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Development of Biodegradable Films Produced from Residues of Nixtamalization of Popcorn

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Abstract: Nejayote and pericarp derived from nixtamalization are an environmental problem. Therefore, there is research interest in using these residues as new compostable and environmentally friendly materials. This work aimed to create, characterize (color, thickness, water solubility, water adsorption capacity, microstructure, and degradability), and apply biodegradable films using residues of nejayote and pericarp of nixtamalized popcorn. Three types of films were compared, pericarp (P), nejayote–pericarp (NP), and nejayote (N), and were applied to avocado stored at room temperature. Results showed that the P film was the best because it was significantly ($p < 0.05$) more transparent (L = 94.75 ± 2.21) and thicker (0.27 ± 0.02 mm). It had higher water adsorption capacity (162.60 ± 14.05%) and higher elastic modulus and toughness (0.004 ± 0.001 MPa, 2.25 ± 0.25 J/m^3) values than the N and NP films; in addition, its microstructure showed a roughness value (Ra) of 6.59 ± 0.57 nm that was intermediate compared to other films. Moreover, the P coating slowed down the maturing process of avocado and showed a visual effect against fungal infection. All films were generally soft and had a degradation time between 11 and 12 days. The novelty of this study is to provide the alternative of using nejayote and pericarp in a film that is compostable and maintains the lifespan of avocados.

Keywords: nixtamalization; nejayote; pericarp; film; avocado; lifespan

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1. Introduction

Over the course of time, the industrialization of nixtamalized products has increased. Nixtamalization is an alkaline cooking of the corn grain process; in addition to that, nejayote is the residual water obtained through this procedure [1]. Later, nejayote is discarded with residues of the pericarp. Salmeron-Alcocer et al. [2] reported that a production of 600 tons of corn per day generates between 1500 and 2000 m^3 of nejayote; accordingly, over 50 million m^3 of it is thrown out a year [3] at drainage sites or rivers. Furthermore, nejayote is wastewater that contains a large load of solids and suspended and dissolved organic matter (COD > 10 200 mg/L and BOD > 2.69 mg O_2/L) [4], which causes the clogging of sewers and drainage, as well as a high content of calcium salts and a pH above 11 that affects the corrosion of the pipes. Consequently, this waste deteriorates the environment

after being dumped into public sewers [1]; therefore, it is considered a global industrial, economic, and environmental problem, which is why many researchers have found a way to use the nejayote and pericarp.

In other respects, nejayote and pericarp contain arabinoxylans, sugars, fibers, phenolic compounds, and calcium, which have the potential for value-added processing and utilization [5]. It was found that they have different uses, such as a culture medium for bacterial growth and for the production of bacteriocins [4], for animal feedstock [6], for the recovery of components with high added technological value (gelling capacity), and for use as bioactive compounds through their incorporation in food products and coatings [7,8]. Additionally, nejayote is a source of microorganisms, which shows its capability to generate high-value molecules from agro-industrial by-products [9]. Because of the technological properties of the components of nejayote and pericarp, it is possible to incorporate and study them on coatings.

In recent years, biodegradable coatings have been used as a means to preserve the quality attributes and extend the postharvest life of fruits [10,11]; they preserve the organoleptic properties of the fruit without affecting it and without the need to keep it under refrigeration to extend its lifetime. A biodegradable coating is based on the concept of packaging materials, since these can prevent food spoilage by reducing the interaction between the food and the surrounding environment, thereby reducing food loss [11]. Currently, biodegradable coatings have also been studied that allow for a simpler and easier way to preserve the quality and prolong the life of the fruits without changing the temperature [10,11]. The coatings have been formulated with various biopolymers, such as pectin [12], cellulose [13], bacterial cellulose [14], and other elements such as beeswax [10,11] nanocomposites [15], and rice starch [16]. Castañeda-Ruelas et al. [8] studied a phenolic extract from nejayote flour applied on alginate-based edible coatings in order to evaluate its antimicrobial activity, and they found that the alginate-based coating with 2.3% nejayote phenolic extract showed total and low growth inhibition for Gram (+) and Gram (−) bacteria, respectively. Additionaly, Yoshida et al. [17] extracted hemicelluloses from corn pericarp and formed a transparent film with mechanical strength and plasticity. Hence, it was demostrated that nejayote and pericarp could be alternatives for the development of biodegradable edible coatings, improving the quality and safety of food; however, more studies about their application on fruits are required.

On the other hand, the avocado (*Persea americana* Mill.) is a highly perishable tropical fruit of culinary relevance in the world, which completes its postharvest ripening process from five to seven days at 25 °C [18]. It has high nutritional value as it is rich in essential oil and bioactive compounds; it also allows protection from oxidative damage and improves the immune system [19]. Its consumption has increased in the last ten years by 15% per year [20]. Therefore, postharvest technologies to maintain and prolong the useful life of this fruit must be found. Hereupon some authors have explored various techniques for this purpose, such as microwave drying, freeze-drying, high-pressure processing [19], and packaging materials evaluation [21]. Conventional plastics (polyethylene (PE), low density polyethylene (LDPE), polystyrene (PS), and polypropylene (PP)) are commercialy used and studied [22], and although these plastics increase the shelf life of avocado, its degradablity is higher. Consequently, the use of biodegradable films on avocado was studied, and these films were created using polymers such as gelatin, starch [23], andelilla wax, pectin, aloe mucilage, and purified polyphenols [24]. However, it is necessary to evaluate more materials in order to provide more conservation alternatives.

Therefore, the objective of this study was to use nejayote and pericarp for the production of biodegradable films. Moreover, our aim was to assess the impact of applying these films on the physicochemical properties of avocados, thereby enhancing their preservation and extending their postharvest life.

2. Materials and Methods

2.1. Elaboration and Characterization of Films

2.1.1. Film Elaboration

The films were made per the method published by Velickova et al. [11] and Ruzaina et al. [10] with some modifications. Beforehand, the pH of nejayote was adjusted to 5 with HCl 1N, and then it was dried in a convection oven (UN 110, Memmert, Bietigheim-Bissingen, Germany) at 40 °C. Three different films were made, where the concentrations of the components of the beeswax (Farmacia Paris, Mexico City, Mexico) 1.5%, glycerin (La Corona, México state, Mexico) 2%, and starch potato (Sigma Aldrich, Steinheim, Germany) 4% were kept constant, while the nejayote and pericarp contents were varied. The formulations are presented in Table 1. All ingredients were mixed into a water bath at 70 °C for 15 min with constant stirring, and subsequently, it was allowed to cool at 22 °C. The avocados were covered by immersion and left to dry at room temperature at 22 °C.

Table 1. Characteristics of the treatments.

Treatments	Beeswax (g/100 g)	Glycerin (g/100 g)	Potato Starch (g/100 g)	Nejayote (g/100 g)	Pericarp (g/100 g)
P	1.5	2	4	0	0.5
NP	1.5	2	4	0.25	0.25
N	1.5	2	4	0.5	0

P: pericarp, NP: nejayote and pericarp, N: nejayote.

2.1.2. Color of Films

The methodology used was reported by Valdespino-León et al. [12]. The parameters "L*", "a*", and "b*" were obtained from the color space CIELab. The parameter of color was measured by a colorimeter (Chroma Meter CR-400, Konica Minolta, Tokyo, Japan), and six films of each treatment studied were taken.

