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Critical Evaluation of Starch-Based Antibacterial Nanocomposites as Agricultural Mulch Films: Study on Their Interactions with Water and Light

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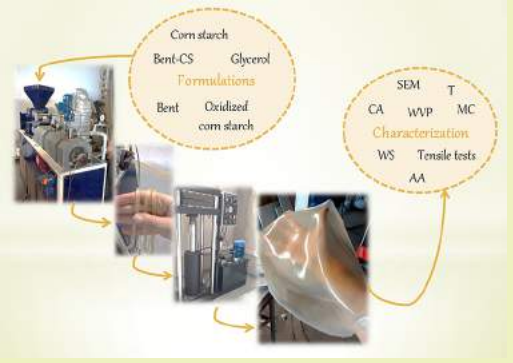
ABSTRACT: In order to evaluate the potentiality of novel formulations based on starch to be used as agricultural mulch films, native and oxidized corn starch nanocomposites were prepared by extrusion using natural (Bent) and chitosan-modified bentonite (Bent-CS) fillers. The nanocomposite interactions with water were studied by means of moisture content (MC) determination, water solubility (WS), water vapor permeability (WVP), and contact angle (CA). The light transmission spectra were analyzed in order to determine the transparency and radiometric properties of films. Mechanical properties are also included and related with the cryo-fractured surface morphology observed by scanning electron microscopy (SEM). Finally, the antimicrobial action of developed nanocomposites was investigated against the phytopathogen bacterium *Pseudomonas syringae* pv *tomato* DC3000 (*Psy*). Results suggest that starch oxidation leads to a reduction in polarity and transparency. The incorporation of nanoclays improved water resistance but did not produce a significant effect in WVP and mechanical properties, and new strategies are required to improve the nanocomposite performance. However, the incorporation of Bent-CS exerted antibacterial activity on nanocomposites, which is an encouraging result.



KEYWORDS: *Corn starch, Bentonite, Chitosan, Nanoclays, Oxidized starch, Mulch films*

INTRODUCTION

Agricultural mulches are thermoplastic films that are placed on the ground before planting to avoid weeds growing and to maintain temperature, humidity, and soil structure, preventing erosion and improving water management. All of these traits are important issues in areas with limited water resources. The use of plastics in agriculture produces an increase in yields over previous harvests, less dependence on herbicides and pesticides, a more efficient use of water, and allows also for the intensive cultivation of poor soils.[1] However, these films are mostly made of polyethylene (PE), a nonbiodegradable polymeric material derived from petroleum. The use of PE films or other nonbiodegradable polymers, such as polyvinyl chloride (PVC) or ethylene vinyl acetate (EVA), presents great environmental and economic disadvantages for responsible farmers.[2] After the harvests, it is necessary to remove the nonbiodegradable mulches to avoid problems with the crops of the next period, and this entails great costs for the farmers who must use labor, equipment, and infrastructure to collect them.[1] In addition, many farmers dispose of them in local landfills, where they are accumulated or burned in the open, causing problems in the environment and to the health of local residents.[3] Others incorporate mulch wastes into the soil in the tillage, which entails a serious risk to the environment since they accumulate agrochemically contaminated PE in the soil[4] and interfere with root growth and development during the next season.[5] In this context, the development of biodegradable or short lifespan films is highly attractive.[1]

In this way, the replacement of petroleum-derived polymers by natural ones, such as starch, is of special importance for this application.[3] Starch is a natural polymer from renewable resources, abundant and cheap, and is capable of forming continuous thermoplastic films by the processing methods currently used for petroleum-derived polymers.[6] However, its hydrophilic character and its poor mechanical properties limit its application.[7,8] Thus, it is necessary to look for strategies that allow its implementation by improving its properties[9−11] and by specifying the advantages of its use, such as biodegradation,

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low cost, and the ability to act as a matrix for phytoactive substances delivery.

In particular, it is known that the addition of nanoclays can improve the thermal, mechanical, and barrier properties of biopolymers.[12−15] Montmorillonite (Mnt) is the type of nanoclay most used for this purpose given its great surface area (of the order of 700−800 m^2/g) and its large aspect ratio (50−1000).[12] The thickness of the clay sheets is in the order of a nanometer and its length can reach several microns. They are characterized by high strength, rigidity, and impermeability.[16] On the other hand, the chemical modification of starch is also an interesting possibility to modify the properties of films. The chemical reactivity of starch is directly related to the presence of hydroxyl groups, which are susceptible to numerous chemical reactions.[17−19] Particularly, during the oxidation process, the hydroxyl groups of the starch are oxidized to carboxyl and carbonyl groups.[20] The presence of carboxyl and carbonyl groups helps to prevent retrogradation,[18,21] which makes this type of modification attractive for the formation of thermoplastic films.

Native and oxidized corn starches were used as matrices, and bentonite (Bent) and chitosan-modified bentonite (Bent-CS) were used as fillers. Bentonite is a nanoclay mostly composed of Mnt, and it is abundant in Argentina.[22] Chitosan is a natural polymer with remarkable properties in agriculture, among which its antimicrobial activity and elicitor capability stand out.[23] Bent-CS nanoclay has demonstrated potential for agricultural application in tomato crops.[24] Thus, starch-based nanocomposites were prepared by extrusion followed by compression molding, and their physicochemical and water interaction properties were analyzed. The benefits and constrains of their utilization as mulch films were critically discussed.

We hypothesized that the oxidation of starch improves the water resistance of thermoplastic films by reducing starch hydrophilicity, and that the formation of nanocomposites with Bent and with Bent-CS improves the mechanical and barrier properties of thermoplastic films of native and oxidized starch. Besides, we hypothesize that the Bent-CS nanoclay incorporation as filler can provide antibacterial activity against the phytopatogenic bacterium, *Psy*. On the basis of this, the mechanical and antibacterial properties, how the water and light interact with the thermoplastic films of native and oxidized corn starch, and its nanocomposites with Bent and Bent-CS were studied.

MATERIALS AND METHODS

Materials. Corn (*Zea mays*) starch used for the preparation of nanocomposites was obtained from the distributor Dos Hermanos, brand Ying Yang (Mar del Plata, Argentina); whereas the glycerol was purchased from Aurum (Mar del Plata, Argentina) and was used as plasticizer. The bentonite used was provided by Minarmco S.A. (Neuquén, Argentina). Its ion exchange capacity was determined by the methylene blue method, and it was 105 mequiv/100 g of clay. The chitosan employed for the Bent-CS nanoclay preparation was purchased from Drogueria Sapporiti (Buenos Aires, Argentina). Its molar mass, determined by the capillary viscosimetry method following the instructions reported by De la Paz et al.,[25] was 531 kDa, and its degree of deacetylation, determined by ^1H nuclear magnetic resonance (NMR) as reported by Lavertu et al.,[26] was greater than 90%.

