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Sustainable and eco-friendly poly (Lactic acid)/cellulose nanocrystal nanocomposite films for the preservation of oxygen-sensitive food

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A B S T R A C T

The development of enzymatic browning constitutes one of the key issues in the preservation of highly perishable fresh-cut and oxygen-sensitive fruits such as avocados. This study explored the use of a packaging solution based on PLA/cellulose nanocrystal (CNC) films produced using a solvent-free cast extrusion process with strong oxygen and UV barrier properties to reduce browning in cut avocados and to prolong shelf life. Specifically, the effects of packaging material types (neat PLA vs PLA/CNC nanocomposite) and storage conditions (temperature and time) on discoloration (browning), microbial growth, and shelf life were assessed. Enzymatic browning was visually evaluated and total discoloration (ΔE^*) quantified as a function of storage time and temperature to estimate the shelf life of the avocados. Compared with neat PLA, nanocomposite packaging reduced the enzymatic browning more and resulted in additional shelf life extension of 1.3 days at 23 °C and 5.4 days at 4 °C. Both PLA-based films reduced the microbial growth. The potential of sustainable and eco-friendly nanocomposite film to prolong the shelf life of food that is oxygen sensitive is clearly demonstrated.

1. Introduction

The development of enzymatic browning constitutes one of the major issues for the preservation of highly perishable fresh-cut and oxygen-sensitive fruits such as avocados (Soliva-Fortuny et al., 2002). This brown discoloration process is catalyzed by polyphenol oxidase (PPO). 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 colored compounds that give a browning appearance on the flesh (Soliva-Fortuny et al., 2002; Pinheiro et al., 2009; Quevedo et al., 2011). The appearance of organoleptic defects such as off-odors or discoloration on fresh-cut fruits and vegetables are easily detectable by the consumer and have been identified as important attributes to monitor their shelf life (Villa-Rodríguez et al., 2011; Corradini, 2018; Velderrain-Rodríguez et al., 2021). Once 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., 2011a, 2011b) and results in shelf life of 1 day if stored at room temperature but 3-4 days if refrigerated at 4 °C (Mercy, 2021; StillTasty LLC, 2021). Since appearance is the first attribute that can lead consumers to decline the product (Corradini, 2018), the ability of packages to preserve the appearance of fresh-cut avocado is highly desirable.

Various protection techniques have been studied to control browning of avocado's flesh. Methods such as the use of PPO inhibitors (Gómez-López 2002; Bustos et al., 2015) and anti-browning agents (Soliva-Fortuny et al., 2002), packaging with modified atmosphere (Gerdes & 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; Velderrain-Rodríguez et al., 2021; Ramos-Villarroel et al., 2011a, 2011b, 2014) have been found to offer some protection against browning in avocados.

However, the method that employs packaging solution based on materials with strong barrier capacities to prevent browning in avocado has not been extensively studied, although materials with strong oxygen and/or UV barrier capacity could also protect packaged foods such as fresh-cut fruits by reducing oxidative photodegradation in the package, thus delaying enzymatic browning on the food (Vilela et al., 2018; Zhang et al., 2021).

The application of bio-based plastics with strong barrier capacities for food packaging has received attention in the scientific community (Stark & Matuana, 2021). Specifically, there have been numerous

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studies investigating the barrier, mechanical, and other properties of PLA/CNC films (Vilarinho et al., 2018; Karkhanis et al., 2018a, 2018b; Silva et al., 2020; Fotie et al., 2020). But there are limited studies that examined the use of these PLA/CNC films to prolong the shelf life of packaged food. Studies have demonstrated strong antimicrobial capacity against *Listeria monocytogenes* in mixed vegetables and meat packaged with bioactive PLA/CNC films containing nisin and oregano antimicrobial agents, respectively (Salmieri et al., 2014a, 2014b). However, the impact of oxygen permeability of these bioactive nanocomposite films was not determined. Eco-friendly PLA/CNC films have also been found effective in retarding the lipid oxidation of the salami at short (15 days) and long storage times (60 days), due to their improved oxygen and water barrier properties (Vilarinho et al., 2021). Recent investigation by Karkhanis and coworkers also proved the ability of poly(lactic acid) (PLA) film filled with 1% cellulose nanocrystals (CNCs) to extend the shelf life of dry foods that are moisture sensitive (e.g., crackers) (Karkhanis et al., 2021). Regardless of the storage relative humidity (61–99%), crackers packaged in the PLA/CNC nanocomposite film had a longer shelf life than counterparts packaged in unfilled PLA film due to the higher moisture barrier of the nanocomposite film compared to the PLA film (Matuana et al., 2016; Karkhanis et al., 2018a, 2018b). PLA/CNC nanocomposite films are sustainable and eco-friendly materials derived from renewable natural resources. Concerns on the toxicity (cytotoxicity, genotoxicity, ecotoxicity, etc.) and migration of CNCs from the nanocomposite films due to their small dimensions have also been investigated. Numerous *in vitro* and *in vivo* studies showed no toxicity in food up to 4% (Ede et al., 2020; Ong et al., 2020; Fotie et al., 2020; Pradhan et al., 2020). Additionally, the migration levels of CNCs from the films were found below the normative limits in polar and nonpolar food simulants (Silva et al., 2020; Fortunati et al., 2012), clearly indicating the food contact appropriateness for the films containing CNCs.

It should also be pointed out from our previous study that the incorporation of 1% CNC into blown PLA film not only decreased its oxygen permeability by approximately 62–75%, but also improved its UV barrier significantly, suggesting that CNC-based nanocomposite films could be desirable in reducing oxidative photodegradation in the package; thus, reducing brown discoloration of food during storage (Karkhanis et al., 2018a). Despite the better oxygen barrier and stronger UV barrier properties of PLA/CNC nanocomposite films than neat PLA film, as well as no toxicity in food up to 4%, to our knowledge, no study has previously investigated the potential of these nanocomposite films on the shelf life of foods that are prone to oxygen deterioration. Consequently, this exploratory study assessed the suitability of PLA/CNC nanocomposite films to reduce browning and prolong the shelf life of fresh-cut avocados.

