

Chitosan coated-phosphorylated starch films: Water interaction, transparency and antibacterial properties

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ABSTRACT

Since there is a current need for developing biodegradable mulch films that keep the advantages of those manufactured with polyethylene but offering earlier biodegradation, new starch-based formulations were proposed and analyzed. In this work, starch was chemically modified by phosphorylation with sodium triphosphate and resulting films were later coated with chitosan. Starch phosphorylation was performed by two different methodologies: chemical modification in aqueous suspension (AS) and reactive extrusion (REx). The content of phosphorus and the degree of substitution (DS) were determined and calculated, respectively, for the purpose of evaluating the efficiency of the phosphorylation reaction. This was used as a reference to analyze its incidence on the structure of the materials tested. Fourier Transform infrared spectroscopy (FTIR) and X ray diffraction (XRD) techniques were used to analyze chemical and structural modifications of the starch. Results show that higher DS was achieved by REx method and that was supported by the analysis of FTIR and XRD spectra. Films characterization indicates that phosphorylation by REx contributed to lower film moisture content and to increase opacity and antimicrobial activity. With regard chitosan (CS) coating, SEM micrographs indicate that it presented a porous structure that produces an increment in hydrophilicity, opacity and antimicrobial activity.

1. Introduction

The development of biodegradable agricultural mulch films is highly attractive and necessary in order to replace the conventional polyethylene (PE) mulch films. The latter, are degradable in at least 100 years, time that exceeds their life use [1]. The use of PE mulch films offers several advantages to farmers: they produce an increase in yields and accelerate crop harvesting time by keeping a proper soil moisture and raising soil temperature [2]. Moreover, mulch films contribute to reduce the amount of herbicide utilization, since they filter the photosynthetically active radiation (PAR) and thus, prevent weed growth [1]. However, the main disadvantages of their use are the economic costs that imply their collection after harvesting and the disposal of great amounts of agrochemically-polluted PE [3]. Recent publications have described that the long-time usage of non-biodegradable mulching can produce soil deterioration [4,5]. At the same time, current trends argue

that the agricultural sector will be required in the coming years due to the continuous increase in the world population and the consequent need to increase food production [6]. This way, the development of new agro-inputs and innovative technologies that let to increase yields, the cultivation of poor soils and that allow to care for the environment and resources, such as water and soils, are urgently required [6]. In this context, starch appears as an interesting polymer that can be used as base of new mulch formulations. Starch is cheap, highly and naturally abundant, and can be processed in a continuous and traditional way in order to obtain thermoplastic films [7]. Their main disadvantages such as, poor mechanical properties and high hydrophilicity can be at least partially overcome by adopting different strategies [8].

Crosslinking reactions are widely used in the chemical modification of starch. In particular, phosphorylation allows to generate intra and intermolecular bonds, and in this way contributes to decrease the rate of retrogradation of their films [9]. In addition, crosslinking through

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phosphate groups is highly attractive for the application of thermoplastic starch (TPS) films as biodegradable and functional agricultural mulches. Plants require large amounts of phosphorus for their growth and development and it is expected that it could be available for plants once the mulch has been biodegraded [10].

Starch phosphorylation can lead to the formation of mono-starch and di-starch phosphates. The latter are obtained when two hydroxyl groups are esterified by the same phosphate [11]. Phosphorylation can occur in any of the carbons C-2, C-3 and C-6 and both amylose and amylopectin can react, although the former has a lower tendency towards crosslinking [9]. The most commonly used crosslinking agents are sodium tripolyphosphate (TPP), sodium trimetaphosphate (TMP), phosphoryl chloride and epichlorohydrin. The last one is highly toxic and therefore its use is becoming less frequent [12].

Starch phosphates can be obtained by different methodologies. One of them, contemplates their modification in dry and consists in heating a reactive mixture of starch, sodium sulfate and TPP, or TMP, at a predetermined pH [11]. Another approach is the modification of starch in aqueous solution. For this purpose, starch dispersion is generally used together with TPP, TMP or a mixture of both, a catalyst such as Na_2SO_4 and the pH, temperature and stirring time are controlled. Finally, the mixture is dried in an oven at 40 °C [13]. Nevertheless, these techniques have great disadvantages when compared to extrusion. Modification in solution involves using large volumes of water and amount of salts (10–30% w/w) to inhibit the gelatinization of the starch and be able to work in a wider range of temperatures. Additionally, the residence time in the reactor can be between 2 and 24 h. These requirements make the process industrially expensive [14]. On the other hand, the dry-mixing followed by oven-heating method usually gives low yields of phosphorylation [13]. Finally, starch phosphates can be obtained by reactive extrusion (REx). The extrusion technique itself offers numerous advantages over other methods of starch modification that must finally be processed by this technique to obtain the TPS [14]. REx allows incorporate perfectly mixed reagents with starch in the extruder and by the temperature, pressure and shear allows the reaction progress simultaneously with the processing of the material [14,15].

The objective of this work was to develop and study the properties of native and phosphorylated thermoplastic corn starch films obtained in two different ways: modification in aqueous suspension with subsequent extrusion and compression molding; and phosphorylation by REx and subsequent compression molding. Additionally, the effect of a chitosan coating on each of the materials developed was studied. CS is a linear chain cationic polymer derived from chitin, the second most abundant polymer in nature [16,17]. It has numerous properties that make it highly attractive for its application in agriculture, among which its antibacterial, antifungal and biostimulant capacity stand out [18]. The interaction between starch-based films and water was studied by measuring its moisture content, moisture absorption, solubility and contact angle. In addition, its superficial morphology was observed by scanning electron microscopy (SEM) and was related with water interaction results. The transparency of each film was also determined by measuring its light transmittance. Antibacterial properties of starch-based films were evaluated against *Pseudomonas syringae* pv. *tomato* DC3000 (*Psy*), a phytopathogenic bacterium that affect tomato crop [19].

It should be noted that the novelty of this research work does not lie in the methodology of phosphorylation by wet chemistry. The novelty of this work was centered in the comparison of the CS-coated modified TPS by means of two methodologies: a traditional one by means of wet chemistry and another one by REx. This last methodology can be considered as a green methodology, since it is energy efficient and does not require water consumption [20]. In particular, the phosphorylation reaction was selected with a view to the application of these materials as agricultural mulch films, which once biodegraded can enrich the soil in a mineral such as phosphorus, which is beneficial for the growth of food crops. Besides, the surface of these materials was covered with

chitosan with the aim of giving antimicrobial protection to the food crops in an ecological way. Thus, the proposed material formulation for application in agriculture as biodegradable mulches has not been previously published and represents an innovative approach for the replacement of traditional ones.

2. Experimental section

2.1. Preparation of phosphorylated corn starch in aqueous suspension

The chemical modification of corn starch was carried out according to the procedure reported by Gutiérrez et al. [21] with some modifications. Briefly, 4 l of distilled water was placed in a 10 l capacity reactor and 1 kg of corn starch was dispersed using stirring at 150 rpm. Then 34.90 g of 0.05 M K_2SO_4 were added and the pH of the dispersion was brought to 11 with 1 M NaOH. Then, 30 g of TPP (3% w/w) was added. Reaction conditions such as pH and phosphate forming agent were chosen as reported by other authors. According to O'Brien et al. [22] pH 11 favors the formation of di-starch phosphates and according to Moad [14], TPP gives better yields than SMP. Once all the reagents were added, the mixture was heated to 45 °C and the stirring was maintained for 3 h. After this time, 2% (v/v) HCl was added to set the pH at 7.0 and the modified starch was allowed to decant for later filtering and drying in an oven at 40 °C for 48 h.

2.2. Preparation of phosphorylated corn starch via reactive extrusion (REx)

The preparation of corn starch phosphate films by REx was carried out by processing a mixture of corn starch with 20% (w/w) glycerol and 10% (w/w) water as plasticizing agents. The water used as a plasticizer was further used to dissolve TPP and K_2SO_4 in the same concentrations used for the modification in solution 3% (w/w) and 0.05 M, respectively and the pH was brought to 11 using 1 M NaOH. The employed extrusion conditions are detailed below.