2.1.3. Moisture and Thickness

The thickness of films was determined by a micrometer. Ten measurements were taken for each film from three different independent samples [25]. The sample's moisture was determined according to the AOAC 942.05 methodology and made using a thermobalance (MB45, Ohaus, Parsippany, NJ, USA).

2.1.4. Water Solubility (WS)

This value was calculated according to the method described by Hernández-Varela et al. [26], using dried film samples (2 cm × 2 cm) (w_i), which were prepared by oven drying the films until a constant weight was reached (105 °C, 24 h). The dried film samples were then submerged in 50 mL of distilled water and stored for 24 h at 25 °C. After this, they were filtered through constant weight filter paper, and the undissolved matter was dried (105 °C for 24 h) and weighed (w_f). Results were reported as % water solubility, using the following formula:

$$\%WS = \left(\frac{w_i - w_f}{w_i}\right) \times 100 \quad (1)$$

where w_i is the initial is weight and w_f is the final weight of each film.

2.1.5. Water Adsorption Capacity (WA)

Each film's water adsorption capacity (WA) was calculated according to the method described by Hernández-Varela et al. [26]. Film samples of 20 × 20 mm were weighed (m_i) and were submerged in 20 mL of distilled water for 30 min at 25 °C. Then, the films were

removed and wiped with tissue paper. Finally, the samples were weighed (m_f). Results were reported as % water adsorption capacity, using the following formula:

$$\%WA = \left(\frac{m_i - m_f}{m_i}\right) \times 100 \quad (2)$$

where m_i is the initial is weight and m_f is the final weight of each film.

2.1.6. Tensile Properties

A texture analyzer (Brookfield CT3, Ametek, Inc., Berwyn, PA, USA) was used to estimate the film tensile properties by a stress–strain test with a set of double T-96 clamps. The test conditions were a 4500 g load cell with an activation load of 450 g, at a speed of 0.3 mm/s, and a return speed of 4.5 mm/s [12,26]. The films were cut in a rectangle with the measurements at 80 × 25 mm. The following formula determined the tensile strength of films (TS):

$$TS = \frac{F}{W * x} \quad (3)$$

where F is the maximum force load (N), x is the thickness (mm), and W is the film width (mm).

Furthermore, with the data obtained from the test, the stress versus strain curves were constructed, plotting the deformation percentage versus the tensile strength up to the breaking point (TS). The elastic modulus (E) and the absorbed energy or toughness were determined by the methodology published by Valdespino-León et al. [12].

2.1.7. Fourier-Transform Infrared Spectroscopy (FT-IR)

The analysis of the secondary structure of the proteins was carried out by FT-IR spectroscopy (Agilent Cary model 630, Santa Clara, CA, USA). A small amount of each sample was placed on the attenuated total reflectance (ATR) diamond crystal of the analyzer. The samples were pressed against the diamond crystal using the attached pressure clamp with a slip clutch press on the clamp that prevents over-tightening. It was operated in the 1000 to 1800 cm^{-1} wavenumber ranges and 64 scans at 4 cm^{-1} of resolution at room temperature (25 °C). For evaluation, a necessary baseline and smoothing correction was performed on the spectrum using OriginPro 8 software (v8.0724, OriginLab Corporation, Northampton, MA, USA). Finally, the deconvolution method was used to evaluate the areas of the regions of interest [27].

2.1.8. Imaging with Atomic Force Microscopy (AFM)

Roughness (Ra) and the films' overall aspect were evaluated using an atomic force microscope (Bruker, Bioscope Catalyts ScanAsyst, Santa Barbara, CA, USA). The films were placed and a silicon cantilever (DNP-10A) of spring constant 0.540 Nm^{-1} was used and a resonant frequency of 1 kHz. Eight images of each film studied and scanned at 2 × 2 μm^2 were captured in ScanAsyst mode in atmospheric conditions. The arithmetic average roughness (Ra) was measured using NanoScope software (NanosScope 1.4, Bruker, Santa Barbara, CA, USA).

2.1.9. Scanning Electron Microscopy (SEM)

Each film was coated with gold using a sputter coater (SPI supplies, West Chester, PA, USA) and observed with a scanning electron microscope (Hitachi, SU3500 I, Santa Clara, CA, USA) at 10.0 kV [27].

2.1.10. Biodegradability Test

The films' biodegradability was determined in accordance with the methodology reported by Hernández-Varela et al. [26] and Valdespino et al. [12] with some modifications. The films were collocated at 25 cm depth with a 1 m^2 surface. The films were dug up at 2, 4,

6, 8, and 10 days. They were collocated in a paper bag and dried in a forced-air stove for 24 h at 45 °C. The weight lost each day was determined by the following formula:

$$\% \text{ weight lost} = \frac{m_i - m_f}{m_i} \times 100 \quad (4)$$

m_i = initial dried weight of the films before the test
m_f = final dried weight of the films

2.2. Physicochemical Properties of Avocado

2.2.1. Avocado Conditioning

The avocados (*Persea americana* Mill.) were bought at wholesale markets in Toluca, Mexico (19.3434° N, 99.5986° W); these were selected without blemishes, external defects, and irregular shapes; therefore, they had a similar size, shape, color (green), and stage of maturity (unripe). All avocados were cleaned with chlorinated water (2%) before they were coated with biodegradable film. The sample coating was made by a brush at a lower temperature to avoid damage to the fruit's peel. Then the coated avocados were dried at room temperature (22 ± 2 °C) for 30 min. Subsequently, the fruits were stored at room temperature (22 ± 2 °C) in a dark room. A total of 120 avocados were evaluated for 25 days.

2.2.2. Color Analysis

The color of the peel and mesocarp of the avocados was measured by a colorimeter (Chroma Meter CR-400, Konica Minolta, Tokyo, Japan); five avocados from each studied film and control were evaluated according to the methodology used by Valdespino-León et al. [12]. The color space CIELab used the parameters "L*," "a*", and "b*".

2.2.3. Weight Loss

The coated avocados and control were weighed on day zero. Then, each week ten fruits were taken and evaluated and were studied for five weeks during the storage period. Weight loss was calculated by:

$$\text{Weight loss} = \frac{w_0 \times 100}{w_1} \quad (5)$$

where w_0 = weight measured on day zero and w_1 = weight final.

2.2.4. Avocado Firmness

The avocado firmness was measured by means of a Texturometer (Brookfield CT3, Ametek, Inc., Berwyn, PA, USA), using a cylindrical probe of 6 mm diameter and a load of 450 g. The penetration speed was set at 30 mm/min until reaching a maximum penetration depth of 9 mm [28]. Three avocados for each coating and control were examined, then three slices of 2 cm thickness were obtained from the perimetral zone of the mesocarp from them, and the evaluation was performed on three different points at a distance of 2 cm from each other in each slice.

