

Production of Starch Films Using Propolis Nanoparticles as Novel Bioplasticizer

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ABSTRACT: Because starch is a biodegradable polymer with low cost and wide availability it is an attractive material for producing edible films for fruits. Films produced with pure starch have the disadvantage of being fragile. To overcome this issue, propolis nanoparticles were used as a novel plasticizer. Mechanical, thermal and morphological properties of the films containing 0.5, 1 and 3 wt.% propolis nanoparticles were evaluated. The best performance was obtained using 0.5 wt.% propolis, increasing the Young's modulus and decreasing the glass transition temperature (T_g), showing their plasticizing effect. The results of scanning electron microscopy (SEM) and atomic force microscopy (AFM) images showed a homogenous material with a low quantity of cracks and higher roughness than the pristine starch film. A more hydrophobic material was obtained due to the resin and wax compounds present in the propolis nanoparticles. This study shows the novel use of propolis as plasticizer for starch films.

KEYWORDS: Propolis, starch, plasticizer, antimicrobial agent, bioplastic

1 INTRODUCTION

Many efforts have been made to produce materials from renewable sources due to the environmental problems related to the non-biodegradable waste generated by petrochemical-based polymers and their amount of residues [1]. In the last decade, there has been an increased interest in the production and use of plastics implementing biopolymers as substitutes for synthetic polymers, especially for short-term applications such as food packaging. Different types of renewable sources have been promoted for the development and application of these plastics such as PLA, PHA, PHB, cellulose, and starch. However, these plastics present several limitations like brittleness, thermal instability, poor mechanical properties, among others [2, 3].

Starch has been suggested as a great alternative for producing bioplastics because of its low price, thermoplastic behavior and abundance [4, 5]. It is mainly composed of amylose, a linear polymer, and

amylopectin, a highly branched polymer [6]. It is a nontoxic and biodegradable compound. However, the films produced using only starch have poor dimensional stability and mechanical properties caused by high intermolecular forces [1, 7]. Consequently, it is necessary to implement chemical or physical modifications to enhance the functionality of the material. Some strategies used to enhance their mechanical properties are crosslinking, oxidation, acid hydrolysis, ultrasound waves, microwave radiation, annealing and mixture with additives like polyols, sugars, antioxidants, lipids and waxes [1, 8]. Therefore, plasticizers increase flexibility by reducing the intermolecular forces of polymer chains. In this study, propolis will be evaluated as a plasticizer to improve the flexibility of the material. Plasticizers are characterized by their low molecular size, allowing them to integrate the intermolecular spaces between the polymer chains. Therefore, increments of plasticizer can increase the free volume and the molecular mobility [9]. The most common plasticizers for biopolymers are glycerol and sorbitol [6, 10, 11]. These substances, when incorporated into the polymer network, increase the flexibility and workability of the material and also decrease the hardness, density and deformation that lead to an

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inevitable decline in the original strength of the material [9, 12]. Plasticizers also lower the water content of the compound, limiting microbial growth [8].

Nowadays, there is an increasing interest in the use of natural-based plasticizers such as vegetable oils from soybean, linseed, castor bean, sunflower and also fatty acid esters, urea, sucrose, xylol, carcadol, etc. [9, 13, 14]. Propolis is a natural resinous substance produced by honeybees (*Apis mellifera*), which is a mixture of chemical compounds that usually contains resins, waxes, essential oils, pollen, among other [15, 16] substances that can provide plasticizing properties. These compounds that propolis possess have been associated with antibacterial, antifungal, anti-inflammatory and antioxidant properties [17]. The antimicrobial properties of propolis are attributed to the presence of flavonoids and phenolic acids [18]. Cornstarch is widely used to produce biofilms, and despite several works that have been done using propolis in starch-glycerol films to provide antimicrobial assets [3, 4, 19], it has not been used as a plasticizer itself. In this study, a starch film for food packaging incorporating propolis as a natural-based plasticizer and antimicrobial agent was developed to substitute the use of other common substances, such as glycerol and sorbitol, that work just as plasticizers, to enhance the post-harvesting life of fruits. According to a search made by the authors, the use of propolis nanoparticles has not yet been documented, only that of ethanoic extract.

2 METHODOLOGY

2.1 Materials

Cornstarch was supplied by Central American Brands Inc. (San José, Costa Rica). Propolis nanoparticles with an approximate diameter of 177 nm were from the National Laboratory of Nanotechnology (LANOTEC), and ethanol reagents were purchased from Sigma-Aldrich and used as received without further purification.

