

In-soil Biodegradation Behavior of Chitosan-Coated Phosphorylated Starch Films

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Abstract

Novel starch-based films were previously proposed as agricultural mulches and their main properties were critically addressed. In this report, the effects of chemical modification by crosslinking (phosphorylation) and surface functionalization with chitosan on corn starch-films biodegradability were studied. The biodegradability assay was performed in soil using a professional substrate. The biodegradation and disintegration of samples were followed by measuring their weight loss (WL), water sorption (WS) and changes in appearance (by photographs). Additionally, changes in surface morphology at microscopic level were investigated by scanning electron microscopy (SEM), while structural and chemical changes were evaluated by Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). Results indicate that neither the chemical modification by phosphorylation, nor the chitosan-functionalization of the surface produced changes in films biodegradation time, which is a positive result. All materials degrade almost completely in three months following a three-step mechanism that involves plasticizer leaching, microbial development, bio-erosion of the surface and the starch molecular weight diminution. Copyright © VBRI Press.

Keywords: biodegradable, environmentally friendly, fungal attack, sustainable agriculture.

Introduction

Since 1948, with the first use of plastic in agriculture as a greenhouse cover to replace glass, plastics have acquired a dominant role in agriculture, which has led to the emergence of the so-called "plasticulture", which consists in the use of plastic materials for greenhouses, tunnels and mulches, among others [1, 2]. Particularly, agricultural mulch films provide significant advantages for the production of fruits and vegetables, such as the reduction of the growth of weeds, the amount of herbicides, soil erosion and the use of water [3]. They produce an increase in the soil temperature and humidity, which produces a beneficial effect for the plants and their root, producing a faster and more productive growth and development [2, 4].

Polyethylene (PE) stands out among the most commonly used polymeric material for that application due to its easy processing, chemical resistance, durability and flexibility [4]. However, the main disadvantage of the use of PE mulches is the handling of plastic waste [5]. These have a great negative impact on the environment since they are impregnated with chemical products used during cultivation. The problem of its fate is serious. Some farmers burn them outdoors, others leave them in the soil and very small part is

collected and recycled but the costs of these procedures are very high [4]. In this context, the development of biodegradable agricultural mulches represents a highly necessary alternative to PE [5].

Starch is a widely available polysaccharide. The main advantages of its use are related to their biodegradability, absence of toxicity and low cost [6]. Moreover, it can be processed by extrusion in presence of an adequate plasticizer in order to obtain thermoplastic starch (TPS). TPS films are usually chemically modified or processed with fillers in order to overcome their high hydrophilicity and poor mechanical properties [7]. Particularly, the chemical modification by crosslink with phosphate groups can give important benefits for the utilization of those films as agricultural mulches. The films can bring phosphorous, which is a common fertilizer for plants, and also improve TPS properties [8]. Moreover, their surface functionalization with chitosan (CS) can provide biological activity to films [8]. CS is a natural polysaccharide derived from chitin, which is the second most abundant polymer in nature [9]. That polymer presents outstanding properties in agriculture: it is antimicrobial (antibacterial, antifungal and antiviral), elicitor and biostimulant [10].

The ASTM and the ISO define degradation as "an irreversible process that leads to a significant change in the structure of a material, characterized by a loss of properties and / or fragmentation" [1]. As explained by Scott 1997 [2], some polymers are initially chemically inert to the attack of microorganisms and require an initial degradation to transform their high molecular weight chains into metabolites of lower molecular weight that can be absorbed by the microbial cells. The initial degradation can occur by enzymatic action or not, and often occurs by the action of abiotic factors such as hydrolysis, photolysis or oxidation [2].

In this way, the breaking of a polymer usually occurs as a combination of biotic and abiotic factors [2] and, in general, the process of biodegradation of a polymer begins with abiotic processes such as photo-oxidation, photolysis and hydrolysis, depending on the chemical structure of the starting polymer [2]. The photo-oxidative processes transform the long polymer chains into carboxylic acids and alcohols that are susceptible to attack by bacteria, fungi and enzymes. That is why polymers such as PE, which are subject to photo-oxidative processes, slowly degrade [2]. Polyesters and polysaccharides such as starch have much shorter biodegradation times [1]. This is due to several reasons, on the one hand, that these polymers are susceptible to hydrolysis which results in accessible monomers or oligomers for the microorganisms. On the other hand, the biodegradation of starch is a consequence of the enzymatic attack to the glycosidic bonds, resulting in the reduction of the chain length to form monomers or oligomers that are then used in biochemical pathways [11]. During biodegradation, the exoenzymes of microorganisms produce depolymerization giving rise to monomers, dimers and oligomers able to cross the semipermeable membranes of bacteria and being used as sources of energy and carbon [1].

