

Isosorbide plasticized corn starch filled with poly(3-hydroxybutyrate-co-3-hydroxyvalerate) microparticles: Properties and behavior under environmental factors

Miguel R. Area ^a, Belén Montero ^{a,*}, Maite Rico ^a, Luis Barral ^{a,b}, Rebeca Bouza ^a, Joaquín López ^a

^a Universidade da Coruña, Campus Industrial de Ferrol, Grupo de Polímeros, Departamento de Física y Ciencias de la Tierra, Escuela Politécnica de Ingeniería de Ferrol, 15403, A Coruña, Spain

^b Cellular and Molecular Cardiology Research Unit, Institute of Biomedical Research (IDIS-SERGAS), University Clinical Hospital, Santiago de Compostela, Spain

ARTICLE INFO

Keywords:

Isosorbide
Thermoplastic starch
PHBV microparticles

ABSTRACT

In this work, new green and fully biodegradable composites, based on corn starch, plasticized with two different amounts of isosorbide and filled by poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) microparticles, were obtained by melt processing. The analysis of their morphologies, crystallinity, structural interactions and dynamomechanical properties as well as the evaluation of their moisture resistance and biodegradability in soil, were performed in function of the plasticizer and/or microparticle amount.

The analysis of morphology, crystallinity and structural interactions showed that the plasticization process was completed under the melting processing conditions used. The microparticles were homogeneously dispersed in the thermoplastic starch matrix without suffering any deformation or breaking during the processing. Biocomposites with adequate storage modulus values were obtained, especially the TPS plasticized with 35% of isosorbide and filled with 5 wt% of PHBV microparticles. The incorporation of PHBV microparticles leads to biocomposites with higher moisture resistance. All the biocomposites were completely biodegraded in soil in a short period of time. The performed study demonstrated that these biocomposites could be used for applications in the packaging industry.

1. Introduction

In spite of the multiple advances and applications that the use of petroleum based thermoplastic materials have provided to society throughout the twentieth century, the harmful incidence of the accumulation of plastic wastes in natural environments as the effects on wildlife and in the human health become evident in recent decades [1,2]. Currently, several research pathways are being developed to obtain eco-sustainable substitutes, from natural and renewable origin, biodegradable and economically competitive.

The starch, formed by monomers of α -glucose organized in two types of polymeric chains: amylose (simple chain) and amylopectin (branched chain), is one of the most abundant biopolymers in nature and complies with all the previously mentioned conditions. However, the starch is not a thermoplastic in a natural way so, it is necessary to convert it into thermoplastic starch (TPS) by the presence of a suitable plasticizing agent, which under temperature and shear conditions, destabilizes the

superstructure of the granules by breaking hydrogen bond interactions between the polymer chains, which are replaced by new starch-plasticizing bonds [3]. The chemical characteristics of the plasticizer, as well as the variations in the amount used, are determining factors on the final properties of TPS. In this work, the isosorbide, which has not yet been extensively studied, was selected as plasticizer for the corn starch for several reasons: First of all, it is considered as a green plasticizer due to its biodegradability and non-toxicity [4] secondly, it allows to obtain TPS by melting processes, which can be scaled up to industrial processing methods and finally, in our previous works, the TPS obtained by using isosorbide as plasticizer had reported not to suffer the known retrogradation phenomena, which is related to the reordering of starch polymer chains into crystalline structures with a consequent loss of properties [5–7].

Usually, the materials obtained from starch have demonstrated some disadvantages such as brittleness and high water sensitivity (i.e., low moisture resistance and hydrophilic character) compared to petroleum-

* Corresponding author.

E-mail address: belen.montero@udc.es (B. Montero).

<https://doi.org/10.1016/j.ijbiomac.2022.01.032>

Received 2 August 2021; Received in revised form 21 December 2021; Accepted 6 January 2022

Available online 13 January 2022

0141-8130/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

based conventional polymers [8]. Nowadays, one of the strategies to improve the TPS properties is the incorporation of micro or nanoparticles from different natural origins, for example: Gutiérrez et al. have given several examples of improvement in mechanical properties of TPS matrixes by addition of cellulosic materials, as cellulose nanoparticles (CNC) [9], Bootklad et al. used eggshell power as filler in thermoplastic cassava starch achieving an improvement in the water resistance and thermal stability of the TPS [10], while Jiang et al. demonstrated the enhancement of mechanical properties as well as barrier properties against water vapor and oxygen by the incorporation of eggshell powder to corn starch matrix [11]. Xie et al. brings several examples of enhancement in mechanical and barrier properties of TPS matrixes by adding different clays and silicates as fillers [12].

One of the novelties of this work lies in the preparation and use of PHBV microparticles as a filler for the TPS matrix, instead the other more conventional micro or nanoparticles from natural resources as those mentioned above. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), was selected due to its environmentally friendly character since it is a biopolymer derived from several types of bacteria, non-toxic, 100% biodegradable, biocompatible with many types of cells and resistant to ultraviolet radiation. PHBV is frequently used in packaging and biomedical applications research and it has been developed on an industrial scale [13,14]. The PHBV microparticles used in this work were prepared following a method tested and refined in a previous work [15]. The use of PHBV microparticles was previously tested in PHBV films [16] and potato starch films [17] with good dispersion in both matrixes reaching an improvement in the mechanical properties and moisture resistance in the first case and in the barrier properties against water vapor, oxygen and carbon dioxide in the second case. In these previous works, the films were prepared by solvent casting at a lab-scale, in the present research, melt processing was used to get the materials with the aim of approaching the processing method of these materials to the methods used at an industry-scale. Moreover, in previous studies [18], these PHBV microparticles demonstrated to be suitable for encapsulating and subsequent release active compounds such as antioxidant, antimicrobial or flavoring substances, which can be highly interesting in applications for food packaging industry.

In this work, the influence of several factors as the proportion of plasticizer used, the addition of PHBV microparticles and the content of the latest in the properties of these corn starch based biocomposites was widely analyzed. The morphology, the structural interactions and the crystallinity were studied and related with the dynamomechanical properties of these biocomposites. Finally, the improvement in the moisture resistance and the biodegradability of the biocomposites obtained were analyzed and corroborated.

2. Experimental

2.1. Materials

The raw corn starch with a 25% amylose content according to the supplier data, was kindly provided by Roquette Freres S.A. (France). The water content was determined by a gravimetric method resulting in a value of 12 wt%. The plasticizer used was isosorbide ($C_6H_{10}O_4$, with a purity of 98%, melting point of 60 °C and molar mass of 146.14 $g \cdot mol^{-1}$) purchased from Acros Organics (Spain).

Microparticles of PHBV, hereinafter PHBVmp, were synthesized from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with 12% of hydroxyvalerate content (HV) and molar mass: 240,000 $g \cdot mol^{-1}$, which was supplied by Goodfellow Cambridge limited (United Kingdom). The preparation of PHBVmp was made following an emulsification/solvent evaporation method previously optimized by Farrag et al. [15]. This method was based on making an oil in water emulsion of a PHBV solution in dichloromethane into an aqueous solution, which contains sodium dodecyl sulfate (SDS) as surfactant. The emulsion was induced by sonication of the organic-aqueous phase mixture at 60 °C

with an agitation rate of 400 rpm. The SDS was removed by centrifugation and the solvents were removed by lyophilization of the solutions. These microparticles showed a spherical morphology with a particle size less than 3 μm calculated by the analysis of FESEM images. The distribution of particle size was non homogeneous and the most of the particles (60%) showed a size less than 0.5 μm . The study made by Differential Scanning Calorimetry in a DSC 7 from Perkin Elmer (USA) revealed two melting peaks at $T_{m1} = 155.0$ °C and $T_{m2} = 162.8$ °C and a crystallization temperature of $T_c = 106$ °C.