2.2.5. Total Soluble Solids (TSS) and Titratable Acidity (TA)

Total soluble solids (TSS) were measured using a refractometer (Atago, Atago Co., Ltd., Tokyo, Japan) and they were expressed as Brix degree. The TSS were determined by the methodology of Rojas-Candelas et al. [28] with a few modifications. Titratable acidity (TA) was determined by titrating with 0.1 N NaOH until obtained at pH 8.2. TA was expressed by the following formula:

$$TA = \frac{V_{NaOH} \times N \times meq \times Vt}{Pm \times Va} \times 100 \quad (6)$$

where V_{NaOH}: Volume of sodium hydroxide spent in the titration, N: Sodium hydroxide normality, meq: Milliequivalents of tartaric acid 0.075, Vt: Final volume, Pm: Sample weight, and Va: Aliquot volume.

2.2.6. Total and Reducing Sugars

The total and reducing sugars were determined according to Rojas-Candelas et al. [27] with some modifications. The total sugar content was determined by adding 1 mL of the sample (0.05% *w*/*v*) with 0.6 mL of phenol (5%) and with 2.6 mL of H_2SO_4; the solution was agitated and rested for 30 min. At the end of this period, it was collocated in cold water for 15 min, and then it was read by a spectrometer (UV-1800, Shimadzu, Kyoto, Japan) at a wavelength of 490 nm.

The reducing sugars content was determined by adding 1 mL of the sample (0.05% *w*/*v*) with the 3.5 dinitro salicylic acid (DNS); it was placed in a water bath for 15 min. At the end of this period, it was collocated in cold water for 15 min, and finally, it was read by a spectrometer (UV-1800, Shimadzu, Kyoto, Japan) at 540 nm.

2.3. Statistical Analysis

All analysis was performed at least in triplicate. The measurements were expressed as average values ± SD. Data were compared using the ANOVA–Tukey test, significant differences were considered when $p < 0.05$, and the mathematical model of biodegradability was determined from the films using XLSTAT software (v. 2020.1.3, Addinsoft, Boston, MA, USA).

3. Results and Discussion

3.1. Characterization of Films

3.1.1. Color

First of all, the color of the films (Table 2) is an important quality characteristic of the product for the consumer, which is why it was determined. It can be seen that the luminosity values (L*) of the nejayote film (N) and the nejayote and pericarp film (NP) were significantly lower ($p < 0.05$) than the pericarp film (P) value, it being the last and the most transparent [29]. Valdespino-León et al. [12] (coffee mucilage films, 94.75 ± 2.21) and Gaona-Sánchez et al. [29] (pectin films, 96.65 ± 0.16) reported similar P film values. À propos of parameter a* (Table 2), the N and NP films had a red tendency (positive values) while the P film had a green tendency (negative values). Regarding parameter b*, all the films (P, NP, and N) tended to have yellow values (positive values), but the value of the NP film was significantly higher ($p < 0.05$) than the N and P film values. Therefore, the P film was the most transparent of all and it had some green and yellow tendencies.

Table 2. Color of the films.

Film	L*	a*	b*
P	94.75 ± 2.21^{b}	-1.96 ± 0.16^{a}	$29.4 \pm 1.13^{a,b}$
NP	85.26 ± 2.09^{a}	1.90 ± 0.10^{b}	31.85 ± 1.67^{b}
N	86.39 ± 1.25^{a}	5.68 ± 0.40^{c}	26.71 ± 2.18^{a}

P: pericarp, NP: nejayote and pericarp, N: nejayote. Results expressed as mean value ± standard deviation. Same letters in the same column indicate that values are not significantly different ($p < 0.05$).

3.1.2. Moisture (M) and Thickness (T)

Table 3 shows the moisture (M) of the films. This parameter did not show a significant difference between the P (10.97 ± 0.79%), NP (12.18 ± 0.87%), and N (11.77 ± 0.12%) film values. Zolfi et al. [30] and Li et al. [31] reported higher values of this parameter (14.28% to 34.35% and 32.40% to 36.44%, respectively) for films based on kefiran exopolysaccharide, and of the TiO_2/whey protein isolate nanocomposite film, separately. This difference was due to the components of the films, the way of preparation, and the amount of used solution. Regarding the thickness (T) of the films (Table 3), the P film value was significantly

higher ($p < 0.05$) than the NP and N film values. Abral et al. [14] reported higher T values (0.83 mm) in films composed of nanofiber cellulose because of their application as food packaging. Hence, the P film was better because its T value was bigger.

Table 3. Parameters of the films.

Film	M (g/100 g)	T (mm)	WS (g/100 g)	WA (g/100 g)	Tensile Strength (MPa)	Elastic Modulus (MPa)	Toughness (J/m^3)
P	10.97 ± 0.79^{a}	0.27 ± 0.02^{b}	22.62 ± 1.78^{a}	162.60 ± 14.05^{c}	0.21 ± 0.001^{b}	0.004 ± 0.001^{a}	2.25 ± 0.25^{a}
NP	12.18 ± 0.87^{a}	0.21 ± 0.01^{a}	23.92 ± 0.77^{a}	136.36 ± 8.87^{b}	0.36 ± 0.08^{c}	0.003 ± 0.001^{a}	2.15 ± 0.33^{a}
N	11.77 ± 0.12^{a}	0.20 ± 0.01^{a}	39.23 ± 2.66^{b}	105.39 ± 1.89^{a}	0.05 ± 0.01^{a}	0.003 ± 0.001^{a}	1.50 ± 0.01^{b}

P: pericarp, NP: nejayote and pericarp, N: nejayote, T: thickness, M: moisture; WS: water solubility; WA: water adsorption capacity; Results expressed as mean value ± standard deviation. Same letters in the same column indicate that values are not significantly different ($p < 0.05$).

3.1.3. Water Solubility (WS)

The water solubility (WS) of the films (Table 3) was affected by the formula, where the N film value was remarkably higher ($p < 0.05$) than the values of the other studied films; the lower values of the P and NP films were probably due to the pericarp presence because it is a semipermeable barrier and contains more insoluble protein than soluble protein [27]. Moreover, these results could be related to the lower values of moisture [30]. Additionally, these data tallied with the results of Colussi et al. [16], who reported values of 18.14% to 20.13% in rice starch films. Insoluble film is useful for food with higher values of moisture because it retains its moisture and increases its lifespan [32], and even more films with pericarp are suitable for this purpose.

3.1.4. Water Adsorption Capacity (WA)

The water adsorption capacity (WA), meanwhile, is an important parameter in packaging food. The results for WA (Table 3) showed that there were notable differences between the values of the studied films ($p < 0.05$). The P film had the highest value of WA (162.60 ± 14.05), probably owing to the hydrophilic sites of the fibers of the pericarp. Similar results were obtained by Abdullah and Dong [15] (WA, 170%) and Hernández-Varela et al. [13] (168.69%), who worked with poly(vinyl) alcohol–starch films and garlic skin–gellan gum-starch films, respectively; these authors indicated that higher values of WA are related to the free sites of the film component that will be occupied by water molecules.

3.1.5. Tensile Properties

Likewise, the mechanical properties of the films are presented in Table 3 and in Figure 1. Figure 1 shows the stress–strain curves, where the P film had significantly smaller tensile strength (TS) values ($p < 0.05$) and a higher deformation percentage (11%) than the N and NP films. Moreover, the P film had a higher elastic modulus value and toughness (Table 3); at the same time, it had a better ability to resist plastic deformation. These differences could be attributed to its thickness and its WA values (Table 3). According to the results of the mechanical properties of films (P, NP, and N), all of them behaved like soft films compared with other reported films [11,12,33] because of their lower TS, E, and toughness values.