Starch Oxidation. Native starch was modified in a 10 L-capacity reactor by using hydrogen peroxide, according to the method described by Gumul, Krystyjan, Buksa, Ziobro, and Zięba.[27] In summary, 1 kg of corn starch was suspended in 4.2 L of distilled water. The dispersion was mechanically stirred at 200 rpm, and then the pH was adjusted to 9 with 2 M NaOH. After that, 126 mL of (20 vol) H_2O_2 was added dropwise. The reaction mixture was stirred for 2 h at room temperature. The dispersion was then removed from the reactor and allowed to settle. The supernatant was removed, and the cake was dried in an oven for 24 h at 45 °C.

Characterization of Starches. Total amylose content in native and oxidized corn starch films was determined by thermogravimetric analysis following the method proposed by Stawski.[28] Results indicate that the apparent amylose content was ∼19 and ∼17% for the native and oxidized corn starch, respectively. During the oxidation process, the hydroxyl groups of starch were oxidized first to carbonyl groups and then to carboxyl groups.[20] The carboxyl and carbonyl contents of oxidized starch were determined according to the procedure described by Yi, Zhang, and Ju,[29] and it was found to be approximately 0.05 and 0.21, respectively. The gelatinization temperature of oxidized starch was 107 °C, less than that of the native starch (121 °C) as reported in our previous work.[30] Figure 1 shows the representation of an oxidized amylopectin molecule in order to illustrate the presence of the new functional groups.

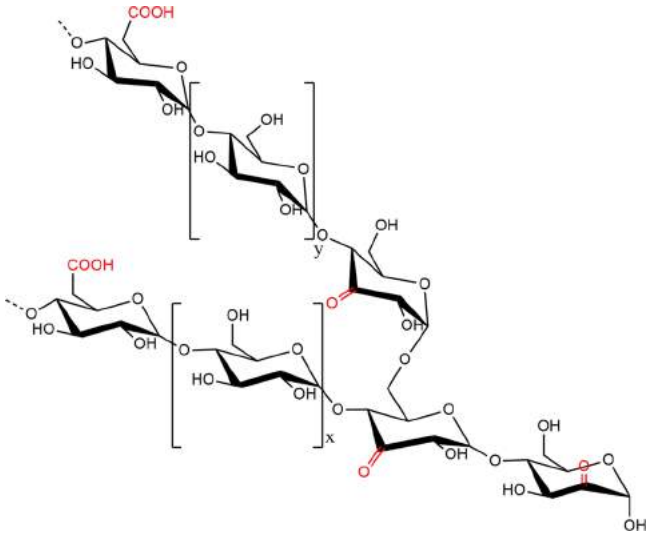

Figure 1. Oxidized amylopectin molecule.

Modification of Filler. Bent-CS was obtained by cationic exchange of Bent with chitosan as reported previously by Merino et al.[24] Briefly, an acid and aqueous CS solution was dropwise added into a Bent dispersion. The stirring was maintained for 30 min, and then Bent-CS nanoclay was centrifuged, washed, and freeze-dried.

Nanocomposites Preparation. Nanocomposite films were prepared from native and oxidized corn starch using 30% (w/w) of glycerol as plasticizer and 4% (w/w) of filler (Bent or Bent-CS). Nanocomposite films were prepared by extrusion in an Extrualex Doble Argentina twin screw extruder (Buenos Aires, Argentina) with six heating zones. The temperature profile used was 90/100/105/110/120/120 °C and the screw rotation speed was 130 rpm. The extruded material was hot-pressed using a hydraulic press at 130 °C and 70 bar for 20 min; thereafter a cooling cycle was applied until obtaining a temperature of 40 °C. The conditions for the manufacturing of the nanocomposite films were established on the basis of previous studies carried out by our research group:[31] percentages greater than 4% (w/w) of nanofiller can deteriorate the mechanical properties of the nanocomposites. The resulting materials were labeled as shown in Table 1.

The film systems were native corn starch (TPS), oxidized corn starch (TPS-Ox), native corn starch with the addition of 4% (w/w) of Bent (TPS-4%Bent), oxidized corn starch with the addition of 4% (w/w) of Bent (TPS-Ox-4%Bent), native corn starch with the addition of 4% (w/w) of Bent-CS (TPS-4%Bent-CS), and oxidized

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Table 1. Nomenclatures and Compositions of the Nanomaterials Developed

name	native corn starch (% w/w)	oxidized corn starch (% w/w)	glycerol (% w/w)	Bent nanoclay (% w/w)	Bent-CS nanoclay (% w/w)
TPS	70		30		
TPS-Ox		70	30		
TPS-4%Bent	66		30	4	
TPS-Ox-4%Bent		66	30	4	
TPS-4%Bent-CS	66		30		4
TPS-Ox-4%Bent-CS		66	30		4

corn starch with the addition of 4% (w/w) of Bent-CS (TPS-Ox-4% Bent-CS).

After preparation, the films were stored in a 60% relative humidity (RH) ambient at 20 °C until they were analyzed as follows.

Films Characterization. *Moisture Content (MC).* The moisture content (MC) was determined by weighing about 0.5 g of sample before and after being placed in an oven at 105 °C for 24 h. The procedure was performed in triplicate for each sample, and the MC was calculated using eq 1

$$\%\mathrm{MC} = \frac{w_i - w_f}{w_i} \times 100\% \quad (1)$$

where w_i and w_f are the initial and final weight of each sample.

Water Solubility (WS). The percentage of dissolution of the films was determined gravimetrically. Approximately 0.5 g of each sample was dried in an oven at 100 °C for 24 h. Then, its initial mass was recorded, and it was placed in 50 mL of distilled water at room temperature for 24 h. After that time, the solution was removed, and the remaining films were placed in an oven at 100 °C for 24 h. Then, their final weight was recorded, and the percentage of solubility was determined using eq 2

$$\%\mathrm{WS} = \frac{w_i - w_f}{w_i} \times 100\% \quad (2)$$

where w_i and w_f are the initial and final weight of each sample.

