CrossMark

RESEARCH ARTICLE

Biodegradable packaging materials conception based on starch and polylactic acid (PLA) reinforced with cellulose

Fatma Masmoudi[1,3,4] · Atef Bessadok[2] · Mohamed Dammak[3] · Mohamed Jaziri[4] ·
Emna Ammar[1]

Abstract The plastic materials used for packaging are increasing leading to a considerable amount of undegradable solid wastes. This work deals with the reduction of conventional plastics waste and the natural resources preservation by using cellulosic polymers from renewable resources (alfa and luffa). Plasticized starch films syntheses were achieved at a laboratory scale. These natural films showed some very attractive mechanical properties at relatively low plasticizers levels (12 to 17 % by weight). Furthermore, mixtures including polylactic acid polymer (PLA) and cellulose fibers extracted from alfa and luffa were investigated by melt extrusion technique. When used at a rate of 10 %, these fibers improved the mixture mechanical properties. Both developed materials were biodegradable, but the plasticized starch exhibited a faster biodegradation kinetic compared to the PLA/cellulose fibers. These new materials would contribute to a sustainable development and a waste reduction.

✉ Emna Ammar
ammarenis@yahoo.fr

[1]   Research Unit Urban and Coastal Environments, National Engineering School of Sfax, BP 1173, 3038 Sfax, Tunisia

[2]   Preparatory Institute for Engineering Studies of Gafsa, Gafsa, Tunisia

[3]   Packaging Technical Centre (PACKTEC), Tunis, Tunisia

[4]   Electrochemistry and Environment Laboratory, National Engineering School of Sfax, Sfax, Tunisia

Introduction

The plastic use has been controversial for some time because of the lack of recycling facilities or the infrastructure, its nonsustainable use (Hopewell et al. 2009), nonrenewability, nonbiodegradability, and its toxic additives incorporation. Current trends indicate that steady growth will occur in the use of biodegradable plastics with increasing availability of suitable materials and because of societal and legislative pressure (Rasato 2009; Urtuvia et al. 2014).

Some of the earliest attempts to solve these problems have focused on blending plastic materials with cheap and biodegradable natural biopolymers, such as starch, cellulose, and polylactic acid (PLA) (Fukushima et al. 2009; Shirai et al. 2013; Araújo et al. 2014). Other works opted for chitin to create new materials with the desired properties (Orhan and Buyukgungor 2000; Degli-Innocenti et al. 2001; Abou-Zeid et al. 2001; Cao et al. 2002; Ishiaku et al. 2002) and to reduce environmental pollution (El Hadji 2008). In particular, biodegradable polymers showed encouraging technical and industrial advantages. Indeed, they can be transformed by most conventional plastics processing techniques such as injection, extrusion, and compounding. However, some adjustments are needed according to the conditions and compounding stage and of the shear controlled orientation in injection molding in the molding process (Mano et al. 2004). Film extrusion, injection molding, blow molding, and thermoforming are the main processing techniques that have used biodegradable polymers and have been introduced mainly in three sectors, i.e.,

packaging (Gilfillan et al. 2016), agriculture (Sharma and Mudhoo 2011), and medicine (Pêgo et al. 2013). As a result, biodegradable polymers applications include not only pharmacological devices, such as matrices for enzyme immobilization and controlled-release devices, but also therapeutic devices, such as temporary prosthesis and porous structures for tissue engineering (Catro et al. 2008; Rudnik 2013). Indeed, because of its biomimetic routes and in vivo biocompatible behavior, it can be used for a broad of applications such as bone fixation/replacement applications, bone cements, drug delivery devices, and tissue engineering scaffolds (Mano et al. 2004).

Furthermore, natural fibers present many environmental and hygienic advantages compared to synthetic ones, especially their relative low molecular weight, and their easy recycling and biodegradation. They are also renewable resources, characterized by relatively high strength and stiffness, and are harmless for human beings. However, these materials present some disadvantages such as their moisture uptake, their low thermal stability, and their properties fluctuations. Many investigations were developed on the natural fibers potential as reinforcements for composites (Huang et al. 2003; Dersch et al. 2004; Li and Xia 2004; Greiner and Wendorff 2007; Reneker et al. 2007; Agarwal et al. 2008). In several cases, the results showed that the natural fiber composites were characterized by high stiffness, but their strength remained lower than those of the glass fiber composites (Oksman et al. 2003).

There are two types of naturally available degradable polymers cellulose and starch. The latter is an organic biosynthesized carbohydrate that is abundant in nature. Besides, it has interesting film-forming properties (Hoover 2001; Averous 2004; Zhang et al. 2007; Averous and Halley 2009; Perez et al. 2009; Xie et al. 2012; Averous 2013). Hence, it would be very interesting to investigate the possibility of merging the plastic polymers and the biodegradable polymers in order to obtain a biodegradable plastic that can rid the environment from these polluting plastics.

According to the American Society for Testing and Materials (ASTM), a biodegradable plastic is a plastic that degrades under the effect of the natural activity of existing microorganisms such as bacteria, fungi, and algae. Therefore, a biodegradable plastic differs from a compostable one. Indeed, during the composting phases, compostable plastic undergoes degradation by biological processes with other known organic materials to yield biomass that releases carbon dioxide, water, and an organic soil amendment rich in humic substances where the microorganisms are at a consistent rate but not visually distinguishable, without any toxic residues. As a result, all compostable plastics are biodegradable, but not the reverse (Kale et al. 2007; Thouand 2014).