2.2 Film Preparation

A suspension of 3 g of starch in 100 g of water was prepared. The solution was gelled by heating to its boiling temperature under magnetic stirring and then left to cool. The propolis nanoparticles were then added at different concentrations of 0.5, 1 and 3 wt.% with respect to the starch mass; and then mixed at 4000 rpm for 5 min using an Ultra Turrax T25 Vortex mixer (IKA, Germany). After mixing, 40 mL of the solution was poured into a Teflon coated plate and dried in an oven at 50 °C for 12 h.

2.3 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was performed using a Q500 instrument (TA Instruments, USA). The samples (approx. 4.4 ± 0.1 mg) were taken in a standard platinum pan. The scan was run at 10 °C/min under a nitrogen flow. Mass change was measured from 25 °C to 700 °C.

2.4 Differential Scanning Calorimetry (DSC)

The glass transition temperature of films was determined using a Q200 differential scanning calorimeter (TA Instruments, USA). The scan was run at 10 °C/min under a nitrogen flow rate of 10 ml/min from 100 °C to 250 °C. Samples were dried at 60 °C for 24 h in order to eliminate the presence of water before analysis.

2.5 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra were recorded using a Nicolet 6700 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) in a range of 500–4000 cm^{-1} and a resolution of 4 cm^{-1} . The starch and the ethanolic propolis extract were analyzed as controls.

2.6 Contact Angle Measurements

The apparent contact angles were measured using a OCA15 Plus goniometer (DataPhysics Instruments, Germany) by sessile drop technique and Milli-Q grade water was used as probe liquid; the volume of the drops was constant (10 μl) for each measurement at a temperature of 21 °C. The contact angle values reported are an average value of at least three separate drops on different substrate areas. The recorded images were analyzed by SCAN 20 DataPhysics software.

2.7 Scanning Electron Microscopy (SEM)

The samples were analyzed using a JEOL JSM-5900 LV scanning electron microscope (Tokyo, Japan) at an acceleration voltage between 7–10 kV and a pressure of 1E^{-4} Pa.

2.8 Atomic Force Microscopy (AFM)

The sample's topography was analyzed using an atomic force microscope (Asylum Research, Santa Barbara, CA) operated in the tapping mode in air. Silicon probes (model Tap150Al-G, back side of the

cantilever covered Al) with resonance frequencies of 150 kHz and force constant of 5 N/m were used. For structure and roughness characterization, the height differences were evaluated.

2.9 Mechanical Analysis

The mechanical properties of the films were evaluated with a TA DHR III rheometer. For the tension test, specimens of $40 \times 2.5 \times 0.3$ mm were prepared using a speed of 1 mm/min. From the experimental data, tension moduli were calculated for each film using TRIOS and Origin 8 software.

3 RESULTS AND DISCUSSION

The films prepared adding propolis nanoparticles were visually homogeneous and considerably easier to manipulate than the pristine starch film, which was very stiff and fragile, showing a positive interaction of the propolis with the starch complex.

3.1 Thermogravimetric Analysis (TGA)

Figure 1 shows the TGA thermograms of the starch and composite films degradation. The curves show three degradation stages. The first between 100 °C and 150 °C, which reveals a mass weight lost associated with water and possibly small molecular compounds contained in the raw material. A second thermal degradation event is presented between 251 °C and 313 °C, which is associated with the degradation of the starch and propolis. The starch films present a degradation temperature at ≈ 320 °C [20] and the propolis a maximum decomposition at 300 °C [21], which

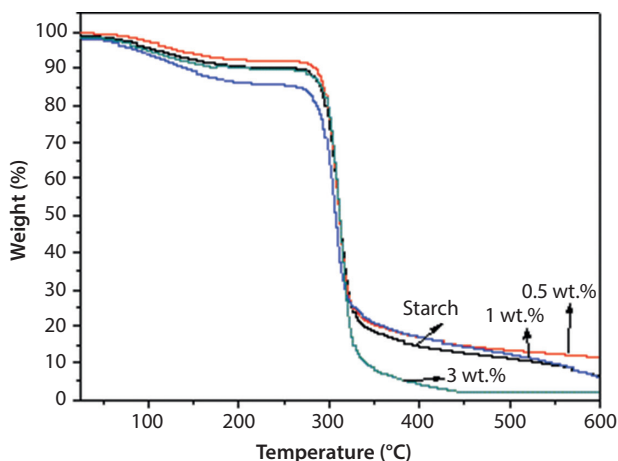


Figure 1 Thermogravimetric analysis of starch film and 0.5 to 3 wt.% propolis nanoparticles/starch films.

is consistent with the results obtained of a maximum peak at 311.5 °C for the starch films containing propolis. The weight loss between 500 °C and 680 °C could be associated with inorganic impurities and residual carbon. Ash content of starch could be related to the presence of phosphate groups, as well as the presence of calcium, magnesium and proteins [22, 23]. No significant shifts in the degradation temperature were observed between the pristine starch film and the composites. The thermal degradation temperature obtained was similar to other biopolymers used for producing edible films such as gelatin, caseinate and PLA-based films present at a temperature of 329 °C, 333 °C and 324 °C, respectively [24, 25].

