged in both PLA and PLA/CNC bags were stored at room temperature (23°C) and chilled temperature (4°C) for shelf life study. The shelf life of unpackaged fresh-cut avocado stored at these conditions were also evaluated.

## 3.6 Visual appearance and color evaluation

The visual appearances of both unpackaged (control) and packaged avocados were observed with digital photos taken periodically, whereas their discolorations were quantified using a Chroma meter (CR-400, Konica Minolta Tokyo, Japan). The samples stored at room temperature were evaluated until 72 hours of storage time. On the other hand, the evaluation proceeded to 240 hours for the sample stored under chilled temperature. The data were recorded as the average readings of L*, a*, and b* taken at five specific locations on the surface of fresh-cut avocado (Figure 3.1).

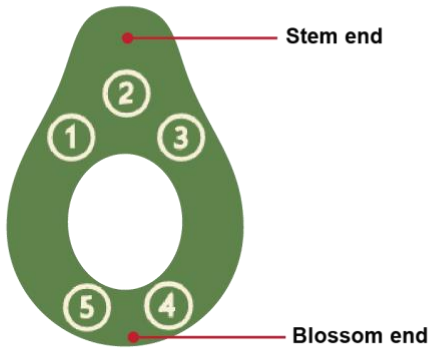


*Figure 3.1* Five specific locations on the surface of fresh-cut avocado that the color measurements were taken

These values were then used to calculate the discoloration ΔE* as follows (López-Malo et al., 1998):

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \tag{3.3}$$

where $\Delta L^* = L^*_{\text{sample}} - L^*_{\text{standard}}$ ; $\Delta a^* = a^*_{\text{sample}} - a^*_{\text{standard}}$ ; and $\Delta b^* = b^*_{\text{sample}} - b^*_{\text{standard}}$ . The sample represent the value at storage time t whereas the standard represents the value at time t = 0 h.

### 3.7 Shelf life estimation

Once the ripened avocado is cut, a browning reaction gradually develops on the surface of avocado flesh, which increases the rate of spoilage (Ramos-Villarroel et al., 2011). The shelf life of fresh-cut and unpackaged avocado is reported to be one day if stored at room temperature but 3-4 days if refrigerated at 4°C (Mercy, 2021; StillTasty LLC, 2021). To estimate the shelf life of packaged avocado, it is necessary to establish the acceptability limits needed for fresh-cut avocado. These limits were defined as the discoloration (ΔE*) exhibited on the unpackaged fresh-cut avocado (control) stored at room temperature for 1 day (RT) and chilled in a refrigerated temperature for 4 days (CT).

The discoloration (ΔE*) of the unpackaged avocado flesh was measured by Chroma meter as described in section 3.6. However, the measurements were taken before storage (0 hour) and after 24 hours storage at room temperature ($\Delta E^*_{RT}$) as well as before storage (0 hour) and after chilling for 96 hours for the refrigerated samples ($\Delta E^*_{CT}$). The acceptability limits were determined to be $\Delta E^*_{RT} = 33.0 \pm 3.6$ and $\Delta E^*_{CT} = 37.4 \pm 2.4$ for unpackaged avocadoes stored at room temperature (RT) and chilled temperature (CT), respectively.

The discoloration data collected on fresh-cut and packaged avocado stored at RT and CT were used to construct plots of discolorations (ΔE*) as a function of storage time. Figure 3.2 illustrates a typical example of a such plot for the packaged fresh-cut avocado stored at room temperature.

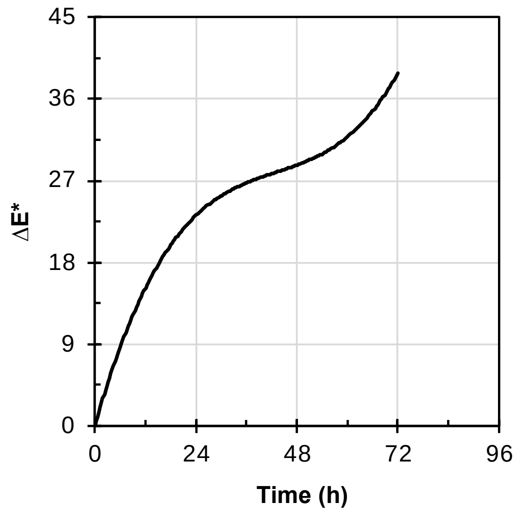


*Figure 3.2 Typical example of a plot of discoloration versus storage time for packaged fresh-cut avocado at room temperature.*

A polynomial third degree regression line was then fitted to the plot using the following type of equation:

$$y = ax^3 + bx^2 + cx + d \tag{3.4}$$

with y and x as the discoloration (ΔE*) and storage time, respectively.

The shelf-life of fresh cut packaged avocado, defined as the time required by the packaged avocado to reach the discoloration acceptability limit, was determined by solving equation 3.4. Since the acceptability limits are known, the shelf life was then estimated by substituting y by ($\Delta E^*_{RT}$) or ($\Delta E^*_{CT}$) in equation 3.4 and solving for the value of x.

## 3.8 Headspace gases analysis

The headspace gases analysis of packaged fresh-cut avocado was measured using vapometer cup assemblies (EZ-Cup Vapometer, Thwing-Albert Instrument Company, New Jersey, USA). The avocados and all equipment that contacted the fruit (vapometer cup, knife, and working area) were sanitized with 91% propyl alcohol. The avocados were cut into 2 by 2 by 1 $cm^3$ pieces and placed in the cups that were closed by placing a 3-inch diameter film samples (PLA and PLA/CNC) between a lightweight aluminum cup and an aluminum threaded flanged ring with two neoprene gaskets with a Teflon seal to hold the films in place as described by Karkhanis and coworkers (Karkhanis et al., 2021). A ¾-inch adhesive septum was attached to the film after tightly closing the cups to prevent leakage of the gas after each measurement. The headspace gases analysis was carried out with the Headspace Oxygen/ Carbon dioxide analyzer, Model 6600 from Illinois Instrument, Inc. (Illinois, USA). The experiments were conducted at room temperature up to 72 hours, by 24 hours increments. The headspace was evaluated every 24 hours with a duration of 10 seconds each time and the results reported as air composition in terms of percent oxygen and percent carbon dioxide.

## 3.9 Weight loss

The weight loss was observed during the headspace gases analysis experiments. The weights of each sample were determined as a function of time by a precision balance (Ohaus Discovery, Ohaus Corporation, New Jersey, USA) and the results reported as a percent weight loss based on the initial fruit weight.

### 3.10 Microbial counts

The total levels of microorganisms on unpackaged and packaged fresh-cut avocados stored at room temperature for 48 h and cold temperature for 180 h were evaluated. Avocados were prepared as mentioned in section 3.4. Portions of avocados (25 grams) were cut and weighed aseptically in a sterile stomacher bag and homogenized with 225 ml of Butterfield's Phosphate Buffer (pH 7.2) for 2 min using a Seward Stomacher 400 Circulator Lab Blender (Seward Laboratory System Inc., USA). The homogenized unpackaged avocado (control) samples were diluted to -1 and -2 while three serial dilutions were made for packaged samples stored at room temperature (-2 to -4) and chilled temperature (-1 to -3). The Plate count agar (PCA) and potato dextrose agar (PDA) were used as a microbiological media. The PCA samples were incubated at 37°C overnight whereas the PDA samples were incubated at 37°C for 48 h before the colony count. Colony counts on packaged avocados were corrected by considering the level of microbes on unpackaged fruit. This was accomplished by subtracting the colony counts of unpackaged avocado from those of packaged counterparts. Two replicate measurements were obtained from each avocado and four avocados were tested for a total of 8 replicates.

### 3.11 Statistical analysis

Statistical analysis was performed using Design-Expert software version 12 (Stat-Ease, Inc, Minneapolis, MN, USA). Significant differences between packaging types (neat PLA versus PLA/CNC nanocomposite) and storage conditions (room temperature versus refrigerated temperature) were determined based on a two-way ANOVA at $p \leq 0.05$.

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# CHAPTER 4

# RESULTS AND DISCUSSIONS

The loss of sensory attributes or the appearance of organoleptic defects such as off-odors or discoloration that are easily detectable by the consumer have been identified as important attributes to monitor the shelf life of highly perishable food products such as fresh-cut fruits and vegetables (Corradini, 2018). Since appearance is highly expected from consumers when acquiring fresh-cut produce, this study selected discoloration as the appearance quality attribute to signal the shelf life of both unpackaged and packaged fresh-cut avocados.

## 4.1 Visual appearance

Avocado is a highly perishable fruit, and its commercialization as a fresh-cut product is difficult due to a rapid loss of quality after processing due to the enzymatic browning and softening (Velderrain-Rodríguez et al., 2021; Villa-Rodríguez et al., 2011). Since appearance is the first attribute that can lead consumers to decline the product, the ability of packages made of neat PLA and PLA/CNC nanocomposite films to preserve the appearance of fresh-cut avocado was assessed visually by following the occurrence of browning on the flesh before and after storages at room temperature for 72 h (Figure 4.1) and at refrigerated temperature for 240 h (Figure 4.2).