The rate of biodegradation of a material under field conditions is usually studied through the measurement of mass loss over the time, making sure that the surface of each sample is clean and that no material is lost [12]. The buried materials are previously placed in a network to facilitate later recovery of the soil [13]. The present article aims to evaluate the processes involved in chitosan-coated phosphorylated starch films biodegradation, previously proposed as mulch film [8, 14]. Biodegradation assays were performed in natural soil, commonly used in horticulture. We propose as hypothesis that performed chemical modification by crosslinking and the coating with CS polysaccharide can slightly produce a delay that does not affect in large way the films biodegradation time. Buried samples were recovered after several times and their biodegradation advance was evaluated by measuring their water sorption and weight loss. Chemical and structural changes were also followed by infrared spectroscopy, thermogravimetric analysis and scanning electron microscopy.

Experimental

Materials

Corn starch was purchased from 'Distribuidora Dos Hermanos' (Mar del Plata, Argentina). Phosphorylated thermoplastic corn starch was then prepared as described in our previous work [8, 14], by extrusion followed by hot-pressing. Briefly, phosphorylation was performed in aqueous suspension (AS) or reactive extrusion (REx) by using sodium tripolyphosphate (3% w/w) as phosphorylation agent, potassium sulfate (0.05 M) as catalyst and a mixture of glycerol (20% w/w) and water (10% w/w) as plasticizing agent [8, 14]. Extrusion was performed using a twin-screw extruder. The temperature profile used was 90/100/105/110/120/120 °C and the screw rotation speed was set at 130 rpm. Chitosan "Parafarm", obtained from Drogeria Saporiti (Mar del Plata, Argentina) of 531 KDa of molecular weight and more than 90% of degree of deacetylation was used for coating starch based films, also as reported previously [8, 14]. Resulted materials, TPS-CS, TPS-P-AS-CS and TPS-P-REx-CS were assayed for in-soil biodegradation.

Biodegradation experimental procedure

In-soil biodegradation assay was performed as previously reported by Alvarez *et al.*, 2006 [12]. Native and phosphorylated starch films were cut into 3 cm x 3 cm pieces and dried in an oven at 40 °C during 48 h. After that, they were weighted and the result was taken as de initial weight, W_0 . Then, film pieces were placed in a PE mesh and buried in a specific substrate, commonly used by horticultures. Biodegradation assay was performed in duplicate and the soil employed was a GROWMIX multipro, purchased from Terrafertil S.A. Their main physicochemical characteristics, provided by the supplier, include a porosity of 80-85%, water retention capacity of 60%, pH of 5.2-5.8, conductivity of 0.3-0.45 mmhos/cm and 85-90% organic matter.

The assay was conducted in pots and in an indoor place in the city of Mar del Plata (Buenos Aires, Argentina). The average temperature during the experiment was 15 °C and the soil moisture content was kept among 55 and 65% with the periodic addition of water.

Samples were removed periodically, carefully cleaned to eliminate rests of soil and microorganisms and weighted in order to obtain their wet weight, W_w . Following, they were dried in an oven at 40 °C during 48 h and weighted again in order to obtain their dry weight, W_t . Finally, their water sorption (WS) and weight loss (WL) was calculated as indicated in the equations (1) and (2):

$$WL (\%) = \frac{W_0 - W_t}{W_0} \times 100\% \quad (1)$$

$$WS (\%) = \frac{W_w - W_t}{W_0} \times 100\% \quad (2)$$

Characterization of samples removed at specific times

Unburied samples were analyzed as follows in order to obtain information about the biodegradation process:

Scanning electron microscopy (SEM). Microscopic observation was carried out in a JEOL JSM-6460 LV scanning electron microscope. Small pieces of each degraded sample were placed directly onto bifaz tape and covered with gold prior observation.