3.2 Differential Scanning Calorimetry (DSC)

The glass transition was measured to demonstrate the effect of the nanoparticles on the polymer mobility. The glass transition temperatures were 61.2 °C, 55.4 °C, 55.8 °C and 62.5 °C for pristine starch, 0.5, 1 and 3 wt.% propolis/starch films, respectively. The films containing 0.5 and 1 wt.% presented a decrease in the T_g . The results are shown in Figure 2. According to Mali *et al.* [6], a reduction in the T_g of an amorphous and/or semicrystalline material reveals a decrease in the intermolecular forces between the polymer chains; increasing the local chain flexibility by lowering the T_g , stimulating the ability of chain rotation and conferring more flexibility to the films. The films containing propolis at 0.5 wt.% presented a decrease in the T_g , suggesting the plasticizing effect of the propolis nanoparticles. Studies made by De Araújo *et al.* [16] reveal that the waxes and

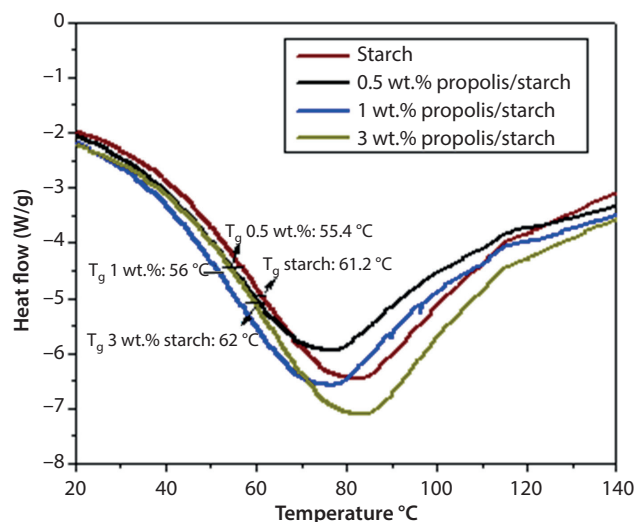


Figure 2 DSC thermograms of starch film and 0.5 to 3 wt.% propolis nanoparticles/starch films.

essential oils contained in the propolis could react as a plasticizer, increasing the mobility of the starch chains and reducing film rigidity. However, factors such as agglomeration on the starch matrix could influence the Tg results. The propolis nanoparticles are not highly soluble in water and a higher concentration as 3 wt.% could result in a non-homogeneous dispersion of the propolis on the starch network due to agglomeration, obtaining a material not as malleable as wanted [26].

Figure 3 shows the spectra of the starch and propolis nanoparticles. The starch spectra reveal a broad band (3347 cm^{-1}) between 3000 cm^{-1} and 3600 cm^{-1} from the OH stretching. The peak at 2927 cm^{-1} is assigned to the C–H stretching and at 1650 cm^{-1} is associated with the water absorbed in the amorphous part of the starch. Finally, the peaks from 1158 cm^{-1} correspond to the C–O bond stretching and at 1073 and 1011 cm^{-1} to C–O bending [27]. The spectra of the propolis nanoparticles show a broad peak at 3600 to 3000 cm^{-1} centered at 3328 cm^{-1} , associated with the OH of phenolic compounds as cinnamic acid, caffeic acid, ferulic

acid and alcoholic C–OH stretching. The peaks at 2927 and 1690 cm^{-1} are associated with the CH_2 and C–O stretching vibration, respectively. The peaks at 1611 and 1447 cm^{-1} are associated with the C=O and C=C groups. The peak at 1041 cm^{-1} corresponds to the C–O stretching of the ester group [28]. The spectra of the starch films containing propolis show similar bands to the pure starch material. The peak centered between 3000 cm^{-1} and 3600 cm^{-1} from the OH stretching of the starch matrix was shifted to lower wavelengths and a broader peak related to the C=O stretching is present in the spectra of the films containing propolis. This could be associated with the hydrogen bonds formed between the propolis and the starch [29, 30].