Water Vapor Permeability (WVP). Water vapor permeability (WVP) was measured according to the desiccant method proposed in ASTM E96-00e1.[32] For that, circular film pieces with a 4.9 cm diameter of each system were used to seal the open mouth of test capsules (exposed area ~18.86 cm^2) containing anhydrous calcium chloride ($CaCl_2$, 0% relative humidity), and then they were introduced into a relative humidity-controlled chamber (65 ± 1%) at 22 °C. The test capsules were weighted with an analytical balance (±0.0001 g) once a day. A plot of weight gain (g) versus time (s) was used to determine the water vapor transmission rate (WVTR, g/s.m^2), which was calculated by dividing the plot slope by the exposed film area (m^2). The WVP of films was then calculated as follows:

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times e}{S \times \mathrm{RH}} \quad (3)$$

where G is the mass gained, e is the thickness of the film, S is the saturated vapor pressure at 22 °C, RH is the relative humidity, t is the time (s), and A is the exposed area of each sample (m^2). Measurements were taken after an initial equilibration period to ensure steady state diffusion and at least five measurements of each sample were realized. All assays were performed in triplicate, reporting the average and standard error in each case.

Contact Angle (CA). The contact angles (θ) were measured using a Ramé–Hart instrument model 100-25-4 equipped with "Drop image advanced" software. CA formed between the liquid–solid interface and the liquid–vapor interface (tangent to the surface of the drop) was determined by placing a 5 μL drop of ultrapure water (Simplicity Water Purification System, Merck Millipore) onto the film surface. Experiments were carried out at 25 °C. At least five measurements per drop were done and the resulting angles were analyzed.

Scanning Electron Microscopy (SEM). The morphology of the top and the cryo-fracture surface of each specimen was investigated using a JEOL JSM-6460 LV instrument. Film samples were mounted on aluminum stubs with double-sided adhesive tape and were coated by sputtering with a thin layer of gold.

Transparency (*T*) and Radiometric Properties. The transparency of the developed films was determined by UV–vis spectrophotometry, using an Agilent 8453 spectrophotometer. The films were cut into rectangular pieces of 4 cm × 2 cm, their thickness was measured at three points, and they were stuck to the measuring cell with adhesive tape. Next, a spectrum was measured for each sample in the wavelength range of 290–1100 nm. Finally, the transmittance at 600 nm was used to calculate the transparency according to eq 4.[33]

$$T = \frac{-\log T_{600}}{x} \quad (4)$$

where T is the transparency, T_{600} is the transmittance percentage at 600 nm, and x is the average thickness of the films in mm. It is of importance to clarify, that high values of T indicate that films are less transparent.

The radiometric properties of the mulch films were evaluated by measuring their direct transmissivity in the wavelength range from 400 to 700 nm (photosynthetically active radiation (PAR) range) using an Agilent 8453 spectrophotometer. Transmissivity coefficients in PAR range (τ_{PAR} were obtained using eq 5, as a weighted function of the spectral distribution of the solar radiation on Earth, according to the values reported in ISO 9050:2003.[34]

$$\tau_{\mathrm{PAR}} = \frac{\sum_{\lambda=400\ \mathrm{nm}}^{\lambda=700\ \mathrm{nm}} S_\lambda \Delta\lambda \tau(\lambda)}{\sum_{\lambda=400\ \mathrm{nm}}^{\lambda=700\ \mathrm{nm}} S_\lambda \Delta\lambda} \quad (5)$$

where S_λ is the spectral distribution of the sun at the wavelength λ, $\Delta\lambda$ is the wavelength range equal to 50 nm, and $\tau\lambda$ is the spectral transmittance at the wavelength λ.

Mechanical Properties. Tensile tests were performed in a universal testing machine INSTRON 3396 at a constant crosshead speed of 1 mm/s for determination of the force (N)–elongation (mm) curves. These curves were transformed into strain (MPa)–deformation curves, which allowed for obtaining the following parameters: Young's modulus (E), maximum stress (σm), and deformation at break (εb). Samples were prepared according to ASTM D882-18[35] and conditioned at room temperature (20 °C) and 60% RH. At least 10 tests were performed for each system.

Antibacterial Properties. The antibacterial activity of the films was evaluated on the viability of the phytopathogenic bacterium *Pseudomonas syringae* pv *tomato* DC3000 (*Psy*). *Psy* was maintained on King's B (KB)-agar medium[36] containing suitable antibiotics, 50 $\mu g\ mL^{-1}$ rifampicin and 50 $\mu g\ mL^{-1}$ kanamycin, according to Mansilla et al.[37] Small pieces (20 mg) of each film were incubated with 500 μL of bacterial cultures from an overnight preculture in an exponential phase (106 cells mL^{-1}) for 24 h at 30 °C. Once this time had elapsed, an aliquot of the cultures was extracted, diluted into fresh KB medium, and plated on KB-agar medium. Colonies were counted after incubation overnight at 30 °C, and the number of CFU in the original culture was calculated. The results were expressed as a percentage with respect to the control treatment without film. Three independent experiments were performed.