The purpose of this paper is to investigate and characterize the properties of new packaging materials produced from vegetal biomass such as cellulose and starch which are renewable and biodegradable.

Materials and methods

Materials

Starch

The three types of starch used in this study were extracted from wheat, corn, and potato. They were purchased from Roquette (France). These three raw materials were characterized by a small particle size inferior to 200 μm and a gelification temperature of 65, 75, and 85 °C for starches from potato, maize, and wheat, respectively. The three starches were stored at 23 °C with 50 % relative humidity (RH) to keep them constant.

PLA

The polylactic acid $(CH_3CHCOO)_n$ (Sigma), a completely biodegradable polymer, was used because it is frequently incorporated into the food packaging materials.

PLA with molecular weight of 96,000 was supplied by NatureWorks (Nebraska, USA).

Alfa

Alfa (*Stipa tenacissima* L.) is an abundant plant which can reach 50 to 80 cm in height. Its fiber diameter ranges from 1 to 2 mm. The alfa used in this study was harvested in Kasserine (Tunisia).

Luffa

Luffa (*Luffa cylindrica*), the liane vegetable sponge, is a plant rich in cellulose, having 45 cm in length and imported from Egypt.

Glycerol

Glycerol or glycerin (Sigma), the most studied organic plasticizer in the field of the biopolymers, was used as a plasticizer. It reduces the viscosity, the modules, and the constraints in the break of the polymers where it is incorporated, and increases the elongation. These properties were particularly salient when proteins and starch were used as polymer.

Bacteria

For biodegradability tests, *Bacillus* strains, the ubiquitous genus, were used. This bacterial genus which is commonly found in the environment is responsible for the biodegradation. The used strains were *Bacillus subtilis* and *Bacillus cereus thermophilus*. These two strains were isolated in the microbiology laboratory of the National Engineering School of Sfax (Tunisia).

Methods

Cellulose extraction

Cellulose fibers were extracted from the alfa stalks. These fibers were cut into small pieces of 3–4 cm (15 g) and then treated with NaOH solution (0.25 M). The hydrolysis process lasted for 6 h at 60 °C. After the cellulose fibers separation, the solution was sieved. Then, the solid phase (the cellulose) was recovered. The fibers were first washed with distilled water and then rewashed with sodium hypochlorite solution at 5 % during 8 h.

After this bleaching step, the fibers were finally washed with distilled water then dried at 60 °C for 12 h. Then, they were crushed and sieved according to the chemical method (Ben Brahim et al. 2001; Bessadok et al. 2009).

The *Luffa cylindrica* fruits were dried during 3 days at 60 °C and then crushed into a powder.

Starch plasticization

The transformation of the native starch into plasticized material was achieved by extrusion processing polyols as plasticizers compounds. The plasticized starch properties were controlled by the transformation conditions (temperature and mechanical energy) and the plasticizer content.

The thermoplastic-like starch (TPS) method was used; this is one of the main research hints for the biodegradable materials manufacturing (Curvelo et al. 2001).

For these plasticizing assays, a total mass of 12 g of starch mixed with plasticizer was added at different percentages (5, 10, 15, 20, 25, and 30 %). The starch was experimented using five investigated plasticizers. These were glycerin, ethylene glycol, PEG_{200}, PEG_{400}, and PEG_{1500}. To select the best plasticizing material, three starches of different origins (wheat, corn, and potato) were experimented. The plasticizing polymer (12 g) was dissolved in 50 ml of distilled water (Averous 2002).

The different constituents were homogenized in a mixer equipped with a heating system, and the temperature was set at 170 °C (Lourdin et al. 1999). The kneading was stopped when the mixture became viscous and transparent. This product underwent degassing in order to eliminate air bubbles produced during the homogenization. The mixture was then displayed over an anti-adhesive pan and left to dry overnight at room temperature.

Biocomposite elaboration by extrusion

Biocomposites were prepared by melt mixing method, using single-screw extruder with $L/D = 28$ ($D = 25$ mm). The rotational speed of the screw was 100 rpm (rotation per minute). The extrusion consists of a thermo mechanical cycle appealing to the temperature and cutting to mix well at a temperature superior to the matrix melting point of the biocomposite constituents.

During the mechanical mixing of the PLA and the cellulose (Alfa/Luffa), a special attention was given to the manner of processing because of its possible impact on fillers dispersion and on the final material properties. The components were introduced through the hopper, and then the material was entrained by a screw into the extruder where the temperature was set at 210 °C. Long filaments were obtained and cut into small pieces to facilitate the further grinding. After drying for 15 min, the small pieces were ground using a crusher (RetschMuhle). Finally, the material was kept under pressure at 70 bars to cross the channel output. The temperature was kept at about 195 °C for 20 min to obtain plates ready for analyses.

Mechanical properties of the starch biofilm and the PLA plates

Starch biofilms

The rectangular specimen was extended along its major axis at a constant speed until it broke or until the constraint and the deformation reached a predetermined value. The load on the specimen and elongation was measured during the test achieved at the Tunisian Packaging Technical Centre (Packtec).