3.3 Contact Angle

Starch is a molecule that contains hydroxyl groups and glucopyranose rings which confer hydrophilic properties. Cornstarch has high levels of amylose compared to starches from other sources like yam and potato. The helix that amylose creates exhibits the OH groups on the exterior, making it a hydrophilic material [31]. Figure 4 shows the images of the films in contact with a drop of deionized water. The nanoparticles changed the surface properties of the films to more hydrophobic domains. This effect could be associated with the wax and resin compounds forming the nanoparticles [17]. Hydrogen and covalent interactions between the starch matrix and the polyphenols interfere with the availability of hydrogen groups to form hydrophilic bonds with water, thus increasing the contact angle of the film [32]. Nevertheless, the films were never considered hydrophobic due to the fact that the contact angle was lower than 90° (Table 1). However, the propolis contains a hydrophilic component that can also act as a barrier to water vapor and gas exchange, making it suitable to be incorporated into food packaging [15]. Micro- and nanostructures can modify the wetting properties of a solid surface, providing an enhancement of the hydrophobicity. This phenomenon

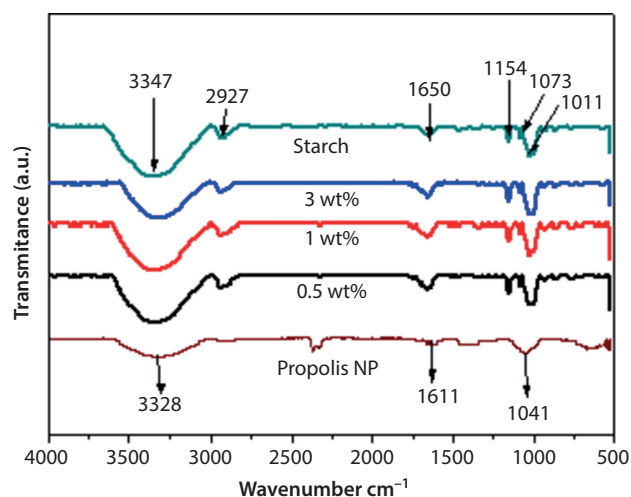


Figure 3 FTIR spectra of the propolis nanoparticles, starch film and 0.5 to 3 wt.% propolis nanoparticles/starch films.

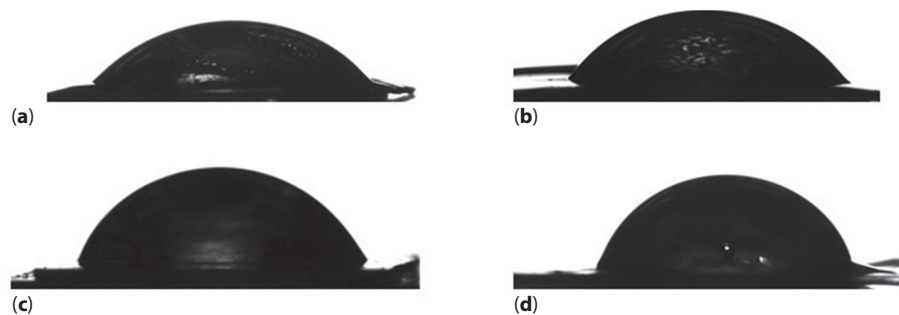


Figure 4 Water drop images of (a) starch film and (b) 0.5 wt.%, (c) 1 wt.% and (d) 3 wt.% propolis nanoparticles/starch films.

is governed by elastic and capillary forces and depends on the elastic properties of the nanoparticles, strength of the interactions and surface patterns [33, 34]. Surface roughness values of the films increase along with their contact angle, as shown in Table 1.

3.4 Mechanical Properties

According to tensile strain assays, Figure 5 shows the strain-stress curves of the starch film and the films with different concentrations of propolis nanoparticles. The propolis nanoparticles had a positive effect on the mechanical properties when the concentration is below to 1 wt.%, which is reflected in the Young's modulus, ultimate tensile strength and elongation at

Table 1 Contact angle values of the starch and starch/propolis based films.