2.3. Preparation of thermoplastic films

The following films were obtained by extrusion and subsequent compression molding: thermoplastic starch (TPS), TPS phosphorylated in aqueous suspension (TPS-P-AS) and TPS phosphorylated by REx (TPS-P-REx). For that, an Extrualux Doble Argentina (Buenos Aires, Argentina) double screw extruder with six heating zones was used. In each case, a mixture of distilled water 10% (w/w) and 20% (w/w) glycerol was used as plasticizer, the screw speed was set at 130 rpm and the following temperature profile was used: 90/100/105/110/120/120 °C. According to Salay et al. [23] subsequent temperature increments (130–175 °C) decrease the amount of phosphate bound to the starch, so that the temperature was kept below these values.

Once extruded, approximately 90 g of each material were pressed using a hydraulic press at 130 °C and 70 Bar for 20 min. The mold was cooled by circulating water and was not opened until its temperature reached 40 °C. An aluminum mold of 30 cm × 30 cm and 0.2 mm of thickness was used to obtain each film.

2.4. CS coating

The TPS, TPS-P-AS and TPS-P-REx films were placed on aluminum foil under a hood and then a solution of CS at 1% (w/v) in 1% (v/v) acetic acid was poured over them and spread over the entire surface with the help of a glass rod. Once dry, the same procedure was carried out on the other side of the films. The resulted samples were labeled TPS-CS, TPS-P-AS-CS and TPS-P-REx-CS and were stored in polyethylene bags at 60% RH.

2.5. Characterization techniques

2.5.1. Determination of phosphorus content and degree of substitution (DS)

The determination of phosphorus content was made following the procedure described at IRAM 15013 [24]. The degree of substitution was then calculated as presented in Eqs. (1) and (2) for the formation of mono- and di-starch phosphates [25], respectively.

$$\text{DS}(\text{mono-starch phosphate}) = \frac{162\%P}{3100 - 97\%P} \quad (1)$$

$$\text{DS}(\text{di-starch phosphate}) = \frac{324\%P}{3100 - 96\%P} \quad (2)$$

where %P is the percentage of phosphorus determined.

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra were measured by attenuated total reflectance (ATR). They were acquired with a Nicolet 6700 Thermo Scientific instrument, in the range of 500 to 4000 cm^{-1} , with 32 scans at a resolution of 4 cm^{-1} and room temperature.

2.5.3. X ray diffraction (XRD)

XRD patterns were acquired in a PANalytical X-Pert Pro diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV and 40 mA. All measurements were conducted at room temperature in the 2–33° (2 θ) range at scanning speed of 1°/min. Crystallinity percentage, X_c (%), was calculated according to Eq. (3), as reported previously by Hermans&Weidinger [26].

$$X_c (\%) = \frac{A_c}{A_T} 100\% \quad (3)$$

where A_c is the area under the crystalline section of the spectrum and, A_T , is the total area under the diffractogram.

2.5.4. Moisture content (MC)

The moisture content was determined thermogravimetrically in samples of approximately 0.5 g placed in an oven at 100 °C for 24 h. The procedure was performed in triplicate for each sample and the moisture content was calculated using Eq. (4):

$$\%MC = \frac{w_i - w_f}{w_i} \times 100\% \quad (4)$$

where w_i and w_f are the initial and final weights of each sample. The results were expressed as mean \pm standard deviation.

2.5.5. Moisture absorption (MA)

Samples of 1 cm \times 1 cm were cut out and dried in an oven at 40 °C for 48 h. Next, the initial weight of each sample was recorded and placed in moisture controlled containers. The moisture absorption of the films was studied at room temperature and in two different environments: at 60% RH, prepared using an aqueous glycerol solution at 72% (w/w); and at 90% RH, prepared using an aqueous solution of 34% (w/w) glycerol. The mass of each sample was recorded regularly for two weeks and it was analyzed in triplicate. The mass gain was determined using Eq. (5):

$$MA (\%) = \frac{w_t - w_0}{w_0} \times 100\% \quad (5)$$

where w_t is the weight of the sample at time of exposure t and w_0 , is the initial weight of the dry sample. The results were expressed as MA_{eq} (%) \pm standard deviation, being MA_{eq} (%), the equilibrium moisture absorption, taken as the maximum value registered for each sample.

2.5.6. Water solubility (WS)

The dissolution percentage of the films was determined gravimetrically. Approximately 0.5 g of each sample was dried in an oven at 100 °C for 24 h. After that, its initial mass was recorded and the sample

was placed in 50 ml of distilled water at room temperature (22 °C) for 24 h. Then, the solution was removed and the remaining films were placed in an oven at 100 °C for 24 h. Finally, its final weight was recorded and the solubility percentage (% WS) was determined using Eq. (6):

$$\%WS = \frac{w_i - w_f}{w} \times 100\% \quad (6)$$

where w_i and w_f are the initial and final weights of each sample. The experiment was carried out in triplicate and the results were expressed as mean \pm standard deviation.

2.5.7. Contact angle (CA)

Contact angles (θ) were measured using a Ramé-Hart goniometer model 100–25–4 by placing a drop of 5 μl of ultra-pure water (Merck Millipore) on the surface of each film and by using the software “Drop image advanced”. The experiments were carried out at 25 °C and at least 5 measurements per sample were carried out. Results are presented as average \pm standard deviation.

2.5.8. Scanning electron microscopy (SEM)

The surface morphology at the top of each sample was observed with a JEV JSM-6460 LV microscope. Small pieces of each film were placed on an aluminum sample holder and coated with a thin layer of gold to ensure electrical conductivity.

2.5.9. Transparency (T)

The transparency was determined using an Agilent 8453 UV–Visible spectrophotometer. The analyzed films were cut to a length of 4 cm and 2 cm in width and fixed to the measuring cell with adhesive tape. Then, the transmittance spectrum was recorded in the wavelength range from 290 to 1100 nm and the transmittance at 600 nm was employed to obtain the transparency according to Eq. (7) [27].

$$T = \frac{-\log T_{600}}{x} \quad (7)$$

where T is the transparency, T_{600} is the transmittance at 600 nm and x is the average thickness of the films in mm.

2.5.10. 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 [56] containing suitable antibiotics, 50 $\mu\text{g ml}^{-1}$ rifampicin and 50 $\mu\text{g ml}^{-1}$ kanamycin according to Mansilla et al. [19]. Small pieces of each film (20 mg) were incubated with cell cultures in an exponential phase (10^6 cells ml^{-1}) for 24 h at 30 °C. Once this time had elapsed, an aliquot of the cultures was extracted, diluted appropriately into fresh KB medium, and plated on KB-agar medium. Colonies were counted after incubation at 30 °C overnight, and the number of CFU in the original culture was calculated from dilution factors. The results were expressed as a percentage with respect to the treatment with TPS. Three independent experiments were performed.

3. Results

3.1. Chemical and structural characterization

The phosphorus content of native and phosphorylated thermoplastic starch (TPS) films (in aqueous suspension and by reactive extrusion - REx), and the degree of substitution by formation of mono- and di-starch phosphates are presented in Table 1.

As can be seen, the REx proved to be more effective than the aqueous suspension phosphorylation method. Similar results were previously reported by Landerito and Wang [13], who worked on the phosphorylation of starch by various techniques and found that the

Table 1
Phosphorous content and degree of substitution (DS) for TPS, TPS-P-AS and TPS-P-REx.