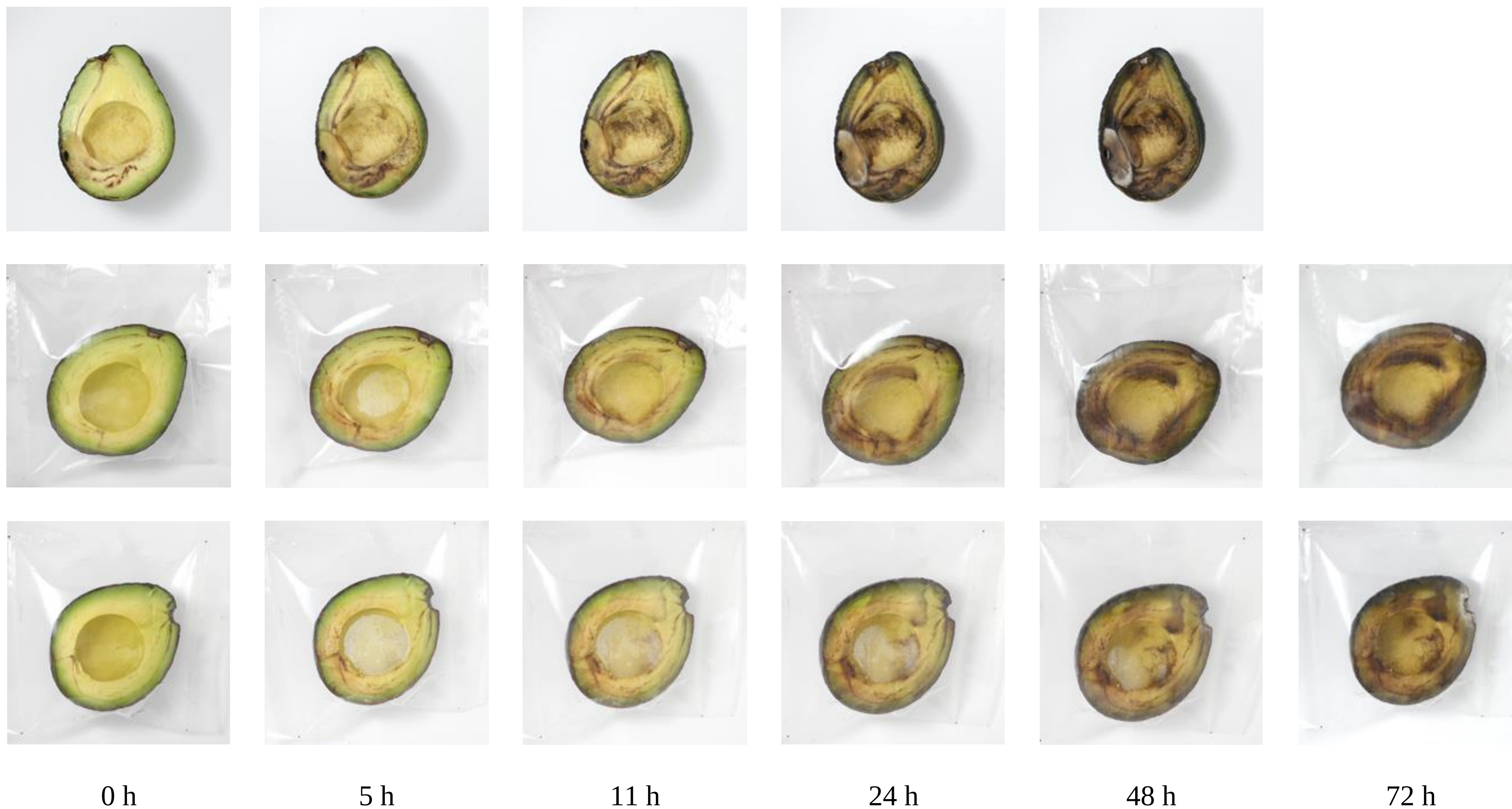
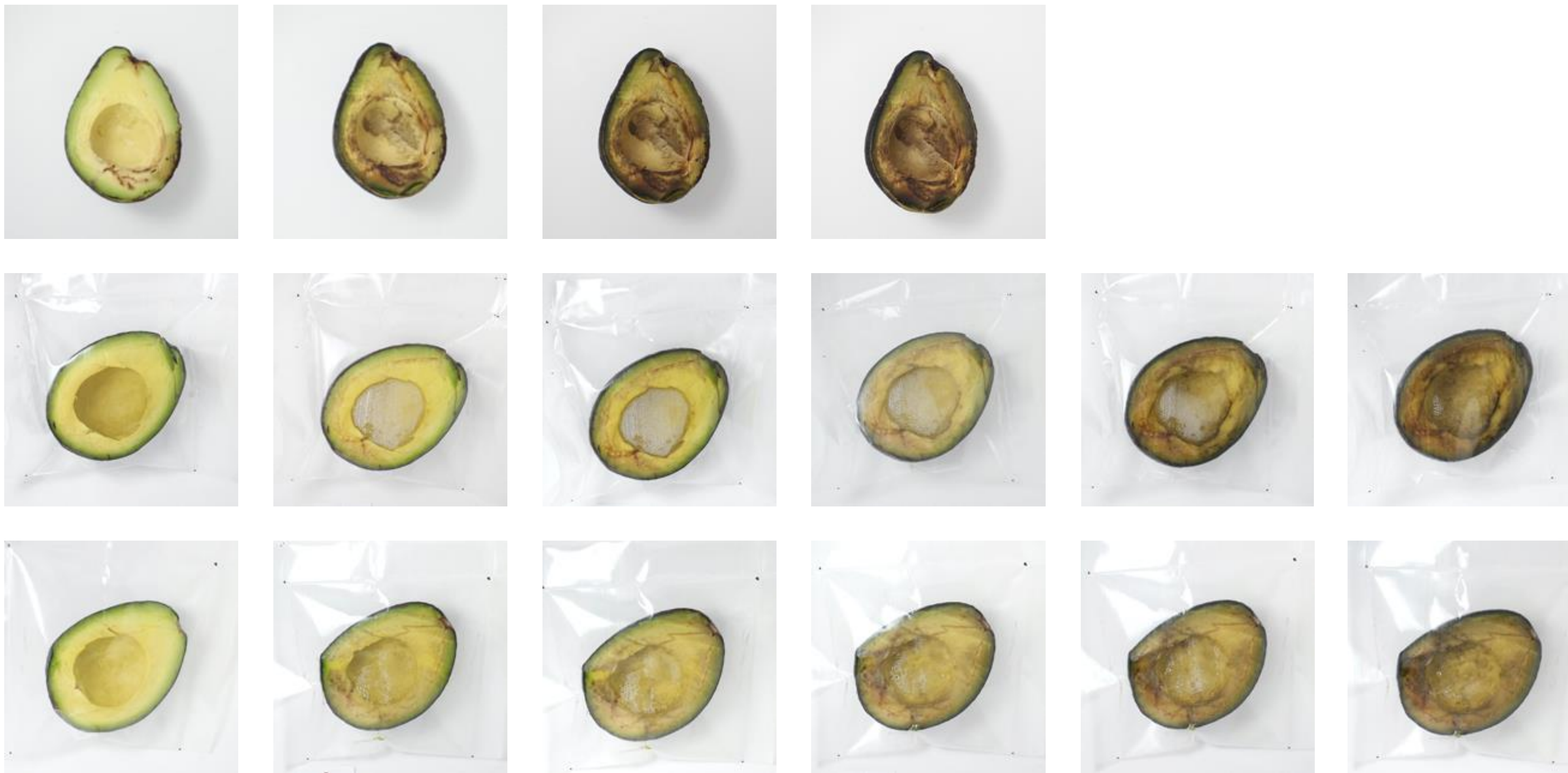



*Figure 4.1 Visual appearances of unpackaged fresh-cut avocados (top row) and avocados packaged with neat PLA film (middle row) and PLA/CNC nanocomposite film (bottom row) stored at room temperature for various time periods.*



0 h | 24 h | 48 h | 96 h | 168 h | 240 h

*Figure 4.2 Visual appearances of unpackaged fresh-cut avocados (top row) and avocados packaged with neat PLA film (middle row) and PLA/CNC nanocomposite film (bottom row) stored at cold temperature for various time periods.*

The flesh of fresh-cut avocados was bright yellow/green in color and unblemished at the beginning of the storage. However, discoloration occurred during storage and the yellow-greenish color become darker, irrespective of storage conditions and package material type. This darkening is attributed to the enzymatic browning caused by polyphenol oxidase (PPO) activity. PPO already present in the avocado catalyzes the oxidation of polyphenols to highly reactive quinones in the presence of an appropriate amount of oxygen, which are then polymerized to brown, red, or black pigments that give a browning appearance on the flesh (Kahn, 1975; Quevedo et al., 2011; Pinheiro et al., 2009; Soliva-Fortuny et al., 2002).

The visual assessment showed that the unpackaged fresh-cut avocados deteriorated very rapidly and the browning on avocados was more severe at any specific storage time than packaged ones, irrespective of storage conditions and material type (Figures 4.1 and 4.2). For products stored at room temperature (Figure 4.1), the extent of enzymatic browning developed on the flesh of the unpackaged avocado stored for 24 h was comparable to those developed on packaged counterparts, but after longer storage periods, i.e., approximately 48 h and more than 48 h for products packaged in neat PLA and PLA/CNC nanocomposite films, respectively. Refrigerated products (Figure 4.2) showed similar trends since browning on the flesh of packaged avocados required longer storage periods (approximately 240 h or more) to reach the browning stage similar to that developed on unpackaged avocado after only 96 h storage (Figure 4.2). While packed avocados stored at 23°C and 4°C remained visually acceptable for longer than 24 h, unpackaged ones had an unappealing visual appearance above 24 h storage (Figures 4.1 and 4.2), implying the protective ability of PLA-based films to prolong the shelf life of avocado, owing to their oxygen barrier characteristic.