Sample	Contact angle	Roughness (nm)
Starch	$53 \pm 1^\circ$	5.7 ± 1.5
0.5 wt.% propolis	$57 \pm 2.1^\circ$	7.0 ± 2.3
1 wt.% propolis	$79 \pm 0.4^\circ$	8.0 ± 1.0
3 wt.% propolis	$78 \pm 1.1^\circ$	6.3 ± 0.6

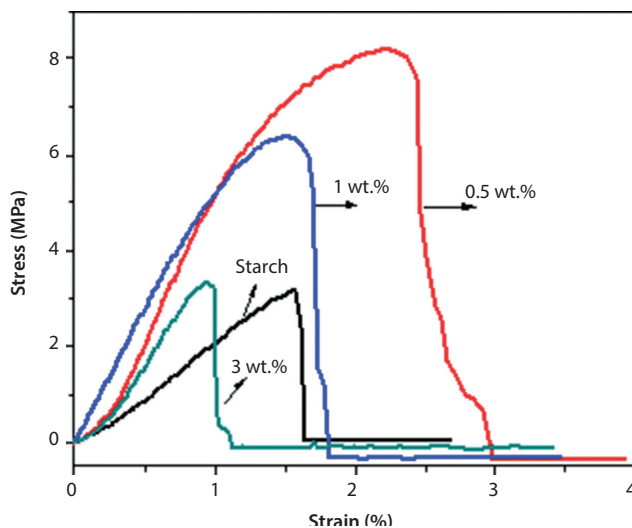


Figure 5 Stress-strain curves of starch and 0.5 to 3 wt.% propolis nanoparticles/starch samples.

break values (Table 2). The addition of propolis nanoparticles at 0.5 and 1 wt.% decrease the glass transition temperature of starch, improving the elongation at break of the films, which is caused by the dispersion of waxes and essential oils characteristic of these compounds, conferring plasticizing properties. The polyphenols forming nanoparticles have adhesive properties which can improve the interface adhesion on the starch matrix [35]. In nature, carbohydrates and polyphenols are excellent partners for forming natural adhesives and gums; for example, mussel adhesive [36, 37]. The high superficial area of propolis nanoparticles and the hydrogen bonds formed could result in strong interfacial adhesion tightening polymer chain-to-chain which improves the resistance to mechanical stress, showing an augmentation of the Young's modulus, which indicates that the material is more rigid [6, 38]. When a higher concentration than 0.5 wt.% was used there was a decrease in the elongation at break but not of the Young's modulus. This could be related to a crosslinking effect and decrease in the free volume [39]. The increase of the Young's modulus is attributed to the high superficial area of propolis nanoparticles and the ability to form strong hydrogen bonds, improving the stiffness of the polymer matrix and contributing to stress distribution [39]. The excess of propolis could cause a decrease in the mechanical properties of the films due to agglomeration; this is also appreciated in the T_g of the films. When analyzing thermal and mechanical properties, 0.5 wt.% propolis is recommended as the concentration where the propolis has a plasticizing effect and the highest improvement in the mechanical properties.

3.5 Morphological Studies Using SEM and AFM

The morphology of the film's surface was studied by SEM and AFM. Figure 6 shows a cross section of the propolis nanoparticles used for the composite formation, of between 75 to 144 nm and roundish-structure.

Figure 7a shows a smooth surface with several cracks, displaying the fragile character of the starch film. The films containing propolis nanoparticles show

Table 2 Mechanical properties from stress-strain analysis of starch film and starch films with different propolis concentration.

Film	Young's modulus (Mpa)	Ultimate tensile strength (Mpa)	Elongation at break (%)
Starch	2.0 ± 0.1	3.1 ± 0.4	1.6 ± 0.1
0.5 wt.% propolis	5.1 ± 0.3	8.2 ± 1.3	2.4 ± 0.9
1 wt.% propolis	5.5 ± 0.2	6.4 ± 0.1	1.6 ± 0.1
3 wt.% propolis	3.2 ± 0.2	3.3 ± 0.2	1 ± 0.1

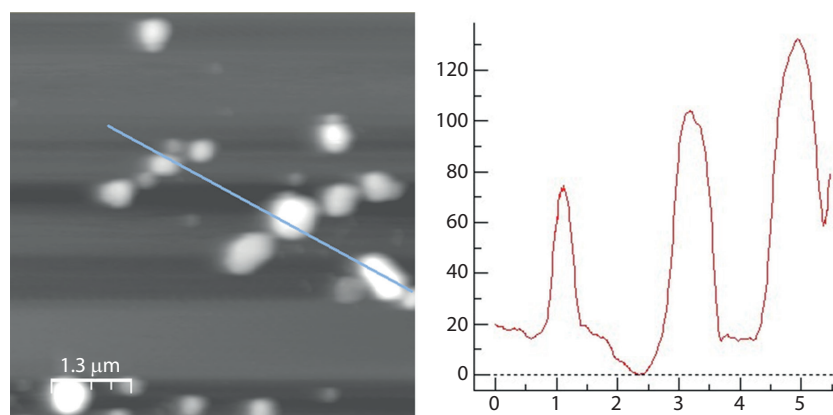


Figure 6 AFM height image and cross section of the propolis nanoparticles used.