	TPS	TPS-P-AS	TPS-P-REx
P (%p/p)	0.01	0.06	0.39
DS (Mono-ester)	$5E^{-4}$	$3E^{-3}$	0.02
DS (Di-ester)	$1E^{-3}$	$6E^{-3}$	0.04

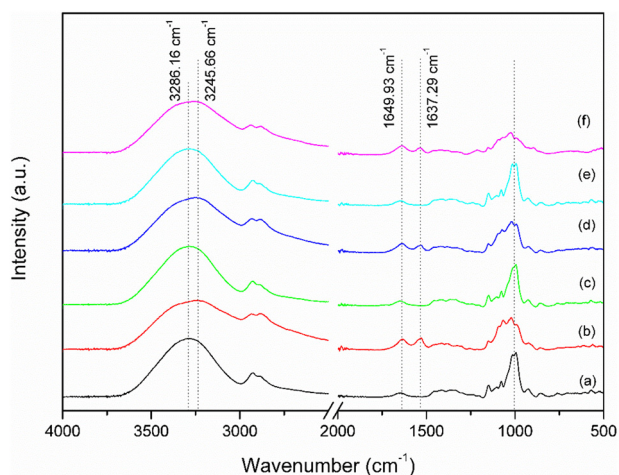


Fig. 1. Infrared absorption spectra of (a) TPS, (b) TPS-CS, (c) TPS-P-AS, (d) TPS-P-AS-CS, (e) TPS-P-REx and (f) TPS-P-REx-CS, obtained by ATR-FTIR in the range of 4000 to 500 cm^{-1} .

modification by REx was more rapid and effective than the methodologies of modification in suspension and oven-heating. Moreover, and according to expectations, it was found that in all cases the degree of substitution by formation of di-starch phosphates was higher than mono-starch phosphates, indicating that have been formed covalent bonds between the starch chains [28]. The values obtained are in the order of those reported by Seker et al. [29] and are higher than those reported by Deetae et al. [25] for the phosphorylation of rice starch.

In order to identify the functional groups associated with the chemical modification of the starch and its coating, the infrared absorption spectra were measured (Fig. 1). The main bands observed, together with their interpretation, are included in Table 2.

According to Table 2, the analyzed samples of TPS, TPS-P-AS and TPS-P-REx, showed the characteristic absorption bands of corn starch. Phosphorylation, either by aqueous suspension or by REx, did not produced significant changes in the appearance of the infrared spectra. The presence of the phosphate groups is identified by the band associated with the stretching of the P=O group that appears approximately at 1244 cm^{-1} [30]. However, the appearance of this band has not been observed, probably due to that the phosphorus concentrations in the modified materials are very small and that the band is expected to appear in the fingerprint area, so it could be probably overlapped

Table 2
Wavenumber at the maximum of the infrared radiation absorption band of the developed materials.

Functional group	TPS	TPS-Q	TPS-P-AS	TPS-P-AS-CS	TPS-P-REx	TPS-P-REx-CS
O-H Stretch of starch, glycerol and water (cm^{-1}) [31,39]	3286.2	3245.7	3286.2	3245.7	3286.2	3245.7
Stretching of C–H in CH_2 (cm^{-1}) [39]	2927.5	2935.2	2927.5	2935.2	2927.5	2942.9
O-H bending adsorbed water (cm^{-1}) [31,42]	1646.9	1637.3	1646.9	1637.3	1646.9	1637.3
C-H bending in CH_2 (cm^{-1}) [39,54]	1365.4	1382.7	1365.4	1382.7	1365.4	1382.7
Stretching of C–O in C-OH (cm^{-1}) [34,39]	1149.4; 1078.0	–	1149.4; 1078.0	–	1149.4; 1078.0	–
Stretching of C–O in C-OC (cm^{-1}) [31,34,54]	1014.4	–	1014.4	–	1014.4	–
Stretching of P=O (cm^{-1}) [30]	–	–	–	–	–	–
N-H bending (Amide II) (cm^{-1}) [33,55]	–	1529.3	–	1533.2	–	1535.1
C=O stretch (Amide I) (cm^{-1}) [33,55]	–	1637.3	–	1637.3	–	1637.3

with other bands. Similar results were found by Galicia-García et al., 2011 [15], who worked on the phosphorylation of corn starch and did not observe this band in their starches.

According to Seligra et al., 2016 [31] the ratio of intensities at the maximum absorption of the O–H group (3286 cm^{-1}) and the band at 1149 cm^{-1} , related to the stretching of the C–O bond in C-OH, gives information about the amount of available hydroxyls in each system. The intensity quotient for TPS, TPS-P-AS and TPS-P-REx gave 4.69, 4.60 and 3.79, respectively. As can be seen, there were no large differences between TPS and TPS-P-AS and, instead, a large decrease in the number of free O–H was observed, which is in line with the results of DS.

According to Sevenou et al. [32], the ATR-FTIR technique is a surface analytical method that allows obtaining short-range order information in starch samples since the penetration of the beam is approximately $2 \mu\text{m}$. In this way, the films that were coated with chitosan presented only their absorption bands. The one corresponding to the stretching of the N–H groups was overlapped with the absorption band of the O–H and appeared approximately at 3245 cm^{-1} [33]. The rest of the typical bands of chitosan are shown in Table 2. Additionally, it was observed that the peak of adsorbed water, which appears at 1646.93 cm^{-1} in the starch, runs towards lower wavenumbers in the films with chitosan coating (1637.29 cm^{-1}) which suggests that the interaction with this one it is even stronger [34].

Starch samples prepared by extrusion and subsequent compression molding may retain a part of the natural crystallinity of the granule, as a result of incomplete gelatinization of the starch, or they may present a new type of crystallinity associated with retrogradation of the starch after processing. Fig. 2 shows the diffractograms of the developed films and their percentages of crystallinity calculated using Eq. (3).

All the systems showed mainly an amorphous fraction with a small crystalline portion in agreement with the diffractograms previously reported in the literature [35]. No differences were found in diffraction peaks positions for the coated and uncoated samples with chitosan. All the developed materials showed characteristic crystallinity patterns of type A, common of cereals [36], and of type V_H , typical of starches whose conformations in double helices have been interrupted due to the presence of glycerol (plasticizer) and formed new simple helical glycerol-amylose complexes [35]. In this way, the peaks that appear at $2\theta \sim 13.4$ and 19.8° (6.5 and 4.4 \AA) are attributed to the organization in V_H structures, while those that appear in $2\theta \sim 17.3$ and 22.2° or 5.1 and 3.9 \AA , respectively, to a type A structure [15,36]. V_H type structures are typical of retrogradation and type A structures may be due to incomplete starch gelatinization and also, to retrogradation [7].

Muhrbeck et al., 1991 [37] studied the effect of the degree of phosphorylation on the crystallinity of native potato starch. This starch possesses ester phosphates directly attached to amylopectin molecules. The phosphorus content is approximately 1 phosphate per 200 glucose units ($\sim 0.1\%w/w$). In their work, these authors reported that the degree of phosphorylation is inversely proportional to the crystallinity and that it would be related to the carbon to which the phosphate group is attached. When this group is attached to the C_6 atom, which has an

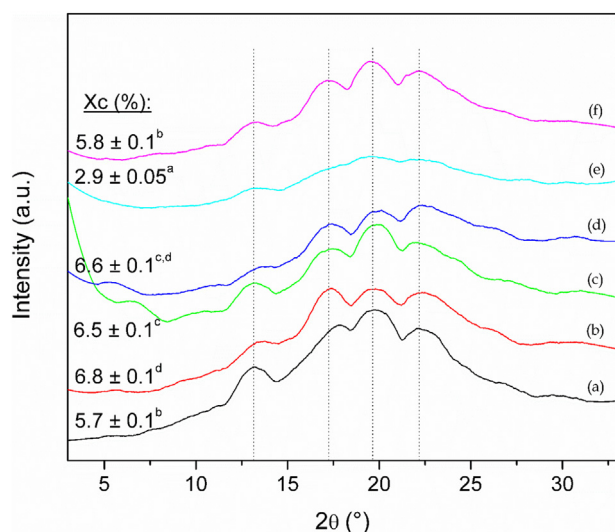


Fig. 2. X-ray diffraction patterns of (a) TPS, (b) TPS-CS, (c) TPS-P-AS, (d) TPS-P-AS-CS, (e) TPS-P-REx and (f) TPS-P-REx-CS, obtained by XRD in the range of 3 to 33°.

outward orientation in the alpha helices, it interferes with the crystallization of amylopectin. On the contrary, the phosphates in C₃ are located towards the interior of the alpha helices and it is believed that they do not interfere with the crystalline structure. At this work, it was found that the phosphorylation of corn starch by REx produced a significant decrease in the crystallinity of the starch which suggests that the modification has been more effective as found by the analysis of the phosphorus content and DS. In the same way, only a slight increase in the crystallinity of TPS-P-AS with respect to TPS was observed by XRD and is correlated with a small increase in the degree of substitution. However, when analyzing the same materials but with chitosan coatings, it was found that the crystallinity of each of them, but mainly that of TPS-P-REx-CS, increased remarkably, which may be due to the fact that during the coating process, the amylose and amylopectin of the starch in contact with the aqueous solution of chitosan could acquire greater mobility and, consequently, accelerate its retrogradation, in such a way that the coated films presented similar crystallinity [38]. In other words, during the coating process, the samples are exposed to an aqueous medium containing chitosan, so the hydrophilic starch absorbs part of this moisture, producing an increment in the free volume of the material and causing the molecules to acquire mobility to organize themselves in a crystalline structure [39].