2. Materials and methods

2.1. Materials

As an oxygen-sensitive product, Mexico 'Hass' avocados, distributed by Mission Produce (Oxnard, CA), were bought from a grocery store in Lansing, Michigan. The avocados were selected as a sizing grade of 40, extra-large size with a total weight of 9.5 - 11.5 oz. Semi-crystalline poly(lactic acid) (PLA), Ingeo biopolymer 4044D grade (D-lactide content < 4%) from NatureWorks LLC (Minnetonka, MN) was used as plastic matrix (Liu & Matuana, 2019). Freeze-dried cellulose nanocrystals (CNCs) (Lot # 2015 - FPL - CNC - 071) with length and diameter of around 100–300 nm and around 5 nm, respectively, were produced and acquired from the U.S. Forest Service (Madison, WI) (Moon et al., 2013).

2.2. Manufacture of PLA and PLA/CNC nanocomposite films

PLA and CNC were oven-dried for at least a day at 60 °C before processing. Then PLA and 2 wt% of CNC were mixed and compounded for about 1 minute in a high intensity mixer. As previously described (Liu & Matuana, 2019), the cast film processing was performed in a conical counter-rotating twin-screw extruder at 30 rpm. Films were produced through a 3.4 mm die gap of a slit die using the processing temperature profile of 180 °C (hopper)- 180 °C- 175 °C- 155 °C (die). Chill rollers were installed at a distance of $\frac{3}{4}$ inch apart from the slit die to stretch the film (thickness of 60 μm), which was then collected by a winder. Neat PLA film was manufactured using similar process conditions.

2.3. Property evaluation of films

Optical property of the films was evaluated using Perkin-Elmer Lambda 25 ultraviolet-visible (UV-Vis) spectrophotometer (Perkin-Elmer Instruments, Beaconsfield, UK). Films' transmission spectra were collected in triplicates over the 200 to 900 nm wavelength range using 240 nm/min scan rate. The average values of UV light (250 to 380 nm) and visible light (380 to 720 nm) of each spectrum were estimated using the procedure given by Karkhanis et al. (2018a, 2018b).

The water vapor transmission rate (WVTR) of at least ten films was measured gravimetrically using the desiccant method (ASTM E 96) at 38 °C and 85% relative humidity (RH). The oxygen transmission rate (OTR) of at least four samples was measured using Mocon Ox-Tran (Model 2/21) permeability tester at 23 °C and 0% RH (ASTM D3985). Water vapor and oxygen permeability coefficients were then calculated from WVTR and OTR data, respectively, as previously described (Natarajan et al., 2022; Karkhanis et al., 2018a, 2018b; Matuana et al., 2016).

2.4. 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 is cut, the discoloration on the flesh occurs slower than a fresh-cut ripe avocado (Villa-Rodríguez et al., 2011; Cox et al., 2004). 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, which are correlated as the avocado ripens (the firmness decreases as the color parameters decrease) (Villa-Rodríguez et al., 2011). This study focused on the ripeness stage that makes the fruits ready to consume. According to Villa-Rodríguez study, the fruit firmness and skin color have to remain below stage 4 to be considered as ready to consume (Table 1). The chroma meter (Model CR-400 from Konica Minolta, Tokyo, Japan) was employed to quantify the L*, a*, and b* color parameters in the CIE LAB color scale. In this scale L* (lightness) is indexed as L = 0 (black) to L = 100 (white). The chromaticity coordinates are from -a* (green) to +a* (red) and from -b* (blue) to +b* (yellow). The data collected as an average of five measurements taken around the fruit equator, were used to calculate the H* (Hue) and C* (Chroma) using the following equations (López-Malo et al., 1998; Soliva et al., 2000; Villa-Rodríguez et al., 2011):

$$H^* = \arctan\left(\frac{b^*}{a^*}\right) \tag{1}$$

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

2.5. Preparation of avocados

Table 1 shows the tracking of avocado skin color collected before cutting. The avocado fruits were purchased with similar skin color and stored at room temperature (23 ± 1 °C) until they were ready to be cut. A chroma meter was used to track the fruits' skin color. Once the avocado skin color reached stage 4, the fruits were stored for an additional 5 days before cutting and packaging.

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

Table 1
The ripeness stage based on avocado skin color.

Ripeness stage based on avocado skin color	Color parameters[a] 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

[a] Fifteen replicates were tested.

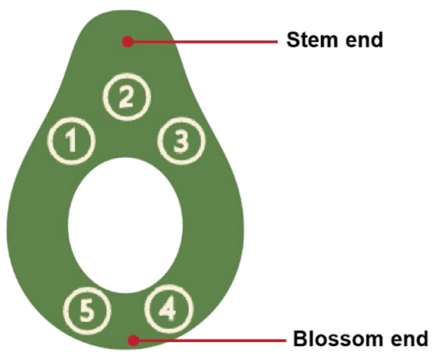

Fig. 1. Five specific locations on the surface of fresh-cut avocado that the color measurements were taken.

2.6. Packaging and storage of fresh-cut avocado

PLA and PLA/CNC packaging bags were manufactured by cutting the films into 12 by 7 inches sheets, folding in half, and sealing two sides of the films 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. Unpackaged fresh-cut avocados as well as those packaged in PLA and nanocomposite bags were stored at room temperature (23 °C) and chilled temperature (4 °C) and periodically evaluated for discoloration and microbial growth.

2.7. Visual appearances and color quantification

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. The samples stored at 23 °C and 4 °C were evaluated for 3 days and 10 days of storage time, respectively. The data was recorded as the average readings of L*, a*, and b* taken at five specific locations on the surface of avocado (Fig. 1).