The list of codes and proportions used for preparing both, the neat TPS and the TPS/PHBVmp biocomposites modified with PHBVmp is shown in Table 1. The starch:isosorbide ratios were 65:35 and 60:40. Neat TPS and TPS filled with 5 wt% and 10 wt% of PHBVmp were prepared and analyzed. All amounts were based on the dry weight of starch. The samples were coded specifying the plasticizer content and the wt% of filler as iNmpN', where N indicates the percentage of plasticizer and N' the percentage of PHBVmp added.

2.2. Processing of neat TPS and TPS/PHBVmp biocomposites

The neat TPS and TPS/PHBVmp biocomposites were prepared by melt processing. The components of each formulation were mixed by manual stirring at room temperature and after, they were processed at 110 °C and 80 rpm for 10 min with 7 min of recirculation by using a co-rotating conical twin-screw micro-extruder MiniLab II HAAKE™ Rheomex CTW5, UK.

2.3. Characterization techniques

2.3.1. Field emission scanning electron microscopy (FESEM)

The morphologies of the fracture surfaces as well as the PHBVmp images were obtained by using a field emission scanning electron microscope FESEM (JEOL-JSM-7200F, Japan) operated at 2.0 kV. The samples were fractured after being cooled in liquid nitrogen and then, they were coated with a gold thin film previously to be analyzed.

2.3.2. X-ray diffraction (XRD)

The X-ray diffraction analysis was performed in a Siemens D5000 diffractometer (Germany). The equipment was operated at a current of 30 mA, voltage of 40 kV and the wavelength of the copper anode was λ ($K\alpha$) = 1.54 Å. Scans were performed from $2\theta = 5^\circ$ to 40° , at room temperature, with a scanning step rate of 0.05° in step time of 4 s.

The crystallinity degree of neat TPS and TPS/PHBVmp biocomposites was calculated as the ratio between the crystalline area under the diffraction peaks and the total area of the diffractogram. The amorphous area was estimated using an iterative smoothing curve following a similar methodology described by Frost et al. [19].

2.3.3. Attenuated total reflection-Fourier transform infra-red (ATR-FTIR)

The ATR-FTIR spectra were collected on a Jasco 4700 spectrometer equipped with a MIRacle™ ZnSe Single Reflection Horizontal ATR

Table 1

Composition of neat TPS and TPS/PHBVmp biocomposites, crystallinity degree (obtained from XRD analysis) and absorbance ratio 995/1018 cm^{-1} (obtained from ATR-FTIR analysis).

Code	Starch: plasticizer (wt %)	PHBV microparticles (wt %)	% Crystallinity	Absorbance ratio 995/1018 cm^{-1}
i35mp0	65:35	0	8.7	0.98
i35mp5	65:35	5	9.9	0.96
i35mp10	65:35	10	10.5	0.94
i40mp0	60:40	0	8.1	0.98
i40mp5	60:40	5	8.9	0.96
i40mp10	60:40	10	10.4	0.95

accessory (Japan). The tests were made with 100 scans between 4000 cm^{-1} and 600 cm^{-1} with a resolution of 1.0 cm^{-1} and subjected to baseline correction.

2.3.4. Dynamic mechanical analysis (DMA)

The dynamomechanical tests were made using a dynamic mechanical analyzer from Perking Elmer, DMA 7 (Massachusetts, USA). Specimens of $15 \times 4 \times 1.15 \text{ mm}^3$ were tested in the tensile mode. The dynamic storage modulus (E') and the loss factor ($\tan \delta$) were measured as a function of temperature from $-70 \text{ }^\circ\text{C}$ to $80 \text{ }^\circ\text{C}$ at a heating rate of $5 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ and a frequency of 1 Hz under a helium atmosphere. Four replicates of each sample were tested.

2.3.5. Moisture absorption

The moisture absorption tests were carried out under four different relative humidity (RH) environments (33%, 54%, 75% and 95%). To create these environments, saturated solutions of magnesium chloride hexahydrate ($\text{MgCl}_2\cdot 6\text{H}_2\text{O}$), magnesium nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$), sodium chloride (NaCl) and potassium nitrate (KNO_3) were respectively prepared as it is stipulated in the UNE-EN ISO 2006:483 in hermetic containers conditioned at $20 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$.

Specimens with dimensions of $8 \times 8 \times 3 \text{ mm}^3$ were cut from each material. They were dried at $70 \text{ }^\circ\text{C}$ for 72 h, then they were cooled and weighed before starting the water absorption tests. These specimens were placed, immediately after being dried and weighed, in the containers with the different RH environments and maintained at room

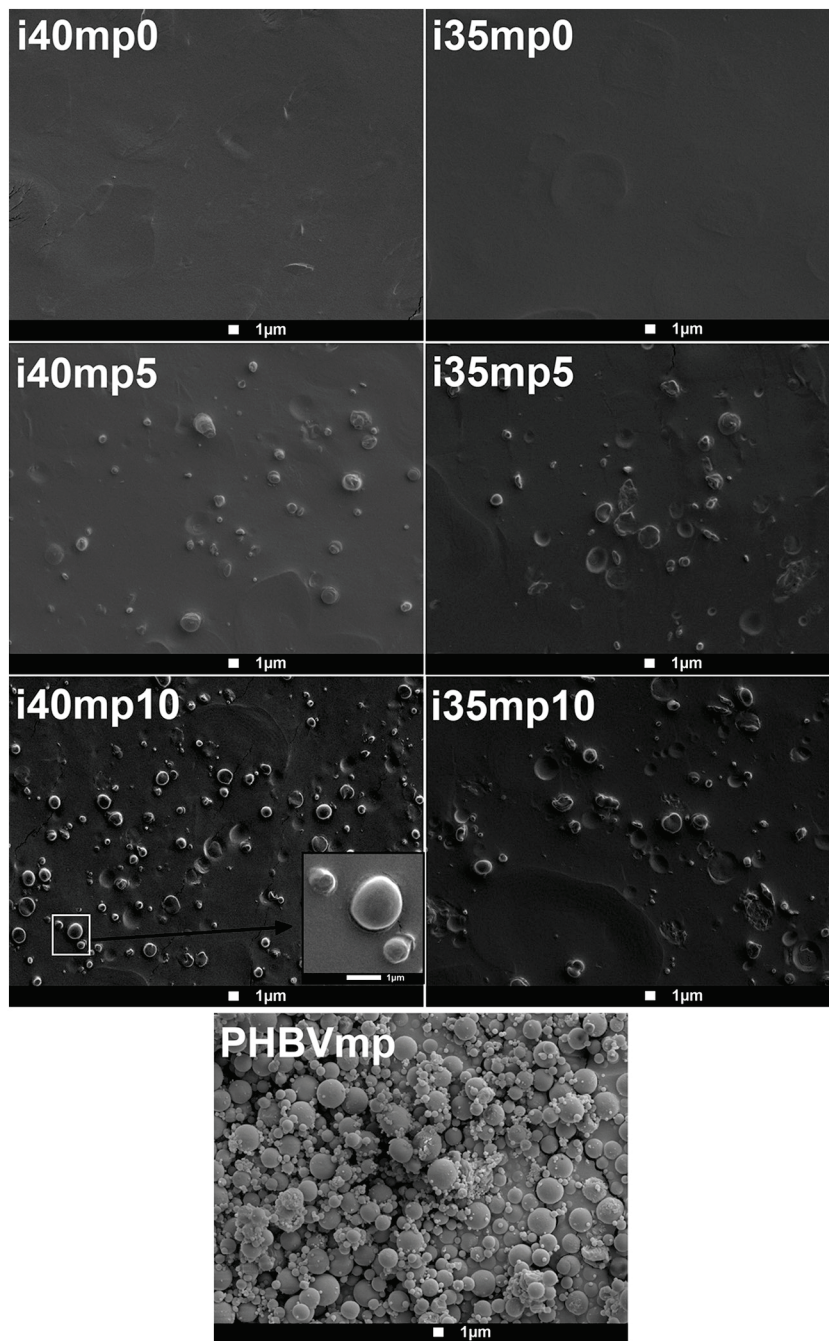


Fig. 1. Fracture surface micrographs of neat TPS and TPS/PHBVmp biocomposites plasticized with isosorbide, as well as the image of PHBV microparticles (PHBVmp).

temperature ($20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$). They were periodically weighed in an analytical balance (with a precision of 0.1 mg) and returned to the containers. The moisture absorption was determined in percentage according to Eq. (1),

$$\text{Moisture absorption (\%)} = \frac{M_t - M_0}{M_0} \times 100 \quad (1)$$

where M_t is the weight at time t and M_0 is the weight of the dry material.