PLA plates

The mechanical properties were studied in traction for the experimented mixtures in order to predict their behavior at real-use conditions.

The mechanical tensile tests were performed on specimens cut from the plates made by the compression molding method and then cut with a numerically controlled machine. The tested specimen was a dumbbell-shaped one in the case of PLA and its mixtures (thickness = 2 mm). However, it was in a rectangular film in the case of starch and its mixtures ($e = 120$ μm) in accordance with standard specifications (NF T 51–034).

Plasticizers

Five plasticizers were used to perform a homogenous plastic film. These were glycerol, ethylene glycol, polyethylene glycol 200 (PEG_{200}), polyethylene glycol 400 (PEG_{400}), and polyethylene glycol 1500 (PEG_{1500}). All of them were purchased from Sigma.

Free radical-scavenging films

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical which can be reduced by an antioxidant, a proton-donating substrate causing the DPPH decolorization and the absorbance at 514-nm reductions. The color decrease rate is an indicator of the DPPH scavenging capacity (Wang et al. 2008).

The films DPPH free radical-scavenging activities were determined as described by Bersuder et al. (1998), with the same modifications. The bioplastic film was dissolved in acetic acid 0.5 M at 1 mg/ml. A volume of 500 μl of each sample was mixed with 500 μl of 99.5 % ethanol and 125 μl of 0.02 mM DPPH in 99.5 % ethanol as a free radical source. The mixtures were then kept for 60 min in the dark at room temperature, and the DPPH radical reduction was measured at 517 nm, using a UV–visible spectrophotometer (UV mini 1240, UV/VIS spectrophotometer, Shimdzu). The control was conducted in the same manner, but distilled water was used instead of the sample. DPPH radical-scavenging activity was calculated as follows:

DPPH free radical-scavenging activity $(\%)$

$$= \frac{\text{Ac} - \text{Ah}}{\text{Ac}} \times 100$$

Ac was the control reaction absorbance and Ah was the hydrolysates absorbance. A low reaction mixture absorbance indicated a high DPPH radical-scavenging activity. Butylated hydroxyanisole (BHA) was used as a standard. Three trials were made.

Biodegradability tests

Biodegradability can occur in diverse environments such as soil and water or through composting or discharging. As a result, the plastic polymer biodegradation is defined as a defragmentation resulting from the microorganism activities that would deteriorate mechanical properties or modify them depending on the chemical aspect. The biotransformed material with simplified structure is a nontoxic residue for human beings and for the environment (Bastioli 2005).

Two methods were used for the biodegradation determination.

- In the invasion method, the polymers invasion by microorganisms evidenced the materials studied sensitivity to microbial attack. The agar sterile medium (23 g/l) poured into sterilized Petri dishes and inoculated with *Bacillus subtilis* or *Bacillus cereus thermophilus*. The plastic film was then deposited on the inoculated agar medium and the plate was incubated at 37 °C for 2 days (Coma 1992).
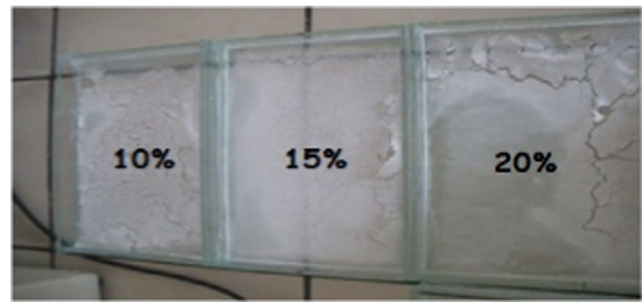

Fig. 1 Films obtained by wheat starch at different PEG$_{200}$ concentrations

- The cell enumeration: Soil microbial flora and that of the compost were enumerated to determine the biomass concentrations before and after the deposited plastic film degradation. Four pots were prepared including a mixture of soil/compost and the biomaterial as follows:

The first pot was filled with sand (1), the second pot with a mixture of sand where a starch potato film was deposited (2). The third pot was filled with compost (3) and the fourth with compost and starch potato film (4).

After 2 days of incubation, a suspension from each pot content was prepared for cell enumeration. First, the count of the total aerobic mesophilic flora was undertaken on a standard agar medium in the Plate Count Agar (PCA, Pronodesia). Second, the total fungal flora was done on the dextrose potato agar (PDA, Pronodesia). Decimal successive dilutions up to 10^{-8} were performed. To estimate the bacterial concentration, the following formula was used:

$$N = \frac{\sum \text{Colonies}}{V \times (\text{n1} + 0.1\ n1) \times d1}$$

N: Number of CFU per gram or per ml of the original product
$\sum$ Colonies: Sum of colonies interpretable dishes $(15 < N < 300)$
n_1: the number of enumerated microorganisms in the first dilution selected
n_2: the number of Petri dishes in the second dilution retained
V: the volume of the deposited solution (1 ml)
d_1: the first dilution factor retained

pH determination: After the biofilm biodegradation, the pH of the substratum used (soil or compost medium) was measured with a pH-meter to evidence the medium modifications. A suspension of 1.5 substratum was prepared and mixed for 2 h. Then, pH value was measured.