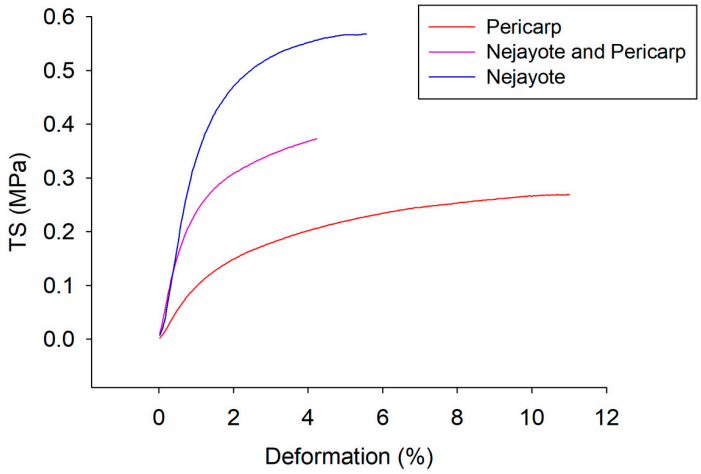


Figure 1. Tensile strength (TS) vs. deformation of films of pericarp, nejayote and pericarp, and nejayote.

3.1.6. Fourier-Transform Infrared Spectroscopy (FT-IR)

During the FT-IR analysis, the presence of the components of the films and their interaction were evaluated. Figure 2 shows the results of the P, NP, and N films. Noticeable peaks around 3299, 2920, and 1000 cm^{-1} were detected. The first peak is related to the presence of the hydroxyl group, which could correspond to water and phenolic compounds [34]. The peak at 2920 cm^{-1} represents methyl (C–H) bonds; it could also be associated with the beeswax presence, which is composed by an alcohol long chain and esters fatty acids. The last peak, measuring around at 1000 cm^{-1}, corresponds to the C-O stretch, which was observed in starch [35]. In addition, another smaller peak was observed around 1691 and 1665 cm^{-1}, which corresponds to the ferulic acid [34]. With respect to Figure 3, it shows the behavior of the amide II region, where just five peaks were obtained through deconvolution. Similar values in the secondary structures of the β-sheet array of the proteins in the films (58.71 to 68.08%) were observed, followed by random coil values ranging from 23.26% to 25.90%, as well as values of α-helix between 12.89% and 15.78%. These results were compared with those reported by Rojas-Candelas et al. [27], who studied only the pericarp. Their results did not show random coil values, but they obtained high values in the α-helix (42.10%), while for twist and β-sheet the values were 21.56% and 36.33% each. These differences were due to the proteins of the pericarp in the film that were probably bound by the other components.

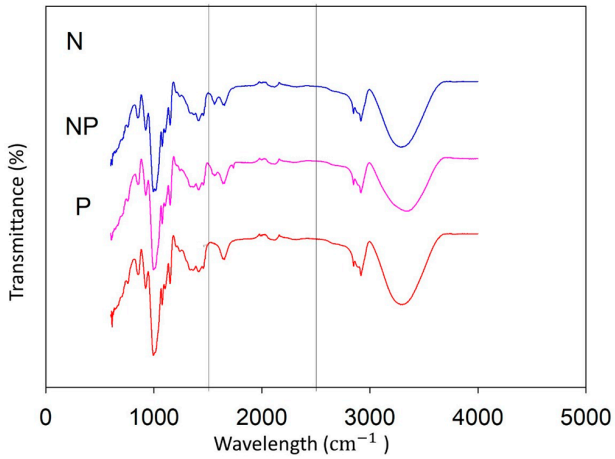
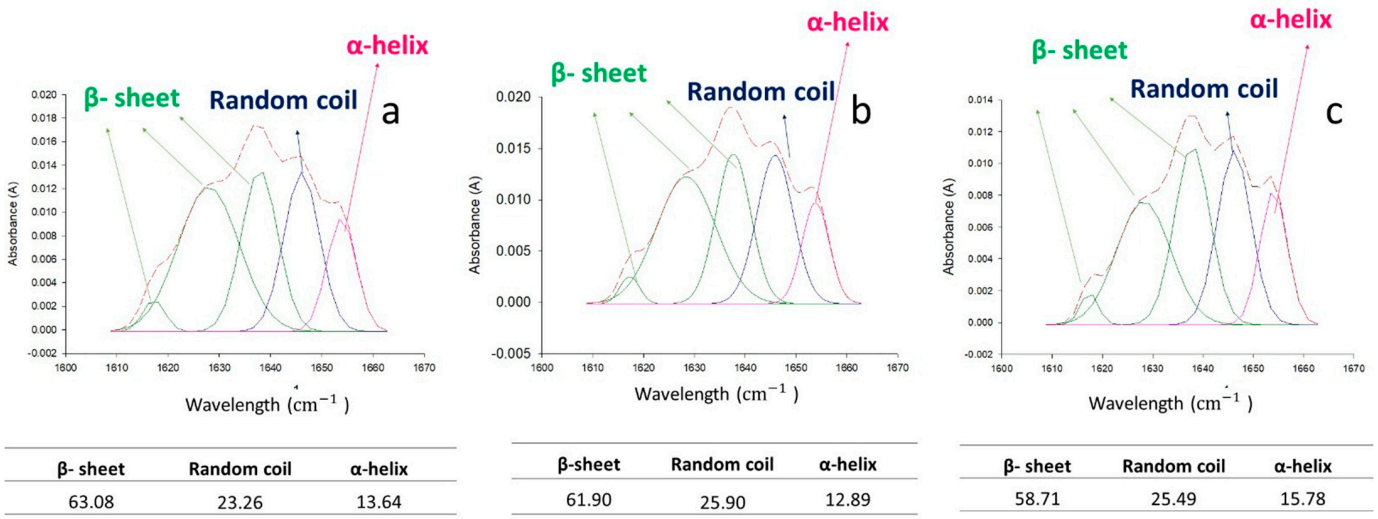


Figure 2. FT-IR spectra of films: (P) pericarp, (NP) nejayote and pericarp, and (N) nejayote.



β- sheet	Random coil	α-helix
63.08	23.26	13.64

β-sheet	Random coil	α-helix
61.90	25.90	12.89

β- sheet	Random coil	α-helix
58.71	25.49	15.78

Figure 3. Changes in FT-IR of films for the amide II band: (**a**) pericarp, (**b**) nejayote and pericarp, and (**c**) nejayote.

3.1.7. Microstructure by Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM)

The microstructure of the films by AFM and SEM are shown in Figures 4 and 5, respectively. Figure 4 shows the 3D height of the studied films and their analysis; the roughness values (Ra) of the films were significantly different ($p < 0.05$). The NP film had the highest Ra value (12.10 ± 0.14 nm) and it had a more heterogeneous surface. On the other hand, the N film showed a lower Ra value (3.74 ± 0.02 nm), whereas the P film had an intermediate Ra value. Furthermore, Figure 5 shows the surface morphology of films by SEM, where the P film has a smooth surface with some irregular particles of the pericarp, the NP film has a more heterogeneous surface with some agglomerations and grooves due to the nejayote and pericarp presence. Lastly, the NP film has heterogeneous surface with small agglomerates. It seems that the P film has a better quality microstructure because of its smooth surface and its intermediate Ra value.