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

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (3)$$

where $\Delta L^* = L^*_{sample} - L^*_{standard}$; $\Delta a^* = a^*_{sample} - a^*_{standard}$; and $\Delta b^* = b^*_{sample} - b^*_{standard}$, with the subscripts "sample" and "standard" representing the values at a specific storage time t and at time t = 0 day, respectively.

2.8. Microbial counts

The total levels of microorganisms on unpackaged (control) and packaged fresh-cut avocados stored at room temperature for 2 days and cold temperature for 7.5 days were evaluated, which correspond to approximately their shelf lives at these storage conditions. Avocados were prepared as mentioned in Section 2.4. Portions of avocados (25 g) 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 minutes using a Seward Stomacher 400 Circulator Lab Blender (Seward Laboratory System Inc., USA). Homogenized samples were plated onto plate count agar (PCA) to measure total aerobic microbes and potato dextrose agar (PDA) to measure yeasts and molds. The PCA plates were incubated at 37 °C overnight whereas the PDA plates were incubated at 37 °C for 48 h before the colony count. The limit of detection was 10 CFU/g for all avocado samples. 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.

2.9. 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 analysis of variance with $p \leq 0.05$.

3. Results and discussion

3.1. Film properties

The influence of CNC addition on water vapor and oxygen barrier of PLA films is summarized in Table 2. Water vapor and oxygen transmission rates of PLA films as well their corresponding permeability coefficients decreased considerably by 48% and 51%, respectively, by incorporating 2% CNC into the films. This enhanced barrier performance is due to the tortuosity effect caused by the existence of highly crystalline CNCs in nanocomposite films that acted as impermeable particles in the matrix (Natarajan et al., 2022; Karkhanis et al., 2018a, 2018b, Liu & Matuana, 2019). Other research groups have also reported decreases in WVP and OP after adding CNCs to PLA. In one study adding 5% CNCs to PLA resulted in a 5% decrease in WVP at 25 °C and 60% RH and 43% decrease in OP at 23 °C and 9% RH (Fortunati et al., 2012). Differences in impact on barrier properties with the data listed in Table 2 can be due to different processing conditions, PLA properties, or testing conditions. CNCs can improve barrier properties in a variety of polymeric matrices due to the tortuosity effect. For example, the WVP of neat poly(caprolactone) (PCL) was reported to be 174.8×10^{-16} kg·m/m²·s·Pa at 25 °C and 60% RH while the OP of neat PCL was 142.9×10^{-20} kg·m/m²·s·Pa at 23 °C and 0% RH. At these testing conditions, adding 5% CNCs to poly(caprolactone) (PCL) decreased the WVP by 19% and the OP by 16% (Khan et al., 2013). Considerable improvement in oxygen barrier property of sugarcane-based LLDPE using CNCs has also been reported recently by Natarajan and coworkers who obtained a significant decline of about 50% in oxygen permeability

Table 2
Optical and barrier properties of PLA and PLA/2%CNC nanocomposite films.

Materials	Optical properties[a]		Barrier properties[a]			
	% UV light(250-380 nm)	% Visible light(380-700 nm)	WVTR[b](10^{-8} kg/m^2·s)	WVP[b](10^{-16} kg·m/m^2·s·Pa)	OTR[c]cm^3/(m^2·d)	OP[c](10^{-20} kg·m/m^2·s·Pa)
PLA	90.0 ± 0.6^A	91.5 ± 0.8^A	217.1 ± 8.1^A	201.8 ± 2.9^A	515.9 ± 79.4^A	353.6 ± 54.4^A
PLA+2%CNC	74.8 ± 4.8^B	77.5 ± 4.0^B	113.9 ± 10.5^B	105.2 ± 9.7^B	254.9 ± 37.6^B	178.8 ± 26.4^B

[a] Different superscript letters within the same column are significantly different ($p \leq 0.05$).
[b] WVTR and WVP were determined at 38 °C and 85% RH.
[c] OTR and OP were determined at 23 °C and 0% RH.

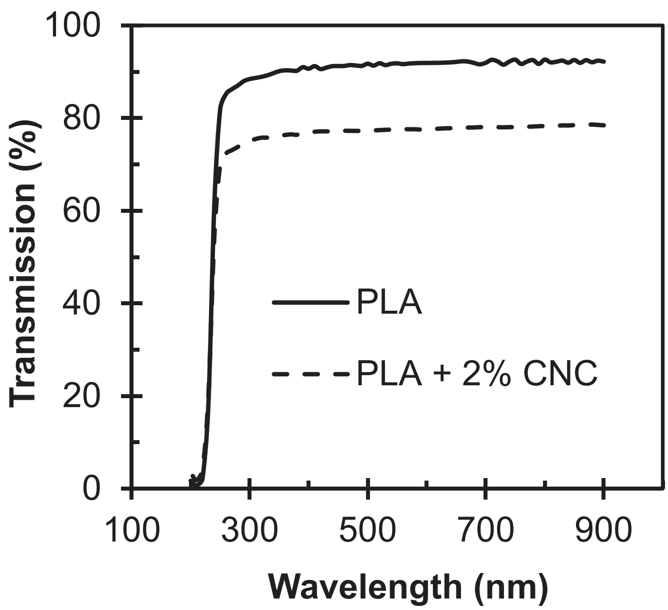

Fig. 2. Ultraviolet-visible (UV-Vis) spectra of PLA and PLA/2%CNC nanocomposite films.

coefficient (from 31.2 to 16.0 × 10^{-17} kg·m/m^2·s·Pa) by adding 2.5 wt% of CNCs into the bio-based LLDPE films (Natarajan et al., 2022).