Fig. 2 Different types and characteristics of the biofilms and the plates ▶ performed. **a** Plastic film wheat starch and plasticizer. **d** Plastic films with potato starch. **e** Plates prepared with cellulose and PLA at different concentrations respectively (0 %, 10 % of luffa, 10 % of alfa). **f** Example of a friable plate (PLA with 30 % alfa)
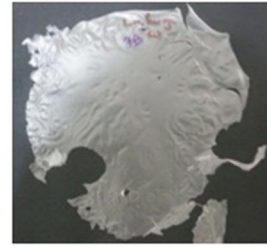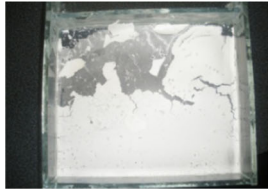
Glycerol 20%   Ethylen glycol 20%

a Plastic film wheat starch and plasticizer

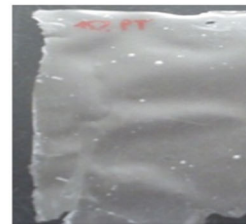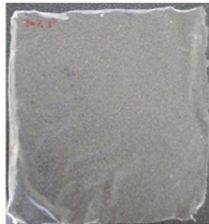**b** Film formed by corn starch mixed with 10% of glycerol

c Film formed by corn starch mixed with 30% of glycerol

30%   20%   15%

d Plastic films with potato starch.

e Plates prepared with cellulose and PLA at different concentrations respectively (0%, 10% of luffa, 10% of alfa).

f Example of a friable plate (PLA with 30% alfa).

Chemical oxygen demand (COD) determination: The COD reflecting the oxygen consumption in potassium dichromate ($K_2Cr_2O_7$) evidenced the residual of organic matter soluble in the water by a chemical oxidation. It is expressed in milligrams O_2 per liter.

The biopolymer was first dissolved in distilled water, and then the solution was subjected to COD determination according to Knechtel (1978). The solution was heated for 2 h at 150 °C and then cooled. The sample optical density was measured at 620 nm and the COD was determined based on a standard solution.

Results and discussion

Plastic biopolymer materials development

Plastic biofilms

To develop a homogeneous plastic film, the three types of starch (wheat, corn, and potato) and the five types of the considered plasticizers were studied.

The obtained plastic films with the PEG at different molecular masses (PEG_{200}, PEG_{400}, and PEG_{1500}) and concentrations (10, 15, and 20 %), mixed with the corn starch at the three experimented concentrations, were dislocated. Therefore, the homogenous films were found for the polymer confectioned with starch extracted from potato, mixed with glycerol, or ethylene glycol at concentrations exceeding 15 %.

Figure 1 shows that the PEG_{200} was a plasticizer incompatible with wheat starch, and the films prepared with different PEG_{200} concentrations were dislocated. This finding could be explained by the high PEG_{200} polymerization degree compared to that of the other plasticizers. Similarly, the mixture of wheat starch and polyethylene glycol with various molecular weights (400 and 1500 g/l) gave nonhomogeneous and dislocated films.

Moreover, at the concentrations of 20 or 30 %, ethylene glycol used as a plasticizer to wheat starch and glycerol produced less homogeneous films, with a medium plasticity as observed in Fig. 2a. Consequently, the polyethylene glycol would not be the best plasticizer, compared to glycerol and ethylene glycol, because of its negative effects on the film. After this first step of compounds performances diagnosis, preliminary tests were carried out using potato or corn starch as polymer at various glycerol and ethylene glycol concentrations (5, 10, 15, 20, and 30 %).

The results found did not show any effective visible difference in the film obtained either with the glycerol or with the ethylene glycol when used as plasticizer. However, corn starch mixed with low plasticizer at the percentages of 5, 10, 15, and 20 % resulted in dislocated films. At the plasticizer concentration of 30 %, the resulting film did not present a uniform appearance (Figs 2b, c). However, the potato starch used at different concentrations (5, 10, 15, 20, and 30 %) showed films of good quality in terms of the aspect, the rigidity, and the transparency criteria (Fig. 2d).

This result differs with a previous study (Angellier et al. 2006). Indeed, those scholars argued that both the potato starch as well as corn starch yielded homogenous films. This difference can be explained by the technical procedure adopted during the biofilm preparation and the native plasticizer used. Indeed, the raw materials as well as experimental conditions (especially relative humidity) seem to have a heavy impact on the conceived biopolymers.

PLA plates

Figure 2e, f shows the plates appearance. They exhibited a compact and crumbly structure. The results showed that the best boards were those prepared with alfa and luffa celluloses at concentrations of 10 and 20 %. Furthermore, at the highest cellulose concentration (30 %) experimented, the conceived material became brittle.
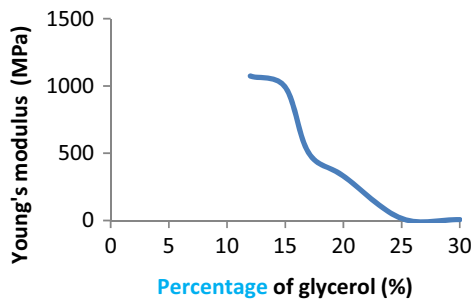
Mechanical properties