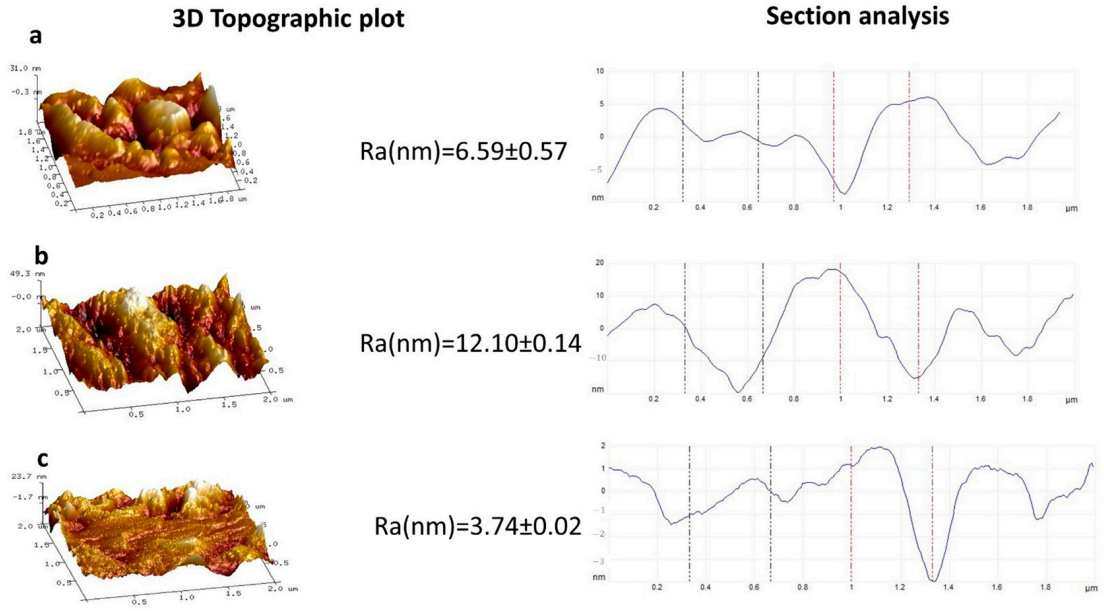


Figure 4. AFM 3D topography images (scale bar 2 µm) and section analysis of films: (**a**) pericarp, (**b**) nejayote and pericarp, and (**c**) nejayote.



Figure 5. Scanning electron microscopy images (SEM) of films: (P) pericarp, (NP) nejayote and pericarp, and (N) nejayote.

3.1.8. Biodegradability of Films

On the other hand, Figures 6 and 7 show the film biodegradability analysis; the images of Figure 6 were taken after 10 days of experimentation in real composting conditions. The study revealed that the biodegradability process began on day 2 (25.58% to 34.68%) (Figure 7), where the N film had the highest value of biodegradability; moreover, the films became white because of the oxidation of the phenolic compounds of the nejayote and pericarp [27]. On day 3, the films became soft, which caused a significant material rupture; the highest values of biodegradability were from the P and N films (53.81 ± 1.11%), while the P film kept its value of 25.23%. On days 5, 6, and 9, the process increased. On day 10, all films had similar biodegradability values between 84.65% and 86.17%. During this period, the extraction of the samples was difficult since the pieces were very small. As a result, we proposed the use of a mathematical model allowing us to predict the day when the biodegradability process of all of the films would finish. The results were the following: for the P film, 10.6 days; for the NP films, 11.05 days; and for the N films, 11.71 days. The value of R^2 was 0.97, which indicated that the model was reliable. As shown below, the next stage of this study was the application of the films on the avocado peel during the postharvest storage. This fruit is highly perishable and completes its ripening process from five to seven days at 25 °C [18]. According to the results of the films' biodegradability, it is possible that the coatings extended the lifetime of avocados one more week, which means a doubling of time; furthermore, the biodegradability test was conducted in wet soil, in the presence of microorganisms, and if the films were subjected to environmental changes in temperature and humidity, this time could be even higher.

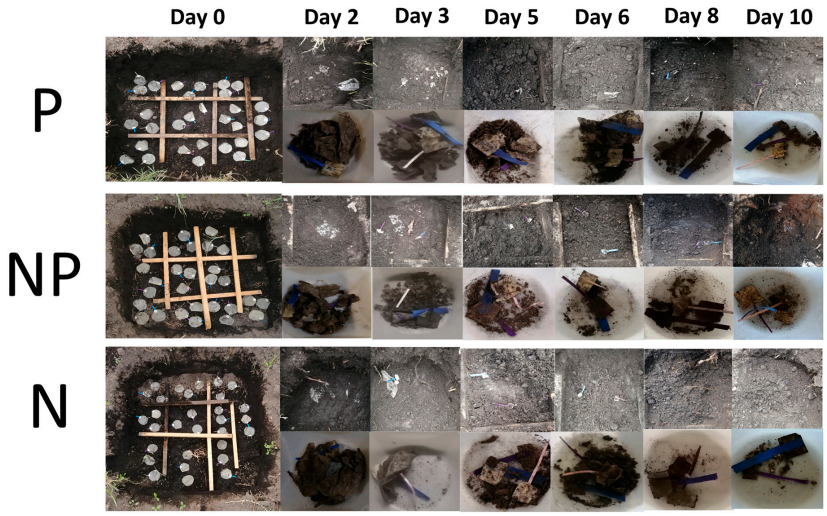


Figure 6. Images of films during biodegradability evaluation at different times (0, 2, 4, 6, 8, 10 days): P: pericarp, NP: nejayote and pericarp, and N: Nejayote.