2.3.6. Biodegradation in soil (burial test)

Soil burial degradation test were carried out according to the method described by Magalhães and Andrade [20]. Specimens of each material were cut into pieces of $45 \times 10 \times 1.25\text{ mm}^3$, weighed, and buried in a commercial soil placed in an open plastic container (5 L). This soil had pH of 5.8 and 75 wt% of organic materials of vegetable origin (mainly peat of *Sphagnum* sp. and composted bark of *Pinus* sp.). The water content of soil was maintained at an average value of 20 wt% during the period of the test. The humidity of the soil was periodically measured and a suitable amount of water was added if necessary. Periodically, each sample was taken out of the soil, cleaned with a smooth brush, and weighed. The biodegradability of the materials was evaluated by measuring the remaining weight of the samples along the time. The percentage of remaining weight was calculated using Eq. (2):

$$\text{Remaining weight (\%)} = \frac{M_t}{M_0} \times 100 \quad (2)$$

where M_0 is the weight of the sample before being buried and M_t is the weight of the sample after a time t buried.

3. Results and discussion

3.1. Morphology (FESEM)

The Fig. 1 shows the images of the manually fractured surfaces for neat TPS and TPS/PHBVmp biocomposites plasticized with isosorbide. The image of PHBVmp is also showed for comparison.

Smooth matrixes, where no starch granules are present, can be observed in the micrographs corresponding to both neat TPS. It suggest the complete loss of ordered structure of the corn starch plasticization under the melting processing conditions used. The use of a higher or lower isosorbide content has not shown significant differences in the morphology of neat TPS.

With respect to the images obtained for the TPS/PHBVmp biocomposites, a homogeneous dispersion of PHBVmp into the TPS matrix is observed, without the presence of aggregates, for both percentages of isosorbide. The good dispersion is maintained independently of the PHBVmp amount added to the TPS/PHBVmp biocomposites. As can be seen by comparison of the images obtained for the PHBVmp and the TPS/PHBVmp biocomposites, the PHBVmp have preserved their size and spherical morphology, without suffering any deformation or breakage during the melting processing by extrusion. The interface between the PHBVmp and the matrix does not show discontinuities or gaps, as can be seen in the detailed image included in Fig. 1, indicating a good interfacial adhesion between the TPS matrix and the PHBVmp, but maybe this interaction is not complete as some holes, with similar size and shape than the PHBVmp, can be seen in the fracture surface of the TPS/PHBVmp biocomposites. Probably the existing interaction between TPS matrix and PHBVmp is only a physical interaction that leads to an homogeneous dispersion of the microparticles into the matrix without existing chemical bonding between them.

3.2. X-ray diffraction analysis (XRD)

The study of the crystallinity was made by X-ray diffraction analysis of neat TPS and TPS/PHBVmp biocomposites plasticized with

isosorbide. The diffractograms obtained for the processed materials are shown in Fig. 2, together with the diffractograms for corn starch granules and PHBVmp.

The native corn starch has shown two diffraction peaks at $2\theta = 15.2^{\circ}$ and 22.9° and a doublet at $17.2^{\circ}/18.1^{\circ}$. This pattern corresponds to the type-A crystallinity, a polymorphism characteristic of starches obtained from cereals [21].

In neat TPS diffractograms, the peaks observed for native starch have disappeared, indicating that the native type-A crystalline structure has been destroyed during the processing. Instead this, a large amorphous scanning halo is observed, typical from semi-crystalline structure polymers with a low crystallinity [19], as well as a single diffraction peak at $2\theta = 17.9^{\circ}$, which typically appears in the plasticized starches. This peak corresponds to the E_h -type crystallinity that is induced during the processing [21]. The percentage of isosorbide used for plasticizing the corn starch has not shown to have any influence in the crystallinity of these materials.

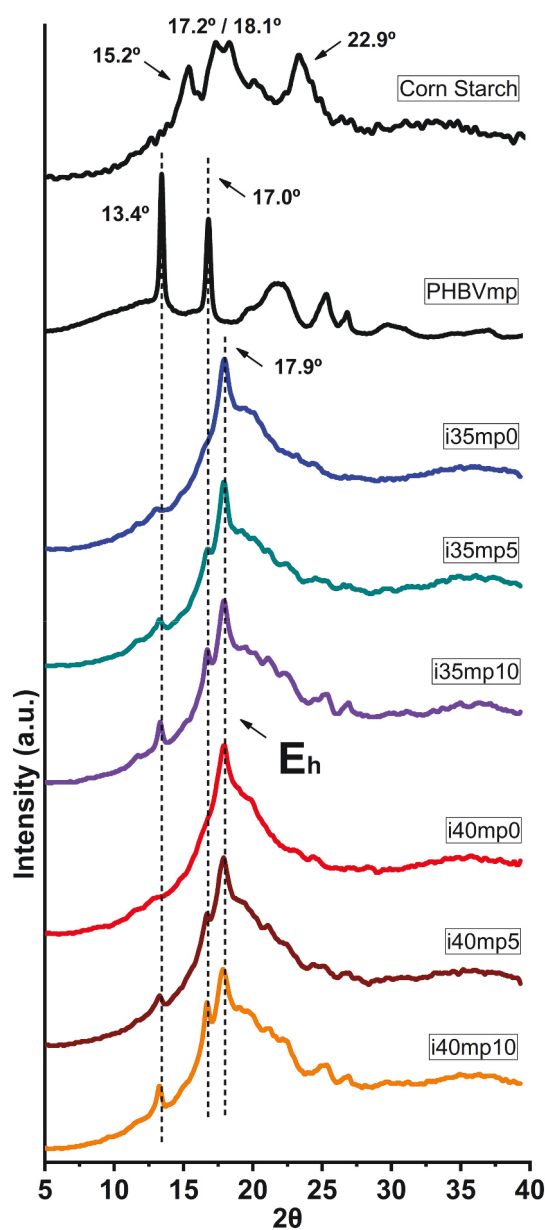


Fig. 2. X-ray diffractograms obtained for corn starch granules, PHBV micro-particles (PHBVmp) and both: neat TPS and TPS/PHBVmp biocomposites plasticized with isosorbide.

The diffractogram corresponding to PHBVmp shows two high intensity peaks located at $2\theta = 13.4^\circ$ and 17.0° , characteristic of the orthorhombic unit cell of PHBV crystals [22]. These two peaks were detected in TPS/PHBVmp biocomposite diffractograms, indicating that PHBVmp have retained their structure during processing, as was observed by FESEM in the previous section. The intensity of these peaks was higher by increasing the microparticles content, as would be expected. In addition, the presence of PHBVmp does not appear to alter the crystallinity of starch as the diffractograms of all TPS/PHBVmp biocomposites are overlapping the patterns shown by corresponding neat TPS (with the exception of the two peaks related to PHBVmp). In the case of the TPS/PHBVmp biocomposites filled with 10% PHBVmp, the two weaker peaks located at higher angles ($2\theta = 25.6^\circ$ and 27.0°) in the PHBV diffractogram can be more clearly observed due to the higher concentration of PHBV present in TPS.