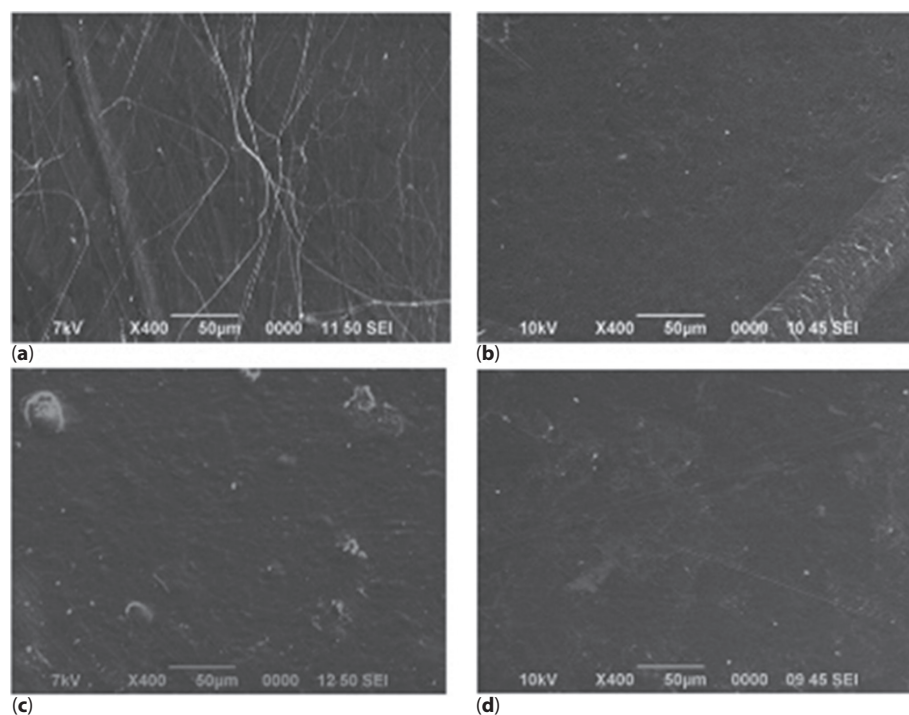


Figure 7 SEM images of (a) starch film and (b) 0.5 wt.%, (c) 1 wt.%, (d) 3 wt.% propolis nanoparticles/starch films (400x).

higher roughness and more homogeneous surface. No phase separation was detected. Similar results were obtained for the three propolis concentrations used, as can be seen in Figure 7b–d. The nanoparticle distribution was studied by AFM due to the low contrast between the carbon-based matrix and the carbon-based nanoparticles to determine the dispersion of the nanoparticles.

Figure 8a shows starch film that presents some waves but no local nanoroughness in the range of 50 nm. Figure 8b presents some lighter local zones that could be related to the nanoparticles dispersion in the starch film containing 0.5 wt.% nanoparticles.

Figure 8c presents the surface of the film containing 3 wt.% propolis, showing zones with agglomeration of nanoparticles. The decrease in the mechanical properties of the films containing 3 wt.% propolis could be associated with the agglomeration of the particles.

The average roughness values of the films were calculated and the results are shown in table 1. The films containing nanoparticles presented structures of less than 100 nm on the surface. A broad value is presented due to the variation in sizes.

Figure 8 shows the AFM images of the films with and without nanoparticles. Figure 8b shows nanoparticulate structures distributed on the film that are not