As the browning reaction is oxygen dependent, the extent of enzymatic browning depended on the material type; avocado packed in neat PLA film exhibited more browning than the counterparts packaged in PLA/CNC nanocomposite film, regardless of storage temperature and time (Figures 4.1 and 4.2). Since the nanocomposite film has better oxygen barrier property than neat PLA film (Liu & Matuana, 2019), it has more potential to reduce the ingress of oxygen in the packaging; thus, delaying enzymatic browning and reducing mesocarp discoloration. Storage temperature also affected the browning of packaged avocados. Refrigerated products discolored less than room temperature stored counterparts for both material types (Figures 4.1 and 4.2), suggesting that cold storage slows the browning reaction on the avocado flesh due to the decreased PPO activity at cold temperature. This result agrees with those of other investigators who also reported a significant decrease in PPO activity throughout the storage temperature at 4°C (Kahn, 1975; Soliva-Fortuny et al., 2002).

## 4.2 Color evaluation

Visual observations of stored avocados showed an uneven browning on the surface of avocado's flesh. This non-uniform browning appearance led to difficulty assessing the discoloration on the sample in some stages during storage. Therefore, color evaluation was needed to quantify the discoloration, which was performed using the L* a* b* coordinates.

The color results of unpackaged and the packaged fresh-cut avocados stored at room (23°C) and chilled temperature (4°C) are summarized in Table 4.1. The initial values of color parameters L, a*, and b* of packaged avocados flesh measured through the films before storage (time = 0 h) were similar to those of unpackaged avocado ($p < 0.05$), indicating a non-effect of the film transparency on color parameters for packaged avocado. All stored avocados darkened irrespective of materials and storage temperature. This was evidenced by the reduced lightness (L*) and changes in color coordinates, which varied from green to red (increased in a*) and yellow to blue (decreased in b*). The darkening effect obtained from color measurements support the visual appearance data illustrated in Figures 4.1 and 4.2 and is attributed to the oxidative enzymatic browning. However, packaging had strong effect on reducing browning since packaged avocado darkened less than the unpackaged fruit.

*Table 4.1 Color parameters of unpackaged and packaged avocado stored for 24 h at room temperature (RT) and 96 h at chilled temperature (CT).*

| **Samples** | **Lightness (L*)**[1] | | | **a***[1] | | | **b***[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| | **$L^*_0$** | **$L^*_{RT24}$** | **$L^*_{CT96}$** | **$a^*_0$** | **$a^*_{RT24}$** | **$a^*_{CT96}$** | **$b^*_0$** | **$b^*_{RT24}$** | **$b^*_{CT96}$** |
| Unpacked | $67.5 \pm 3.6^a$ | $37.7 \pm 3.6^a$ | $33.7 \pm 2.6^a$ | $-4.0 \pm 1.5^a$ | $3.2 \pm 1.1^a$ | $4.1 \pm 0.9^a$ | $25.9 \pm 1.4^a$ | $14.0 \pm 2.5^a$ | $12.3 \pm 2.0^a$ |
| PLA | $69.9 \pm 2.9^a$ | $47.7 \pm 5.6^b$ | $46.9 \pm 6.1^b$ | $-4.8 \pm 1.8^a$ | $1.4 \pm 1.2^b$ | $2.0 \pm 1.9^b$ | $26.1 \pm 1.0^a$ | $14.1 \pm 4.7^a$ | $14.2 \pm 5.3^a$ |
| PLA/CNC | $69.1 \pm 3.4^a$ | $51.0 \pm 3.4^c$ | $49.2 \pm 5.6^b$ | $-5.0 \pm 1.5^a$ | $1.1 \pm 0.4^b$ | $1.8 \pm 1.8^b$ | $25.3 \pm 1.1^a$ | $16.0 \pm 2.5^a$ | $14.5 \pm 4.0^a$ |

[1] ANOVA test with $\alpha = 0.05$. Different superscript letters within the same column indicate significant differences. The numbers of avocados evaluated were 8, 3, and 5 for samples tested at 0 h, 24 h , and 96 h, respectively.

It should be noted that the values of lightness L* had more influence determining the darkening of avocado flesh than the chromaticity coordinates a* and b* given their ability to monitor the differences in lightness/darkness on avocados packaged with different materials and stored under different conditions (Table 4.1). The results showed that avocados in PLA/CNC nanocomposite package exhibited lighter surfaces than those packaged in neat PLA film ($p < 0.05$) particularly at room temperature, whereas the color coordinates a* and b* showed no significant differences between the two packaging materials, regardless of storage conditions (Table 4.1). This was expected since the L* axis, which run from L* = 100 for white to L* = 0 for black, is the most indicative parameter associated with the enzymatic browning of avocado (Gómez-López, 2002; Ramos-Villarroel et al., 2011). As the surface color on fresh-cut avocado changed from a light yellow/green to dark brown during storage, the values of a* and b* also changed accordingly but without revealing significant difference in browning between the two different packaging materials probably due to the uneven browning of the flesh. Since the color measurements were taken at five specific locations (Figure 3.1) on the flesh of fresh-cut avocado, the large standard deviations in a* and b* values could be attributed to the gradual browning of the yellow portion of flesh, which darkened the fastest at the blossom end and the slowest at the stem end of the avocado (Figures 4.1 and 4.2).Therefore, difference in lightness/darkness ($\Delta L^*$) values that indicate how much a standard and sample differ from one another in L* as well as the total color difference ($\Delta E^*$) values were selected in this study to monitor discoloration on avocado flesh. Since $\Delta E^*$ is a single value that considers the differences between L*, a*, and b* of the sample and standard, it was used as a standard scale for comparison of discoloration on packaged avocado where a higher value of $\Delta E^*$ indicating more browning on the flesh.

The effects of package material types and storage conditions on the lightness ($\Delta L^*$) and total discoloration ($\Delta E^*$) of fresh-cut avocado are illustrated in Figure 4.3 and 4.4, respectively. It is obvious that the darkness and discoloration of fresh-cut avocados increased with the increased storage time for both packaging materials and storage temperatures. This was evidenced by the decreased values of $\Delta L^*$ (Figure 4.3) and increased values of $\Delta E^*$ (Figure 4.4), indicative of increased level of browning on the avocado flesh. Avocado packaged in neat PLA film darkened more than the counterparts packaged in PLA/CNC nanocomposite film, regardless of storage temperature and time (Figure 4.3 and 4.4), indicating the nanocomposite films inhibited darkening when compared to neat PLA film. As the packaging atmosphere significantly influences the PPO activity depletion (Soliva-Fortuny et al., 2002), the higher oxygen barrier of PLA/CNC film effectively prevented packaged product from exposure to oxygen, which then reduced PPO activity and the subsequent browning of avocado. Additionally, refrigerated avocados discolored less than counterparts stored in room temperature, irrespective of package material type due to the decreased PPO activity at cold temperature (Pinheiro et al., 2009; Kahn, 1975; Soliva-Fortuny et al., 2002). Longer storage times were needed for refrigerated products to reach for examples the darkness values of $\Delta L^* = -20$ and discoloration value of $\Delta E^* = 40$ when compared to room temperature stored avocados, implying that the samples stored in cold temperature preserved their fresh appearance longer than those in the room temperature. These results corroborate those obtained from visual appearance (Figures 4.1 and 4.2).

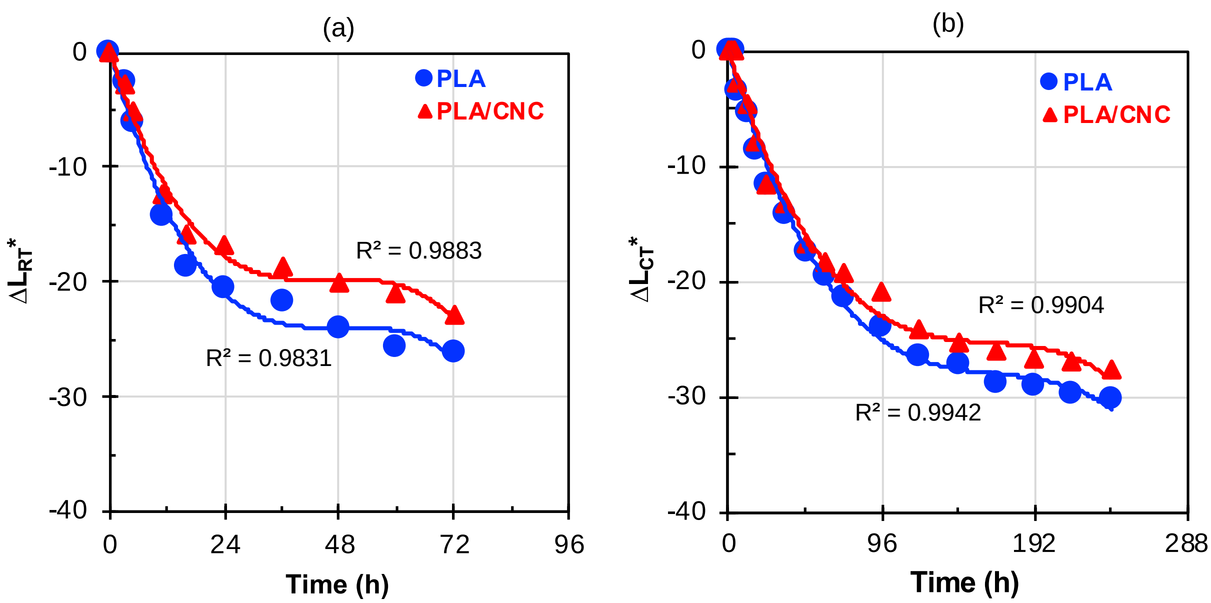



*Figure 4.3 ΔL* values of fresh-cut avocado packaged in PLA and PLA/CNC nanocomposite bags as a function of storage time under: (a) room temperature. (b) chilled temperature.*