Fourier transform infrared spectroscopy (FTIR). Absorption spectra were acquired in a PerkinElmer Spectrum 100 spectrometer in attenuated total reflectance (ATR) configuration. The resolution was 4 cm^{-1} and the range was $4000\text{--}600\text{ cm}^{-1}$.

Thermogravimetric analysis (TGA). Thermograms were obtained in a TA Auto-MTGA Q500 Hi-Res in the temperature range of $30\text{--}600\text{ }^{\circ}\text{C}$, using nitrogen atmosphere (in order to avoid the thermo-oxidative oxidation) and a heating rate of $10\text{ }^{\circ}\text{C}/\text{min}$.

Statistical analysis

Results were informed as mean \pm standard deviation. In order to establish significant differences among samples One-way-ANOVA with Tukey's test was used as statistical tests. Samples were taken as significantly different with a 95% confident ($p < 0.05$).

Results and discussion

The biodegradability test of the films was carried out in spring of 2017 in the city of Mar del Plata where the average temperature was $15\text{ }^{\circ}\text{C}$ and the humidity of the pots was maintained at 55 - 65% RH. The samples were placed in individual supports constructed with $1\text{ mm} \times 2\text{ mm}$ mesh polyethylene nets, properly labeled and buried at the same time in $20\text{ cm} \times 22\text{ cm} \times 80\text{ cm}$ pots. At certain periods the samples and their duplicate were removed. Using Eq. (1) and (2), WL and WS were calculated and used to perform the curves shown in Fig. 1 and Fig. 2.

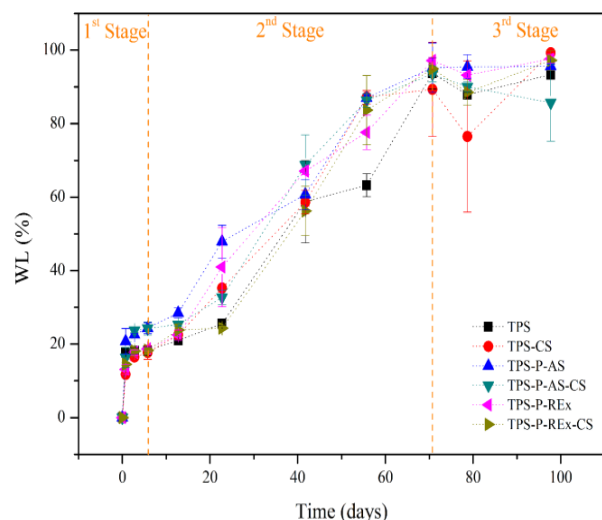


Fig. 1. Weight loss as a function of biodegradation time (days) for ■ TPS, ● TPS-CS, ▲ TPS-P-AS, ▼ TPS-P-AS-CS, ◆ TPS-P-REx and ◀ TPS-P-REx-CS.

As previously reported [15, 16], starch materials degraded mainly in three stages. The first one involves the loss of glycerol incorporated as plasticizer. As shown in Fig. 1 and Table 1, during the first 24 h, the mass losses were around 18%, except for the TPS-P-AS and TPS-P-AS-CS samples, which in this period had a mass loss that exceeded 20%. Next, the system remained without apparent weight loss until the next stage began. During this second stage, which lasted until 70 days of testing, mass losses of all systems reached 95% without showing significant differences. This stage is described in bibliography as a slower stage [16] characterized by microbial development and bio erosion of the surface of the material. On the other hand, the last stage of biodegradation was much slower than the previous one and the registered mass losses reached 99% for almost 100 days of testing and indistinctly for all the samples, showing that the chemical modification by phosphorylation and the functionalization of the surface with chitosan does not significantly affect the biodegradability of the matrix.

Table 1. Weight loss and water sorption registered for each biodegradation stage.