Improved barrier performance was achieved at the expense of film transparency since the transmission of visible light in PLA film (380–720 nm) (Fig. 2) decreased significantly from 91.5% to 77.5% with the addition of 2% CNCs (Table 2). This deleterious effect is due to the presence of CNC nanoparticles that scatter light in nanocomposite films (Karkhanis et al., 2018a, 2018b). Interestingly, the decreased transparency of PLA film caused by CNCs addition did not impact its optical clarity (Fig. 3). Furthermore, the addition of CNCs into PLA was beneficial in reducing the UV light transmission (250–380 nm) (Fig. 2) from 90% to 74.8% (Table 2). These results imply that CNCs obstruct UV light and their addition into PLA results in nanocomposite films that are desirable for packaging applications needing UV light protection.

3.2. Discoloration evolution

Fresh-cut avocados are highly perishable food products and 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 their shelf life (Corradini, 2018). Since appearance is the first attribute that can lead consumers to decline the product (Corradini, 2018), this study selected discoloration as the appearance quality attribute to signal the shelf life of fresh-cut avocados.

3.2.1. Visual appearance

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 3 days (Fig. 4) and at refrigerated temperature for 10 days (Fig. 5).

The flesh of the fresh-cut avocados was bright yellow/green in color and unblemished at the beginning of storage. However, discoloration occurred during storage and the yellow-greenish color became darker, irrespective of storage conditions and packaging material type. Several investigators have attributed this darkening to the enzymatic browning caused by polyphenol oxidase (PPO) activity (Soliva-Fortuny et al., 2002; Pinheiro et al., 2009; Quevedo et al., 2011). 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 (Soliva-Fortuny et al., 2002; Pinheiro et al., 2009; Quevedo et al., 2011).

The visual assessment showed that the unpackaged fresh-cut avocados deteriorated very rapidly and the browning on unpackaged avocados was more severe at any specific storage time than for packaged ones, irrespective of storage conditions and material type (Figs. 4 and 5). For room temperature-stored products (Fig. 4), the extent of enzymatic browning developed on the flesh of the unpackaged avocado stored for 24 h was comparable to enzymatic browning on the flesh after 48 h when packaged in neat PLA and for longer than 48 h when packaged in PLA/CNC nanocomposites. Cold storage conditions (Fig. 5) showed similar trends but browning on the flesh of packaged avocados required longer storage periods. In this case, browning that was apparent after 96 h of storage for unpackaged avocado was similar to browning at 240 h or longer when packaged in PLA or PLA/CNC composites (Fig. 5). Packaging had a strong effect on reducing browning as packaged avocado darkened less compared to the unpackaged fruit. While packaged 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 (Figs. 4 and 5), implying the protective ability of PLA-based films to prolong the shelf life of avocado, owing to the moderate and high oxygen barrier of PLA and nanocomposite films, respectively (Table 2).

As the browning reaction is oxygen dependent, the extent of enzymatic browning depended on the packaging 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 (Figs. 4 and 5). Since the nanocomposite film has better oxygen barrier property than neat PLA film (Table 2), it has more potential to reduce the exposure of the avocado flesh to oxygen in the package; 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 packaging materials (Figs. 4 and 5), suggesting that cold storage slows the browning reaction on the avocado flesh due to the decreased PPO activity at cold temperatures. This result agrees with those of other investigators who also reported a significant

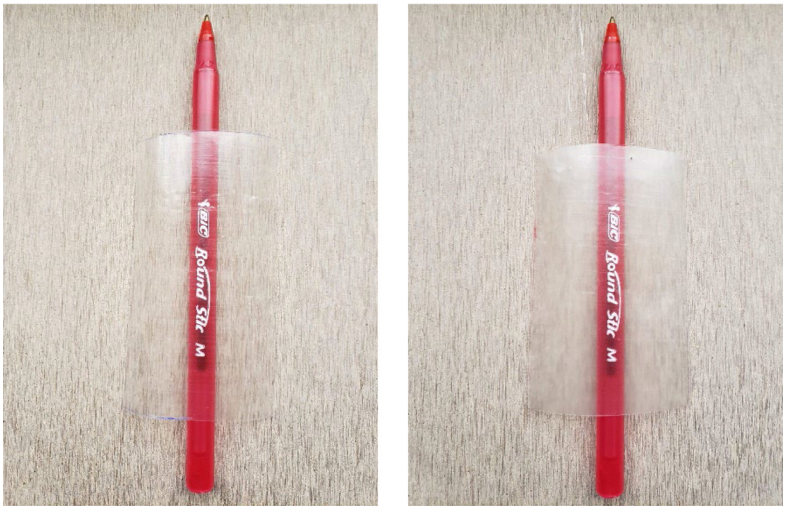

Fig. 3. Visual clarity of neat PLA film (left) and PLA/2%CNC nanocomposites film (right).