The crystallinity degree for neat TPS and TPS/PHBV biocomposites was calculated and the values obtained are shown in Table 1. The crystallinity degree is higher for TPS with minor content of isosorbide, it can be due to a higher shear reached during the plasticization process. Whereas, the addition of PHBVmp has increased the degree of crystallinity developed in TPS/PHBVmp biocomposites. It was higher when a higher percentage of microparticles was used and this increase is attributed to the high crystallinity of the PHBVmp.

3.3. Attenuated total reflection-Fourier transform infra-red (ATR-FTIR)

ATR-FTIR analysis allows us to characterize and analyze the interactions established in the ordered structure of the thermoplastic starch by identifying the vibration of the different bond types specific to each functional group. Fig. 3, section a) shows the spectra corresponding to raw corn starch granules, PHBVmp and neat TPS plasticized with 35% and 40% of isosorbide. The spectra from neat TPS and TPS/PHBVmp biocomposites are shown in section b) for materials plasticized with 35% of isosorbide and in section c) for materials with 40% of isosorbide.

The PHBVmp spectra presents a band at 1720 cm^{-1} attributed to the stretching vibration of hydrogen bonds with the carbonyl (C=O) functional groups present in the polymeric structure of PHBVmp [23]; a band at 1378 cm^{-1} is related with CH_3 symmetric deformation and it corresponds to the helical structure deformation [24]; a set of bands in the region $1280\text{--}1050\text{ cm}^{-1}$ are attributed to C—O—C stretching mainly and CH and CH_2 bending modes [24–26] and finally a band located at 980 cm^{-1} corresponding to C—C stretching vibration of sensitive crystalline regions of PHBV [27].

With respect corn starch, neat TPS and TPS/PHBVmp biocomposites, the spectra analysis were focused on the more significant regions, such as the abroad band located in the $3700\text{--}3000\text{ cm}^{-1}$ region, linked to stretching vibration of OH groups related to inter- and intra-molecular hydrogen bonds in starch chains [28], the slight band at 1646 cm^{-1} , corresponding to the OH bending vibration of absorbed water in amorphous regions of starch [29,30] and the group of bands located in the range $1050\text{--}950\text{ cm}^{-1}$, which is sensible to short range-order structural changes in double helix of starch and so, it can be related with the thermoplasticization process [31]. These bands are associated to C—O stretching vibration corresponding to C—O—C bonds in the anhydroglucose rings [32]. The band at 995 cm^{-1} showed by raw corn starch and TPS is attributed to structural arrangements in crystalline domains of them, while the band at 1018 cm^{-1} , only present on TPS, appears with plasticization and it implies the formation of hydrogen bonds between both oxygen atoms in C—O—H and C—O of anhydroglucose ring [33]. Therefore, by means of the absorbance ratio between these two bands (absorbance ratio $995/1018\text{ cm}^{-1}$) it is possible to evaluate the plasticization evolution. The values obtained are shown in Table 1 [34,35].

With respect to the hydroxyl band ($3700\text{--}3000\text{ cm}^{-1}$), the raw corn starch and neat TPS spectra have shown a wide band of high intensity due to the high number of hydroxyl groups present in polymeric chains

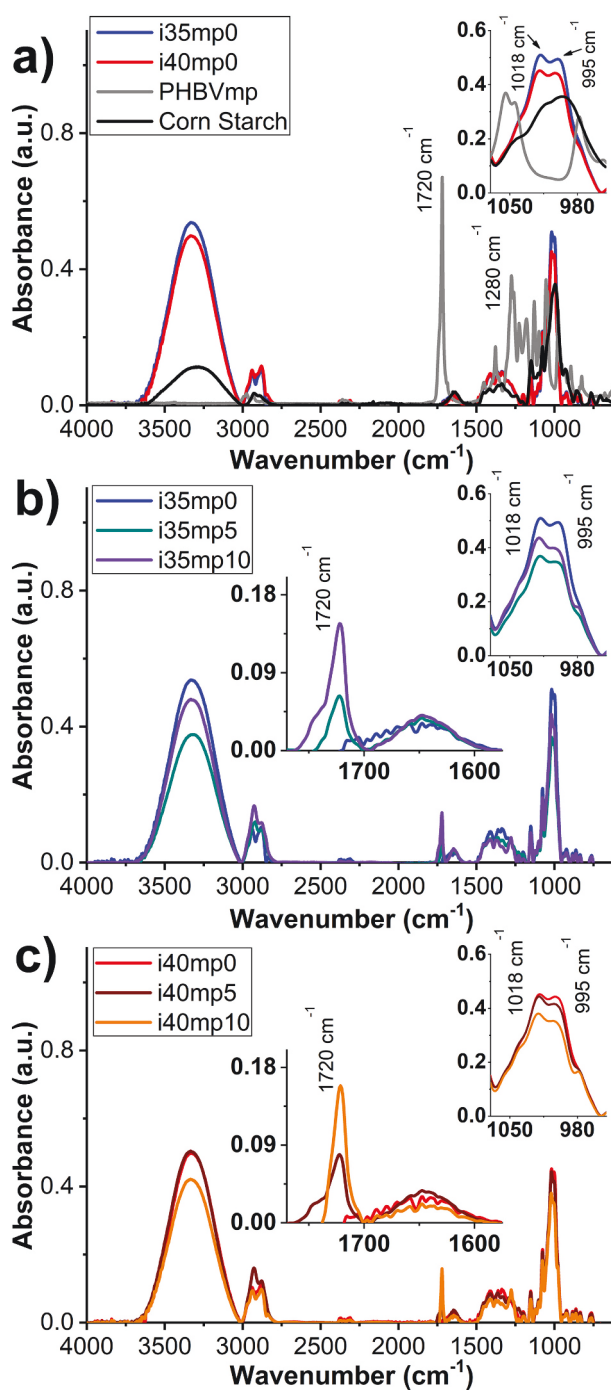


Fig. 3. Spectra obtained by ATR-FTIR analysis corresponding to neat TPS, raw corn starch and PHBV microparticles (PHBVmp) (a), TPS/PHBVmp biocomposites plasticized with 35 wt% isosorbide (b) and plasticized with 40 wt% isosorbide (c).

of starch (Fig. 3, section a). The slightly minor intensity observed in this band when a higher percentage of isosorbide was used would be due to the formation of H-bonds between the plasticizer and the starch matrix is impeded by a high number of hindering isosorbide molecules [6].

The characteristic band of PHBV located at 1720 cm^{-1} is not present in neat TPS but has been detected in TPS/PHBVmp biocomposites, with an increase in intensity proportional to the increase PHBVmp content and regardless of the percentage of isosorbide used, as can be seen in Fig. 3 section b) and section c). This increase is therefore correlated with the higher number of carbonyl groups present in the biocomposites as

the content of microparticles increases. As for the bands located in the region 1280–1050 cm^{-1} in the spectra of TPS/PHBVmp biocomposites have been overlapped with the starch-isosorbide matrix, no significant differences have been observed between the different materials.