Sample	WL (%)*			WS (%)*		
	1 st stage	2 nd stage	3 rd stage	1 st stage	2 nd stage	3 rd stage
TPS	18.2 \pm 1.1 ^a	93.7 \pm 4.3 ^a	93.4 \pm 0.4 ^a	57.3 \pm 0.8 ^c	24.8 \pm 22.9 ^a	17.7 \pm 9.3 ^b
TPS-CS	17.7 \pm 1.9 ^a	89.4 \pm 12.9 ^a	99.3 \pm 0.1 ^a	54.9 \pm 1.0 ^c	6.0 \pm 0.4 ^a	0.8 \pm 0.3 ^a
TPS-P-AS	24.3 \pm 1.5 ^b	95.2 \pm 6.7 ^a	95.6 \pm 0.6 ^a	43.5 \pm 2.4 ^a	12.9 \pm 18.3 ^a	7.1 \pm 2.1 ^{a,b}
TPS-P-AS-CS	24.3 \pm 1.8 ^b	93.5 \pm 2.2 ^a	85.7 \pm 10.4 ^a	48.0 \pm 2.0 ^{a,b}	16.8 \pm 0.2 ^a	16.8 \pm 8.0 ^{a,b}
TPS-P-REx	18.7 \pm 0.5 ^a	97.2 \pm 0.9 ^a	97.7 \pm 1.2 ^a	65.8 \pm 0.5 ^d	7.9 \pm 5.4 ^a	13.6 \pm 1.1 ^{a,b}
TPS-P-REx-CS	18.3 \pm 0.01 ^a	94.9 \pm 0.4 ^a	97.2 \pm 2.7 ^a	54.1 \pm 1.7 ^{b,c}	12.6 \pm 3.0 ^a	0.9 \pm 3.4 ^a

*Equal letters in a column indicate that there are not statistical differences among samples ($p < 0.05$).

On the other hand, as can be seen from Table 2, the observed biodegradation time was higher than previously reported by Torres *et al.*, (2011) [15] and Zain *et al.*, (2018) [16] but lesser to the reported by Magalhaes and Adrade (2009) [17]. The observed differences could be attributed to the biodegradation media chosen for the performance of each test, among other factors such as the thickness of the sample, the solubility in water and the temperature of the test [18]. In this work, the substrate is one commonly used by horticulturists. Thus, reliable results could be obtained for the biodegradation times of the proposed materials in conditions closest to the real ones, since this type of material must be designed to fulfill its function and degrade at the place of use.

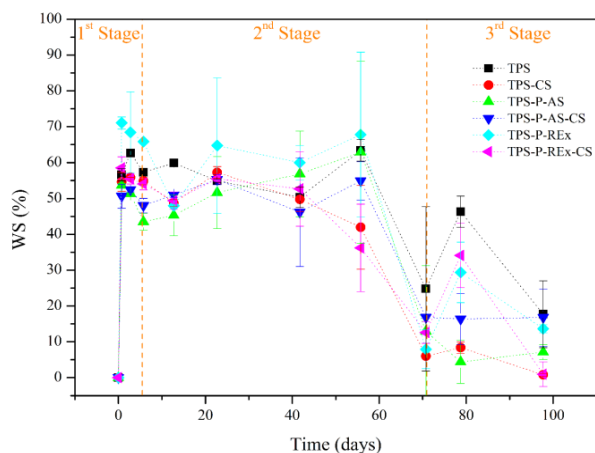


Fig. 2. Water sorption curves as a function of biodegradation time (days) for ■ TPS, ● TPS-CS, ▲ TPS-P-AS, ▼ TPS-P-AS-CS, ◆ TPS-P-REx and ◀ TPS-P-REx-CS.

The WS curve, **Fig. 2** with data in **Table 1**, accompanies the results of mass loss as it is directly related to the microbial development. As previously reported, the swelling and absorption of moisture by the samples favors the development of microorganisms that are responsible for their degradation [19]. The analyzed samples did not show significant differences in moisture sorption in a controlled environment at 60% H.R. [8], and therefore it is expected that they do not present significant differences in WS during the biodegradation test. Only small differences in moisture sorption were observed for the first stage. In addition, moisture sorption decreases with the test time and this is directly related to the progress in the biodegradation process of each sample, since it was referred to the initial weight of the sample as can be seen in **Eq. (2)**.

The degradation of a material in soil can also be described in terms of the physical changes that the samples experienced [13]. With the advance of the biodegradation process, a progressive increase in the roughness of the surface, the formation of pores and cracks, fragmentation and changes in color are commonly observed [13].

Table 2. Previously reported similar studies on starch based films biodegradation.