Additionally, Gutierrez et al., 2015 [35] claim that an increase in the crystallinity of a semicrystalline material is directly related to a decrease in moisture content, so it is expected to find a lower moisture content for TPS-P-Rex sample. Regarding the results found by FTIR, this sample would have a lower amount of free OH groups, so both results would be suggesting lower moisture content. The results of this determination are presented later.

3.2. Films interaction with water

As mentioned earlier, agricultural mulches help maintain soil moisture by reducing the rate of water evaporation. In this way, films that have a rather hydrophobic character, such as PE, tend to have very low moisture absorption and water vapor permeability. In the particular case of starch films, its behavior is rather hydrophilic, a characteristic that can be modified to a certain degree, by replacing part of its hydroxyl groups with new functional groups.

The interaction between water and the developed materials was studied through tests of determination of moisture content, moisture absorption in different environments, water solubility and contact

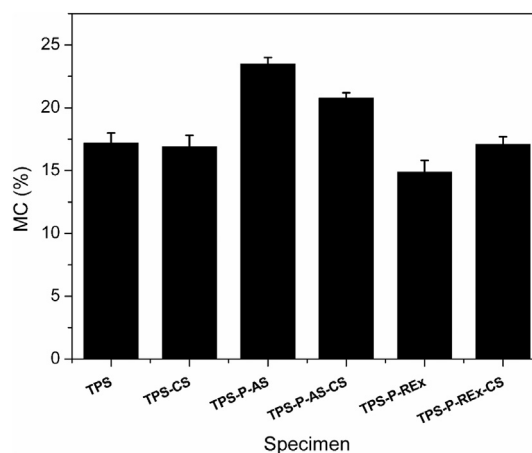


Fig. 3. Percentage of moisture content (MC) present in TPS, TPS-CS, TPS-P-AS, TPS-P-AS-CS, TPS-P-REx and TPS-P-REx-CS.

angle.

The moisture content results are presented in Fig. 3. It can be seen that phosphorylation in aqueous suspension produced films with a higher moisture content than those of native starch and phosphorylated obtained by REx.

The ability to bind water molecules by native and modified starch depends on the availability of binding sites for these molecules. According to Sukhija et al., 2016 [12] the new functional groups introduced during the chemical modification of the starch weaken the hydrogen bonds present in it, thus facilitating the penetration of the water molecules. In this way, it can be explained why the starch reacted in aqueous suspension had a higher moisture content. The TPS and the TPS-P-REx have been obtained directly by extrusion.

The decrease in MC occurs as a consequence of the decrease in OH groups. The sample of starch modified by REx presented a MC slightly lower than that of TPS and this is in agreement with the results of the analysis of the ATR-FTIR spectra and XRD, and coincides with that reported by Gutiérrez et al., 2015 [35], who worked with the phosphorylation of cassava starch. In this way, the decrease in MC of TPS-P-REx, with respect to TPS, suggests that part of the hydroxyl groups of the starch have been replaced in the phosphorylation reaction, as was found with the calculation of the DS.

Regarding the chitosan coating, it was found that it does not show a clear trend in the moisture content of the films. TPS and TPS-CS did not show significant differences, while there was a decrease in MC with respect to TPS-P-AS and a slight increase in moisture content with respect to TPS-P-REx. These irregularities could be associated with inhomogeneities in the coating appearance. Surface morphology was analyzed by SEM and results are presented later in the text.

The moisture absorption curves of the films were measured in controlled environments at 60 and 90% RH and they are shown in Fig. 4. In both cases, the films initially exhibited a rapid absorption of moisture and then reached a plateau after 200 and 50 h, for each environment, respectively. The different samples did not show significant differences in moisture absorption when they were in a controlled environment at 60% RH. In Fig. 5, equilibrium moisture absorptions are shown for each case, taken as the maximum of MA for each curve.

When comparing the absorption at 90% RH of TPS with the phosphorylated samples, it was found that TPS-P-AS had a lower absorption and, on the contrary, no significant differences were observed in the MA of TPS and TPS-P-REx. In this way, the TPS-P-AS films seemed to be the more resistant to water. Regarding the effect of the coating with chitosan, it was found that in all cases, the coating increased the water absorption capacity of the samples, which can be related with FTIR results that suggested that chitosan interaction with water is stronger than that with starch.

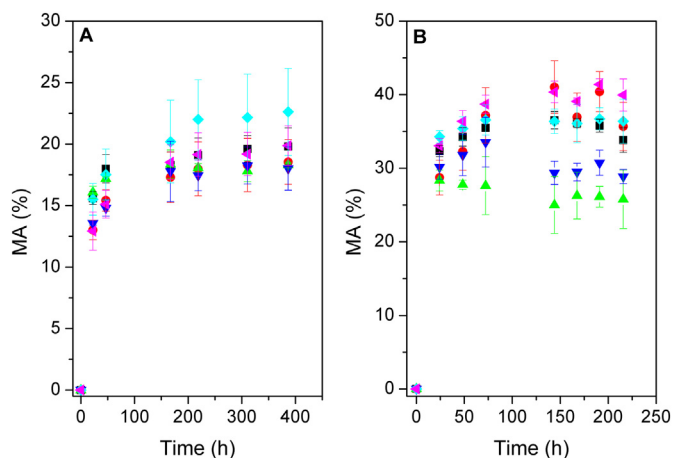


Fig. 4. Moisture absorption curves as a function of exposure time in environments at: A: 60% RH, and B: 90% RH, for ■ TPS, ● TPS-CS, ▲ TPS-P-AS, ▼ TPS-P-AS-CS, ◆ TPS-P-REx and ◀ TPS-P-REx-CS.

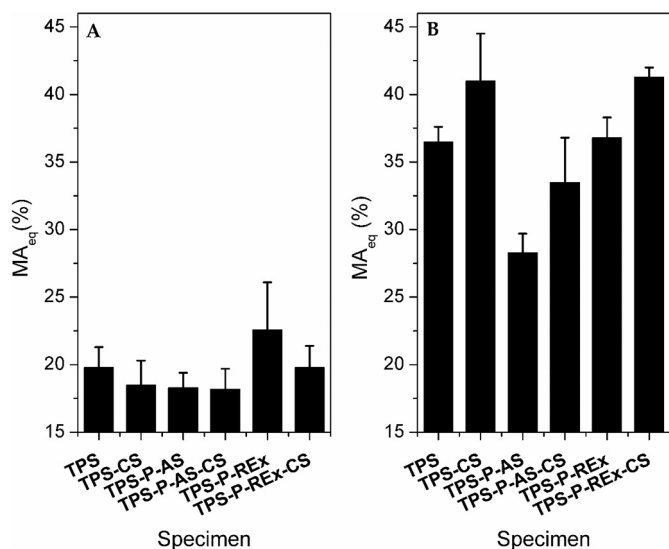


Fig. 5. Equilibrium moisture absorption (MA_{eq}) in environments at: A: 60% RH and; B: 90% RH for TPS, TPS-CS, TPS-P-AS, TPS-P-AS-CS, TPS-P-REx and TPS-P-REx-CS.