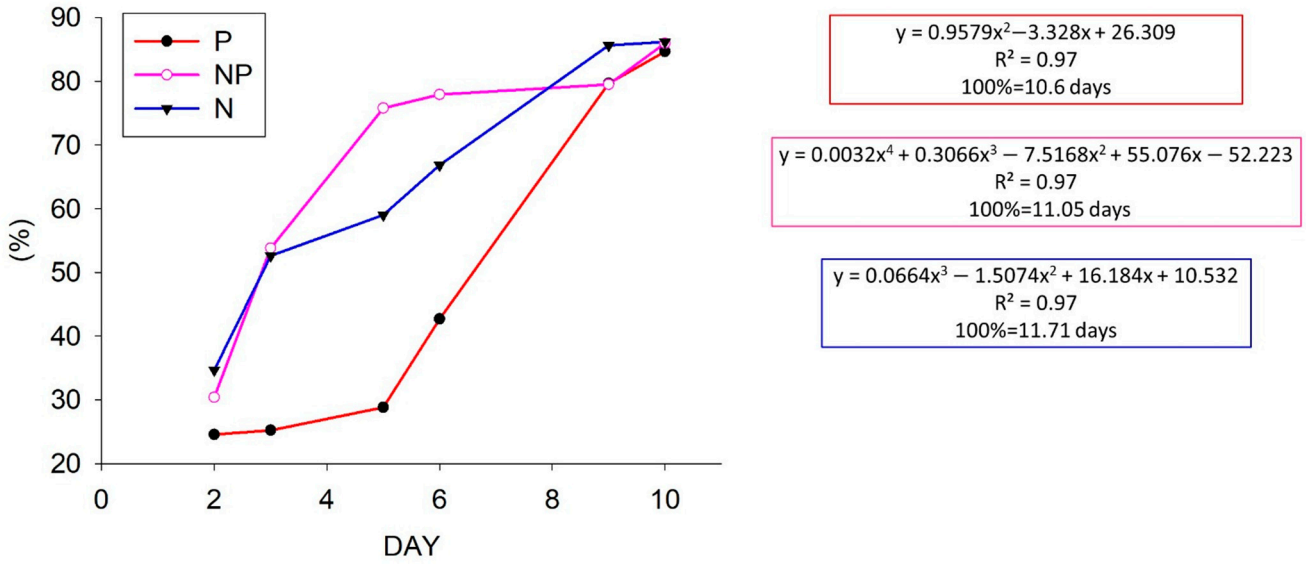


Figure 7. Kinetics of biodegradability of films and their model to predict the day of 100% of degradability. P: pericarp, NP: nejayote and pericarp, and N: nejayote.

3.2. Physicochemical Characterization of Avocado

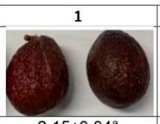
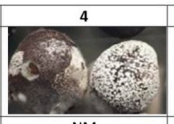
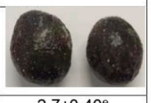
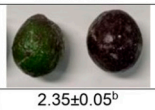
3.2.1. Color Analysis

Figure 8 shows the change in the color of the avocado peels during their storage. The L* value of the control (C) and coated avocados decreased significantly ($p < 0.05$) at the first week compared to the initial time (0 weeks). In all cases, a tendency to dark color was shown, but coated samples presented some green areas, which could be associated with the protection of the coating from chlorophyll degradation [36]. When observing the a* value in week 0, the result was negative, which revealed a green tendency since the avocados were unripe. Then, in the first week, it changed to positive values in all samples, showing a red trend. These results coincided with those of Lancaster et al. [37], who reported an initial a* value of −6.0 (green skin) and thereafter a value of 2.0 (purple skin). In addition, the b* value decreased significantly ($p < 0.05$) from week 0 to the first week; this tendency was stated by Lancaster et al. [37]. After the initial phase of ripening, the color change was associated with the synthesis of cyanidin 3-O-glucoside, so the anthocyanin and carotenoid content increased in the fruit as well as chlorophyll degradation [37,38].

From the second to the fourth week of storage, the C, NP, and N avocados presented some dents and remarkable microbiological deterioration without noticeable changes in the L*, a* and b* parameters; by contrast, the P avocados kept some green areas without an initial fungi growth on their surface until the fifth week. In addition, their L*, a*, and b* parameters did not change significantly, the ripening of P avocados being slower than that of the other samples.

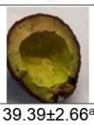
On the other hand, the color of the mesocarp of avocados is shown in Figure 9. In the first week of storage, the L* values of all samples decreased significantly ($p < 0.05$) compared to C in week 0, having a dark tendency; the a* value only increased significantly ($p < 0.05$) in C, manifesting a lower green tonality; these results were due to the protection of the coating. At this time, the yellow tonality (b* values) decreased significantly ($p < 0.05$) in all samples because of enzymatic darkening [39]. From the second to the third week, the changes in color were represented by the reduction in the L* (dark color tendency) of the C, P, and NP avocados, while the value of N held constant. The a* values showed a red tendency, while the b* values presented a reduction in yellow color. On the third week, the C, NP, and N avocados presented more quality deterioration than the P avocado, and on the fourth week, the C, NP, and N avocados presented fungi on the surface of the fruit skin. It is noteworthy that no pictures or measurements were taken during this week; however, it was observed that the P avocados kept the color properties as well as the C

avocados in week 0. Later, in the fifth week, the color of the P avocado was darker, less green and yellow.

Sample	Weeks					
	0	1	2	3	4	5
C						
L*	5.57±0.4^{d}	2.15±0.04^{a}	2.76±0.13^{b}	2.42±0.20^{a}	NM	
a*	−9.29±0.76^{d}	3.18±0.28^{a}	2.28±0.09^{a}	2.41±0.20^{a}	NM	
b*	3.26±0.05^{d}	0.78±0.07^{a}	0.74±0.02^{a}	0.70±0.05^{a}	NM	
P						
L*		2.13±0.03^{a}	2.28±0.16^{a}	2.40±0.11^{a}	2.47±0.19^{e}	2.7±0.40^{e}
a*		3.15±0.07^{a}	2.53±0.17^{a}	2.56±0.15^{a}	2.59±0.21^{e}	2.47±0.21^{e}
b*		0.72±0.05^{a}	0.92±0.06^{b}	0.86±0.07^{b}	0.98±0.21^{e}	0.89±0.03^{e}
NP						
L*		2.35±0.05^{b}	2.18±0.08^{a}	2.36±0.22^{a}	NM	
a*		3.04±0.20^{a}	2.53±0.20^{a}	2.61±0.19^{a}	NM	
b*		1.06±0.08^{b}	0.76±0.07^{a}	0.77±0.05^{ab}	NM	
N						
L*		2.70±0.17^{c}	2.43±0.17^{a}	2.39±0.08^{a}	NM	
a*		2.22±0.22^{b}	2.52±0.17^{a}	2.66±0.23^{a}	NM	
b*		1.72±0.25^{c}	1.10±0.09^{b}	0.77±0.05^{ab}	NM	

Values are the average ± standard deviation. NM: Not measurable. Different letters in the same parameter and column from 1 to 3 week indicate that the values are significantly different ($p < 0.05$). The parameters of 4 and 5 weeks of P avocado were compared to the control at 0 weeks where different letters indicate that the values are significantly different ($p < 0.05$).

Figure 8. Images and color parameters of the peels of coated (P: pericarp, NP: nejayote and pericarp, and N: nejayote) and control (C) avocados stored at room temperature at different sampling times (1, 2, 3, 4 and 5 weeks).