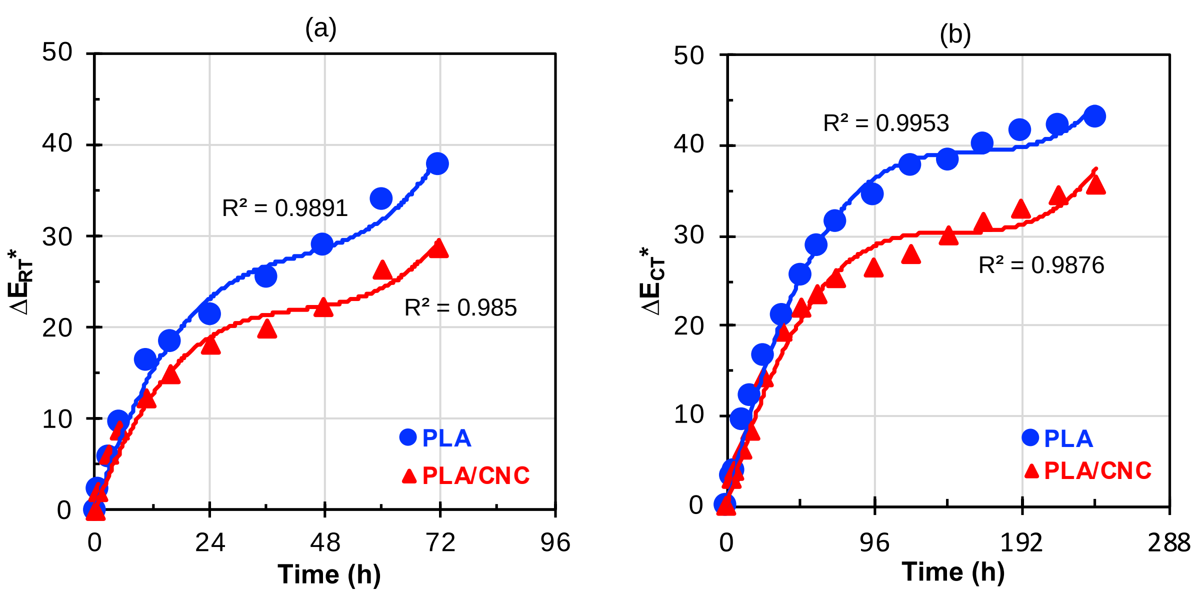



*Figure 4.4 Discoloration (ΔE*) of fresh-cut avocado packaged in PLA and PLA/CNC nanocomposite bags as a function of storage time under: (a) room temperature, and (b) chilled temperature.*

Browning of avocado could also be attributed to the $O_2$ and $CO_2$ contents inside the package since packaging in film bags creates a modified atmosphere within the package. The gaseous compositions of the package headspace are illustrated in Figure 4.5. The consumption of oxygen (decreased $O_2$ content) and production of carbon dioxide (increased $CO_2$ content) with storage time indicates avocado tissue respiration occurred irrespective of package materials (Figure 4.5). However, avocados in neat PLA package had significantly higher respiration rates ($p < 0.05$) than those in nanocomposite package during storage due to their lower $O_2$ and higher $CO_2$ contents. The increase in avocado respiratory activity has been reported to also increase the enzymatic metabolic process that leads to browning of the product (Sudheesh & Sunooj, 2020; González-Aguilar et al., 2000). Therefore, the high level of browning of avocados in neat PLA package could be attributed to the combined rapid reduction of $O_2$ and increase of $CO_2$ concentrations in the package during storage (Figure 4.5). Packaging with nanocomposite film significantly delayed the respiration rate in the package. Similar results were reported for fresh-cut mangoes treated with a combination of anti-browning agents, which included a D-isoascorbic acid as a reducing agent, 4-hexylresorcinol as a competitive PPO inhibitor, and potassium sorbate as an antimicrobial compound. This treatment was found effective in reducing browning because of higher $O_2$ but lower $CO_2$ levels in its in-package atmosphere compared the untreated mangoes, which browned and deteriorated more (González-Aguilar et al., 2000).

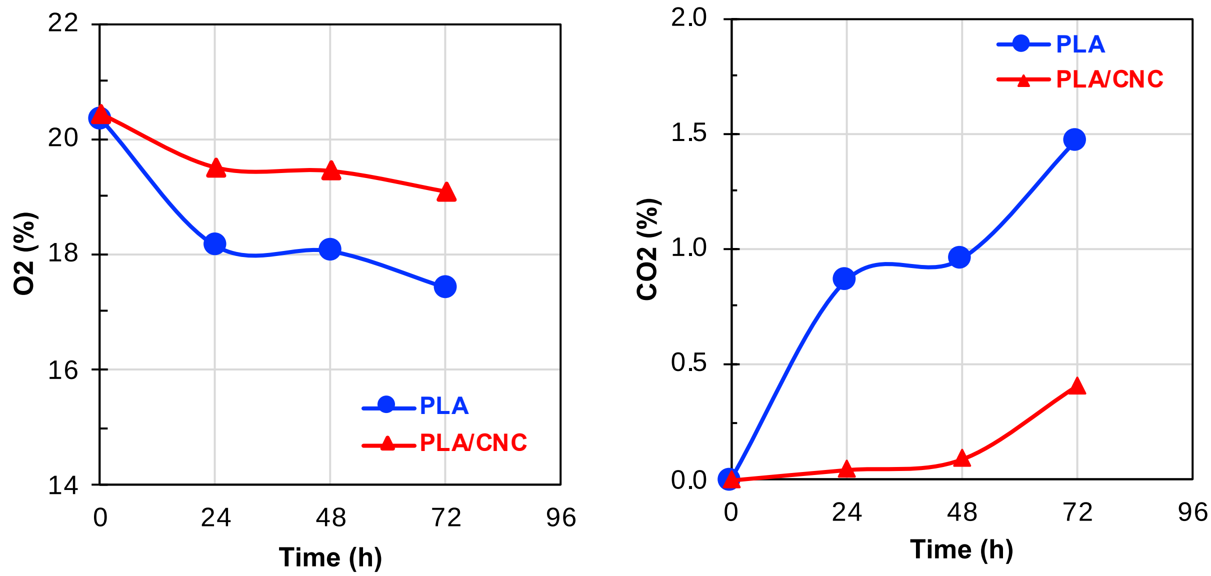



*Figure 4.5 The headspace gas compositions (% $O_2$ and % $CO_2$) as function of storage time for fresh-cut avocados packaged in PLA and PLA/CNC bags stored at room temperature.*

Browning of fresh-cut fruit has also been related to the degradation of the tissue produced by dryness (González-Aguilar et al., 2000). Consequently, retaining a high humidity atmosphere inside the package is beneficial to lessen tissue dryness; thus, reducing browning. The level of humidity inside the packages was monitored by measuring the percent weight loss of packaged avocados (Figure 4.6) because the moisture loss occurred due to transfer of water vapor from the product to air. Avocados in neat PLA package exhibited a significantly higher percent weight loss than that the counterparts in PLA/CNC nanocomposite packaged during the entire storage period ($p < 0.05$), suggesting a less humid atmosphere inside the package. This was expected because the lower moisture barrier of neat PLA film compared to nanocomposite allowed most of the moisture to escape the package to the environment, thus reducing the weight of the product (Karkhanis et al., 2018a, 2018b, 2021). Therefore, browning of avocado was reduced when packaged in nanocomposite bags, which created a high humidity atmosphere inside the package.

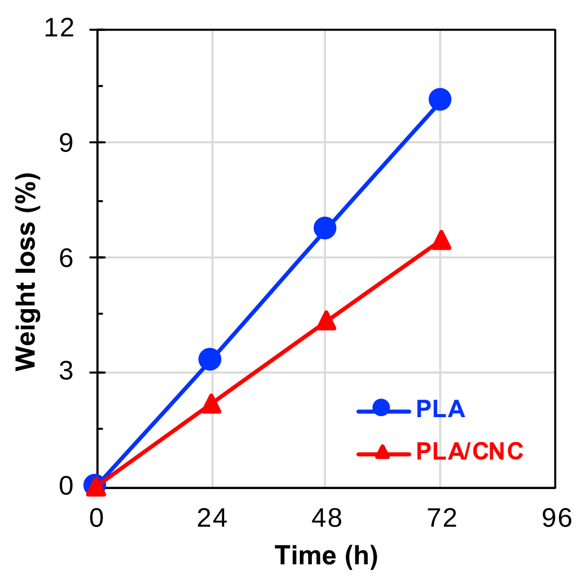



*Figure 4.6 Changes in moisture loss as a function of storage time for fresh-cut avocados packaged in PLA and PLA/CNC nanocomposite films stored at 23°C.*

## 4.3 Shelf life estimation

Overall, PLA/CNC nanocomposite package performed better in delaying the enzymatic browning on packaged avocado flesh than neat PLA package, insensitive of the storage conditions. Storage at cold temperature also delayed browning. Therefore, the potential of these films to prolong the shelf life of avocado was evaluated.

The discoloration data collected on packaged fresh-cut avocados stored at room temperature (RT) and chilled temperature (CT) were used to construct plots of discolorations (ΔE*) as a function of storage time (Figure 4.4). Polynomial third degree regression lines were then fitted to the plots using the type of equation 3.4 to estimate the shelf life as described in section 3.7.

The shelf-life of packaged fresh cut avocado was defined as the storage time required by the packaged avocado to reach the discoloration acceptability limits, i.e., the discoloration (ΔE*) exhibited on the unpackaged fresh-cut avocado (control, 5 replicates) stored at room temperature for 24h ($\Delta E^{*}_{RT} = 33.0 \pm 3.6$) and refrigerated for 96 h ($\Delta E^{*}_{CT} = 37.4 \pm 2.4$) (Table 4.2) Since the acceptability limits were known, the shelf life was then estimated by substituting the variable y by ($\Delta E^{*}_{RT}$) or ($\Delta E^{*}_{CT}$) in equation 3.4 and solving for the value of x, i.e., the storage time.