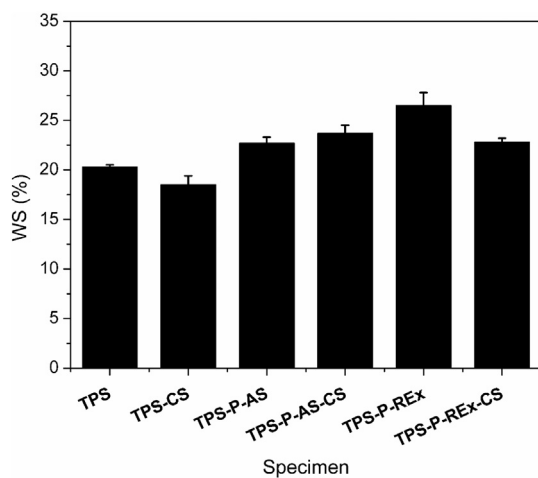


Fig. 6. Water solubility of TPS, TPS-CS, TPS-P-AS, TPS-P-AS-CS, TPS-P-REx and TPS-P-REx-CS.

Fig. 6 shows the results of the water solubility test. The results indicate that the phosphorylation of corn starch produces a slight increase in the solubility of its films, both for TPS-P-AS and for TPS-P-REx, with WS being a bit higher for the latter. In the literature, opposite trends have been reported regarding the solubility of phosphorylated starch films. Some authors argue that cross-linking produces a decrease in solubility [12,29,39,40], which they attribute to the formation of covalent bonds between starch chains that would act strengthening the structure and limiting the mobility of the chains in the amorphous zone; while other authors found an opposite trend [35,41], thus attributing the increase in solubility to a greater interaction with water, proposing that phosphorylation makes the matrices more hydrophilic. Regarding the effect introduced by the presence of the chitosan coating, it was found that it seems to contribute to decrease the percentage of dissolution of the TPS and TPS-P-REx samples. However, no significant differences were found for the phosphate starch in aqueous suspension.

The measurement of the contact angle formed by the water with the surface of the films makes it possible to evaluate the hydrophilic character of each material. The greater the attraction between water and surface, the smaller the contact angle observed and therefore, the more hydrophilic the material [40].

The contact angles determined are shown in Fig. 7. Results indicate that there were no significant differences in the water contact angle of TPS, TPS-P-AS and TPS-P-REx. However, when studying the effect of the coating, the TPS-P-AS-CS and TPS-F-RE-CS films presented an inferior angle than their uncoated matrices, suggesting that CS-coated films surface presented high hydrophilicity, while no significant differences were found in the TPS and TPS-CS results. In general, a lot of variation was found between the measurements, possibly due to the heterogeneity of the films surface as suggested also by MC results.

Previously, it has been reported that films prepared with modified starches should have a different contact angle from that of their unmodified matrix [42]. However, the contact angle is a technique for measuring the surface hydrophilicity of films and, as observed by ATR-FTIR, the surface of the modified starch films is quite similar to the native one.

Fig. 8 shows the SEM micrographs at the top surface of the developed materials. The images of the uncoated films are presented in the figures (a), (c) and (e). At first glance, there were no large differences in their surfaces. These presented a homogeneous aspect but with some irregularities mainly in the case of TPS and TPS-P-AS. Regarding micrographs (b), (d) and (f) of the chitosan-coated films, it was observed that the coating showed high porosity, with different sizes and shapes.

These results can be related mainly with the results of contact angle and with those of moisture absorption. As it is observed, the surfaces

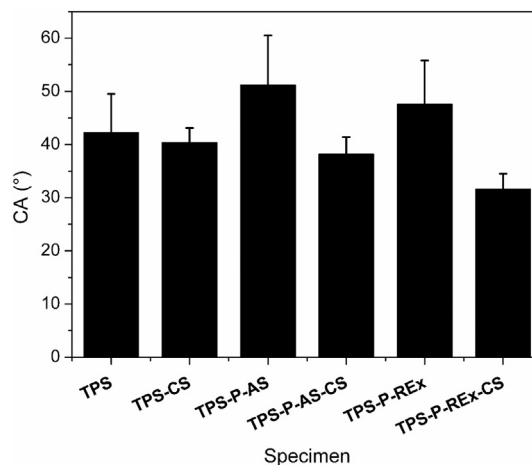


Fig. 7. Contact angles of TPS, TPS-CS, TPS-P-AS, TPS-P-AS-CS, TPS-P-REx and TPS-P-REx-CS with water.

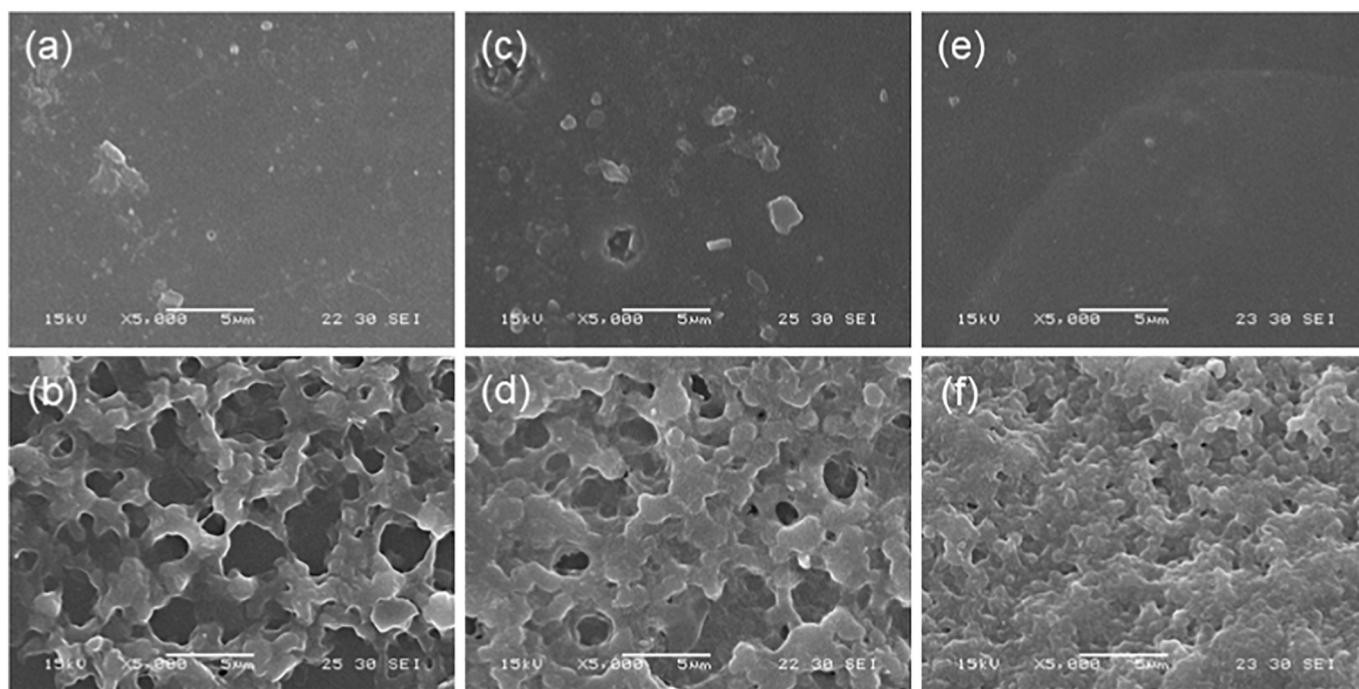


Fig. 8. Micrographs obtained by SEM for (a) TPS, (b) TPS-CS, (c) TPS-P-AS, (d) TPS-P-AS-CS, (e) TPS-P-REx, (f) TPS-P-REx-CS. Bar = 5 µm.

were not uniform and it may be for this reason that the determination of the contact angle presented a high degree of dispersion. In addition, the MA_{eq} of the chitosan-coated films was higher than that of the uncoated films and this can be explained considering the greater absorption surface represented by the porous coatings of chitosan.

3.3. Films interaction with light

Fig. 9 shows the transparency results of the developed materials determined by UV-Visible spectroscopy and by using of Eq. (7). It is important to mention that the large values of transparency in Fig. 9 refer to less transparent films. The results show that the modification of the starch, either by REx or by the aqueous suspension method, led to the obtaining of films with a higher value of T (greater opacity).