Starch and its derived

Figures 3a, b show that the glycerol was a starch solvent. Indeed, at low concentrations (from 0 to 15 %), glycerol acted as a plasticizer. It was inserted between the polymer chains and therefore destroyed locally the interactions responsible for the material cohesion. As a result, a slight reduction in the rigidity and the tensile strength of the starch was noticed. This fact would be assigned to the appearance of movements amplification exhibited at a given deformation.
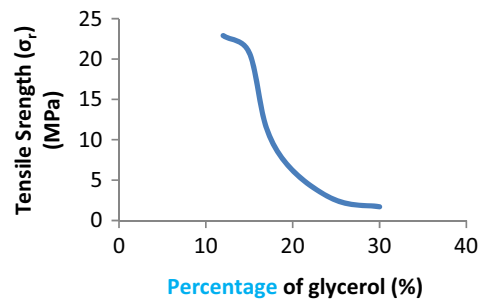
For higher levels of glycerol (from 15 to 25 %), a significant decrease in the material deformation resistance was noticed. In this concentration range, more interchain interactions destruction would occur as the glycerol rate increased, until a total macromolecule constituent dissociation took place, leading to the tangle intra-chain and interchain entire destruction. This fact would be a chain reports slip because of is molecular structure. It can be explained by the sliding of the chains between them.

At a concentration exceeding 30 %, starch seemed to be completely dissolved in glycerol and did not interact with the film microstructure. Figure 3c shows the film elongation progress at breaking point: Until a concentration of about 15 %, the

Fig. 3 Mechanical properties of different materials. **a** Evolution of the Young's modulus of the starch mixtures according to the glycerol percentage. **b** Change in tensile strength of starch mixtures according to the glycerol percentage. **c** Change in elongation at break of blends of starch with the glycerol percentage. **d** Variation of Young's modulus of PLA blends based on the cellulose percentage. **e** Change in tensile strength of blends of PLA based on the cellulose percentage. **f** Variation of elongation at break of PLA blends with cellulose percentage

a Evolution of the Young's modulus of the starch mixtures according to the glycerol percentage.

b Change in tensile strength of starch mixtures according to the glycerol percentage.
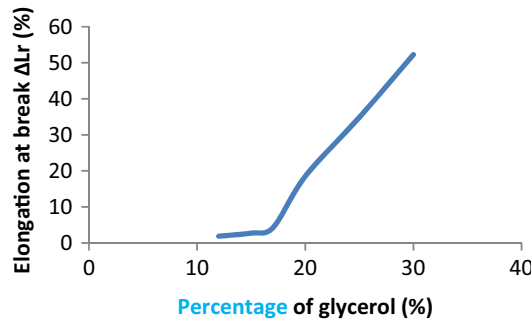
c Change in elongation at break of blends of starch with the glycerol percentage.

d Variation of Young's modulus of PLA blends based on the cellulose percentage.

e Change in tensile strength of blends of PLA based on the cellulose percentage.

f Variation of elongation at break of PLA blends with cellulose percentage.
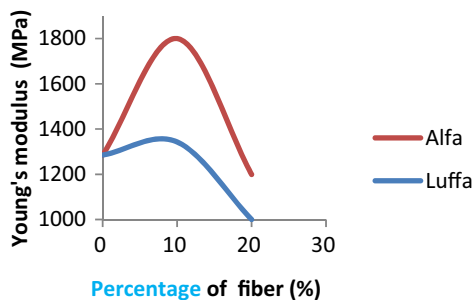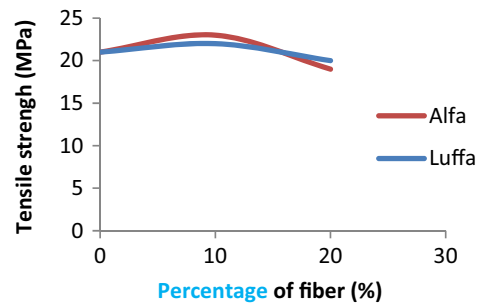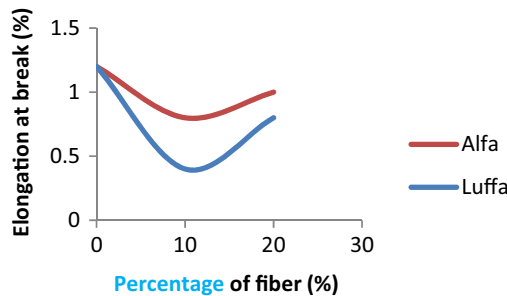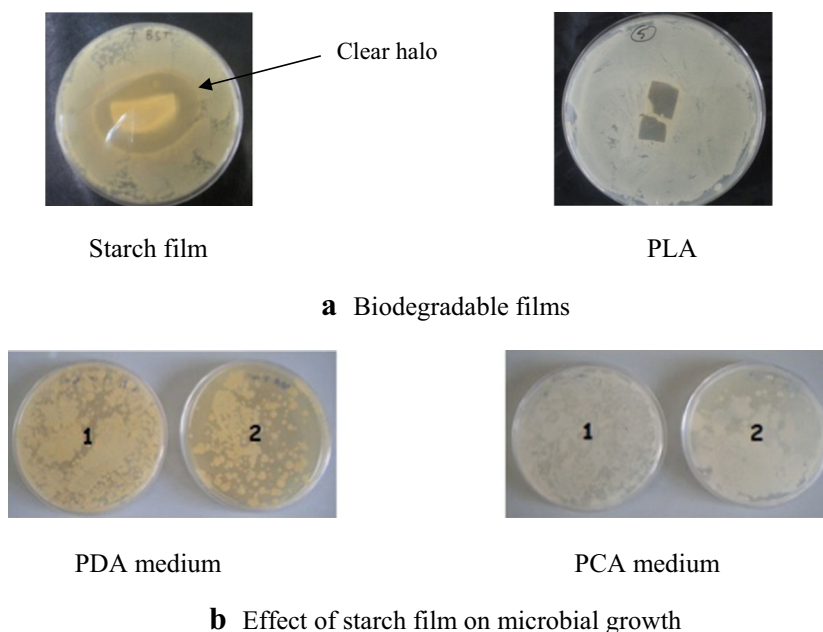
Fig. 4 Microbial effect on starch film. **a** Biodegradable films. **b** Effect of starch film on microbial growth