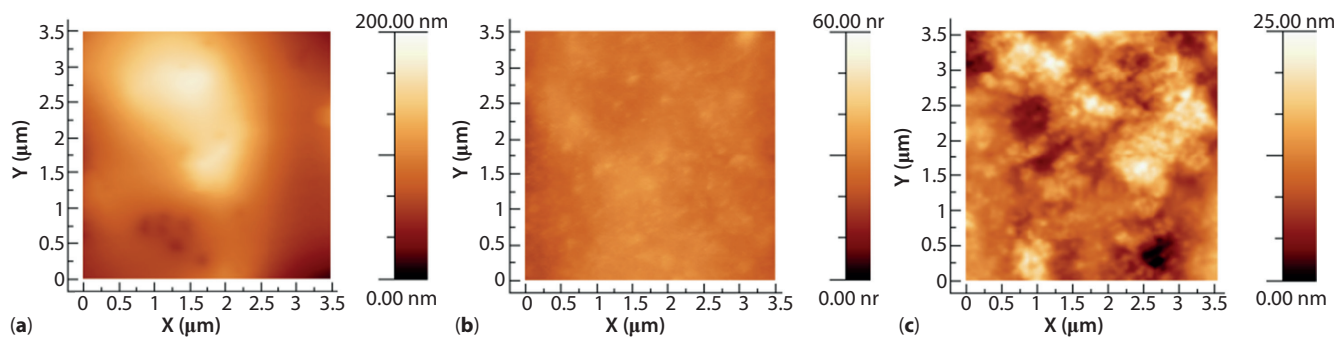


Figure 8 AFM height images of (a) starch film and (b) 1 wt.%, (c) 3 wt.% propolis nanoparticles/starch films, 3.5 μm × 3.5 μm.

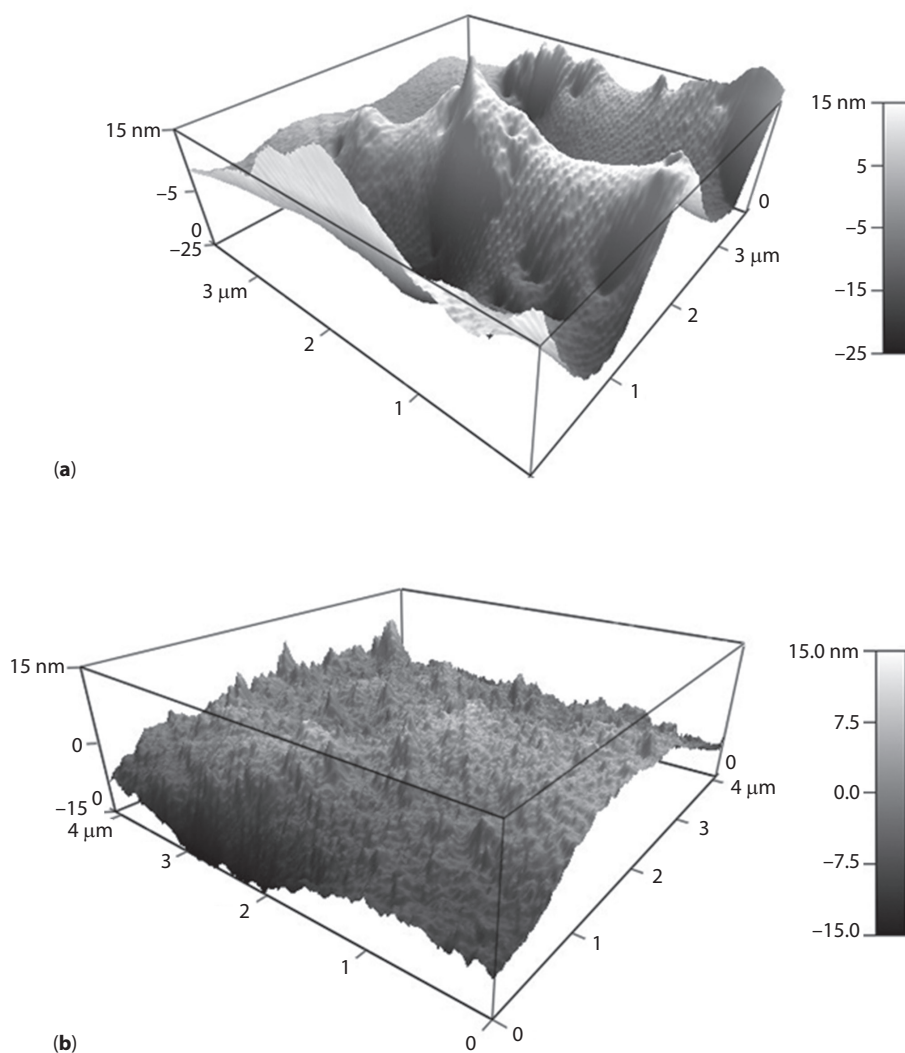


Figure 9 AFM height images of (a) starch film, 3.5 μm × 3.5 μm, and (b) 0.5 wt.% propolis/starch film, 4 μm × 4 μm.

presented on the pristine starch film, as can be seen in Figures 9a and Figure 9b. Figure 8b shows an increase in the roughness of the films when the nanoparticles were added. These features reveal the change in surface structure due to the addition of propolis. A

similar 4 μm × 4 μm area was analyzed. Table 1 shows a decrease in the roughness which can be related to a lesser amount of particles per area.