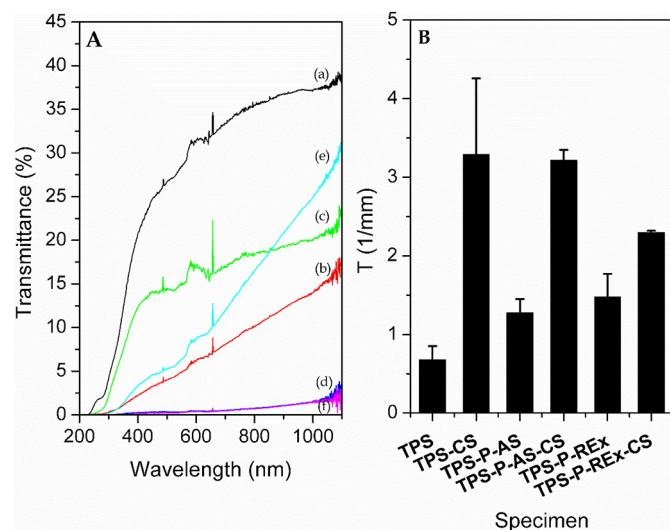


Fig. 9. A: Transmittance percentage of light in the range of 200 to 1100 nm, and B: Transparency (T) for (a) TPS, (b) TPS-CS, (c) TPS-P-AS, (d) TPS-P-AS-CS, (e) TPS-P-REx, (f) TPS-P-REx-CS. High values of T represents low transparent films.

According to Gutiérrez et al. (2017) [43], the crosslinking sites act as radiation scattering sites. Similar results have been previously reported by Gutiérrez et al. [44] who worked on the phosphorylation of corn starch. Regarding the coating with chitosan it was found that in all cases, the opacity increases and this may be due to the light being scattered by the presence of the coating.

3.4. Antibacterial properties

The results of the antibacterial activity of TPS films are shown in Fig. 10. In this test, approximately 20 mg of each film were incubated with *Psy* cell cultures for 24 h and then the colony forming units were counted.

As shown in Fig. 10, it was found that the phosphorylated starches produced an inhibition of the bacterium growth, being higher in TPS-P-

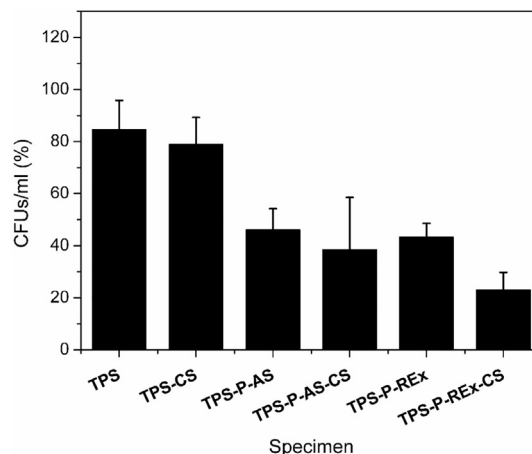


Fig. 10. Antibacterial activity of the different thermoplastic starch films assayed on *Psy*. Cells suspended in KB medium containing 20 mg of each film were incubated with shaking for 24 h at 30 °C. The number of CFU for each experimental condition was then quantified. The results were expressed as a percentage with respect to the TPS treatment. Data are mean values (\pm standard deviation) of 3 independent experiments.

REx-CS-treated cells. According to Sitohy & Ramadan 2001 [45] and Wei et al., 2011 [9], starch phosphates are less susceptible than native starch to be degraded by bacteria since they have a lower susceptibility to hydrolysis caused by α -amylases. Sitohy & Ramadan 2001 [45] argue that the presence of phosphate groups acts by inhibiting the action of these enzymes and that the inhibition is higher, the higher the degree of substitution of the starch, which is in line with the reported results.

4. Discussion

The effects produced by the use of mulches in soil moisture not only depend on rainfall and climatic factors, but also on the type of material used for mulching [4]. In this work, the results of interaction between water and the phosphorylated materials did not show very large changes with respect to the properties of the native starch. The materials developed may not reach the levels of soil moisture that are obtained with the use of PE mulches. However, as previously reported, in general, the use of mulches is more beneficial in this sense than the uncovered soil (bare soil) [5,46–48]. Additionally, plastic agricultural mulches maintain even more moisture in the soil than the widely organic ones [46]. Moreover, several studies have shown that various biodegradable agricultural mulches showed lower moisture retention and, nevertheless, crop yields were the same as those obtained with PE. For example, corn starch and poly (butylene adipate-co-terephthalate) (PBAT) based mulches produced the same yield of strawberries [1], similar results were obtained for a cultivation of grapes with TPS-PBAT and PLA-PBAT mulches [49] and in another work, biodegradable mulches of different colors produced the same tomato yields as those obtained with PE films [50]. Apparently, the greatest importance of the use of these materials lies in the protection of the seed at the beginning of its development. In this first stage is when the plant needs soil moisture to germinate and for this reason, mulches developed in this work could be used in short crops in order to protect the plant in the early stages of its development [49]. A benefit that could be presented by starch mulches is that they could allow the passage of water from rainfall, since they are more permeable than PE. In this way the mulch would represent a physical barrier for the evaporation of water and at the same time would have a greater permeability that would allow to incorporate part of the water of precipitations [48].

The use of agricultural mulch in tomato production is associated with increases in yields, better fruit quality and less weeds presence. These benefits are related to changes in the microclimate of the plant such as soil heating and moisture content [51]. This first factor is directly related to the interaction of light with mulch films [52]. The composition of the light that manages to cross the agricultural mulch has a great influence on the growth and quality of the product [52]. The insulating effect of the mulch depends on the ability to reflect and transmit solar energy. It absorbs the sun's radiation and reduces the heat loss from the soil with a consequent increase in its humidity and temperature [4]. For its part, photosynthetically active radiation (PAR) is used to perform photosynthesis by plants and contemplates radiation of wavelengths between 400 and 700 nm. It is important that the films are opaque to this radiation to prevent the growth of weeds that then compete for the nutrients with the plant of interest [53]. In this sense, both the phosphorylation and the use of a coating of chitosan contribute to improve the optical properties of the mulch films developed.

The antibacterial action presented by the developed materials appears as a benefit that would keep a very low inoculum of the phytopathogenic tomato bacteria. *Psy*, bacterium produces the bacterial spot in tomato and is the main bacterial disease responsible of large economic losses for this crop [19].

5. Conclusions

Starch phosphorylation was carried out by two methodologies: AS and REX. REX resulted in the more effective since it produced higher

phosphorous contents and DS than that obtained with AS method. FTIR and XRD techniques were in line with those results. Water interaction analysis suggested that REX produces a decrease in moisture content and no significant differences in MAeq were found with TPS. REX presented higher WS but this was improved by coating with CS. No significant differences in CA were observed when compared with TPS. Films obtained by REX presented also higher opacity and antimicrobial activity which represent an interesting advantage for the proposed application.

CS coated films presented stronger water interaction that uncoated films which was related to the higher MA_{eq} at 90% RH of coated films and minor CA. Thus, the coating increased the water adsorption capacity of the films and makes them more hydrophilic. By analyzing CS-coated top surface of films could be seen that it presented high porosity and that different samples presented differences in porous sizes and shapes. CS coating produces also more opaque films. Only in the case of TPS-P-REx-CS the presence of CS produced and increment in the antimicrobial activity.

Despite no greater changes were observed in thermoplastic phosphorylated starch films water interaction, encouraging results were obtained by transparency and antimicrobial analyses. Future work includes the report of other critical characteristics necessary for mulching such as mechanical and barrier properties and also, their biodegradation behavior.