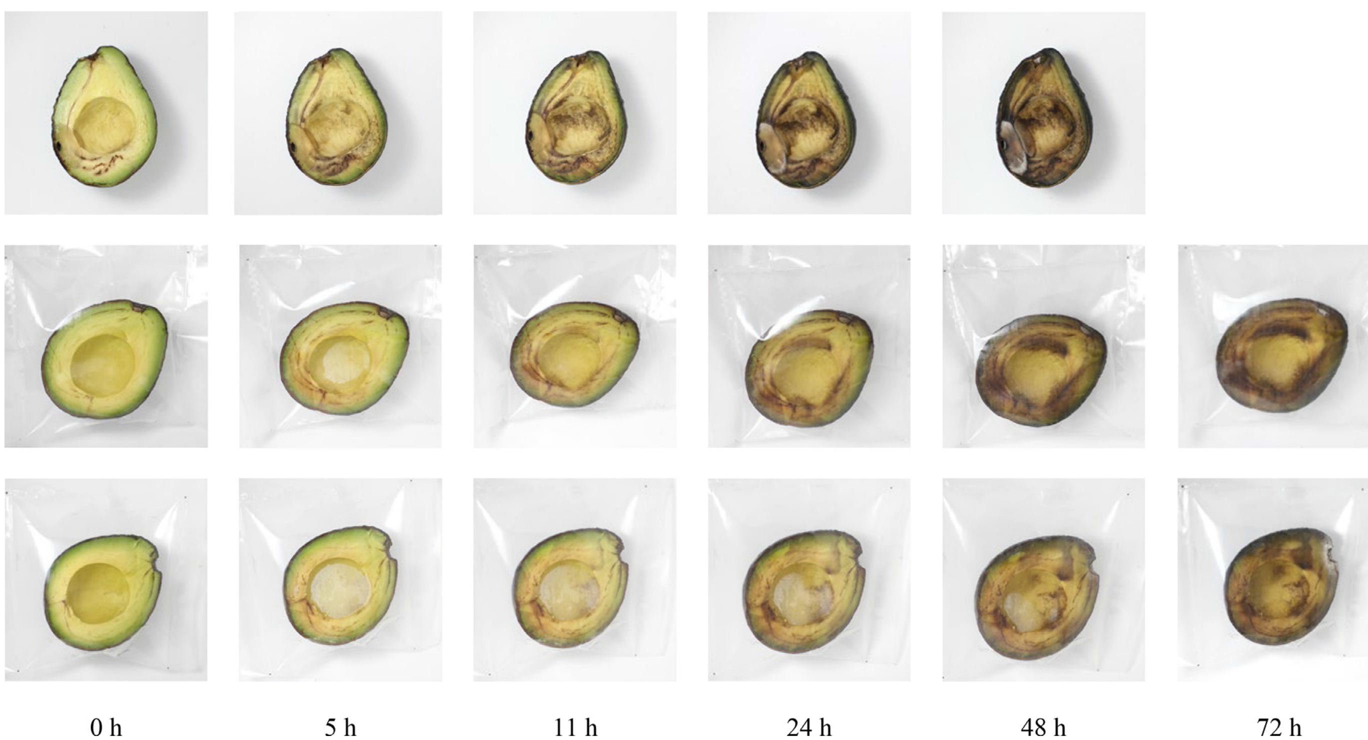

Fig. 4. Visual appearances of unpackaged fresh-cut avocados (top row) and avocados packaged with neat PLA film (middle row) and PLA/CNC nanocomposite film (bottom row) stored at room temperature for various time periods.

decrease in PPO activity throughout the storage temperature at 4 °C (Soliva-Fortuny et al., 2002).

3.2.2. *Color evaluation*

Visual observations of stored avocados showed an uneven browning on the surface of avocado's flesh, which darkened the fastest at the blossom end and the slowest at the stem end of the avocado (Figs. 4 and 5). 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 as described in Section 2.7.

Because the addition of CNC slightly affected the transparency of PLA film (Fig. 2 and Table 2), it was crucial to determine whether the presence of CNC in the film altered the measured color of the avocado flesh. This was verified by measuring the color of a white calibration plate through the films as a standard background (Table 3). The L*, a*, and b* color parameters of both films measured on white calibration plate were similar to those of the white calibration plate itself, verifying no impact of the film transparency on the measured color. The color of unpackaged and the packaged fresh-cut avocados were then measured (Table 3). Once again, the values of the three color parameters L, a*, and b* of packaged avocados measured through the films were comparable to those of unpackaged avocado ($p \leq 0.05$), validating the color measurements for packaged avocado.

Fig. 6 illustrates the variation in lightness ΔL* as well as the chromaticity coordinates Δa* and Δb* of packaged fresh-cut avocados as a function of storage times. The lightness ΔL* and Δb* of packaged avocados decreased considerably with storage time whereas Δa* increased significantly and then levelled off after approximately 1 day and 4 days storage at 23 °C and 4 °C, respectively. These results indicate that all

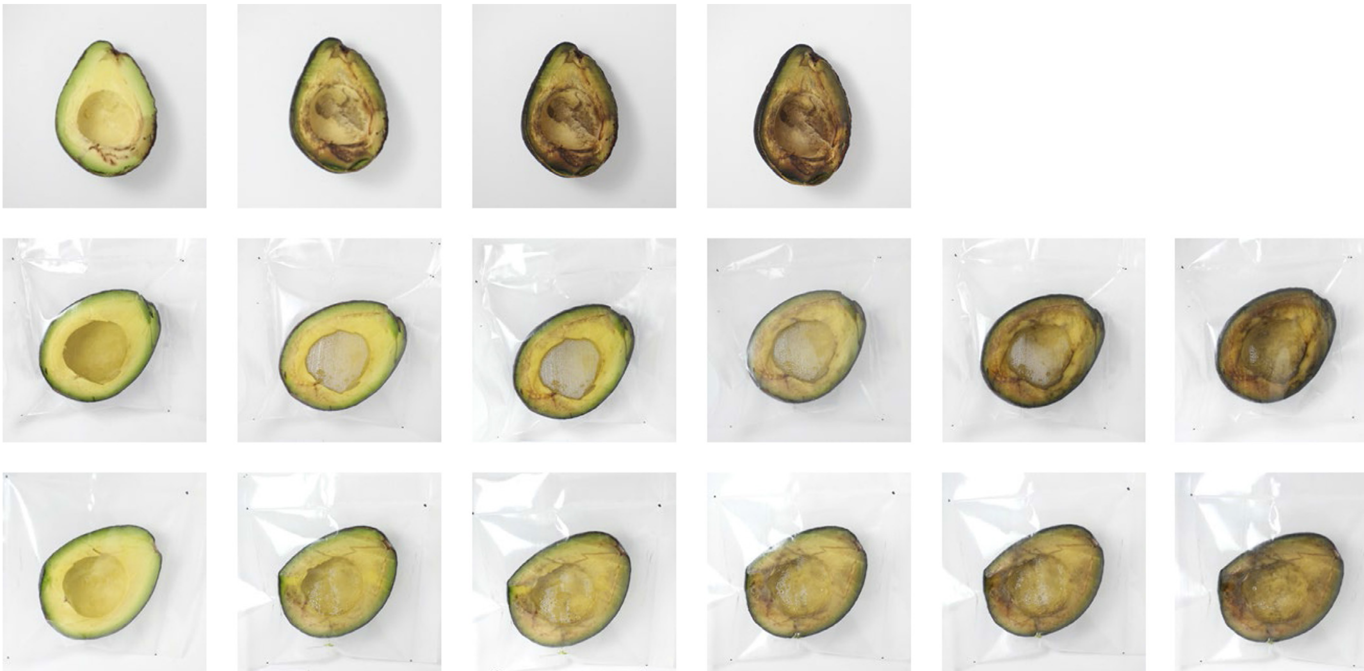

Fig. 5. Visual appearances of unpackaged fresh-cut avocados (top row) and avocados packaged with neat PLA film (middle row) and PLA/CNC nanocomposite film (bottom row) stored at cold temperature for various time periods.