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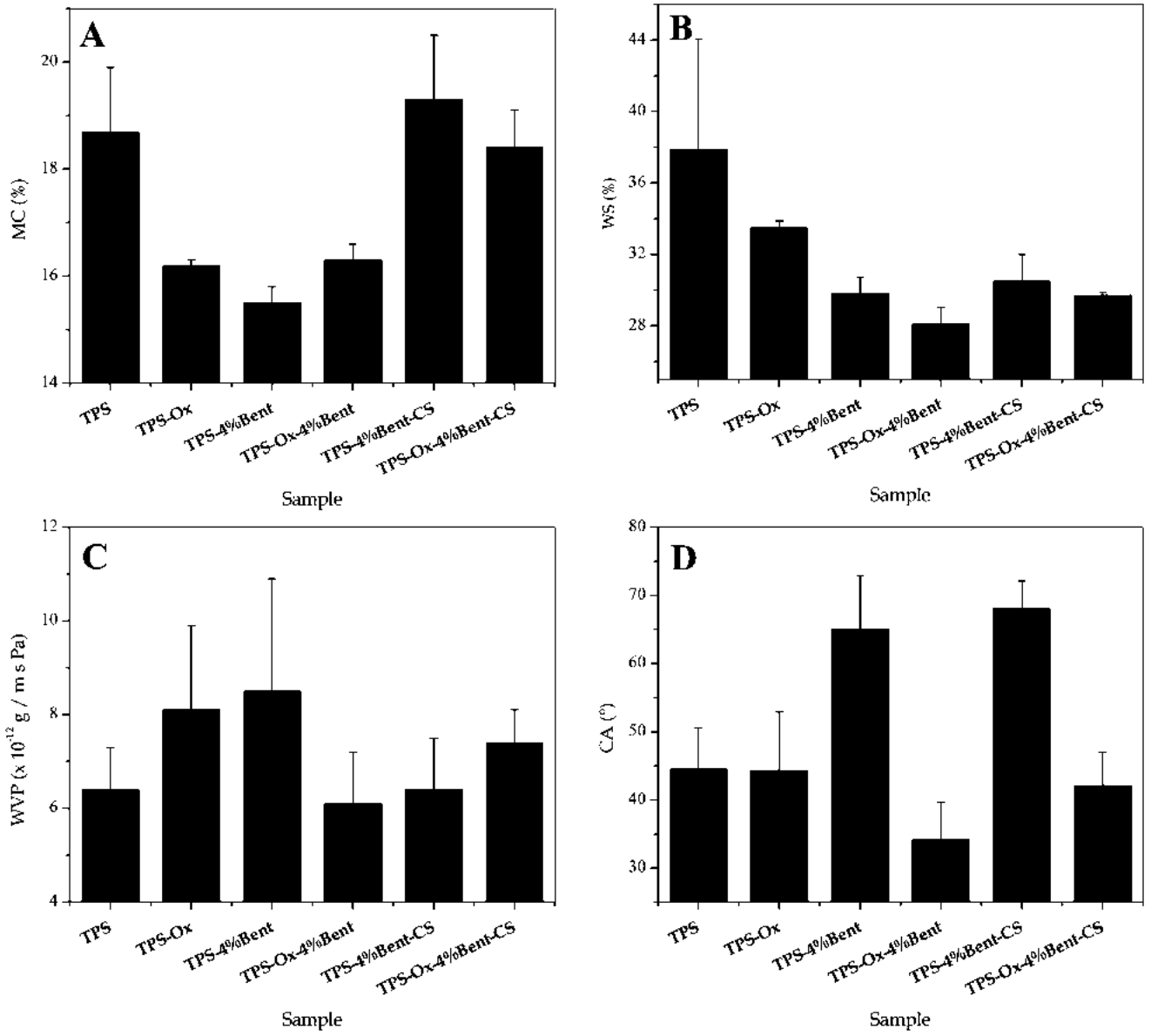

Figure 2. Film interactions with water. (A) MC, (B) WS, (C) WVP, and (D) CA of TPS, TPS-Ox matrices, and its nanocomposites with Bent and Bent-CS.

■ RESULTS AND DISCUSSION

Currently, the possibility of combining the benefits of mulch films with their biodegradation is a highly attractive and challenging goal. Since, on one hand, the biodegradability of a polymeric material requires a certain degree of hydrophilicity to occur in a reasonably short time,[5] and that, on the other hand, agricultural mulches must have a very low WVP to keep soil moisture,[38] the design of mulching materials results from the compromise between, at least, these two properties. Additionally, the wide variability associated with climatic conditions and rainfall that would eventually produce changes in soil moisture and mulch properties, making this process highly complex. The agricultural mulches fulfill the function of maintaining soil moisture, which means that the water is efficiently used, since they ideally act as a film that trap and retain water in soil, making it available for the plants when necessary.[39] Thus, in order to estimate the usefulness of the starch-based mulch films, it is important to know their interactions with water.

The determination of the MC of the films was carried out to study the effect of starch oxidation and the use of different nanoclays in the final material/water interactions. The results are presented in Figure 2A and indicate that the TPS-Ox matrix has an MC lower than that of TPS.

According to Pandey et al.,[40] the plasticization of starch increases its hydrophilicity since it breaks the hydrogen bonding interactions between the starch chains and exposes its hydroxyl groups, thus facilitating their interaction with water molecules. Therefore, the higher MC of TPS compared to TPS-Ox could indicate a higher degree of plasticization.

Regarding the nanocomposites, it was found that TPS-4% Bent has lower MC than the pure matrix, TPS, while TPS-4% Bent-CS did not present significant differences. According to Seligra et al.,[6] the amount of moisture present in the films is directly related to the presence of free OH groups. According to Cyras et al.,[15] the decrease in MC in nanocomposites may

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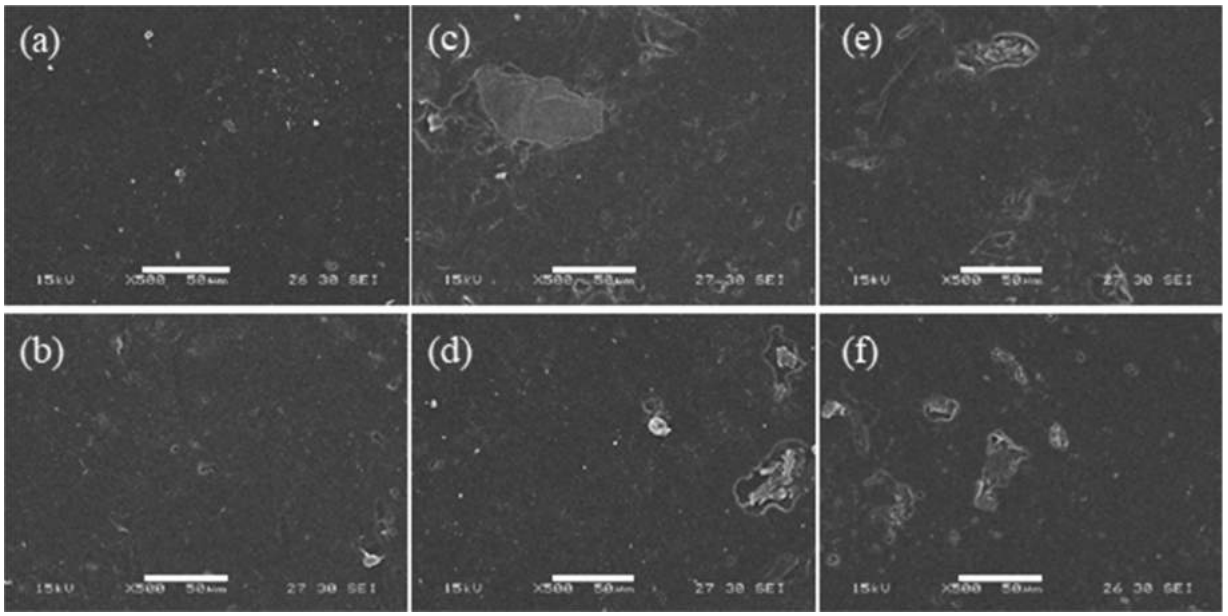

Figure 3. Micrographs of the surface of the films obtained by SEM for (a) TPS, (b) TPS-Ox, (c) TPS-4%Bent, (d) TPS-Ox-4%Bent, (e) TPS-Ox-4%Bent-CS, and (f) TPS-Ox-4%Bent-CS. Bar = 50 μm.

be due to the interaction by hydrogen bonds between the starch and the hydroxyl groups in the nanoclays, which form a network that hinders the diffusion of water molecules.