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References

- [1] A.P. Bilck, M.V.E. Grossmann, F. Yamashita, Biodegradable mulch films for strawberry production, *Polym. Test.* 29 (2010) 471–476, <https://doi.org/10.1016/j.POLYMERTESTING.2010.02.007>.
- [2] D. Briassoulis, Analysis of the mechanical and degradation performances of optimised agricultural biodegradable films, *Polym. Degrad. Stab.* 92 (2007) 1115–1132, <https://doi.org/10.1016/j.polymdegradstab.2007.01.024>.
- [3] M.M. Moreno, A. Moreno, M.M. Moreno, A. Moreno, Effect of different biodegradable and polyethylene mulches on soil properties and production in a tomato crop, *Sci. Hortic. (Amsterdam)* 116 (2008) 256–263, <https://doi.org/10.1016/j.SCIENTA.2008.01.007>.
- [4] M.A. Kader, M. Senge, M.A. Mojid, K. Ito, Recent advances in mulching materials and methods for modifying soil environment, *Soil Tillage Res.* 168 (2017) 155–166, <https://doi.org/10.1016/J.STILL.2017.01.001>.
- [5] Z. Steinmetz, C. Wollmann, M. Schaefer, C. Buchmann, J. David, J. Tröger, K. Muñoz, O. Frör, G.E. Schaumann, Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Sci. Total Environ.* 550 (2016) 690–705, <https://doi.org/10.1016/J.SCITOTENV.2016.01.153>.
- [6] (FAO) Food and Agriculture Organization of the United Nations, Food and Agriculture: Driving action across the 2030 Agenda for Sustainable Development, <http://www.fao.org/3/a-i7454e.pdf>, (2017) (accessed July 9, 2017).
- [7] M. Mitrus, TPS and its nature, *Thermoplast. Starch*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2010, pp. 77–104, <https://doi.org/10.1002/9783527628216.ch4>.
- [8] K. Sathya Modification of biodegradable polymers through reactive extrusion-II, in: A. Jasim, T. Brijesh K., I. Syed (Eds.), *Starch-Based Polym. Mater. Nanocomposites*, CRC Press, 2016: pp. 133–166.
- [9] M. Wei, Y. Liu, B. Liu, X. Lv, P. Sun, Z. Zhang, F. Zhang, S. Yin, Z. Liu, Preparation and application of starch phosphate with a low degree of substitution, *Phosphorus Sulfur Silicon Relat. Elem.* 186 (2011) 974–982, <https://doi.org/10.1080/10426507.2010.537714>.
- [10] H. Rouached, Phosphorus in agriculture: need for efficient use and re-use, *J. Crop Res. Fert.* (2016) 1–2, <https://doi.org/10.17303/jcrf.2016.103>.
- [11] N.A. Landerito, Y.-J. Wang, Preparation and properties of starch phosphates using waxy, common, and high-amylose corn starches. I. Oven-heating method, *Cereal Chem. J.* 82 (2005) 264–270, <https://doi.org/10.1094/CC-82-0264>.
- [12] S. Sukhija, S. Singh, C.S. Riar, Effect of oxidation, cross-linking and dual