Table 3
Color parameters of PLA and PLA/CNC films measured on white calibration plate of a colorimeter and fresh-cut avocado as background.

Materials	Color parameters[a]		
	L*	a*	b*
Calibration plate			
White calibration plate (WCP)	96.8 ± 0.3^A	0.0 ± 0.1^A	2.0 ± 0.3^A
PLA film on WCP	95.8 ± 0.6^A	0.0 ± 0.1^A	2.3 ± 0.3^A
PLA/2% CNC film on WCP	95.7 ± 0.4^A	0.0 ± 0.1^A	2.5 ± 0.3^A
Fresh-cut avocados			
Unpackaged	67.5 ± 3.6^A	-4.0 ± 1.5^A	25.9 ± 1.4^A
Packaged in PLA film	69.9 ± 2.9^A	-4.8 ± 1.8^A	26.1 ± 1.0^A
Packaged in PLA/2% CNC film	69.1 ± 3.4^A	-5.0 ± 1.5^A	25.3 ± 1.1^A

[a] Similar superscript letters within the same column indicates no significant differences ($p \leq 0.05$). Average and standard deviation measured on 11 and 8 replicates for WCP and avocado background, respectively.

stored avocados darkened regardless of packaging materials and storage conditions as evidenced by the reduced lightness (-ΔL*) and changes in color coordinates, which varied from green to red (+Δa*) and yellow to blue (-Δb*). The darkening effect obtained from color data supports the visual appearance shown in Figs. 4 and 5 and is attributed to the oxidative enzymatic browning.

Although all color parameters (L* a* b*) were good darkening indicators, it should also be noted that ΔL* and Δb* were more effective distinguishing the protective capability of different packaging materials to reduce browning. The values of ΔL* and Δb* clearly show that avocados in nanocomposite package exhibited lighter surfaces than those packaged in neat PLA film after 0.5 day and 2-4 days storage at 23 °C and 4 °C, respectively (Fig. 4 and Fig. 5). This was expected because nanocomposite film provided more oxygen barrier, which decreased oxygen availability in the package, thus limiting to a certain extent the catalytic action of polyphenol oxidases present in the avocado. In contrast, the evolution of Δa* values showed no significant differences between nanocomposite and neat PLA films, suggesting that Δa* was not an effective color parameter to reveal significant difference in the darkening of avocado packaged in two different packaging materials. Therefore, the total color difference (ΔE*) was selected in this study to monitor discoloration on avocado flesh. Since ΔE* is a single value that considers all three color parameters (ΔL*, Δa*, and Δb*), it was used as a standard scale for comparison of discoloration on packaged avocado where a higher value of ΔE* indicating more browning on the flesh.

Fig. 7 shows the effect of packaging material types on total discoloration (ΔE*) of fresh-cut avocado during storage at 23 °C and 4 °C. It is obvious that the discoloration of fresh-cut avocados increased with the increased storage time for both packaging materials and storage temperatures, indicative of increased level of browning on the avocado flesh.

Packaged avocados discolored at similar rates irrespective of packaging type at times less than 0.5 day at 23°C and 2 days at 4 °C. This was expected because the oxygen barrier capacities of both materials were adequate to reduce the ingress of oxygen in the package at such short storage times. Nevertheless, above these storage times, avocado packaged in neat PLA film discolored more than the counterparts packaged in PLA/CNC nanocomposite film, regardless of storage temperature (Fig. 7), indicating the nanocomposite film reduced discoloration when compared to neat PLA film. As the packaging atmosphere significantly influences the PPO activity depletion (Soliva-Fortuny et al., 2002), the