With regard to TPS-Ox matrix nanocomposites, no significant differences in MC were observed for TPS-Ox-4% Bent, but an increase in MC was observed for TPS-Ox-4% Bent-CS, probably due to the presence of OH groups of chitosan. These results suggest that there is interaction between matrices and reinforcements.

The WS of films is shown in Figure 2B. As it can be seen, there are no significant differences between the dissolution percentage of both TPS and TPS-Ox matrices. When considering the WS of nanocomposites, it was found that TPS-4%Bent presented a decrease in its solubility, while TPS-4%Bent-CS did not present statistically significant differences. For TPS-Ox4%Bent and TPS-Ox-4%Bent-CS nanocomposites, it was found that their solubility decreases. The possible interaction by hydrogen bonds between the matrices and the nanoclays used reinforces the stability of the nanocomposites compared with their matrices. Both hydroxyl and carboxyl groups can form hydrogen bonds with the hydroxyls of clays, resulting in these interactions, and a dense structure would be formed that would produce a decrease in solubility.[14] In this way, it can be concluded that the addition of both fillers to the TPS and TPS-Ox matrices improved the water resistance of the composite films.

Meanwhile, Figure 2C shows the WVP values of developed films. WVP is a key property that must be evaluated since it is expected that mulch films act by reducing the losses of water by evaporation and, in this way, keeping the soil moisture.[41]

As it can be seen, no significant differences were found in the WVP of the different matrices and their nanocomposites. Thus, the results suggest that there is not a good enough dispersion of the filler in the matrix to reduce the WVP of the nanocomposites. It is expected that, when the nanoclays are incorporated into a polymer, forming an exfoliated nanocomposite, the water molecules are forced to move through the material following a tortuous path around them, thus causing an increase in the path length increases and producing an improvement in the barrier properties of the material.[42] Therefore, compounds with an intercalated-exfoliated nanostructure would be expected to reduce their permeability.[43] Similar results were reported by Romero-Bastida et al.,[13] who have suggested that this is consequence of the greater starch−glycerol interaction, thus reducing the polar attractions between starch and clay and consequently the barrier effect of them.

On the other hand, the permeability values reported were lower than those obtained by Ma et al., -1.23×10^{-10} g Pa^{-1} s^{-1} m^{-1},[38] and Ao et al., -3.1×10^{-10} g Pa^{-1} s^{-1} m^{-1},[44], who developed different agricultural mulches on the basis of the waste from the fermentation industry, so that the starch-based films developed would offer a greater capacity for water retention. However, the starch, being a hydrophilic polymer, has a WVP relatively higher than that of the PE, so that the maintenance of humidity is relatively lower.[38] Nevertheless, in literature, it has been reported that biodegradable mulches that have a WVP higher than those of PE, presented fruit (grapes) and wood production, such as those obtained with any of the studied mulch modalities.[45] Similar results were found for the cultivation of strawberries.[41]

Meanwhile, the surface hydrophilicity of the films was evaluated by CA measurements with ultrapure water. Results are shown in Figure 2D.

As it can be seen, no significant differences in the surface polarity of the TPS and TPS-Ox films or in the nanocomposites TPS-Ox-4%Bent and TPS-Ox-4%Bent-CS with its matrix TPS-Ox were found. However, it was found that the TPS nanocomposites with Bent and Bent-CS led to an increase in the CA, which could be due to the roughness of the material. According to Medina et al.,[46] a greater roughness produces an increase in the CA and consequently in the hydrophobicity of the material. The surface of the developed materials was analyzed by SEM, and their results are described below.

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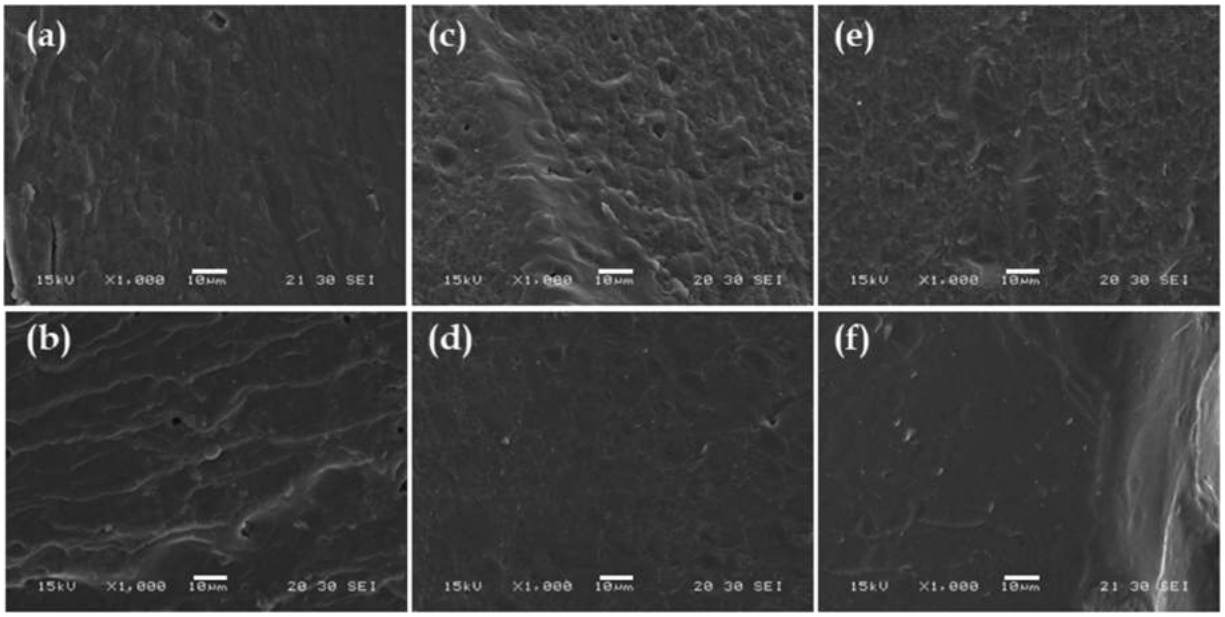

Figure 4. Micrographs of the cryofractured surface of the films obtained by SEM for (a) TPS, (b) TPS-Ox, (c) TPS-4%Bent, (d) TPS-Ox-4%Bent, (e) TPS-Ox-4%Bent-CS, and (f) TPS-Ox-4%Bent-CS. Bar = 10 μm.