The wavenumber range of 1050–950 cm^{-1} is one of the regions of greatest interest in the study of thermoplastic starches, where they present a high intensity double band, due to the vibration of double helix conformation of the starch polymer chains in crystalline (band at 995 cm^{-1}) or amorphous form (band at 1018 cm^{-1}). The comparison of absorbance ratio 995/1018 cm^{-1} calculated for TPS and TPS/PHBVmp biocomposites and collected in Table 1, indicates a predominance of amorphous structures in all cases, without significant differences in relation to the percentage of isosorbide used but favored by addition of PHBVmp. This trend observed in the absorbance ratio 995/1018 cm^{-1} would indicate that the PHBVmp hinder the rearrangement of the starch matrix promoting the formation of amorphous structures. In addition, the apparition of a weak band located at 980 cm^{-1} , in biocomposites with 5% or 10% PHBVmp respectively, together with the presence in the spectra of the bands located at 1720 cm^{-1} and 1280 cm^{-1} (previously approached), confirm the preservation of the crystalline integrity of the microparticles of PHBV.

3.4. Dynamic mechanical analysis (DMA)

The influence of the isosorbide proportion and the PHBVmp addition on the dynamomechanical properties of neat TPS and TPS/PHBVmp biocomposites were analyzed by DMA. Table 2 shows the values of storage modulus (E') obtained for all materials. The storage modulus (E') values have been recorded at room temperature (20 °C). These materials are initially designed for applications of food packaging at environmental temperature, so the storage modulus value at 20 °C is particularly interesting.

According to the storage modulus values obtained for neat TPS plasticized with isosorbide and considering that the storage modulus is a measure of the stiffness of a material, the use of higher isosorbide contents for plasticizing the corn starch clearly lead to less rigid TPS. The higher amount of small plasticizer molecules probably increases the starch chains mobility reducing material stiffness [36]. The addition of PHBVmp to the TPS/PHBVmp biocomposites caused a decrease in the storage modulus for all the samples studied indicating a loss of rigidity in the TPS/PHBVmp matrixes, probably due to a higher mobility of polymer chains caused by an increase in the free volume into the matrix because of the presence of PHBVmp.

The damping properties are reflected in $\tan \delta$ curves as a function of temperature. The curves obtained for neat TPS and TPS/PHBVmp biocomposites are shown in Fig. 4. Two main relaxation phenomena can be observed, which indicate that TPS materials are partially miscible heterogeneous systems with two separated phases [37]. These relaxation phenomena detected in $\tan \delta$ curves may be associated to the glass transition of the plasticizer-rich phase (relaxation located at lower temperatures, $T_{g\text{plasticizer}}$) and with the main glass transition correspondent to starch-rich phase (relaxation located at higher temperatures, $T_{g\text{starch}}$). The temperature values, corresponding to both transitions, obtained for TPS and TPS/PHBVmp biocomposites, are collected in Table 2.

Table 2
Dynamic mechanical parameters for neat TPS and TPS/PHBVmp biocomposites.

Sample	$T_{g\text{plasticizer}}$ (°C)	$T_{g\text{starch}}$ (°C)	E' at 20 °C (10^8 Pa)
i35mp0	−17.9	63.1	9.78 ± 1.02
i35mp5	−20.7	59.7	9.10 ± 1.42
i35mp10	−29.9	51.2	4.23 ± 0.59
i40mp0	−16.3	54.4	4.22 ± 0.22
i40mp5	−15.9	50.9	3.19 ± 0.45
i40mp10	−14.1	50.7	2.81 ± 0.03

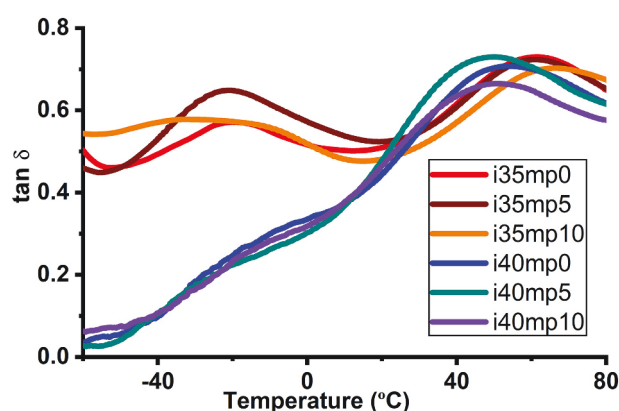


Fig. 4. $\tan \delta$ curves obtained by DMA for neat TPS and TPS/PHBVmp biocomposites.

The main relaxation corresponding to starch-rich phase has occurred at higher $T_{g\text{starch}}$ values when a lower percentage of isosorbide was used. This is related to a reduction in starch chain mobility and improved material stiffness as was expected because the higher number of plasticizer molecules interacting with starch promote the mobility of the starch polymeric chains [6]. With respect to the relaxation corresponding to the plasticizer-rich phase, the amount of plasticizer used hardly affects to the $T_{g\text{plasticizer}}$ values for neat materials. However, the peak intensity of these peaks is affected by the amount of plasticizer added. With a minor content of plasticizer, the intensity and the area under this peak is much higher, indicating higher mobility of plasticizer molecules in the plasticizer-rich phase.

With respect to the starch-rich phase, there are hardly changes in $T_{g\text{starch}}$ values. The intensity of the peaks and the area under the curves barely vary. These facts indicate the PHBVmp do not affect to the starch chains mobility. With respect to the plasticizer-rich phase, when PHBVmp were added to the biocomposites plasticized with 40% of isosorbide, $T_{g\text{plasticizer}}$ values are similar to the obtained for the neat. The intensity of these peaks follow being very small indicating poor mobility of isosorbide molecules in this phase. However, in case of biocomposites modified with 35% of isosorbide, the addition of increasing content of PHBVmp leads to a decreasing in $T_{g\text{plasticizer}}$ values. These biocomposites have shown a relatively intense relaxation in the plasticizer-rich phase for neat TPS and biocomposites with contents of PHBVmp of 5 wt% but, they show a peak of lower intensity when 10 wt% of microparticles is added, where a marked decrease in the intensity of the peak is also observed, suggesting that a higher content of microparticles diminish the free volume and avoid the mobility of plasticizer molecules.

3.5. Moisture absorption

In order to evaluate the moisture resistance of TPS and the influence of adding PHBVmp, dry samples of neat TPS and TPS/PHBVmp biocomposites plasticized with isosorbide were subjected to four environments with different relative humidity (33, 54, 75 and 95% RH). The moisture absorption of each material under each one of the four environments was recorded over time until moisture absorption equilibrium was reached, which is considered as the maximum moisture absorption value for each biocomposite. The moisture absorption curves obtained are shown in Fig. 5 and the final values of moisture absorption at equilibrium are reported in Table 3.

The moisture absorption curves show similar evolution for all the materials. The percentage of moisture absorbed increases linearly during the early hours of exposure to the humidity environment. From that moment, all the materials reach the maximum moisture absorption value reaching a plateau zone indicating an equilibrium in moisture absorption. Finally, and after having reached the maximum moisture