Material	WL (%)	Time of assay (days)	Medium of Assay	Ref
Starch films from 12 varieties of Andean crops	>90	30	Organic compost	[15]
Modified thermoplastic starch films via esterification with citric acid and ascorbic acid	>60	30	Organic Compost	[16]
Thermoplastic corn starch plasticized with glycerol and reinforced with sodium montmorillonite or Cloisite 30B	87	180	Soil mixed with bovine manure	[17]

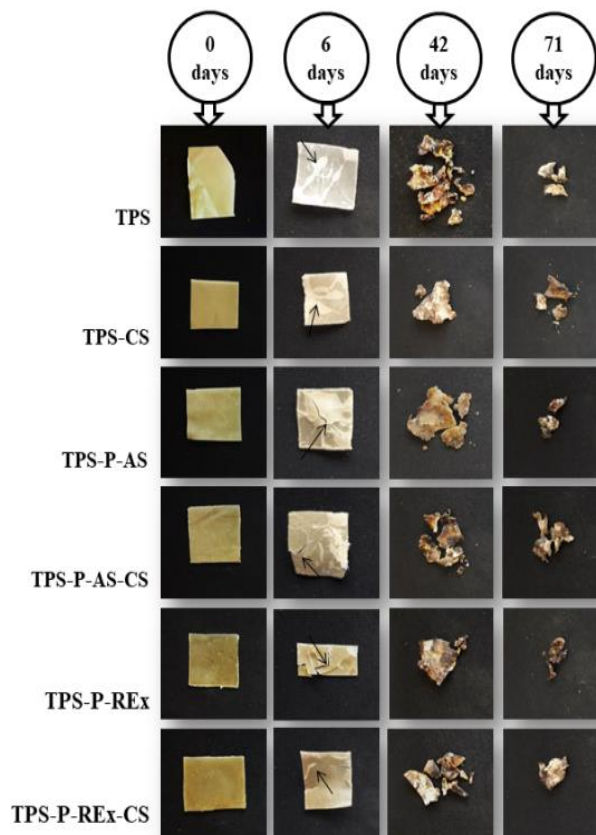


Fig. 3. Photographs of the films appearance along the biodegradation process (initial, 6, 42 and 71 days of assay) for samples: TPS, TPS-CS, TPS-P-AS, TPS-P-AS-CS, TPS-P-REx and TPS-P-REx-CS. In pictures taken at 6 days of assay, arrows are signaling the presence of cracks.

Fig. 3 shows a series of photographs taken at each stage of degradation time analyzed. As it was observed, during the course of the first stage the samples take on a fragile and brittle appearance, with the presence of the first cracks. During the second stage, the samples changed color significantly and fragmented, indicating the microbial action [13]. The last stage is characterized by having a highly reduced sample area and a darker coloration. The observed behavior was similar for all samples.

To obtain more detail of the biodegradation process, mainly during the second stage, the surfaces of the samples were observed with a scanning electron microscope. The obtained micrographs are shown in **Fig. 4**. Again, it was observed that after 6 days of testing the samples presented a cracked surface, in agreement with what previously reported Alvarez *et al.*, 2006 [12]. Additionally, the appearance of the first fungal structures on the surface of the materials that did not have chitosan coating was observed. With respect to the coated samples, it can be observed that during this first stage the coating begins to be lost, as can be seen in the sample TPS-CS. At the beginning the surface of TPS-CS is rough, with the presence of pores, in agreement with what was previously reported [8]. After the first stage, the surface became more smooth and with the presence of cracks.