Figure 3 shows the SEM micrographs of the surface of matrices and nanocomposites. These images were taken with the purpose of studying the superficial morphology of the films.

The surfaces of the TPS and TPS-Ox matrices are smooth and homogeneous, which suggest a complete gelatinization of the starch.[47] In the micrographs of nanocomposites with Bent and Bent-CS fillers, the presence of irregularities was observed. According to Cyras et al.,[15] these are attributed to the presence of poorly dispersed clay that forms agglomerates, as it was proposed after the WVP analysis. The presence of these irregularities on the surface of the nanocomposite films could also explain the results obtained from CA measurements.

In Figure 4, the SEM micrographs of the cryofractured surfaces of the developed materials are presented. In all cases, the cryofractured surface of TPS and its nanocomposites was smooth and homogeneous, while that of TPS-Ox and its nanocomposites was slightly rough. Additionally, a slightly porous morphology was found for films. According to Mitrus,[48] materials obtained from corn starch usually have some porosity as a result of the excessive expansion of the granule under pressure and temperature conditions suffered during processing.

In any case, the presence of typical granular starch structures was observed, thus suggesting a complete disruption of the granule during extrusion.[49] In the case of nanocomposites, clay aggregates were not observed, even at higher magnifications (not shown).

It is known that the passage of light through a given medium modifies the spectral distribution of the radiation that passes through it. Films used as agricultural mulches can prevent the passage of photosynthetically active radiation (400−700 nm), and thus, they can limit the light that reaches the ground, preventing weed growth and, consequently, the future competition with the crop for solar radiation and nutrients.[50] To determine the transmittance of UV and vis radiation of the developed materials, their transmission spectrum was measured in the wavelength range of 290−1100 nm. It was found from the spectra analysis, that films absorb UV radiation, with a wavelength up to 300 nm, by 99.99%, and more than 95% in the area of photosynthetically active radiation (400−700 nm),[51] depending on the particular film. In this way, developed films act as blockers of UV−vis radiation. After 400 nm, all of the spectra showed an increase in their transmittance by increasing the wavelength of the radiation.

The wavelength of 600 nm is commonly used to determine the transparency of films, according to the results shown in Figure 5 and bearing in mind that high values of transparency

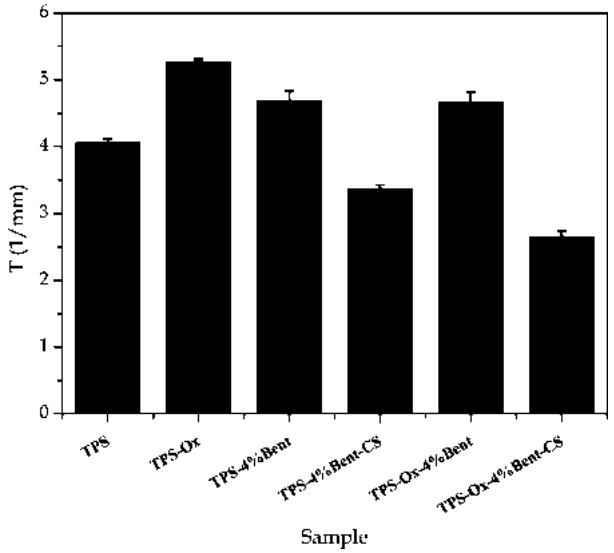

Figure 5. Transparency of TPS, TPS-Ox, and its nanocomposites with Bent and Bent-CS.

refer to more opaque materials. The lower transparency or greater opacity was obtained for the TPS-Ox film. In general, the addition of Bent and Bent-CS produced a decrease in the opacity of the films, but this was more significant for the TPS-Ox matrix. Only an increase in the opacity of the TPS films

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Table 2. Direct Transmissivity Coefficients in the Photosynthetically Active Radiation Region, τ_{PAR}

materials	TPS	TPS-Ox	TPS-4%Bent	TPS-4%Bent-CS	TPS-Ox-4%Bent	TPS-Ox-4%Bent-CS
τ_{PAR} (%)	0.18	0.07	0.19	0.39	2.72	1.32