Sample	Weeks					
	0	1	2	3	4	5
C						
L*	55.74±3.59^{e}	33.73±2.74^{a}	39.39±2.66^{a}	32.28±3.46^{a}	NM	NM
a*	−10.23±0.67^{e}	−7.78±0.80^{a}	−12.28±1.06^{a}	9.99±0.16^{a}	NM	NM
b*	65.38±3.00^{e}	44.01±2.10^{ab}	52.73±4.69^{a}	31.73±1.79^{a}	NM	NM
P						
L*		34.71±1.27^{a}	46.87±2.48^{b}	44.53±1.69^{b}	51.85±2.02^{e}	28.78±2.66^{f}
a*		−12.16±0.85^{b}	−8.28±1.21^{b}	−7.33±0.49^{b}	−10.37±0.72^{e}	4.09±0.25^{f}
b*		48.29±1.80^{bc}	65.68±2.99^{b}	41.66±3.17^{b}	67.58±1.42^{e}	36.40±1.66^{f}
NP						
L*		32.57±1.61^{a}	41.36±3.94^{ab}	31.50±1.86^{a}	NM	NM
a*		−11.64±1.14^{be}	−4.36±0.14^{c}	11.73±1.63^{a}	NM	NM
b*		43.42±2.65^{a}	56.92±4.97^{a}	33.85±3.00^{a}	NM	NM
N						
L*		41.38±3.34^{b}	43.00±4.25^{ab}	47.01±3.62^{b}	NM	NM
a*		−10.53±1.41^{be}	2.79±0.14^{d}	−7.12±0.57^{b}	NM	NM
b*		54.23±3.35^{c}	16.78±0.42^{c}	60.98±5.51^{c}	NM	NM

Values are the average ± standard deviation. NM: Not measurable. Different letters in the same parameter and column from 1 to 3 week indicate that the values are significantly different ($p < 0.05$). The parameters of 4 and 5 weeks of P avocado were compared to the control at 0 weeks where different letters indicate that the values are significantly different ($p < 0.05$).

Figure 9. Images and color parameters of the mesocarp of coated (P: pericarp, NP: nejayote and pericarp, and N: nejayote) and control (C) avocados stored at room temperature at different sampling times (1, 2, 3, 4 and 5 weeks).

3.2.2. Weight Loss

The respiration process of the fruit during its ripening led to a weight loss [10]. The fruit takes oxygen then produces carbon dioxide and water vapor, which shrinks the fruit and promotes its weight loss. Therefore, the thickness of the peel is an important parameter to control the weight loss because the thinner the skin is, the faster the fruit loses water, more so than a thicker peel of fruit [11]. The peel of the avocado is normally thicker and irregular, which allows protecting the fruit from the weight loss. Figure 10 shows the results of the weight loss during the storage of the avocados, where all of them exhibited a continuous weight loss over the storage period. The values of this parameter were similar until the second week; however, in the third week of storage, the weight loss of C was significantly ($p < 0.05$) higher (35.26%) than the other coated avocados (around 10–15%). These results were due to the P, NP, and N coating containing beeswax and glycerin that acted as a physical barrier to water loss [10]. In the fourth and the fifth weeks, only the values of the P avocados were obtained because the fungi growth of the other samples prevented their preservation. Regarding the weight loss of coating strawberries, it was studied by Velickova et al. [11], who reported values around 15–20% in seven days. In another study by Ruzaina et al. [10], who covered guavas with films based on palm stearin and palm kernel olein, values of 45.89% were reported in around 14 days. This study demonstrated the better values of the P avocados in the literature because their weight loss was slower.

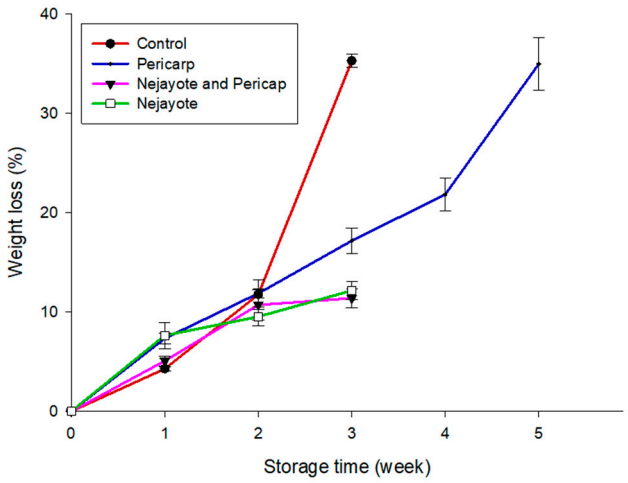


Figure 10. Weight loss of coated (pericarp, nejayote and pericarp, and nejayote) and control avocados stored at room temperature.

3.2.3. Avocado Firmness

It is important to remark that avocado firmness plays an essential role in consumer acceptance. This parameter changes with the ripening, so we observed its behavior during its storage. Figure 11 shows the results of the firmness of the mesocarp of avocados, where this parameter decreased significantly ($p < 0.05$) in all the samples until the second week. In the third week, this reduction was maintained in coated avocados but not in C avocados, whose firmness value increased significantly ($p < 0.05$). The last result was related to the highest weight loss (Figure 10) of the sample. Regarding the coated avocados, the P coating showed the slower firmness loss rate until the third week, which demonstrated the positive effect of this covering as it avoided the texture damage of the fruit. According to Goulao and Oliveira [40] and Sakurai and Nevins [41], the loss of firmness is due to the loss of galactose and arabinose, which decreases the molecular size of pectin and other components of the cell wall, in addition to the depolymerization, rearrangement, and solubilization of polysaccharides.

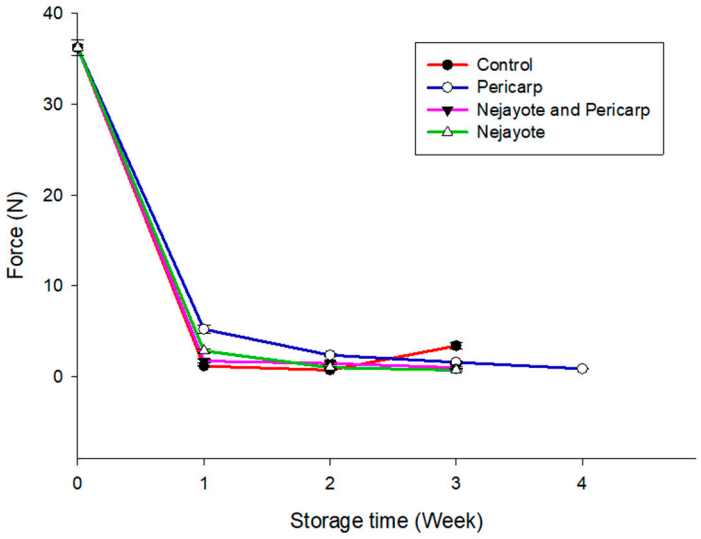


Figure 11. Mesocarp firmness of coated (pericarp, nejayote and pericarp, and nejayote) and control avocados stored at room temperature.

3.2.4. Titratable Acidity (TA) and Total Soluble Solids (TSS)

The results of TA of avocados during their storage are shown in Figure 12. The values of C and the coated avocados increased significantly ($p < 0.05$) during the first week; at that moment, the highest values of TA corresponded to the NP avocados while the lowest values belonged to the P and C samples. Then, from the second to the third week a reduction in the values of TA was noticeable, excepting the NP avocado; this reduction during ripening was due to the decrease in total acids such as tartaric, malic, citric, and ascorbic [20]. Furthermore, from the fourth to the fifth weeks, the TA of the P avocados decreased slowly and it did not reach the value of C in the third week, demonstrating in that way a preservation advantage of this coating. With respect to TSS results (Figure 13), in the first week these values increased slowly, but in the second week, they increased significantly ($p < 0.05$), without an important difference between the samples. However, in the third week the value of C decreased significantly ($p < 0.05$) compared to the coated avocados' value. The P avocados kept the value of TSS until the fourth week of storage; after that, their TSS value decreased markedly. According to Sierra et al. [38], the reduction in TSS is related to fruit respiration because TSS act as a substrate in the CO_2 production.