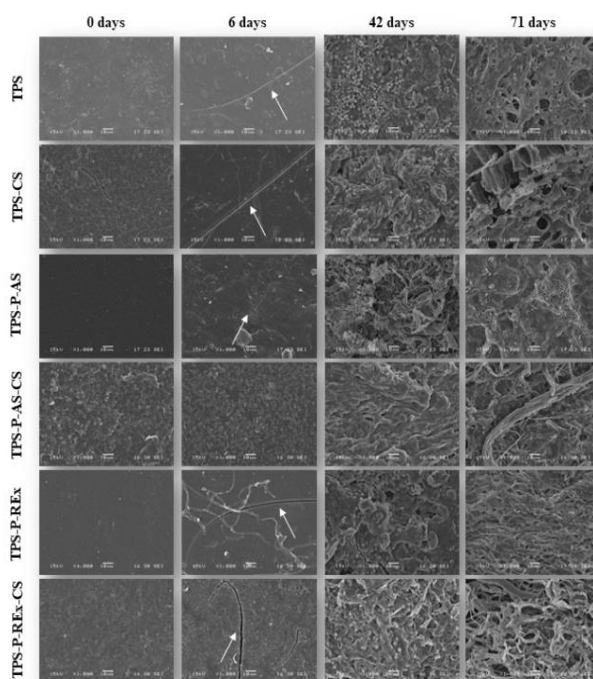


Fig. 4. SEM micrographs of the films surface along the biodegradation process (initial, 6, 42 and 71 days of assay) for samples: TPS, TPS-CS, TPS-P-AS, TPS-P-AS-CS, TPS-P-REx and TPS-P-REx-CS. In micrographs taken at 6 days of assay, arrows are signaling the presence of cracks.

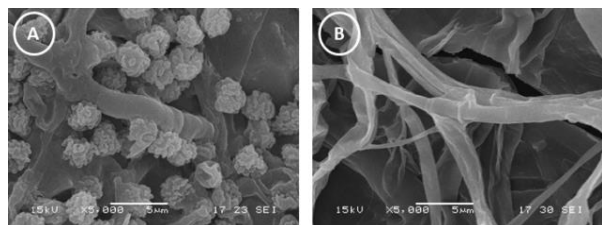


Fig. 5. SEM micrographs of A) TPS and B) TPS-P-REx at 23 days of assay. Micrographs obtained at 5000 x of magnification.

During the second stage of biodegradation, it can clearly be seen how the surface of all the samples becomes colonized by fungal structures identified by the presence of mycelia and spores [20], as can be seen in more detail in **Fig. 5**. On the other hand, the appearance of the samples during the third stage was characterized by presenting a covered surface very similar to the second stage.

A subsequent analysis by FTIR and TGA of the samples tested confirmed the loss of glycerol in the first stage of biodegradation, in accordance with other authors publications [12, 15]. In **Fig. 6** are presented the normalized FTIR spectra of films. The normalization was performed by dividing all absorption spectra by the intensity of the C-H stretching band at 2929 cm^{-1} . Then, it was observed that the uncoated samples showed a decrease in the intensity of the stretch band of the OH group present in the starch, glycerol and water molecules at 3287 cm^{-1} [15], while the samples that presented chitosan coating indicated that the coating begins to lose during this first stage since in some cases

the shift of the overlapping stretch band of OH and NH in chitosan at 3245 cm^{-1} towards 3287 cm^{-1} is observed, typical of the stretch OH in the starch.

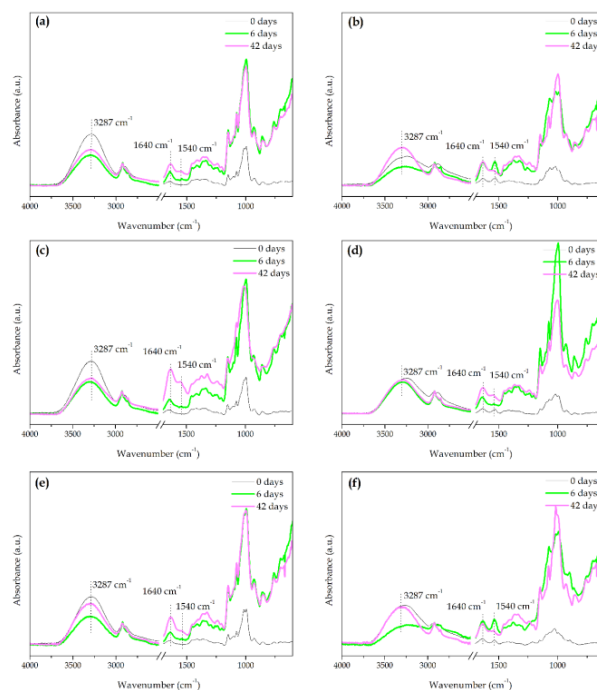


Fig. 6. FTIR spectra evolution along the biodegradation progress (initial, 6 and 42 days of assay) for (a) TPS, (b) TPS-CS, (c) TPS-P-AS, (d) TPS-P-AS-CS, (e) TPS-P-REx and (f) TPS-P-REx-CS.