Irrespective of material type, packaging avocado with PLA-based materials extended its shelf life in both room and cold temperature storages ($p < 0.05$) (Table 4.2). PLA/CNC nanocomposite package extended the shelf life of avocado by 54 h when compared with neat PLA package in room temperature due to its higher oxygen barrier, which delayed discoloration. The nanocomposite film remarkably doubled the shelf life of packaged avocado compared to neat PLA at room temperature. Storage at cold temperature provided additional shelf life extension since the nanocomposite package prolonged the shelf life of avocado by 98.4 h than neat PLA, due to the decreased PPO activity at cold temperature.

*Table 4.2 Predicted shelf life of packaged avocado stored at room temperature and cold temperature.*

| Storage conditions | | Acceptability limits | Shelf life (h)[1] | | Shelf life improvement |
|---|---|---|---|---|---|
| Temperature (°C) | Time (h) | ($\Delta E^*$) | PLA film | PLA/CNC film | (h) |
| 23.0 ± 1.0 | 24 | 33.0 ± 4.0 | 50.4 ± 3.0$^{a}$ | 104.4 ± 7.2$^{b}$ | 54.0 |
| 4.0 ± 1.0 | 96 | 37.4 ± 2.4 | 184.6 ± 64.1$^{c}$ | 283.1 ± 44.3$^{d}$ | 98.4 |

[1]A two-way t-test with an $\alpha$ of 0.05 comparing the effects of package material types and storage temperature on the shelf life of fresh-cut avocados. Different superscript letters in the same row (PLA vs PLA/CNC nanocomposite) or column (room vs chilled temperature) indicate significant difference. Three to five replicates were tested.

Validation tests were carried out to verify the accuracy of the shelf life predicted from the polynomial third degree regression equation. This was achieved by comparing the predicted storage time versus the experimental one needed by the packaged avocado to reach a specified discoloration at room temperature and cold temperature (Table 4.3). The experimental data confirmed the predicted results since both methods yielded similar storage times. Therefore, the model using polynomial regression equations accurately predicted the shelf life of packaged avocados. The results clearly demonstrate the potential of PLA/CNC nanocomposite film to extend the shelf life of oxygen-sensitive food product.

*Table 4.3 Model validation through the comparison of predicted versus experimental storage times needed to reach a specified discoloration at different storage conditions.*

| **Temperature (°C)** | **Experimental discoloration (ΔE)** | **Time (h) needed to reach (ΔE)** | |
|---|---|---|---|
| | | **Experimental ($t_{exp}$)** | **Predicted ($t_{pred}$)** |
| 23.0 ± 1.0 | 31.6 ± 4.7 | 48 | 48.7 ± 3.0 |
| 4.0 ± 1.0 | 36.1 ±5.6 | 192 | 196.5 ± 27.1 |

## 4.4 Microbial count

Both the plate count agar (PCA), a non-selective bacteriological substrate used for determination of the total number of microorganisms (fungi, mold, bacteria, etc.) in a sample, and the potato dextrose agar (PDA), a more selective medium for growing yeasts and mold, were employed to estimate the levels of microorganisms on the unpackaged and packaged fresh cut avocados (Table 4.4). The microbial counts (log CFU/g) on the unpackaged fresh cut avocado were $1.3 \pm 0.2$ in both PCA and PDA media, clearly indicating that molds are the type of microorganisms mostly growing on the avocado. The package material's type did not affect the growth of microbes, irrespective of their estimated shelf lives storage conditions. However, cold storage significantly prevented mold growth compared to room temperature storage, insensitive to material types. The effect of storage temperature was well observed using corrected data (Table 4.4), which showed no evidence of mold growth during cold storage as expected since the enzyme activities decrease by lowering storage temperature. These data clearly support the role of cold temperature in reducing the enzymatic activities causing discoloration on fresh-cut avocado as previously discussed. It should also be pointed out that the levels of mesophilic microorganism counts above $10^6$ CFU/g (6 log CFU/g) associated with the end of shelf life of perishable products (Corradini, 2018) were not reached by the packaged fresh-cut avocados, irrespective storage conditions, demonstrating the potential of PLA-based films to package oxygen-sensitive foods.

*Table 4.4 Levels of microorganisms on packaged fresh-cut avocados stored at room temperature for 48 h ($RT_{48}$) and cold temperature for 180 h ($CT_{180}$).*

| **Package material types** | **Levels of microorganisms without correction (log CFU/g)[1]** | | | | **Levels of microorganisms with correction (log CFU/g)[1]** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Plate count agar[2]** | | **Potato dextrose agar[2]** | | **Plate count agar** | | **Potato dextrose agar** | |
| | **$RT_{48}$** | **$CT_{180}$** | **$RT_{48}$** | **$CT_{180}$** | **$RT_{48}$** | **$CT_{180}$** | **$RT_{48}$** | **$CT_{180}$** |
| PLA | $2.1 \pm 0.2^{a}$ | $1.1 \pm 0.2^{b}$ | $2.4 \pm 0.7^{a}$ | $1.1 \pm 0.2^{b}$ | 2.0 ± 0.2 | - | 2.3 ± 0.8 | - |
| PLA/CNC | $2.0 \pm 0.1^{a}$ | $1.2 \pm 0.4^{b}$ | $2.1 \pm 0.2^{a}$ | $1.1 \pm 0.2^{b}$ | 2.0 ± 0.1 | - | 2.0 ± 0.2 | - |

[1]The microbial counts (log CFU/g) on the unpackaged fresh cut avocado were 1.3 ± 0.23 for both PCA and PD tests.
[2]A two-way t-test with an α of 0.05 comparing the effects of package material types and storage temperature on the microbe counts of fresh-cut avocados. Different superscript letters in the same row (room vs chilled temperature) or column (PLA vs PLA/CNC nanocomposite) indicate significant difference (n = 8). Three to five replicates were tested.

# APPENDICES

**APPENDIX A: Ripeness stage of avocado fruit based on skin color in L*C*H* values**

*Table A.1 Skin color of avocado fruits.*

| Number of Replicates | Color at purchase | | | Color after reaching stage 4 | | | Color at experimental stage | | |
|---|---|---|---|---|---|---|---|---|---|
| | **L*** | **C*** | **H*** | **L*** | **C*** | **H*** | **L*** | **C*** | **H*** |
| 1 | 25.1 | 4.4 | 106.0 | 24.4 | 3.4 | 94.5 | 21.8 | 1.5 | 53.2 |
| 2 | 25.2 | 5.7 | 99.1 | 24.1 | 4.5 | 97.3 | 22.1 | 1.9 | 51.8 |
| 3 | 25.0 | 4.8 | 96.0 | 23.7 | 3.8 | 90.1 | 21.9 | 2.4 | 62.0 |
| 4 | 24.5 | 4.3 | 96.2 | 23.9 | 3.9 | 88.8 | 21.8 | 2.2 | 67.3 |
| 5 | 24.6 | 5.3 | 94.3 | 24.1 | 4.8 | 59.3 | 21.9 | 3.3 | 40.4 |
| 6 | 24.8 | 4.7 | 82.8 | 23.4 | 3.7 | 64.0 | 21.9 | 2.9 | 46.2 |
| 7 | 25.7 | 5.8 | 91.0 | 24.2 | 4.5 | 70.5 | 23.0 | 3.7 | 59.1 |
| 8 | 25.1 | 6.1 | 97.8 | 23.2 | 4.3 | 77.9 | 22.0 | 2.7 | 58.5 |
| 9 | 26.7 | 6.8 | 93.3 | 25.2 | 5.5 | 75.2 | 23.3 | 4.1 | 60.4 |
| 10 | 25.4 | 6.8 | 103.1 | 24.3 | 4.8 | 81.4 | 21.9 | 3.0 | 53.1 |
| 11 | 25.4 | 6.2 | 101.2 | 23.8 | 3.3 | 82.2 | 21.9 | 2.0 | 50.4 |
| 12 | 25.0 | 4.8 | 97.4 | 23.7 | 3.7 | 90.2 | 21.7 | 1.3 | 50.4 |
| 13 | 25.1 | 5.5 | 95.5 | 23.9 | 3.7 | 89.9 | 22.5 | 2.7 | 64.0 |
| 14 | 24.9 | 4.5 | 95.0 | 23.5 | 3.5 | 81.0 | 21.7 | 1.9 | 36.8 |
| 15 | 25.1 | 4.6 | 99.2 | 23.9 | 4.1 | 98.4 | 21.9 | 2.4 | 62.0 |

**APPENDIX B: Color measurements of films through calibrated plate in L*a*b* values**

*Table B.1 Color measurements of calibrated plate and films through calibrate plate.*

| **Measurements** | **Calibrate plate** | | | **PLA** | | | **PLA/CNC** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| 1 | 96.6 | 0.0 | 2.1 | 96.8 | -0.1 | 1.8 | 96.5 | -0.1 | 1.9 |
| 2 | 96.6 | 0.0 | 2.2 | 95.6 | 0.1 | 2.6 | 95.4 | 0.1 | 2.6 |
| 3 | 97.0 | 0.0 | 1.8 | 96.1 | 0.0 | 2.4 | 95.9 | 0.0 | 2.4 |
| 4 | 96.8 | 0.0 | 2.0 | 95.8 | 0.1 | 2.7 | 95.6 | 0.1 | 2.7 |
| 5 | 96.8 | 0.0 | 2.0 | 95.7 | -0.1 | 2.4 | 95.7 | 0.0 | 2.6 |
| 6 | 97.1 | -0.1 | 1.6 | 96.6 | -0.1 | 1.7 | 96.3 | -0.1 | 1.9 |
| 7 | 97.3 | -0.1 | 1.6 | 95.6 | 0.1 | 2.5 | 95.7 | -0.1 | 2.7 |
| 8 | 96.3 | 0.1 | 2.4 | 95.8 | 0.1 | 2.5 | 95.1 | 0.0 | 2.7 |
| 9 | 96.6 | 0.0 | 2.1 | 94.8 | -0.1 | 2.2 | 95.6 | 0.0 | 2.6 |
| 10 | 96.7 | 0.0 | 2.0 | 95.5 | 0.0 | 2.4 | 95.5 | 0.1 | 2.6 |
| **Average** | **96.8** | **0.0** | **2.0** | **95.8** | **0.0** | **2.3** | **95.7** | **0.0** | **2.5** |
| **SD** | **0.3** | **0.1** | **0.3** | **0.6** | **0.1** | **0.3** | **0.4** | **0.1** | **0.3** |