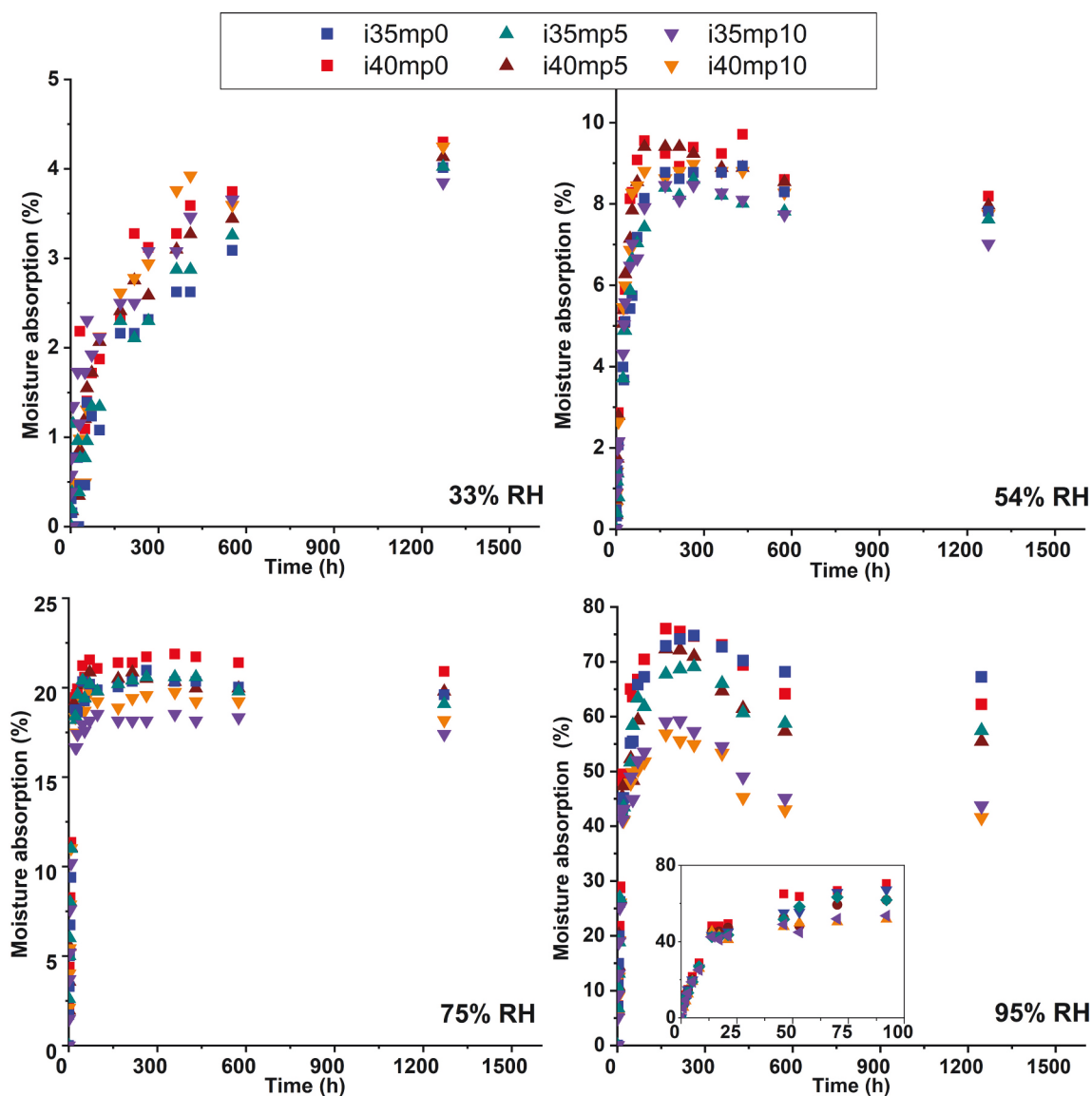


Fig. 5. Moisture absorption curves versus time obtained for neat TPS and TPS/PHBVmp biocomposites under atmospheres with different relative humidities (33, 54, 75 and 95% RH).

Table 3

Moisture absorption at equilibrium in different RH for neat TPS and TPS/PHBVmp biocomposites plasticized with isosorbide.

Sample	Moisture absorption at equilibrium (wt%)			
	33% RH	54% RH	75% RH	95% RH
i35mp0	4.0	7.8	19.6	67.2
i35mp5	4.0	7.6	19.1	57.4
i35mp10	3.8	7.0	17.4	43.7
i40mp0	4.3	8.2	20.9	62.2
i40mp5	4.1	8.0	19.8	55.5
i40mp10	4.2	7.7	18.2	41.6

absorption capacity, the moisture absorption starts decreasing as the time of exposition increases. The moisture absorption percentages at the equilibrium are collected in Table 3 for all the materials tested. As was expected, the exposure to environments with higher relative humidity increases the amount of moisture absorbed in all cases. The neat TPS and TPS/PHBVmp biocomposites plasticized with 40% of isosorbide showed values slightly higher than the corresponding plasticized with 35% of isosorbide. The presence of higher content of plasticizer probably leads

to a higher mobility of starch chains into the starch-based matrix, as was seen in the previous section, favoring the diffusion of moisture into the material.

The moisture absorption values at equilibrium diminish in all cases with the addition of PHBVmp to TPS plasticized with isosorbide. This improvement in the moisture resistance of the materials is higher when the content of PHBVmp increases. This phenomenon is more pronounced for environments with higher RH values. This effect is attributed to the greater hydrophobicity of PHBVmp together to the crystalline structure of PHBVmp, which offers geometrical impediments, diminishing the accessibility to water vapor molecules diffusion throughout the matrix more than in the more amorphous and branched matrix of neat TPS [38].

3.6. Biodegradation in soil (burial test)

Samples of neat TPS and TPS/PHBVmp biocomposites plasticized with isosorbide were subjected to a biodegradation test by soil burial in order to know the biodegradability of these materials, where weight loss is a reflection of the biodegradation process by the action of

microorganisms in the humid soil [10]. The moisture present in soil is necessary for growing and multiplication of microorganisms, that through metabolic or enzymatic processes, disintegrate the materials into low molecular weight compounds. This biodegradation process is supposed to occur basically in aerobic condition according to the experiment conditions used. In these cases, CO₂ and water are the final products of this process. [39].

The weight loss of all, the neat TPS and the TPS/PHBVmp biocomposites, was determined over time and the weight remaining as a function of time is shown in Fig. 6.

The initial increase in mass observed for all the neat TPS and TPS/PHBVmp biocomposites during the first week of testing is attributed to the absorption of water present in the soil during the period of colonization and adaptation of the microorganisms before the beginning of biodegradation in soil [6,29,40]. The increase in the mass during this first week is higher for materials plasticized with 35% of isosorbide, so the water absorption is less favored in this case due to the higher rigidity of the matrix. The incorporation of PHBVmp leads to a minor water absorption reaching values similar to the obtained from materials plasticized with 40% of isosorbide. These trends are consistent with the results observed above for the higher relative humidity environment (95%) during the moisture absorption study.

After the first week of testing, there was a decrease in sample mass due to the degradation of materials by starch hydrolysis and microbial action. During this biodegradation stage, neat TPS showed a faster mass loss rate than TPS/PHBVmp biocomposites. A higher content of water absorbed by neat TPS favors the matrix hydrolysis and the diffusion of microorganisms through unfilled materials. The TPS/PHBVmp biocomposites present a greater difficulty for the absorption of water and the action of microorganisms due to the higher hydrophobicity of the PHBVmp. Moreover, the crystalline structure of the PHBVmp offers a greater resistance to the biodegradation in relation to the starch matrix.

The biodegradation curves show a similar degradation rate independently of the PHBVmp content in the first 30 days, only varying on the quantity of water absorbed during the first week. However, after 30 days, the higher resistance to biodegradation in TPS/PHBVmp biocomposites plasticized with isosorbide and modified with 10% of PHBVmp has become evident, so a higher content in PHBVmp slows down the biodegradation processes of these biocomposites. In any case, all the compositions analyzed were completely biodegraded or not distinguishable from the rest of the soil components after 60 days.

4. Conclusions

In this work, new green and environmentally friendly biocomposites have been obtained from corn starch plasticized with two different amounts of isosorbide and filled with 5 wt% and 10 wt% of PHBVmp. The starch plasticization was achieved by using the melt processing technique. The plasticization process was considered completed because the images obtained by SEM of neat TPS are homogeneous and, also for all neat TPS and TPS/PHBVmp analyzed, the amorphous halo appears in XRD analysis and the bands characteristic of amorphous zones can be seen in ATR-FTIR spectra. The SEM images showed a good physical interaction between the PHBVmp and the thermoplastic matrix. The dispersion of the microparticles was homogeneous and they were not deteriorated during processing, maintaining their spherical shape.