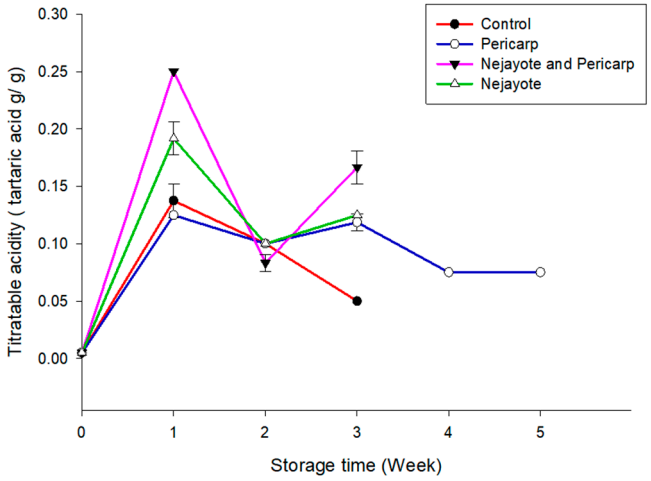


Figure 12. Titratable acidity of coated (pericarp, nejayote and pericarp, and nejayote) and control avocados stored at room temperature.

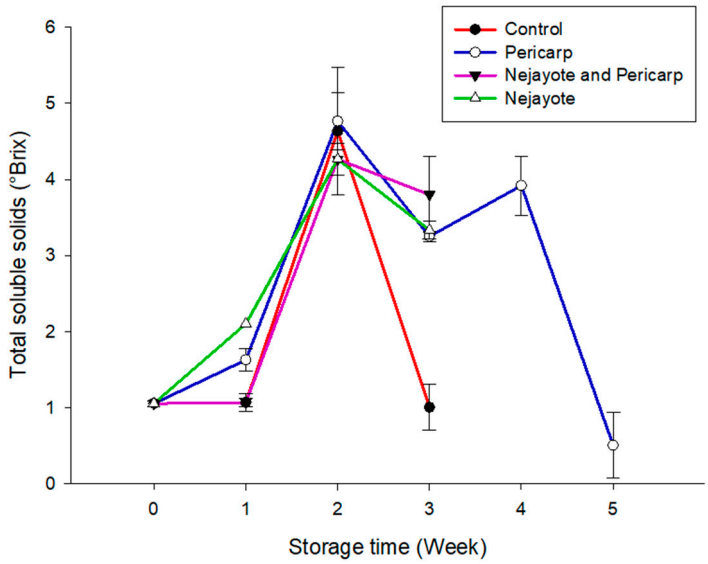


Figure 13. Total soluble solids of coated (pericarp, nejayote and pericarp, and nejayote) and control avocados stored at room temperature.

3.2.5. Total and Reducing Sugars

It is necessary to remark that the avocado mesocarp contains sugars such as D-manoheptulose, perseitol, sucrose, glucose, and fructose, which have antioxidant capacity and, at the same time, they are carbon and energy sources. Likewise, sugars are the primary substrate for fruit respiration [42,43], and they are also broken down by α and β-amylases. For example, sucrose is used for carbon transport, glucose for cellulose synthesis, and fructose for lipid synthesis [43], while C7 (D-manoheptulose and perseitol) sugars are regulators of carbon flux and they prevent oxidative damage [44]. Thus, depending on the treatment we used, the total sugar content in the avocados was heterogeneous (Table 4). During the first week, this parameter decreased significantly ($p < 0.05$) in all samples, excepting the N avocado value; this reduction tendency was observed in the NP and N avocados until the second week. In contrast, the values of the C and P samples increased in the second week, but the value of the P avocado decreased significantly ($p < 0.05$) until the fourth week.

Table 4. Total and reducing sugars contents of control and coated avocados.

Total Sugar (g/100 g)				
Week	Control	Pericarp	NP	Nejayote
0	66.3 ± 2.20^{a}			
1	59.6 ± 0.86^{b}	59.40 ± 2.12^{bc}	52.30 ± 3.11^{e}	62.5 ± 3.17^{ab}
2	71.0 ± 1.24^{d}	67.10 ± 5.68^{acd}	42.50 ± 0.59^{f}	60.00 ± 0.67^{b}
4		22.70 ± 0.38^{g}		
Reducing sugar (g/100 g)				
0	2.53 ± 0.15^{a}			
1	2.38 ± 0.03^{a}	1.19 ± 0.15^{d}	1.19 ± 0.15^{d}	1.54 ± 0.22^{cd}
2	0.69 ± 0.02^{e}	1.4 ± 0.06^{d}	1.39 ± 0.11^{d}	2.04 ± 0.19^{b}
4		1.84 ± 0.09^{bc}		

NP: nejayote and pericarp. Values presented are the average ± standard deviation. Different letters indicate that the values are significantly different ($p < 0.05$).

In the case of the reducing sugars values (Table 4), the results of coated avocados decreased significantly ($p < 0.05$) in the first week in comparison to the C sample. During the second week the value of the control decreased; in contrast, the N avocado value

increased significantly ($p < 0.05$). In the fourth week, the reducing sugar was only measured in the P avocado, whose value was significantly ($p < 0.05$) higher. These changes in sugar concentration occurred because of the generation of cellulose, which requires an increase in glucose concentration. This increase stimulated the synthesis of abscisic acid. Subsequently, the concentration of sugars decreased, accompanied by an increase in oil [43].

4. Conclusions

In summary, this study provides evidence of the physicochemical, mechanical, and microstructural properties of biodegradable films based on pericarp and nejayote. Pericarp film (P film) is the best because it is significantly more transparent and thicker. It also has a higher water adsorption capacity, a higher elastic modulus and toughness values. Its microstructure showed intermediate roughness value compared to other films. Moreover, the P film extended the useful life of avocados until four weeks at room temperature because their maturing process (peel and mesocarp color, weight, firmness, titratable acidity, total soluble solids, and total sugar losses) was slower compared to control and to the other studied films. Furthermore, the P film showed a visual effect against fungal infection; however, more studies on this effect are required. Future studies are needed on the toxicity of nejayote and nixtamalized pericarp, the diffusion of these materials through the peel of avocado, and the sensory analysis of fruit. This information could be valuable to understand the interaction between the film components and avocado (peel and mesocarp) and could possibly reinforce the findings of this study.

Author Contributions: Conceptualization, M.D.-R., M.d.l.P.S.-C. and G.C.-D.; Methodology, A.A.R.-A., J.V.M.-M., G.C.-D. and M.G.-V.; Validation, J.V.M.-M.; Formal analysis, L.E.R.-C., R.G.C.-M. and A.d.J.C.-S.; Investigation, L.E.R.-C., R.G.C.-M., A.d.J.C.-S. and M.G.-V.; Resources, M.d.l.P.S.-C.; Writing—original draft, L.E.R.-C.; Writing—review & editing, M.D.-R.; Supervision, M.D.-R. and J.V.M.-M. All authors have read and agreed to the published version of the manuscript.

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