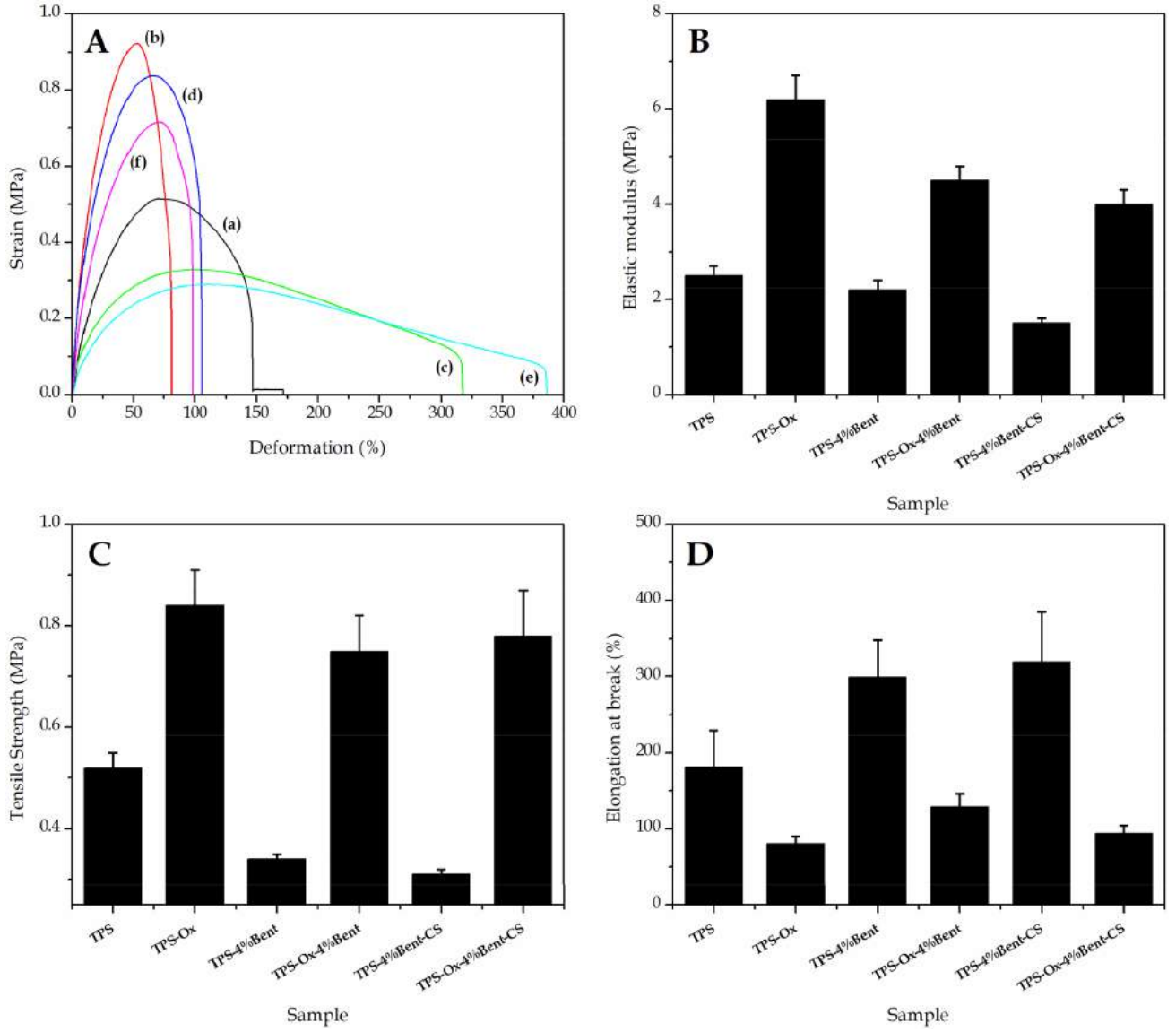

Figure 6. Mechanical properties. (A) Strain curves (MPa) vs deformation (%) for (a) TPS, (b) TPS-Ox, (c) TPS-4%Bent, (d) TPS-Ox-4%Bent, (e) TPS-4%Bent-CS, and (f) TPS-Ox-4%Bent-CS. (B) Elastic modulus (MPa), (C) tensile strength (MPa), and (D) elongation at break (%) for TPS, TPS-Ox, TPS-4%Bent, TPS-Ox-4%Bent, TPS-4%Bent-CS, and TPS-Ox-4%Bent-CS.

with 4% (w/w) Bent was observed with respect to the TPS matrix.

Similar results were obtained by Slavutsky et al.[52] for corn starch films reinforced with Mnt. These authors observed that the exfoliated nanocomposites showed a decrease in the opacity of their films, and that, when the nanocomposites were intercalated, they gave rise to higher values than those of the unfilled matrix. In this way, the results suggest that Bent has a better interaction with TPS-Ox than with TPS, as well as in all cases, the nanoclay Bent-CS presented a better dispersion than Bent in the native and modified matrices. However, Heydari et al.[53] reported opposite results for the opacity of corn starch nanocomposites with different Mnt contents. Bharadwaj et al.[54] sustained that the degree of dispersion and the orientation of the clay sheets determine the optical clarity of the films. They argued that if the dispersion is good and there are exfoliated nanocomposites, there is good optical clarity.

Meanwhile, Heydari et al. and Rhim[53,55] described that the transmittance of the films drops with the addition of nanoclays, so that these become more opaque, indicating that the clay is not completely dispersed, and that it forms agglomerates, which prevents the passage of light. As Rhim[55] indicates, the transparency of nanocomposites would be expected not to change with the addition of nanoclays as long as they are well dispersed and exfoliated, since these have a thickness less than the wavelength of the radiation and do not prevent the step of light.

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Direct transmissivity coefficients of developed materials in the PAR range were obtained by using eq 5. Results included in Table 2 indicated that mulches presented potential results for the control of weeds. Scarascia-Mugnozza et al.[56] informed values higher than 5% for different biodegradable agricultural mulch films. As reported by Briassoulis and Giannoulis,[57] the measured coefficients satisfy the requirements introduced in the project of standard EN 17033,[58] which considers a relative light transmission inferior of 3% for black or opaque mulches.

As a result of the loads that are applied to the material during the manual or mechanical application of the mulches, the films are subject to stresses and deformations.[59] As described by Briassoulis,[59] it is important that agricultural mulches maintain their integrity during this process and preferably throughout their useful life.

As shown in Figure 6, oxidation of the TPS matrix to give TPS-Ox produced a statistically significant increase in Young's modulus and tensile strength with a decrease in elongation at break. These results could be explained by considering that in TPS there are a greater number of starch−glycerol interactions and a lower number of starch−starch interactions compared to what occurs in TPS-Ox. These results suggest that an antiplasticizing effect could occur in the TPS-Ox matrix, such that there would be a strong oxidized starch−oxidized starch interaction and therefore, an increase of the Young's modulus and the maximum tension would be observed.[60,61] In TPS, the plasticizing effect promotes the decrease of these parameters and the increase of elongation at break. The latter is due to the fact that the plasticizer produces the disruption of the starch−starch interactions allowing the mobility of its chains and thus reducing the rigidity of its films.[62] When the plasticizer agent cannot fulfill that function, a phase separation is generated where there are highly plasticized zones, rich in glycerol, and starch rich areas, and the latter interact strongly producing a reduction in the elongation at break.[62]

Previously, when talking about the plasticizer we always refer to glycerol, which was added for that purpose. However, the present water molecules also act as plasticizers and, for this reason, to carry out a more accurate analysis, the MC present in the films must also be considered.[63] The MC of TPS was higher than that of TPS-Ox, and in this way, the mechanical properties of the TPS and TPS-Ox matrices present the expected results when considering a greater plasticization.

The results of the uniaxial tensile tests for the materials described above indicated that they present an anomalous behavior. Generally, in the polymeric nanocomposites reinforced with clays of the Mnt type, an increase in the modulus and the tensile strength and a decrease in the elongation at break are usually observed.