- modification on physicochemical, crystallinity, morphological, pasting and thermal characteristics of elephant foot yam (*Amorphophallus paeoniifolius*) starch, *Food Hydrocoll.* 55 (2016) 56–64, <https://doi.org/10.1016/j.foodhyd.2015.11.003>.
- [13] N.A. Landerito, Y.-J. Wang, Preparation and properties of starch phosphates using waxy, common, and high-amylose corn starches. II. Reactive extrusion method, *Cereal Chem. J.* 82 (2005) 271–276, <https://doi.org/10.1094/CC-82-0271>.
- [14] G. Moad, Chemical modification of starch by reactive extrusion, *Prog. Polym. Sci.* 36 (2011) 218–237, <https://doi.org/10.1016/J.PROGPOLYMSCI.2010.11.002>.
- [15] T. Galicia-García, F. Martínez-Bustos, O. Jiménez-Arevalo, A.B. Martínez, R. Ibarra-Gómez, M. Gaytán-Martínez, M. Mendoza-Duarte, Thermal and microstructural characterization of biodegradable films prepared by extrusion–calendering process, *Carbohydr. Polym.* 83 (2011) 354–361, <https://doi.org/10.1016/J.CARBPOL.2010.07.050>.
- [16] X. Wang, N. Chi, X. Tang, Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting, *Eur. J. Pharm. Biopharm.* 70 (2008) 735–740, <https://doi.org/10.1016/j.ejpb.2008.07.005>.
- [17] A.A. Zubareva, A.V. Ilyina, A.N. Levov, V.S. Zueva, E.V. Svirshchevskaya, V.P. Varlamov, Protein delivery by nanoparticles formed by chitosan-N-acyl derivatives, *Prog. Chem. Appl. Chitin Its Deriv.* XVI (2011) 61–70 <https://pbn.nauka.gov.pl/polindex-webapp/browse/article/article-132d29a4-0c50-40f7-8929-9e48f517098b>, Accessed date: 18 January 2017.
- [18] P.L. Kashyap, X. Xiang, P. Heiden, Chitosan nanoparticle based delivery systems for sustainable agriculture, *Int. J. Biol. Macromol.* 77 (2015) 36–51, <https://doi.org/10.1016/j.ijbiomac.2015.02.039>.
- [19] A.Y. Mansilla, L. Albertengo, M.S. Rodríguez, A. Debbaudt, A. Zúñiga, C.A. Casalongué, Evidence on antimicrobial properties and mode of action of a chitosan obtained from crustacean exoskeletons on *Pseudomonas syringae* pv. *Tomato DC3000*, *Appl. Microbiol. Biotechnol.* 97 (2013) 6957–6966, <https://doi.org/10.1007/s00253-013-4993-8>.
- [20] L.A. Toro-Márquez, D. Merino, T.J. Gutiérrez, Bionanocomposite films prepared from corn starch with and without nanopackaged jamaica (*Hibiscus sabdariffa*) flower extract, *Food Bioprocess Technol.* (2018) 1–19, <https://doi.org/10.1007/s11947-018-2160-z>.
- [21] T.J. Gutiérrez, N.J. Morales, E. Pérez, M.S. Tapia, L. Famá, Physico-chemical properties of edible films derived from native and phosphated cush-cush yam and cassava starches, *Food Packag. Shelf Life* 3 (2015) 1–8, <https://doi.org/10.1016/J.FPSL.2014.09.002>.
- [22] S. O'Brien, Y.-J. Wang, C. Vervaet, J.P. Remon, Starch phosphates prepared by reactive extrusion as a sustained release agent, *Carbohydr. Polym.* 76 (2009) 557–566, <https://doi.org/10.1016/J.CARBPOL.2008.11.024>.
- [23] E. Salay, C.F. Ciacco, Production and properties of starch phosphates produced by the extrusion process, *Starch* 42 (1990) 15–17, <https://doi.org/10.1002/star.19900420106>.
- [24] IRAM 15013-1, Productos de la industria pesquera. Método de determinación del fósforo, por la técnica volumétrica, <http://web.iram.org.ar/index.php?vernorma&id=1D2E>, (1985), Accessed date: 29 January 2018.
- [25] P. Deetae, S. Shobsngob, W. Varanyanond, P. Chinachoti, O. Naivikul, S. Varavit, Preparation, pasting properties and freeze–thaw stability of dual modified crosslink-phosphorylated rice starch, *Carbohydr. Polym.* 73 (2008) 351–358, <https://doi.org/10.1016/J.CARBPOL.2007.12.004>.
- [26] P.H. Hermans, A. Weidinger, On the determination of the crystalline fraction of polyethylenes from X-ray diffraction, *Die Makromol. Chemie.* 44 (1961) 24–36, <https://doi.org/10.1002/macp.1961.020440103>.
- [27] T. Woggon, P. Sirivongpaisal, T. Wittaya, Properties and characteristics of dual-modified rice starch based biodegradable films, *Int. J. Biol. Macromol.* 67 (2014) 490–502, <https://doi.org/10.1016/J.IJBIOMAC.2014.03.029>.
- [28] L. Kaur, J. Singh, N. Singh, Effect of cross-linking on some properties of potato (*Solanum tuberosum* L.) starches, *J. Sci. Food Agric.* 86 (2006) 1945–1954, <https://doi.org/10.1002/jsfa.2568>.
- [29] M. Seker, H. Sadikoglu, M. Ozdemir, M.A. Hanna, Cross-linking of starch with reactive extrusion and expansion of extrudates, *Int. J. Food Prop.* 6 (2003) 473–480, <https://doi.org/10.1081/JFP-120021453>.
- [30] G. Li, J. Zeng, H. Gao, X. Li, Characterization of phosphate monoester resistant starch, *Int. J. Food Prop.* 14 (2011) 978–987, <https://doi.org/10.1080/10942910903514669>.
- [31] P.G. Seligra, C. Medina Jaramillo, L. Famá, S. Goyanes, Biodegradable and non-retrogradable eco-films based on starch–glycerol with citric acid as crosslinking agent, *Carbohydr. Polym.* 138 (2016) 66–74, <https://doi.org/10.1016/j.carbpol.2015.11.041>.
- [32] O. Sevenou, S. Hill, I. Farhat, J. Mitchell, Organisation of the external region of the starch granule as determined by infrared spectroscopy, *Int. J. Biol. Macromol.* 31 (2002) 79–85, [https://doi.org/10.1016/S0141-8130\(02\)00067-3](https://doi.org/10.1016/S0141-8130(02)00067-3).
- [33] Y.X. Xu, K.M. Kim, M.A. Hanna, D. Nag, Chitosan–starch composite film: preparation and characterization, *Ind. Crop. Prod.* 21 (2005) 185–192, <https://doi.org/10.1016/J.INDCROP.2004.03.002>.
- [34] M.-F. Huang, J.-G. Yu, X.-F. Ma, Studies on the properties of Montmorillonite-reinforced thermoplastic starch composites, *Polymer (Guildf.)* 45 (2004) 7017–7023, <https://doi.org/10.1016/j.polymer.2004.07.068>.
- [35] T.J. Gutiérrez, M.S. Tapia, E. Pérez, L. Famá, Structural and mechanical properties of edible films made from native and modified cush-cush yam and cassava starch, *Food Hydrocoll.* 45 (2015) 211–217, <https://doi.org/10.1016/J.FOODHYD.2014.11.017>.
- [36] H.F. Zobel, Starch crystal transformations and their industrial importance, *Starch* 40 (1988) 1–7, <https://doi.org/10.1002/star.19880400102>.
- [37] P. Muhrbeck, E. Svensson, A.-C. Eliasson, Effect of the degree of phosphorylation on the crystallinity of native potato starch, *Starch* 43 (1991) 466–468, <https://doi.org/10.1002/star.19910431204>.
- [38] X. Ma, P. Chang, J. Yu, Plasticized starch, *Starch-Based Polym. Mater. Nanocomposites*, CRC Press, 2012, pp. 69–84, <https://doi.org/10.1201/b11848-5>.
- [39] M.B.K. Niazi, A.A. Broekhuis, Surface photo-crosslinking of plasticized thermoplastic starch films, *Eur. Polym. J.* 64 (2015) 229–243, <https://doi.org/10.1016/j.eurpolymj.2015.01.027>.
- [40] K. Wilpiszewska, A.K. Antosik, T. Szychaj, Novel hydrophilic carboxymethyl starch/montmorillonite nanocomposite films, *Carbohydr. Polym.* 128 (2015) 82–89, <https://doi.org/10.1016/j.carbpol.2015.04.023>.
- [41] E. Pérez, X. Segovia, M.S. Tapia, M. Schroeder, Native and cross-linked modified *Dioscorea trifida* (cush-cush yam) starches as bio-matrices for edible films, *J. Cell. Plast.* 48 (2012) 545–556, <https://doi.org/10.1177/0021955X12445603>.
- [42] J. Colivet, R.A. Carvalho, Hydrophilicity and physicochemical properties of chemically modified cassava starch films, *Ind. Crop. Prod.* 95 (2017) 599–607, <https://doi.org/10.1016/j.indcrop.2016.11.018>.
- [43] T.J. Gutiérrez, Effects of exposure to pulsed light on molecular aspects of edible films made from cassava and taro starch, *Innov. Food Sci. Emerg. Technol.* 41 (2017) 387–396, <https://doi.org/10.1016/J.IFSET.2017.04.014>.
- [44] T.J. Gutiérrez, M.S. Tapia, E. Pérez, L. Famá, Edible films based on native and phosphated 80:20 waxy:normal corn starch, *Starch - Stärke* 67 (2015) 90–97, <https://doi.org/10.1002/star.201400164>.
- [45] M.Z. Sitohy, M.F. Ramadan, Degradability of different phosphorylated starches and thermoplastic films prepared from corn starch phosphomonoesters, *Starch - Stärke* 53 (2001) 317–322, [https://doi.org/10.1002/1521-379X\(200107\)53:7<317::AID-STAR317>3.0.CO;2-N](https://doi.org/10.1002/1521-379X(200107)53:7<317::AID-STAR317>3.0.CO;2-N).
- [46] D. Chakraborty, S. Nagarajan, P. Aggarwal, V.K. Gupta, R.K. Tomar, R.N. Garg, R.N. Sahoo, A. Sarkar, U.K. Chopra, K.S.S. Sarma, N. Kalra, Effect of mulching on soil and plant water status, and the growth and yield of wheat (*Triticum aestivum* L.) in a semi-arid environment, *Agric. Water Manag.* 95 (2008) 1323–1334, <https://doi.org/10.1016/J.AGWAT.2008.06.001>.
- [47] H. Zhao, R.-Y. Wang, B.-L. Ma, Y.-C. Xiong, S.-C. Qiang, C.-L. Wang, C.-A. Liu, F.-M. Li, Ridge-furrow with full plastic film mulching improves water use efficiency and tuber yields of potato in a semiarid rainfed ecosystem, *F. Crop. Res.* 161 (2014) 137–148, <https://doi.org/10.1016/J.FCR.2014.02.013>.
- [48] S.E. Wortman, I. Kadoma, M.D. Crandall, Assessing the potential for spunbond, nonwoven biodegradable fabric as mulches for tomato and bell pepper crops, *Sci. Hortic. (Amsterdam)* 193 (2015) 209–217, <https://doi.org/10.1016/J.SCIENTA.2015.07.019>.
- [49] F. Touchaleaume, L. Martin-Closas, H. Angellier-Coussy, A. Chevillard, G. Cesar, N. Gontard, E. Gastaldi, Performance and environmental impact of biodegradable polymers as agricultural mulching films, *Chemosphere* 144 (2016) 433–439, <https://doi.org/10.1016/J.CHEMOSPHERE.2015.09.006>.
- [50] M.M. Moreno, A. Moreno, Effect of different biodegradable and polyethylene mulches on soil properties and production in a tomato crop, *Sci. Hortic. (Amsterdam)* 116 (2008) 256–263, <https://doi.org/10.1016/J.SCIENTA.2008.01.007>.
- [51] D.R. Decoteau, M.J. Kasperbauer, D.D. Daniels, P.G. Hunt, Plastic mulch color effects on reflected light and tomato plant growth, *Sci. Hortic. (Amsterdam)* 34 (1988) 169–175, [https://doi.org/10.1016/0304-4238\(88\)90089-1](https://doi.org/10.1016/0304-4238(88)90089-1).
- [52] G. Liling, Z. Di, X. Jiachao, G. Xin, F. Xiaoting, Z. Qing, Effects of ionic crosslinking on physical and mechanical properties of alginate mulching films, *Carbohydr. Polym.* 136 (2016) 259–265, <https://doi.org/10.1016/J.CARBPOL.2015.09.034>.
- [53] E. Schettini, G. Vox, B. De Lucia, Effects of the radiometric properties of innovative biodegradable mulching materials on snapdragon cultivation, *Sci. Hortic. (Amsterdam)* 112 (2007) 456–461, <https://doi.org/10.1016/J.SCIENTA.2007.01.013>.
- [54] K.M. Dang, R. Yoksan, Development of thermoplastic starch blown film by incorporating plasticized chitosan, *Carbohydr. Polym.* 115 (2015) 575–581, <https://doi.org/10.1016/j.carbpol.2014.09.005>.
- [55] T. Bourtoom, M.S. Chinnan, Preparation and properties of rice starch–chitosan blend biodegradable film, *LWT Food Sci. Technol.* 41 (2008) 1633–1641, <https://doi.org/10.1016/j.lwt.2007.10.014>.
- [56] E.O. King, M.K. Ward, D.E. Raney, Two simple media for the demonstration of pyocyanin and fluorescein, *Translat. Res.* 44 (2) (1954) 301–307.