Both, the glass transition temperatures of plasticized-rich and starch-rich phases and the storage modulus values showed that the TPS/PHBVmp biocomposites plasticized with 35% of isosorbide are more rigid than the plasticized with 40% of isosorbide. Moreover, the resistance to moisture was higher with the addition of PHBVmp to the TPS matrix improving the performance of the biocomposites in this regard. The biodegradation in soil was not affected by the addition of PHBVmp to the biocomposites. All the compositions were completely biodegraded after 60 days.

In view of these results, it can be concluded that these biodegradable

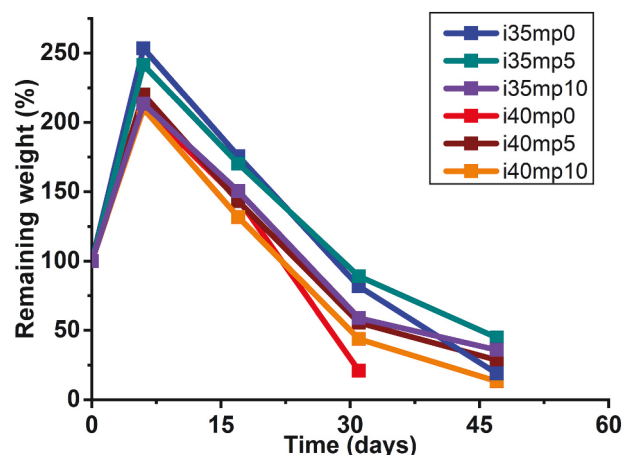


Fig. 6. Remaining weight as a function of soil burial time for neat TPS and TPS/PHBVmp biocomposites.

TPS/PHBVmp biocomposites, obtained by melt processing, could be used for packaging applications.

CRediT authorship contribution statement

Miguel R. Area: Methodology, Investigation, Formal analysis, Writing - original draft.

Belén Montero: Conceptualization, Methodology, Validation, Writing - review & editing.

Maite Rico: Conceptualization, Methodology, Validation, Writing - review & editing.

Luis Barral: Validation, Funding acquisition, Supervision.

Rebeca Bouza: Funding acquisition, Project administration.

Joaquín López: Validation, Supervision.

Declaration of competing interest

None.

Acknowledgements

This work was supported by the Xunta de Galicia Government and FEDER: program of consolidation and structuring competitive research units [grant number: ED-431C 2019/1]. The authors would like to thank ROQUETTE for kindly supplying corn starch. Funding for open access charge was provided by Universidade da Coruña/CISUG.

References

- [1] J.R. Jambeck, R. Geyer, C. Wilcox, T.R. Siegler, M. Perryman, A. Andraday, R. Narayan, K.L. Law, Plastic waste inputs from land into the ocean, *Science* 347 (2015) 768–771, <https://doi.org/10.1126/science.1260352>.
- [2] R.C. Thompson, C.J. Moore, F.S. Vom Saal, S.H. Swan, Plastics, the environment and human health: current consensus and future trends, *Philos. Trans. R. Soc. B-Biol. Sci.* 364 (2009) 2153–2166 (doi:10.1098%2Frbst.2009.0053).
- [3] A.M. Nafchi, M. Moradpour, M. Saeidi, A.K. Alias, Thermoplastic starches: properties, challenges, and prospects, *Starch/Stärke*. 65 (2013) 61–72, <https://doi.org/10.1002/star.201200201>.
- [4] J. Keskinvalli, S. Rautiainen, M. Heikkilä, T.T.T. Myllymäki, J. Karjalainen, K. Lagerblom, M. Kernell, M. Vehkamäki, K. Meinander, T. Repo, Isosorbide synthesis from cellulose with efficient and recyclable ruthenium catalyst, *Green Chem.* 19 (2017) 4563–4570, <https://doi.org/10.1039/C7GC01821E>.
- [5] M.R. Area, M. Rico, B. Montero, L. Barral, R. Bouza, J. López, C. Ramírez, Corn starch plasticized with isosorbide and filled with microcrystalline cellulose: processing and characterization, *Carbohydr. Polym.* 206 (2019) 726–733, <https://doi.org/10.1016/j.carbpol.2018.11.055>.
- [6] M.R. Area, B. Montero, M. Rico, L. Barral, R. Bouza, J. López, Properties and behavior under environmental factors of isosorbide-plasticized starch reinforced with microcrystalline cellulose biocomposites, *Int. J. Bio. Macromol.* 164 (2020) 2028–2037, <https://doi.org/10.1016/j.ijbiomac.2020.08.075>.