**APPENDIX C: Color measurements of unpackaged fresh-cut avocado**

*Table C.1 Values of lightness ΔL* and chromaticity parameters Δa* and Δb* of unpackaged fresh-cut avocados at different storage temperatures.*

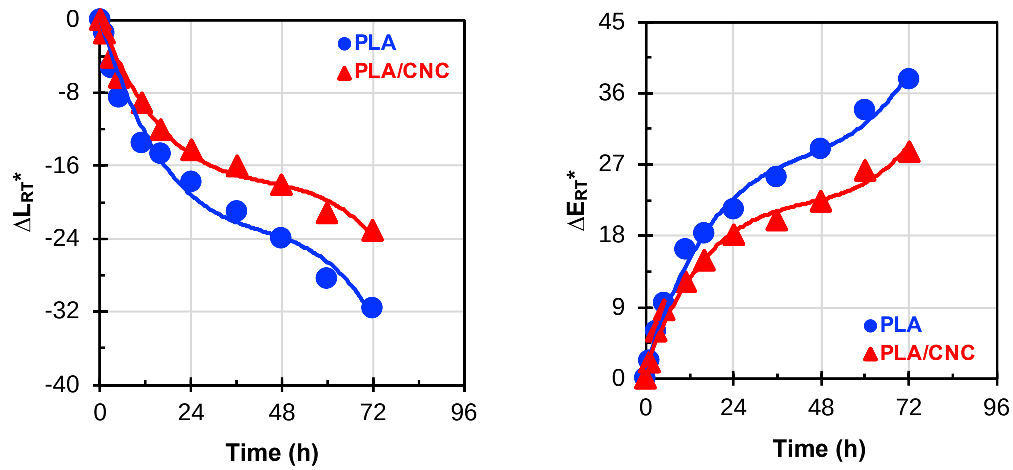
| Temperatures | Replicates | ΔL* | Δa* | Δb* | ΔE* |
|---|---|---|---|---|---|
| Room temperature after 24 hours | 1 | -25.6 | 7.4 | -9.3 | 28.21 |
| | 2 | -26.5 | 5.6 | -11.7 | 29.49 |
| | 3 | -31.7 | 6.8 | -14.8 | 35.65 |
| | 4 | -31.2 | 8.8 | -10.9 | 34.24 |
| | 5 | -34.2 | 7.2 | -13.7 | 37.56 |
| | **Average** | **-29.9** | **7.2** | **-12.1** | 33.03 |
| | **SD** | **3.7** | **1.1** | **2.2** | 4.02 |
| Chilled temperature after 96 hours | 1 | -33.8 | 9.4 | -13.3 | 37.53 |
| | 2 | -31.6 | 5.5 | -13.1 | 34.62 |
| | 3 | -35.1 | 7.7 | -16.0 | 39.31 |
| | 4 | -36.3 | 11.2 | -13.1 | 40.12 |
| | 5 | -32.5 | 6.8 | -12.0 | 35.31 |
| | **Average** | **-33.8** | **8.1** | **-13.5** | 37.38 |
| | **SD** | **1.9** | **2.2** | **1.5** | 2.41 |

## APPENDIX D: Color measurements of packaged avocados stored at room temperature

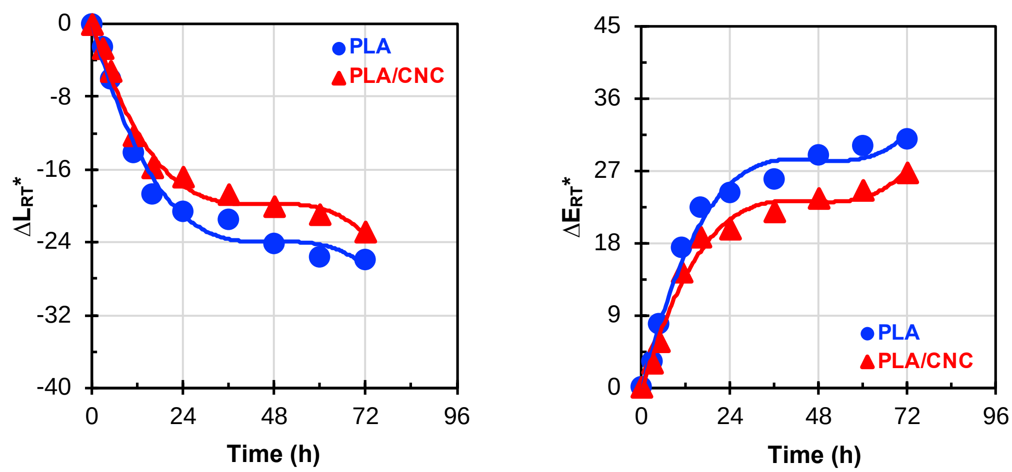
*Table D.1 Effect of packaging material types on the lightness ΔL*, chromaticity parameters Δa* and Δb*, and total discoloration ΔE*of fresh-*cut avocados stored at room temperature for various periods of time.

| **Replicates** | **Times (h)** | **PLA** | | | | **PLA/CNC** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **ΔL*** | **Δa*** | **Δb*** | **ΔE*** | **ΔL*** | **Δa*** | **Δb*** | **ΔE*** |
| 1 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | -8.4 | 3.0 | -3.2 | 9.5 | -6.3 | 5.5 | -2.5 | 8.7 |
| | 11 | -13.5 | 6.9 | -6.1 | 16.3 | -9.1 | 7.0 | -4.3 | 12.3 |
| | 16 | -14.7 | 7.3 | -8.2 | 18.3 | -12.0 | 7.5 | -5.1 | 15.0 |
| | 24 | -17.8 | 7.6 | -8.9 | 21.3 | -14.3 | 8.8 | -6.9 | 18.1 |
| | 48 | -23.9 | 9.5 | -13.2 | 28.9 | -18.0 | 10.3 | -8.3 | 22.3 |
| | 72 | -31.6 | 10.3 | -18.0 | 37.8 | -23.1 | 10.6 | -13.2 | 28.7 |
| 2 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | -6.1 | 3.6 | -3.6 | 7.9 | -5.2 | 2.5 | -0.3 | 5.8 |
| | 11 | -14.2 | 6.6 | -7.7 | 17.5 | -12.3 | 5.1 | -5.6 | 14.5 |
| | 16 | -18.7 | 7.9 | -9.6 | 22.4 | -15.8 | 6.4 | -7.7 | 18.7 |
| | 24 | -20.6 | 7.9 | -10.2 | 24.3 | -16.8 | 6.9 | -7.9 | 19.8 |
| | 48 | -24.1 | 9.2 | -13.1 | 29.0 | -20.0 | 7.8 | -9.7 | 23.6 |
| | 72 | -26.0 | 9.4 | -13.9 | 31.0 | -22.8 | 8.2 | -11.3 | 26.8 |
| 3 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | -9.5 | 3.5 | -7.3 | 12.5 | -8.4 | 2.2 | -5.0 | 10.0 |
| | 11 | -16.6 | 5.0 | -10.8 | 20.5 | -13.3 | 3.3 | -8.1 | 15.9 |
| | 16 | -21.2 | 6.1 | -14.1 | 26.2 | -15.8 | 4.2 | -9.0 | 18.6 |
| | 24 | -25.9 | 6.4 | -17.1 | 31.7 | -19.3 | 4.6 | -12.3 | 23.4 |
| | 48 | -30.9 | 6.7 | -19.4 | 37.1 | -24.2 | 5.0 | -16.1 | 29.5 |
| | 72 | -32.8 | 6.6 | -20.6 | 39.3 | -28.1 | 5.3 | -18.1 | 33.8 |

Replicate 1




Replicate 2




Replicate 3

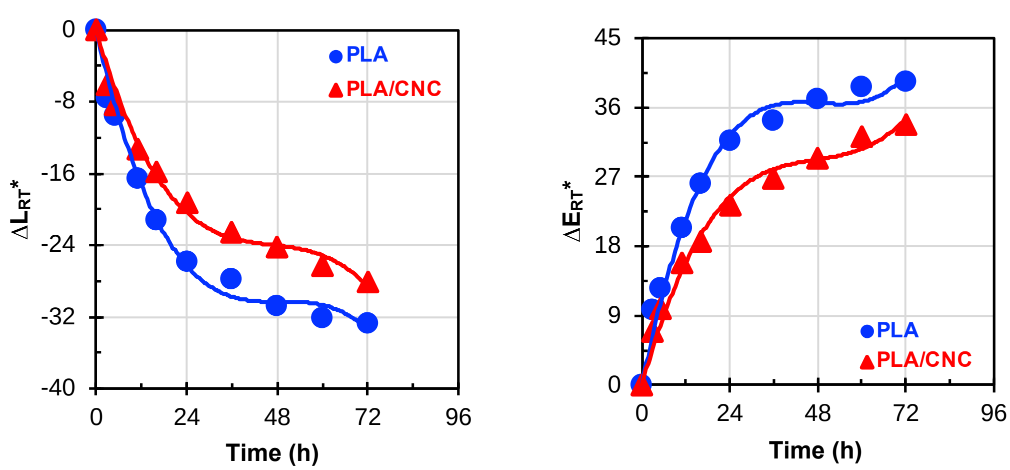



*Figure D.1 Plots of ΔL* and ΔE* versus time for packaged samples stored at room temperature.*