Figure 10b shows an increase of the roughness of the films when the nanoparticles were added. These features

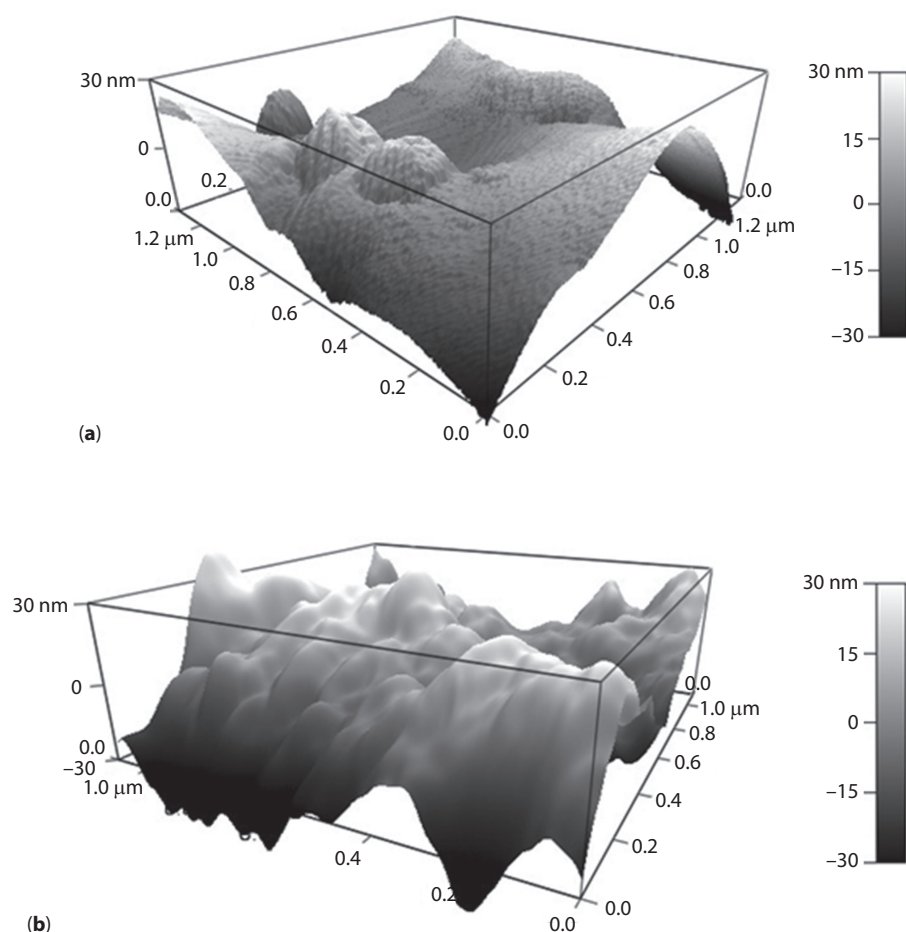


Figure 10 AFM height images, $1.1\ \mu\text{m} \times 1.1\ \mu\text{m}$, of (a) starch film and (b) 0.5 wt.% propolis/starch films, respectively.

reveal a change on the surface structure due to the addition of propolis. The propolis nanoparticles changed the bulk and surface properties of the starch films, acting as plasticizers in a concentration of 0.5 wt.%.

For a complete understanding of the influence of the material in nanoparticulate form and the arrangement of the nano-polymer matrix, the composition of the non-particulate extract will be done in future studies. Therefore, complementary studies related to microhardness. Biobased films with antioxidant and antimicrobial properties have been developed for improvement of the problems related to microbial contamination [40]. Further studies will be done to determine the capability of the propolis starch films as coatings able to decrease moisture loss and protect food from external microorganisms.

4 CONCLUSIONS

The present study has revealed the use of propolis as an attractive bioplasticizer to be used in eco-friendly starch matrix, leading to high renewable content materials.

The propolis nanoparticles changed the bulk and surface properties of the starch films. The propolis starch films showed higher elongation at break and Young's modulus than the pristine starch films when the propolis was added in concentrations of 0.5 wt.% and 1 wt.%. The commonly used plasticizers decrease the T_g and the Young's modulus. The use of propolis nanoparticles not only decreased the T_g but also increased the material matrix strength. The nanoparticles could be used as a new plasticizing agent to improve film strength and flexibility. Morphological studies showed that the nanoparticles were well dispersed in the starch matrix until 1 wt.%, increasing the roughness in nanometer range and the hydrophobicity of the surface. Propolis nanoparticles are well known as antimicrobial agents. Further studies will be done to determine their antibacterial properties and performance as coating for food packaging.

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