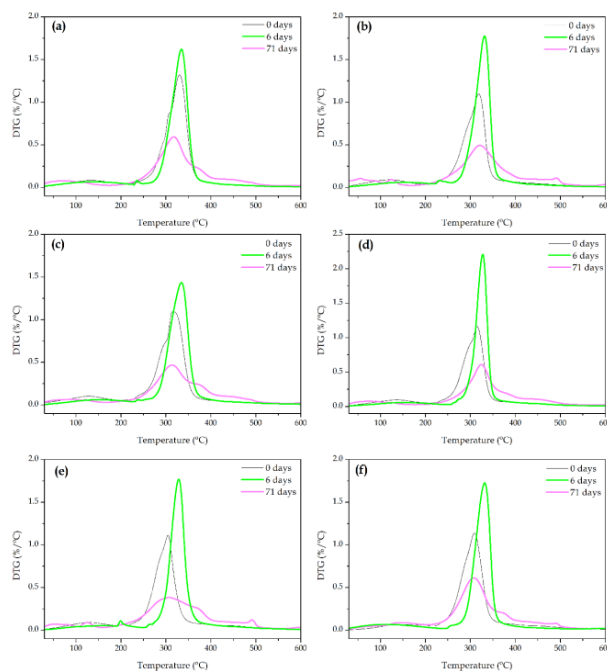


Fig. 7. DTGA curves evolution along the biodegradation progress (initial, 6 and 71 days of assay) for (a) TPS, (b) TPS-CS, (c) TPS-P-AS, (d) TPS-P-AS-CS, (e) TPS-P-REx and (f) TPS-P-REx-CS.

Even in **Fig. 7**, it can be observed how the shoulder of the starch degradation peak disappears, associated with the presence of a glycerol-rich phase in the uncoated samples as previously suggested [21]. As seen by SEM, during the second stage the degradation of the

sample occurs due to the action of microorganisms mainly of the fungal type [20, 22]. As reported by N. Lucas *et al.*, 2008 [23] microbial species adhere to surfaces by secreting a substance composed of carbohydrates and proteins that acts as a glue. This substance allows protecting microorganisms from adverse conditions and contributes to alter the size and distribution of pores and causes cracks. The presence of this substance can be observed in the infrared spectra thanks to the appearance of bands at 1640 and 1540 cm^{-1} associated with the stretching bands of amide I (stretching C = O) and II (stretching of the CN bond and NH bending) [24]. These bands are observed initially in the coated materials since chitosan presents this functional group in its structure. Finally, we see from TGA that as the biodegradation process progresses, the peak degradation of the starch becomes smaller and wider. This may be correlated with the decrease in molecular weight caused by biotic decomposition [25]. During the process of biodegradation, the microorganisms naturally present in the soil degrade the material through the enzymatic attack on the glycosidic bonds, producing a reduction in the length of the chain leading to the formation of sugars of lower molecular weight that are used by these microorganisms in various metabolic pathways [11].

Conclusion

Phosphorylated thermoplastic corn starch was obtained by traditional aqueous suspension and also by reactive extrusion. Additionally, films developed as mulch films were functionalized by performing a chitosan coating. The effect of chemical modification and surface functionalization on the biodegradability was studied by in-soil burial tests using a professional substrate. From the WS and WL curves it was observed that the biodegradation process follows a three step mechanism. Later characterizations by SEM, FTIR and TGA allowed identify that the first stage involves a great weight loss due to the glycerol leaching. At the second stage, the weight loss rate was inferior due to the development of microorganisms and surface bio-erosion and at the last stage the biodegradation showed the lesser biodegradation rate since the mayor molecular weight diminution was detected. In general, non-differences were observed among samples and all of them degrade almost completely in three months, being indicative that the modifications carried out to improve the performance of that material had no delayed the biodegradation time. Future work must be performed to add an induction period that prevents rapid biodegradation of starch films in contact with soil in order to extend their shelf-life for allowing it act as mulch film.

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Author's contributions

Conceived the plan: VAA; Performed the experiments: DM; Data analysis: DM; Wrote the paper: DM. Authors have no competing financial interests.

Conflicts of interest

There are no conflicts to declare.

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