*Table D.2 Polynomial third degree regression equations derived from plots of ΔE* (y-axis) versus storage time (x-axis) used to predict the shelf life of avocados packaged in neat PLA and nanocomposite films stored at room temperature.*

| Samples | Replicates | Regression equations | $R^2$ | Predicted shelf life |
|---|---|---|---|---|
| PLA | 1 | $y = 0.0003x^3 - 0.0345x^2 + 1.6387x$ | 0.9891 | 49.6 |
| | 2 | $y = 0.0003x^3 - 0.0387x^2 + 1.8278x$ | 0.9835 | 53.7 |
| | 3 | $y = 0.0003x^3 - 0.0485x^2 + 2.3234x$ | 0.9904 | 47.8 |
| PLA/CNC | 1 | $y = 0.0002x^3 - 0.0301x^2 + 1.3767x$ | 0.985 | 97.1 |
| | 2 | $y = 0.0002x^3 - 0.0334x^2 + 1.5332x$ | 0.9869 | 111.5 |
| | 3 | $y = 0.0002x^3 - 0.0341x^2 + 1.6957x$ | 0.9895 | 104.5 |

**APPENDIX E: Color measurements of packaged avocado at chilled temperature**

*Table E.1 Effect of packaging material types on the lightness ΔL*, chromaticity parameters Δa* and Δb*, and total discoloration ΔE*of fresh*-cut *avocados stored at chilled temperature for various periods of time.*

| **Replicates** | **Times (h)** | **PLA** | | | | **PLA/CNC** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **ΔL*** | **Δa*** | **Δb*** | **ΔE*** | **ΔL*** | **Δa*** | **Δb*** | **ΔE*** |
| 1 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 11 | -2.2 | 1.9 | -0.2 | 2.9 | -3.0 | 0.8 | 1.8 | 3.6 |
| | 24 | -5.0 | 2.6 | -1.4 | 5.8 | -7.6 | 2.5 | -1.4 | 8.1 |
| | 48 | -9.0 | 4.5 | -3.1 | 10.6 | -10.7 | 4.2 | -3.7 | 12.1 |
| | 96 | -14.1 | 6.6 | -5.8 | 16.6 | -14.8 | 6.4 | -7.7 | 17.9 |
| | 168 | -23.0 | 8.4 | -10.7 | 26.7 | -19.5 | 9.3 | -9.2 | 23.5 |
| | 240 | -29.0 | 8.5 | -15.8 | 34.1 | -22.3 | 8.2 | -11.3 | 26.3 |
| 2 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | -4.2 | 1.9 | 0.9 | 4.7 | -3.1 | 1.3 | 0.9 | 3.5 |
| | 11 | -6.2 | 2.9 | -0.4 | 6.8 | -11.5 | 4.8 | -2.0 | 12.6 |
| | 16 | -18.7 | 8.3 | -6.9 | 21.6 | -21.8 | 7.9 | -7.3 | 24.3 |
| | 24 | -29.5 | 10.0 | -14.9 | 34.5 | -27.7 | 9.5 | -12.0 | 31.7 |
| | 48 | -34.6 | 9.2 | -18.0 | 40.1 | -31.4 | 10.4 | -13.3 | 35.6 |
| | 72 | -39.7 | 8.7 | -19.9 | 45.2 | -34.1 | 10.7 | -14.5 | 38.6 |
| 3 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | -8.8 | 2.6 | -2.6 | 9.5 | -5.7 | 2.5 | -0.9 | 6.3 |
| | 11 | -15.0 | 3.4 | -6.6 | 16.7 | -12.8 | 3.6 | -5.5 | 14.4 |
| | 16 | -23.1 | 4.8 | -9.9 | 25.6 | -19.2 | 4.4 | -9.8 | 22.0 |
| | 24 | -30.5 | 6.8 | -14.5 | 34.5 | -22.6 | 5.8 | -12.4 | 26.5 |
| | 48 | -35.7 | 7.1 | -16.9 | 40.1 | -27.8 | 6.7 | -13.2 | 31.5 |
| | 72 | -38.1 | 6.5 | -19.0 | 43.1 | -31.4 | 6.5 | -15.7 | 35.7 |

Table E.1 (cont'd)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | -5.2 | 1.2 | -1.4 | 5.6 | -4.7 | 2.0 | -2.1 | 5.5 |
| | 11 | -11.6 | 2.0 | -4.9 | 12.7 | -11.6 | 3.8 | -7.4 | 14.2 |
| 4 | 16 | -17.3 | 3.4 | -8.7 | 19.7 | -16.6 | 5.3 | -9.5 | 19.8 |
| | 24 | -23.8 | 4.5 | -15.3 | 28.6 | -20.8 | 5.2 | -13.8 | 25.5 |
| | 48 | -28.7 | 4.9 | -19.4 | 34.9 | -25.9 | 5.4 | -17.8 | 31.8 |
| | 72 | -30.2 | 5.0 | -20.3 | 36.7 | -27.6 | 5.3 | -18.6 | 33.7 |
| | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | -3.7 | -1.7 | -0.3 | 4.1 | -3.2 | 1.1 | -1.7 | 3.8 |
| | 11 | -8.3 | 0.3 | -3.7 | 9.1 | -7.3 | 1.5 | -3.4 | 8.2 |
| 5 | 16 | -14.5 | 1.0 | -6.8 | 16.0 | -13.8 | 4.0 | -7.1 | 16.0 |
| | 24 | -19.7 | 2.6 | -8.6 | 21.7 | -17.4 | 5.5 | -8.7 | 20.2 |
| | 48 | -26.9 | 4.9 | -10.1 | 29.1 | -23.6 | 7.0 | -10.8 | 26.9 |
| | 72 | -32.2 | 5.4 | -13.7 | 35.4 | -26.0 | 6.9 | -13.6 | 30.1 |

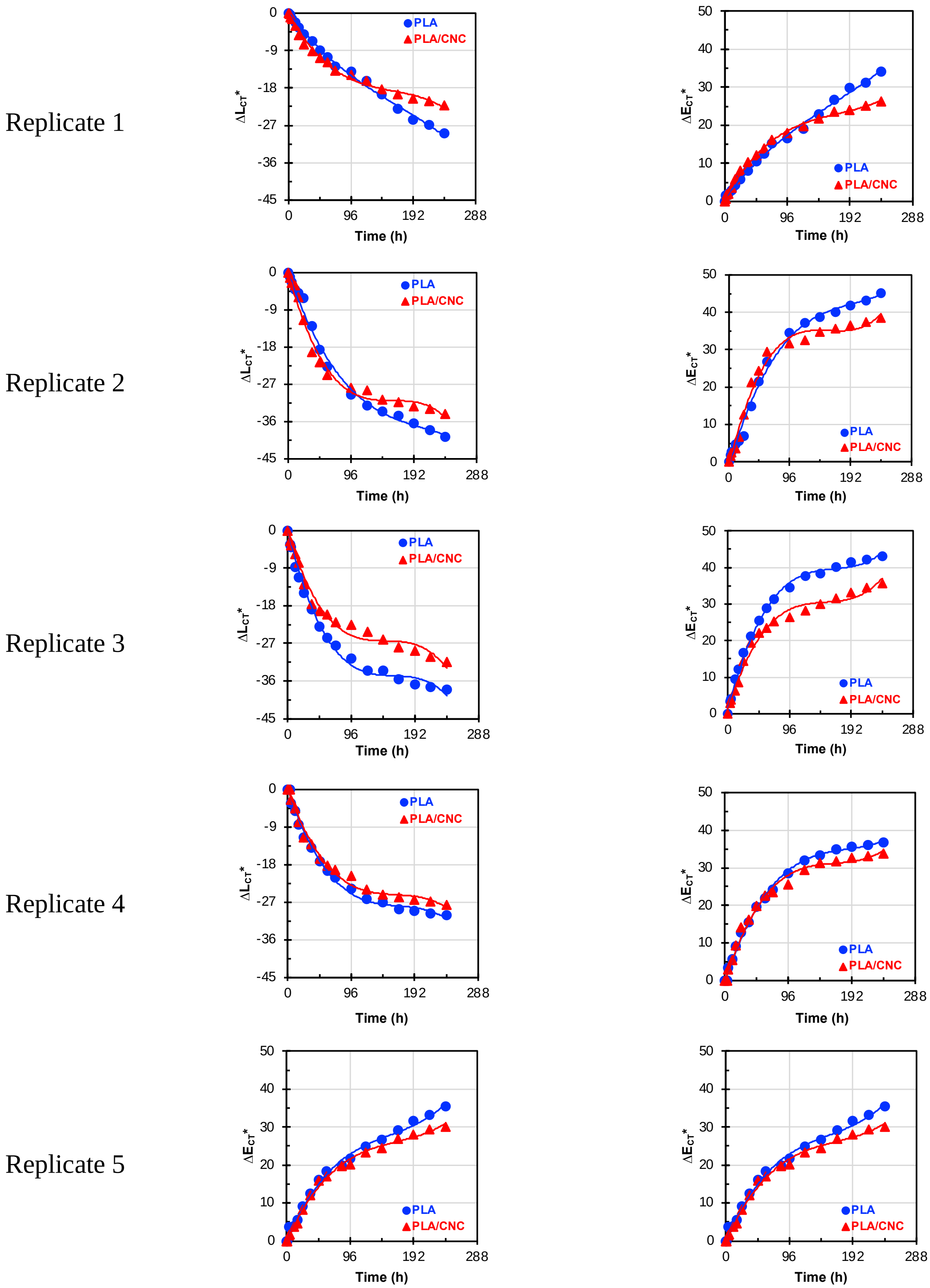



*Figure E.1 Plots of ΔL* and ΔE* versus time for packaged samples stored at cold temperature.*