- [7] D. Battezzore, S. Bocchini, G. Nicola, E. Martini, A. Frache, Isosorbide, a green plasticizer for thermoplastic starch that does not retrograde, *Carbohydr. Polym.* 119 (2015) 78–84, <https://doi.org/10.1016/j.carbpol.2014.11.030>.
- [8] S. Punia, W.S. Whiteside, Nano-cellulose reinforced starch bio composite films—a review on green composites, *Int. J. Biol. Macromol.* 185 (2021) 849–860, <https://doi.org/10.1016/j.ijbiomac.2021.07.017>.
- [9] T.J. Gutiérrez, V.A. Alvarez, Cellulosic materials as natural fillers in starch-containing matrix-based films: a review, *Polym. Bull.* 74 (2016) 2401–2430, <https://doi.org/10.1007/s00289-016-1814-0>.
- [10] M. Bootklad, K. Kaewtatip, Biodegradation of thermoplastic starch/eggshell powder composites, *Carbohydr. Polym.* 97 (2013) 315–320, <https://doi.org/10.1016/j.carbpol.2013.05.030>.
- [11] B. Jiang, S. Li, Y. Wu, J. Song, S. Chen, X. Li, H. Sun, Preparation and characterization of natural corn starch-based composite films reinforced by eggshell powder, *CyTA—J. Food.* 16 (2018) 1045–1054, <https://doi.org/10.1080/19476337.2018.1527783>.
- [12] F. Xie, E. Pollet, P.J. Halley, L. Avérous, Starch-based nano-biocomposites, *Prog. Polym. Sci.* 38 (2013) 1590–1628, <https://doi.org/10.1016/j.progpolymsci.2013.05.002>.
- [13] A.L. Rivera-Briso, A. Serrano-Aroca, Poly(3-hydroxybutyrate-co-3-hydroxyvalerate): enhancement strategies for advanced applications, *Polymers.* 10 (2018) 732, <https://doi.org/10.3390/polym10070732>.
- [14] Y. Wang, R. Chen, J. Cai, Z. Liu, Y. Zheng, H. Wang, Q. Li, N. He, Biosynthesis and thermal properties of PHBV produced from levulinic acid by *Ralstonia eutropha*, *PLoS One* 8 (2013), e60318, <https://doi.org/10.1371/journal.pone.0060318>.
- [15] Y. Farrag, B. Montero, M. Rico, L. Barral, R. Bouza, Preparation and characterization of nano and micro particles of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) via emulsification/solvent evaporation and nanoprecipitation techniques, *J. Nanopart. Res.* 20 (2018) 71, <https://doi.org/10.1007/s11051-018-4177-7>.
- [16] S. Malmir, B. Montero, M. Rico, L. Barral, R. Bouza, Y. Farrag, Novel self-reinforced films based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV) and PHBV microparticles, *Polym. Eng. Sci.* 59 (2018) E120–E128, <https://doi.org/10.1002/pen.24882>.
- [17] S. Malmir, B. Montero, M. Rico, L. Barral, R. Bouza, Y. Farrag, Effects of poly(3-hydroxybutyrate-co-hydroxyvalerate) microparticles on morphological, mechanical, thermal and barrier properties in thermoplastic potato starch films, *Carbohydr. Polym.* 194 (2018) 357–364, <https://doi.org/10.1016/j.carbpol.2018.04.056>.
- [18] N. Pettinelli, S. Rodríguez-Llamazares, Y. Farrag, R. Bouza, L. Barral, S. Feijoo-Bandín, F. Lago, Poly(hydroxybutyrate-co-hydroxyvalerate) microparticles embedded in κ -carrageenan/locust bean gum hydrogel as a dual drug delivery carrier, *Int. J. Biol. Macromol.* 146 (2020) 110–118, <https://doi.org/10.1016/j.ijbiomac.2019.12.193>.
- [19] K. Frost, D. Kaminsky, G. Kirwan, E. Lascaris, R. Shanks, Crystallinity and structure of starch using wide angle X-ray scattering, *Carbohydr. Polym.* 78 (2009) 543–548, <https://doi.org/10.1016/j.carbpol.2009.05.018>.
- [20] N.F. Magalhães, C.T. Andrade, Properties of melt-processed poly(hydroxybutyrate-co-hydroxyvalerate)/starch 1:1 blend nanocomposites, *Polímeros.* 23 (2013) 366–372, <https://doi.org/10.4322/polimeros.2013.034>.
- [21] J.J.G. Van Soest, S.H.D. Hulleman, D. de Wit, J.F.G. Vliegthart, Crystallinity in starch bioplastics, *Ind. Crop. Prod.* 5 (1996) 11–22, [https://doi.org/10.1016/0926-6690\(95\)00048-8](https://doi.org/10.1016/0926-6690(95)00048-8).
- [22] M.D. Slongo, S.D.F. Brandolt, T.S. Daitx, R.S. Mauler, M. Giovanela, J.S. Crespo, L. N. Carli, Comparison of the effect of plasticizers on PHBV-and organoclay-based biodegradable polymer nanocomposites, *J. Polym. Environ.* 26 (2018) 2290–2299, <https://doi.org/10.1007/s10924-017-1128-9>.
- [23] H. Yu, B. Sun, D. Zhang, G. Chen, X. Yang, J. Yao, Reinforcement of biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with cellulose nanocrystal/silver nanohybrids as bifunctional nanofillers, *J. Mater. Chem. B* 2 (2014) 8479, <https://doi.org/10.1039/C4TB01372G>.
- [24] H. Sato, R. Murakami, A. Padermshoke, F. Hirose, K. Senda, I. Noda, Y. Ozaki, Infrared spectroscopy studies of CH₂-O hydrogen bondings and thermal behaviour of biodegradable poly(hydroxyalcanoate), *Macromolecules.* 37 (2004) 7203–7213, <https://doi.org/10.1021/ma049117o>.
- [25] S. Bloembergen, D.A. Holden, Studies of composition and crystallinity of bacterial poly(β -hydroxybutyrate-co- β -hydroxyvalerate), *Macromolecules* 19 (1986) 2865–2871, <https://doi.org/10.1021/ma00165a034>.
- [26] S. Luo, A.N. Netravali, A study of physical and mechanical properties of poly(hydroxybutyrate-hydroxyvalerate) during composting, *Polym. Degrad. Stabil.* 80 (2003) 59–66, [https://doi.org/10.1016/S0141-3910\(02\)00383-X](https://doi.org/10.1016/S0141-3910(02)00383-X).
- [27] S. Luo, J. Cao, A.G. McDonald, Interfacial improvements in a green biopolymer alloy of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and lignin via in situ reactive extrusion, *ACS Sustain. Chem. Eng.* 4 (2016) 3465–3476, <https://doi.org/10.1021/acssuschemeng.6b00495>.
- [28] J. Raquez, Y. Nabar, M. Srinivasan, B. Shin, R. Narayan, P. Dubois, Maleated thermoplastic starch by reactive extrusion, *Carbohydr. Polym.* 74 (2008) 159–169, <https://doi.org/10.1016/j.carbpol.2008.01.027>.
- [29] S. Karimi, A. Dufresne, P.M. Tahir, A. Karimi, A. Abdulkhani, Biodegradable starch-based composites: effect of micro and nanoreinforcements on composite properties, *J. Mater. Sci.* 49 (2014) 4513–4521, <https://doi.org/10.1007/s10853-014-8151-1>.
- [30] R. Kizil, J. Irudayaraj, K. Seetharaman, Characterization of irradiated starches by using FT-Raman and FTIR spectroscopy, *J. Agric. Food Chem.* 50 (2002) 3912–3918, <https://doi.org/10.1021/jf011652p>.
- [31] J.J.G. Van Soest, H. Tournois, D. de Wit, J.F.G. Vliegthart, Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy, *Carbohydr. Res.* 279 (1995) 201–214, [https://doi.org/10.1016/0008-6215\(95\)00270-7](https://doi.org/10.1016/0008-6215(95)00270-7).
- [32] A. Orue, M.A. Corcuera, C. Peña, A. Eceiza, A. Arbelaz, Bionanocomposites based on thermoplastic starch and cellulose nanofibers, *J. Thermoplast. Compos. Mater.* 29 (2016) 817–832 (doi:10.1177/2F0892705714536424).
- [33] H. Schmitt, A. Guidez, K. Prashantha, J. Soulestin, M.F. Lacrampe, P., Krawczak studies on the effect of storage time and plasticizers on the structural variations in thermoplastic starch, *Carbohydr. Polym.* 115 (2015) 364–372, <https://doi.org/10.1016/j.carbpol.2014.09.004>.
- [34] I. Capron, P. Robert, P. Colonna, M. Brogly, V. Planchot, Starch in rubbery and glassy states by FTIR spectroscopy, *Carbohydr. Polym.* 68 (2007) 249–259, <https://doi.org/10.1016/j.carbpol.2006.12.015>.
- [35] N.L. García, L. Ribba, A. Dufresne, M. Aranguren, S. Goyanes, Effect of glycerol on the morphology of nanocomposites made from thermoplastic starch and starch nanocrystals, *Carbohydr. Polym.* 84 (2011) 203–210, <https://doi.org/10.1016/j.carbpol.2010.11.024>.
- [36] X. Qiao, Z. Tang, K. Sun, Plasticization of corn starch by polyol mixtures, *Carbohydr. Polym.* 83 (2011) 659–664, <https://doi.org/10.1016/j.carbpol.2010.08.035>.
- [37] P.R. Chang, R. Jian, P. Zheng, J. Yu, X. Ma, Preparation and properties of glycerol plasticized starch (GPS)/cellulose nanoparticle (CN) composites, *Carbohydr. Polym.* 79 (2010) 301–305, <https://doi.org/10.1016/j.carbpol.2009.08.007>.
- [38] M. Babae, M. Jonoobi, Y. Hamzeh, A. Ashori, Biodegradability and mechanical properties of reinforced starch nanocomposites using cellulose nanofibers, *Carbohydr. Polym.* 132 (2015) 1–8, <https://doi.org/10.1016/j.carbpol.2015.06.043>.
- [39] R.A. Ilyas, S.M. Sapuan, M.R. Ishak, E.S. Zainudin, Development and characterization of sugar palm nanocrystalline cellulose reinforced sugar palm starch bionanocomposites, *Carbohydr. Polym.* 202 (2018) 186–202, <https://doi.org/10.1016/j.carbpol.2018.09.002>.
- [40] N.F. Magalhães, C.T. Andrade, Thermoplastic corn starch/clay hybrids: effect of clay type and content on physical properties, *Carbohydr. Polym.* 75 (2009) 712–718, <https://doi.org/10.1016/j.carbpol.2008.09.020>.