Clear halo

Starch film · PLA

a Biodegradable films

PDA medium · PCA medium

b Effect of starch film on microbial growth

polymer was characterized by a relatively low elongation rate, but at the break pressure, the material reflected some stiffness and fragility.

Beyond 15 %, the glycerol presence would induce some flexibility of the material structure, following the solvation effect. The latter would increase significantly the plasticizing effect with the glycerol rate.

Phan et al. (2009) reported tensile strength (TS) and elongation at break (EB) values of 42.11 MPa and 3.27 %, respectively, for 3 % agar-based films plasticized with 15 % glycerol. We can say that the starch mixed with glycerol yields better mechanical properties than the agar.

PLA and its mixtures

In the PLA, the cellulose effect when used as a filler could be significant in terms of mechanical properties. This was investigated based on the tensile mechanical properties successively in the elastic region and in the area where the large deformations occurred.

In Fig. 3d, e, it can be observed that the most important values for the stiffness (E) and strain (σe) were obtained for the composition including a fiber content of 10 % cellulose. For this polymer composition, an optimum of the mechanical characteristics was assigned to the appropriate interface state

allowing the best constraints transmission from the PLA matrix to the cellulose filler.

Below this maximum transmission, the mechanical performance increased with the fibers percentage integrated in the mix. As a result, the filler contributed effectively to improve the deformation resistance and to raise the energy required to break the material.

Beyond 10 %, the dispersed fibers partially adhered to the PLA matrix, and consequently, their clear structural defects could be associated to the low density of these interactive forces. These were located at the components interface and would promote the material premature rupture under the biasing action.

Furthermore, the elongation at break was minimal for a polymer formulation with 10 % of cellulose fibers. Each minimum value found would be explained by the high and strong adhesion, reaching the saturation between the filler and the matrix. This effect could be attributed on both the interface improvement when increasing forces of the interactive density and to the interfacial tension reduction between the two phases.

At a filler rate exceeding 10 %, there was an increase in the dispersed filler surface. This could be interpreted as a transmission of most applied stresses to the cellulose fibers and would justify the more rigid structure obtained when 10 % of cellulose fibers were used.

Figure 3f shows that beyond 10 % as a filler rate, the easy sliding chains (PLA and fiber of cellulose) generated by

Table 1 The microbial flora (ufc/g) in different environments

Substrate	Soil	Soil + starch	Compost	Compost + starch
Mesophilic bacterial flora	4.4×10^4	2.2×10^6	1.2×10^4	2.6×10^4
Fungal flora	1.8×10^4	4.2×10^4	2.1×10^3	1.3×10^5

Table 2 The pH and COD values for the different tested environments

Parameter	Soil	Soil + starch film	Compost	Compost + starch film
pH	8.16 ± 0.01	8.01 ± 0.01	7.47 ± 0,01	7.36 ± 0.06
Soluble COD (mg O$_2$/l)	0.344	0.686	0.209	0.781

reducing the adhesion was amplified. In addition, the elongation at break increased with the increase of the fibers rate into the material.

Biopolymer biodegradability

Microbial biodegradation in vitro

The biodegradability of the starch and the PLA was assessed based on the invasion method. After the incubation for 2 days, a clear halo around the biostarch film was noted and exhibited the bacterial capacity to grow on the polymer biofilm, degrading both its compound and structure. Indeed, it was clearly observed that the invasive bacterial growth by *Bacillus subtilis* and *Bacillus cereus thermophilus* was well marked on the biopolymer and decreased the biopolymer area by around 8 % as compared to the initial state. For the PLA, we observed a beginning of a halo formation and a little degradation of the material after 1 month incubation (Fig. 4a).

Previous works argued that the starch can be readily metabolized by a range of microorganisms leading to various fermented products such as ethanol (Jamai et al. 2007; Bai et al. 2008), hydrogen (Tadasa and Takeda 1986; Yang and Shen 2006), and methane (Amon et al. 2007). Furthermore, the widespread microbial species are able to degrade aliphatic polyesters, such as PHA and PLA (Chiellini et al. 1999).

Furthermore, several studies have been conducted on the biodegradation of starch–polyvinyl alcohol (PVOH) blends or their nanocomposites. Chen et al. (1997) observed that the biodegradation rate of starch–PVOH cast films was negatively correlated to the PVOH content in the compost. However,

films including both the starch and the PVOH were degraded much faster than pure PVOH.