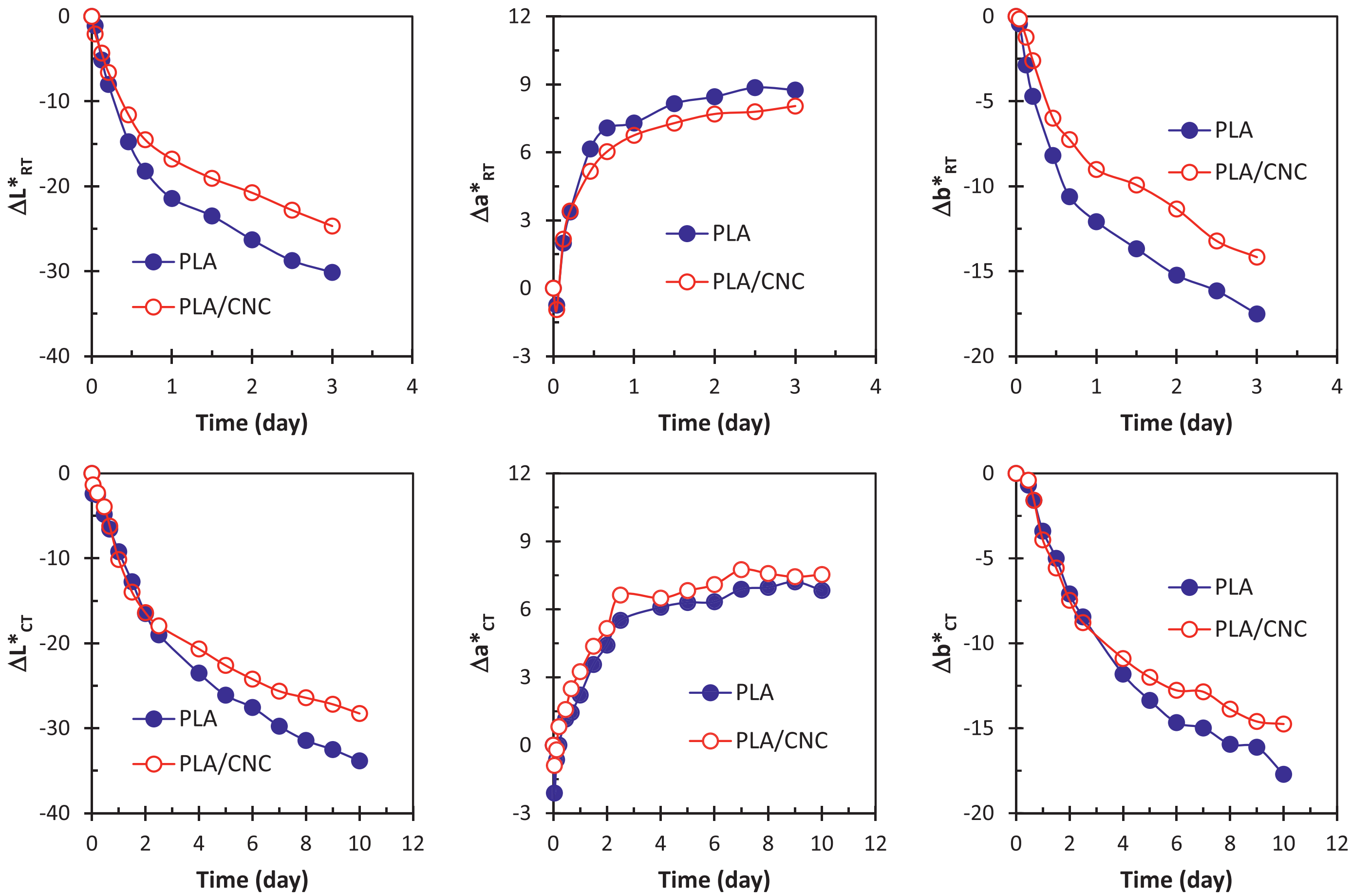

Fig. 6. Lightness ΔL^* as well as the chromaticity coordinates Δa^* and Δb^* of fresh-cut avocado packaged in PLA and PLA/CNC nanocomposite bags as a function of storage time at room temperature (top row) and chilled temperature (bottom row).

higher oxygen barrier of PLA/CNC film effectively prevented packaged product from exposure to oxygen, which then reduced PPO activity and the subsequent discoloration of avocado. Additionally, refrigerated avocados discolored less than counterparts stored in room temperature, irrespective of packaging material due to the decreased PPO activity at cold temperature (Soliva-Fortuny et al., 2002; Pinheiro et al., 2009). Longer storage times were needed for refrigerated products to reach, for example, a discoloration value of $\Delta E^* = 30$ when compared to room temperature stored avocados (Fig. 7), implying that cold storage further reduces the discoloration of packaged avocados. These results corroborate those obtained from visual appearance (Figs. 4 and 5).

3.2.3. Shelf life estimation

The shelf life of unpackaged fresh-cut avocado is 1 day at room temperature but 3-4 days at 4 °C (Mercy, 2021; StillTasty LLC, 2021). A large part of shelf life of highly perishable foods such as fresh-cut avocados is attributed to discoloration, which is easily detected by the consumer and can cause rejection of the product (Corradini, 2018).

From this information, the discoloration (ΔE^*) of unpackaged avocado (control) stored for 1 day at room temperature (ΔE^*_{RT}) and chilled for 4 days at 4 °C (ΔE^*_{CT}) were measured and their corresponding values of $\Delta E^*_{RT} = 33.0 \pm 3.6$ and $\Delta E^*_{CT} = 37.4 \pm 2.4$ were used as discoloration acceptability limits for the estimation of the shelf life of packaged avocado. Using these limits as reference, the shelf life of packaged avocado, defined as the time needed for the discoloration of packaged avocado to reach the acceptability limit (dotted lines in Fig. 7) was then predicted by solving the linear regression (Y=ax+b) of the last portion of the discoloration (ΔE^*) versus storage time plots by substituting the variable y with (ΔE^*_{RT}) or (ΔE^*_{CT}) in the regression equation and solving for the value of x, i.e., the storage time.

Packaging avocado with PLA-based materials extended its shelf life in both room and cold temperature storages ($p < 0.05$) (Table 4). When compared with neat PLA, PLA/CNC nanocomposite package extended the shelf life of fresh-cut avocado by an additional 1.3 days at 23 °C and 5.4 days at 4 °C due to the higher oxygen barrier of nanocomposite film, which reduced discoloration.

3.4. Microbial count

Both the plate count agar (PCA), a non-selective bacteriological substrate used for determination of the total number of aerobic microorganisms (fungi, mold, bacteria, etc.) in a sample, and the potato dextrose agar (PDA), a more selective medium for growing yeasts and molds, were employed to estimate the levels of microorganisms on the unpackaged and packaged fresh-cut avocados (Table 5). The microbial counts (CFU/g) on the unpackaged fresh-cut avocado were 20.0 in both PCA and PDA media, clearly indicating that molds are the type of microorganisms mostly growing on the avocado. The growth of microbes was not affected by the packaging material's type, irrespective of their storage conditions. However, cold storage significantly reduced mold growth compared to room temperature storage, insensitive to material types. Indeed, no evidence of mold growth was observed during cold storage as indicated by the negative values of corrected microbe counts. This was expected since the enzyme and microbe activities decrease by lowering storage temperature (Soliva-Fortuny et al., 2002). It should also be pointed out that the levels of mesophilic microorganism counts