*Table E.2 Polynomial third degree regression equations derived from plots of ΔE* (y-axis) versus storage time (x-axis) used to predict the shelf life of avocados packaged in neat PLA and nanocomposite films stored at chilled temperature.*

| Samples | Replicates | Regression equations | $R^2$ | Predicted shelf life |
|---|---|---|---|---|
| PLA | 1 | $y = 0.000002x^3 - 0.0009x^2 + 0.2537x$ | 0.9961 | 224.2 |
| | 2 | $y = 0.000004x^3 - 0.0023x^2 + 0.5286x$ | 0.9913 | 121.2 |
| | 3 | $y = 0.000009x^3 - 0.0044x^2 + 0.7162x$ | 0.9953 | 108.4 |
| | 4 | $y = 0.000005x^3 - 0.0027x^2 + 0.5162x$ | 0.9966 | 238.9 |
| | 5 | $y = 0.000005x^3 - 0.0022x^2 + 0.4038x$ | 0.9939 | 230.4 |
| PLA/CNC | 1 | $y = 0.000003x^3 - 0.0017x^2 + 0.3304x$ | 0.9972 | 355.5 |
| | 2 | $y = 0.000008x^3 - 0.0041x^2 + 0.6579x$ | 0.9874 | 275.8 |
| | 3 | $y = 0.000009x^3 - 0.004x^2 + 0.6029x$ | 0.9876 | 234.1 |
| | 4 | $y = 0.000006x^3 - 0.0031x^2 + 0.5359x$ | 0.9904 | 273.3 |
| | 5 | $y = 0.000004x^3 - 0.002x^2 + 0.3824x$ | 0.995 | 276.6 |

**APPENDIX F: Headspace gas composition and percent weight loss of packaged avocados**

*Table F.1 Percent oxygen in the headspace of packaged avocado stored at room temperature.*

| **Replicates** | **$O_2$ content (%)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **PLA** | | | | **PLA/CNC** | | | |
| | **0 h** | **24 h** | **48 h** | **72 h** | **0 h** | **24 h** | **48 h** | **72 h** |
| 1 | 20.5 | 17.1 | 17.2 | 17.4 | 20.5 | 19.2 | 19.3 | 19.3 |
| 2 | 20.3 | 17.8 | 16.6 | 13.0 | 20.6 | 19.5 | 19.1 | 17.7 |
| 3 | 20.5 | 18.8 | 19.1 | 18.6 | 20.2 | 19.9 | 19.9 | 19.6 |
| 4 | 20.3 | 19.2 | 18.9 | 18.8 | 20.4 | 19.3 | 19.3 | 18.7 |
| 5 | 20.4 | 18.1 | 18.3 | 18.5 | 20.6 | 19.5 | 19.6 | 19.8 |
| 6 | 20.1 | 17.8 | 18.2 | 18.2 | 20.3 | 19.5 | 19.5 | 19.4 |
| **Average** | **20.4** | **18.1** | **18.1** | **17.4** | **20.4** | **19.5** | **19.5** | **19.1** |
| **SD** | **0.2** | **0.8** | **1.0** | **2.2** | **0.2** | **0.2** | **0.3** | **0.8** |

*Table F.2 Percent carbon dioxide in the headspace of packaged avocado stored at room temperature.*

| **Replicates** | **$CO_2$ content (%)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **PLA** | | | | **PLA/CNC** | | | |
| | **0 h** | **24 h** | **48 h** | **72 h** | **0 h** | **24 h** | **48 h** | **72 h** |
| 1 | 0.0 | 2.0 | 1.9 | 1.9 | 0.0 | 0.2 | 0.2 | 0.1 |
| 2 | 0.0 | 1.1 | 2.4 | 4.9 | 0.0 | 0.0 | 0.2 | 1.5 |
| 3 | 0.0 | 0.5 | 0.2 | 0.9 | 0.0 | 0.0 | 0.0 | 0.3 |
| 4 | 0.0 | 0.1 | 0.3 | 0.6 | 0.0 | 0.0 | 0.1 | 0.5 |
| 5 | 0.0 | 0.8 | 0.7 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6 | 0.0 | 0.8 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Average** | **0.00** | **0.86** | **0.96** | **1.47** | **0.00** | **0.04** | **0.09** | **0.41** |
| **SD** | **0.00** | **0.65** | **0.94** | **1.79** | **0.00** | **0.09** | **0.11** | **0.57** |

*Table F.3 Percent weight loss of packaged fresh-cut avocado stored at room temperature.*

| **Replicates** | **Weight loss (%)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **PLA** | | | | **PLA/CNC** | | | |
| | **0 h** | **24 h** | **48 h** | **72 h** | **0 h** | **24 h** | **48 h** | **72 h** |
| 1 | 0.0 | 2.3 | 4.9 | 7.0 | 0.0 | 1.7 | 3.6 | 5.1 |
| 2 | 0.0 | 3.4 | 6.8 | 10.3 | 0.0 | 2.6 | 5.0 | 7.5 |
| 3 | 0.0 | 4.0 | 8.0 | 12.6 | 0.0 | 2.2 | 4.3 | 6.7 |
| 4 | 0.0 | 4.0 | 8.1 | 11.9 | 0.0 | 2.1 | 4.3 | 6.4 |
| 5 | 0.0 | 3.4 | 6.7 | 9.4 | 0.0 | 2.5 | 4.7 | 6.7 |
| 6 | 0.0 | 2.8 | 5.9 | 9.3 | 0.0 | 2.0 | 4.0 | 6.4 |
| **Average** | **0.0** | **3.3** | **6.7** | **10.1** | **0.0** | **2.2** | **4.3** | **6.5** |
| **SD** | **0.0** | **0.6** | **1.2** | **2.0** | **0.0** | **0.3** | **0.5** | **0.8** |

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## REFERENCES


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# CHAPTER 5

# CONCLUSIONS

## 5.1 Conclusions

The development of enzymatic browning catalyzed by polyphenol oxidase is one of the main problems in the preservation of fresh-cut and oxygen sensitive fruits such as avocados. This study employed packaging solution based on PLA/cellulose nanocrystal (CNC) films with strong barrier and UV capacities to prevent browning in avocado and prolong its shelf life. Specifically, the effects of package material types (neat PLA vs PLA/CNC nanocomposite) and storage temperature (23°C vs 4°C), and storage time on browning, in-package humidity level and gaseous compositions, and shelf life were assessed. Visual appearances of unpackaged and packaged avocados were observed with digital photos taken periodically to qualitatively assess enzymatic browning, whereas their lightness ($\Delta L^*$) and total discoloration ($\Delta E^*$) were quantified as function of storage time and temperature to estimate the shelf life of the avocados using polynomial third-degree regression equation. The following conclusions were drawn from the experimental results:

1. The nanocomposite package inhibited the browning of fresh-cut avocado when compared to neat PLA package due to the higher oxygen barrier of nanocomposite film, which effectively prevented packaged avocados from exposure to oxygen.

2. Fresh-cut avocados packaged with PLA/CNC nanocomposite film maintained a high humidity atmosphere and an appropriate gas evolution (% $O_2$ and % $CO_2$) inside the package that slowed its respiration down compared to neat PLA package.

3. Slowing the avocado respiration rate down was beneficial in delaying the enzymatic browning since fresh-cut avocados in nanocomposite package discolored less and preserved their color better than the counterparts in neat PLA package, irrespective of storage conditions.

4. Cold temperature (4°C) preserved the color of packaged fresh-cut avocado longer than those stored at the room temperature (23°C) due to the decreased oxidation activity at cold temperature.

5. PLA/CNC package extended the shelf life of fresh-cut avocados by additional 54 h and 98 h compared to neat PLA package, when stored at room temperature and refrigerated, respectively. Combination of nanocomposite package and refrigerated storage could be used to inhibit browning and extend the shelf life of fresh-cut avocados.

6. None of the packaged fresh-cut avocados reached the minimum level of mesophilic microorganism counts of $10^6$ CFU/g (or log CFU/g = 6) associated with the end of shelf life of perishable products, irrespective storage conditions.

The experimental results have shown that packaging solution based on PLA/CNC films with strong barrier and UV capacities is an effective method to delay browning in fresh-cut avocado, prevent the microbial growth, and prolong its shelf life. Therefore, the potential of PLA/CNC film to extend the shelf life of oxygen-sensitive food products has been clearly demonstrated.

## 5.2 Future work

The potential of PLA/CNC film to extend the shelf-life of oxygen-sensitive food products was clearly demonstrated using only discoloration as the quality attribute to signal the shelf life of packaged fresh-cut avocados, a highly perishable food product. Other important characteristics that could limit the shelf life of foods such as the loss of sensory quality, appearance of organoleptic defects like off-odors, loss of nutritional value, or the growth of microorganisms (microbial spoilage) must also be investigated. This could expand the application of the nanocomposite films to the packaging of foods with intermediate or long shelf lives. Additionally, this study employed packaging solution based on PLA/cellulose nanocrystal (CNC) films with strong barrier and UV capacities to prevent browning in avocado and prolong its shelf life. These materials could also be combined with other protection techniques that have been found effective in preventing browning in avocados. This could include combination of PLA/CNC film with use of anti-browning agents in the package or packaging with modified atmosphere.