Long-term biodegradability in soil

In the present study, we focused on the bacterial counts about the existing telluric flora in the soil/compost before and after the degradation of the starch film to evidence the bioplastic progress in soil/compost. At a first sight, we noticed that pots containing only soil or compost were less concentrated in microorganisms than those included in the film (Fig. 4b).

The total bacterial flora enumeration and that of the yeasts and the fungi in different environments (soil, soil + starch, compost, and compost + starch) are presented in Table 1.

The results showed that the colonies concentration in the environment containing the film of starch was higher than in that containing only the soil or the compost. Indeed, the starch film would be a source of metabolized carbon for bacteria (PCA medium) and fungi (PDA medium). As a result, the microorganisms would use the bioplastic film (starch + glycerol) as a substrate to grow.

pH changes and soluble COD reduction

The pH and the soluble COD determinations are presented in Table 2. The results showed that the pH of the medium containing the starch film was relatively more acidic than that without starch. This could be explained by the acids release by bacteria through carbohydrate assimilation in the environment. Furthermore, the COD increased with the starch film

Fig. 5 Radical scavenging activity on DPPH free radical of the bioplastic films
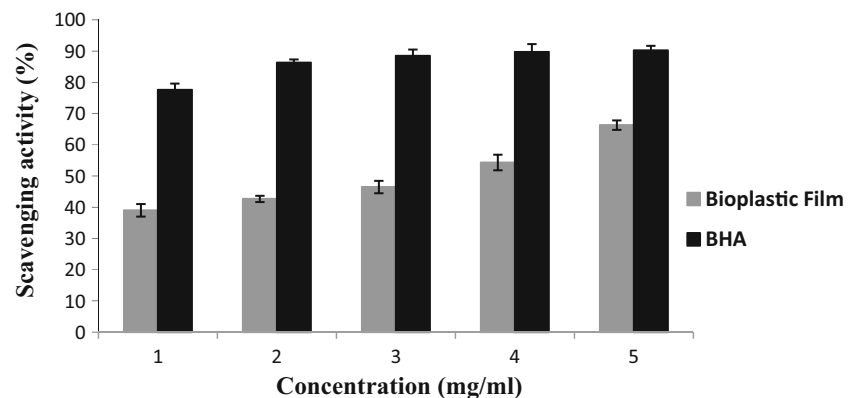
presence, this substrate enriched the soil/compost with organic matter, enhancing the biopolymer biodegradation.

Hence, when the biopolymer degrades, it is converted by the active telluric bioflora and could be dissolved in the carbon structure involving an increase of the soluble COD in the medium. In light of these findings, it would be fairly consistent to conclude that the biopolymer was more rapidly biodegradable in soil than in the compost.

Antioxidant activities

The radical scavenging capacities of bioplastic film increased with the increase of the biofilm concentration (Fig. 5). However, the bioplastic film exhibited a lower radical-scavenging activity than the BHA used as positive control, at the same concentrations. The antioxidant activities of the bioplastic film at 4 and 5 mg/ml concentrations were 54.31 and 66.28 %, respectively. However, the DPPH scavenging activities displayed by the bioplastic film at a concentration of 1 g/ml were 39 %. These findings are in line with previous works. Indeed, Jridi et al. (2013) reported that the DPPH scavenging activity of gelatine film at the same concentration was 31 %. The IC_{50} value of the bioplastic film was 3.48 mg/ml.

Hence, it can be concluded that the bioplastic film might act as an electron or hydrogen donator to scavenge the DPPH.

Conclusion

In the present work, the adopted approach was a biomass substitution of the conventional plastic to preserve and control the ecosystem. To achieve these goals, two alternatives were proceeded. The first was a biopolymer synthesis from different kinds of starch (wheat, corn, and potato); these may be used as a material for "flexible packaging." The film was developed with glycerol as a plasticizer. The most attractive mechanical properties were found for samples including low plasticizer levels (12 to 17 % of glycerol by weight). The second alternative was established based on a commercial plastic (PLA), in order to enhance its mechanical performances and to preserve its biodegradability. This material was reinforced with natural fibers (cellulose from alfa and luffa). To be used as materials for "thick package," these composites were reinforced with fibers at a rate of 10 %, leading to a significant gain in the stiffness as well as in the tensile strength and therefore improving the mixture mechanical properties while being biodegradable.

The study showed that the conceived biopolymers were produced from available raw materials, especially starch, at a relatively low cost. Comparing to previous published data, the present findings show a concordance but with a relatively high biodegradability registered based on microbial enumeration and COD determination.

Consequently, safe, high value-added biopolymers, with potential applications for food packaging, can be produced from the experimented starch and PLA reinforced by natural fibers. These would reduce waste and environmental impact, and generate practical uses contributing to a sustainable system development. Furthermore, it is noteworthy that although the objectives of this study dealt with the conception prototypes (film and plates) to be developed from raw materials, the film production could be potentially scaled up using tape casting as reported by de Moraes et al. (2016).

Future work will focus on the optimization of biodegradability test with different inocula to improve the biomaterial biodegradability, and therefore, it will be possible to contrast the results of the different experimental operations making easier the impact assessment (Sweetlove et al. 2016).