According to Huang et al.,[63] a part of the hydrogen bond interactions between the starch chains and between starch and glycerol breaks down, and new interactions are formed by hydrogen bonding with the hydroxyl of Mnt, providing an improvement in stiffness and strength and exerting an obstructive action for the sliding of polymer chains during elongation. Additionally, other factors that affect the mechanical properties of nanocomposites are the degree of intercalation of the polymer in the galleries of the clay and the degree of dispersion of the latter in the matrix. The rigidity of the material increases with the interlaminar spacing of the clay, and therefore the exfoliated nanocomposites present the greatest increases in modulus.[40,64] On the other hand, an increase in the tensile strength indicates that the plasticization is sufficient to allow the movement of the segments of the starch chains.[40] According to Colivet and Carvalho,[65] an increase in the elongation at break occurs when the mobility of the chains increases in the amorphous region of the film, and as mentioned above, this is related to the MC of the matrix. To increase the elongation at break by the presence of water molecules, a decrease in the modulus should be observed since it acts as a plasticizer.[49] When comparing the results of uniaxial traction with those of MC for the nanocomposites, it was found that both results well correlate.

Chung et al.[49] worked with corn starch nanocomposites reinforced with sodium Mnt and Mnt previously modified with CS, both nanocomposites obtained by casting and subsequent hot pressing. In their work, they reported important improvements in the Young's modulus and the tensile strength of starch nanocomposites with sodium Mnt, attributing this to the good dispersion of the clay in the starch matrix. On the contrary, they found that the Young's modulus and the tensile strength of the CS-modified Mnt nanocomposites were not significantly different from those of the pure starch matrix and justified this difference considering the presence of large agglomerates of modified Mnt with CS, which reflects the greatest difficulty in dispersing the modified clay, given the strong interaction between CS and clay.

The results obtained in this work indicate that oxidized starch chains in TPS-Ox do not interact well enough with glycerol as native starch does, TPS, and an antiplasticizing effect may occur. Then, the addition of the Bent and Bent-CS nanoclays to both matrices did not produce changes or produced a decrease in the mechanical properties of the nanocomposites. This fact can be attributed to a low interaction of the fillers with the matrices, which produces an insufficient intercalation of the matrix and a consequent poor dispersion of the filler.

Regarding the Young's modulus, strength, and elongation values obtained for the materials analyzed, it was found that these were much lower than those reported by Briassolius[7] for Mater-Bi and PE mulches. However, as Briassoulis[59] explains, the suggested minimum values, included in the European Standard EN 13655,[66] that these materials should present have been determined arbitrarily, and they are not related with the conditions to which the films will be exposed. Although its mechanical properties can be defined by a standard. such as PE, it is known in advance the success of its functions because these properties tend to be much higher than those actually required.[67] In fact, it has been previously reported in the literature that although biodegradable mulches begin to break before the end of the growing season, they still fulfill the desired function.[45] The previously mentioned, although it would be in favor of the use of agricultural mulches with properties inferior to those of PE, does not reach to justify the properties found for the developed starch mulches since these are well below the values that are used for these functions. It is still necessary to continue working in this direction to improve the properties of these films, probably through the formation of mixtures with other polymers, always taking into account the biodegradability and the possibility of including new functionalities tending to improve the use of resources and care the environment.

Finally, the results of the antibacterial activity of thermoplastic starch films are shown in Figure 7. Approximately 20 mg of each film was incubated with *Psy* cell cultures for 24 h, and then the colony forming units were counted. As shown in

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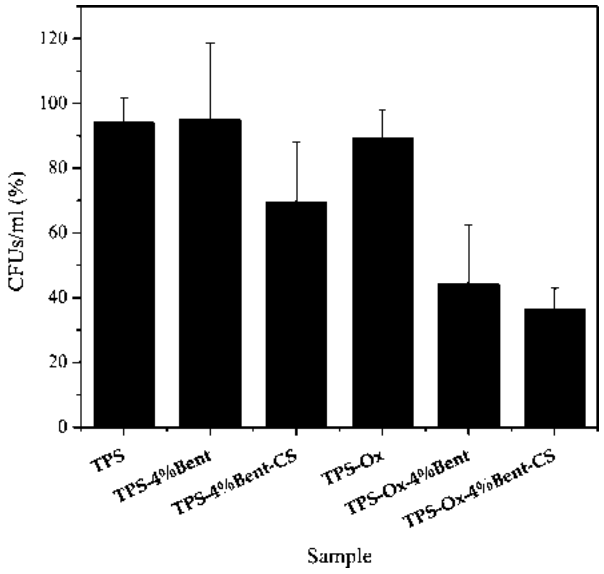

Figure 7. Antibacterial activity of the different thermoplastic starch films assayed on *Psy*. Cells suspended in KB medium containing 20 mg of each film were incubated with shaking for 24 h at 30 °C. The number of CFU for each experimental condition was then quantified. The results were expressed as a percentage with respect to the control treatment without film. Data are mean values (±SD) of three independent experiments.

Figure 7, the addition of Bent-CS to starch films slightly inhibits the growth of *Psy*. However, oxidized starch with Bent and Bent-CS produced a significant inhibition of the bacterial growth, being higher in TPS-Ox-4%Bent-CS-treated cells. The antimicrobial activity of Bent-CS nanoclay was previously demonstrated onto two phytopathogens, *Psy* and the necrotrophic fungus *Fusarium solani* f. sp. *Eumartii* (Merino et al.[24]). Nevertheless, the inclusion of Bent-CS in starch films can affect the antibacterial activity by decreasing the available amino groups of CS capable of interacting with negatively charged surface components of many fungi and bacteria. Similarly, Vásconez et al.[68] demonstrated that the antimicrobial activity of CS was reduced by starch in chitosan−tapioca starch-based edible films.[68] The antimicrobial activity was greater in the oxidized starch films than in the native ones. Moreno et al.[69] described the influence of starch oxidation on the functionality of gelatin-based films. They found that films with oxidized starch also showed bactericidal effect against *Escherichia coli* and *Listeria innocua*, due to the antimicrobial properties of Maillard compounds obtained by the reaction between carbonyls of reducing sugar and protein amino groups.[70,71]

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Notes

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ABBREVIATIONS

Mnt: montmorillonite
Bent: bentonite
CS: chitosan
TPS: thermoplastic starch
TPS-Ox: oxidized thermoplastic starch
MC: moisture content
WS: water solubility
WVP: water vapor permeability
CA: contact angle
T: transparency

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