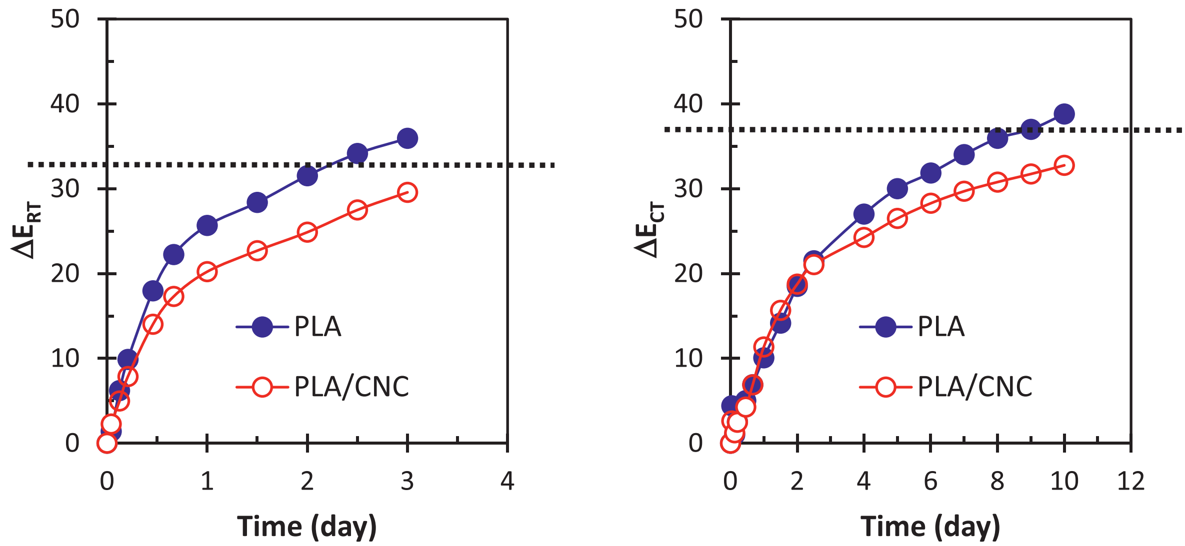

Fig. 7. Discoloration (ΔE*) of fresh-cut avocado packaged in PLA and PLA/CNC nanocomposite bags as a function of storage time at room temperature (left) and chilled temperature (right). The dotted lines are the acceptability limits for the shelf life of packaged avocados.

Table 4
Predicted shelf life of packaged avocado stored at room and cold temperatures.

Storage temperatures	Predicted shelf life (days) Unpackaged avocado[a]	Avocado packaged in PLA[b]	Avocado packaged in PLA/2%CNC[b]
23°C	1.0	2.1 ± 0.5	3.4 ± 0.6
4°C	4.0	8.4 ± 1.4	13.8 ± 1.8

[a] The shelf life values for unpackaged avocado are from the literature (Mercy, 2021; StillTasty LLC, 2021).
[b] Averages and standard errors of four (23 °C) and five (4 °C) replicates.

Table 5
Corrected microbial counts on packaged fresh-cut avocados stored at room temperature for 2 days (RT_2) and cold temperature for 7.5 days ($CT_{7.5}$).

Package material types	Microbial counts (CFU/g)[a] Plate count agar[b]		Potato dextrose agar[b]	
	RT_2	$CT_{7.5}$	RT_2	$CT_{7.5}$
PLA	105.0 ± 66.1[A]	-10.0 ± 0.0[B]	80.0 ± 0.0[A]	-8.6 ± 3.5[B]
PLA/CNC	92.5 ± 33.1[A]	-10.0 ± 0.0[B]	117.5 ± 69.6[A]	-10.0 ± 0.0[B]

[a] The microbial counts (CFU/g) on the unpackaged fresh-cut avocado were 20.0 ± 14.1 for both PCA and PD tests.
[b] 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.

above 10^6 CFU/g related to the end of shelf life of perishable products (Corradini, 2018) were not reached by the packaged fresh-cut avocados, irrespective of storage conditions, indicating the suitability of PLA-based films to package oxygen-sensitive foods.

4. Conclusions

Fresh-cut avocado packed in PLA/CNC nanocomposite film exhibited less browning than the counterparts packaged in neat PLA film due to the higher oxygen barrier of nanocomposite film, which effectively reduced the ingress of oxygen in the package. Refrigerated products discolored less than room temperature-stored counterparts for both packaging materials because of the decreased oxidation activity at cold temperature. Compared with neat PLA, packaging with nanocomposite film resulted in additional shelf life extension of 1.3 days at 23 °C and 5.4 days at 4 °C. Interestingly, none of the packaged fresh-cut avocados reached the minimum level of mesophilic microorganism counts of 10^6 CFU/g associated with the end of shelf life of perishable products, irrespective of storage conditions.

The experimental results of this exploratory study have shown that packaging based on PLA/CNC films with high oxygen and UV barrier is an effective method to reduce discoloration in fresh-cut avocado, reduce microbial growth, and prolong its shelf life. Discoloration was selected as a determining parameter based on its importance to the avocado shelf life. However, discoloration is not the sole appearance quality attribute to signal the shelf life of fresh-cut avocados packaged in nanocomposite film. Other attributes that affect the shelf life of highly perishable foods such as product firmness, loss of sensory attributes, the appearance of off-odors, etc. will be evaluated in future studies to gain a better understanding of the suitability of the nanocomposite films for food shelf life extension.

This study clearly demonstrates that PLA/CNC nanocomposite films with strong oxygen and UV barrier capacity have enormous potential for food packaging applications. Particularly, they could protect packaged foods such as fresh-cut fruits by reducing the oxidative photodegradation in the package due to their high oxygen barrier, thus inhibiting the discoloration of the product.

Ethical statement - studies in humans and animals

This exploratory study does not involve human or animal testing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Data Availability

Data will be made available on request.

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