References

Abou-Zeid DM, Muller RJ, Deckwer WDL (2001) Degradation of natural and synthetic polyesters under anaerobic conditions. J Biotechnol 86:113–126

Agarwal S, Wendorff JH, Greiner A (2008) Use of electrospinning technique for biomedical applications. Polymer 26:5603–5621

Amon T, Amson B, Karyvoruchko V, Machmuller A, Hopfner-Sixt K, et al. (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour Technol 98:3204–3212

Angellier H, Molina-Boisseau S, Dole P, Dufresne A (2006) Thermoplastic strach–waxy maize strach nanocrystals nanocomposites. Biomacromolecules 7:531–539

Araújo A, Oliveira M, Oliveira R, Botelho G, et al. (2014) Biodegradation assessement of PLA and its nanocomposites. Environ Sci Pollut Res 21:9477–9486

Averous L (2002) Etude de système polymers multiphasés: approche des relations matériaux-procédés-propriétés. Habilitation à diriger des recherches, Université de Reims Champagne-Ardenne. p 46

Averous L (2004) Biodegradable multiphase systems based on plasticized starch: a review. J Macromol Sci C Polym Rev 44:231–274

Averous L (2013) Synthesis, properties, environmental and biomedical applications of polylactic acid. In: Ebnesajjad S (ed) Handbook of biopolymers and biodegradable plastics. Elsevier, Boston

Averous L, Halley PJ (2009) Biocomposites based on plasticized starch. Biofuels Bioprod Biorefin 3:329–343

Bai FW, Anderson WA, Moo-Young M (2008) Ethanol fermentation technologies from sugar and starch feedstocks. Biotechnol Adv 26:89–105

Bastioli C (2005) Handbook of biodegradable polymers

Ben Brahim S, Ben Cheikh R, Baklouti M (2001) The alfa fibres in composite materials, ID1620, In: International conference on composite materials. 13:25–29

Bersuder P, Hole M, Smith G (1998) Antioxidants from a heated histidine-glucose model system I: investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography. J Am Chem Soc 75:181–187

Bessadok A, Langevin D, Gouanvé F, Chappey C, et al. (2009) Study of water sorption on modified agave fibres. Carbohydr Polym 76:74–85

Cao A, Okamura T, Ishiguro C, Nakayama K, et al. (2002) Polymer 43: 671–679

Catro G, Panilaitis B, Kaplan D (2008) Emulsan, a tailorable biopolymer for controlled release. Bioresour Technol 99:4566–4571

Chen L, Iman SH, Stein TM, Gordon SH (1997) Starch-polyvinyl alcohol cast film-performance and biodegradation. Polym Prepr 37:461–462

Chiellini E, Corti A, Solaro R (1999) Biodegradation of poly(vinyl alcohol) based blown films under different environmental conditions. Polym Degrad Stab 64:305–312

Coma V (1992) Proposition d'une méthodologie pour l'estimation de la biodégradabilité de matériaux d'emballage, Thèse de l'Université Champagne-Ardenne

Curvelo AA, de Carvalho A, Agnelli JAM (2001) Thermoplastic starch–cellulosic fibers composites: preliminary results. Carbohydr Polym 45:183–188

de Moraes CT, Haas Costa TM, de Oliveira RA, Hickmann Flôres S (2016) Valorization of food-grade industrial waste in the obtaining active biodegradable films for packaging. Ind Crop Prod 87:218–228

Degli-Innocenti F, Bellia G, Tosin M, Kapanen A (2001) Detection of toxicity released by biodegradable plastics after composting in activated vermiculite. Polym Degrad Stab 73:101

Dersch R, Greiner R, Wendorff JH (2004) Polymer nanofibers by electrospinning. In: Dekker Encyclopedia of Nanoscience and Nanotechnology. New York, USA

El Hadji BLY (2008) Nouveaux matériaux composites thermo formables à base de fibres de cellulose. Thèse de Doctorat Université de Grenoble. p 19–21

Fukushima K, Abbate C, Tabuani D, Gennari M (2009) Biodegradation of poly(lactic acid) and its nanocomposites. Polym Degrad Stab 94: 1646–1655

Greiner A, Wendorff JH (2007) Electrospinning: a fascinating method for the preparation of ultrathin fibers. Angew Chem Int Ed :5670–5703

Gilfillan WN, Moghaddam L, Bartley J, Doherty WOS (2016) Thermal extrusion of starch film with alcohol. J Food Eng 170:92–99

Hoover R (2001) Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. Carbohydr Polym 45: 253–267

Hopewell J, Dvorak R, Kosior E (2009) Plastics recycling: challenges and opportunities. Philos Trans R Soc B 364:2115–2126

Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S (2003) Modeling inelastic and strength properties of textile laminates: a unified approach. Compos Sci Technol 63:2223–2253

Ishiaku US, Pang KW, Lee WS, Ishak ZAM (2002) Mechanical properties and enzymic degradation of thermoplastic and granular sago starch filled poly(ε-caprolactone). Eur Polym J 38:393–401

Jamai L, Ettayebi K, El Yamani J, Ettayebi M (2007) Production of ethanol from starch by free and immobilized Candida tropicalis in the presence of α-amylase. Bioresour Technol 98:2765–2770

Jridi M, Souissi N, Mbarek A, Chadeyron G, et al. (2013) Comparative study of physico-mechanical and antioxidant properties of edible gelatin films from the skin of cuttlefish. Int J Biol Macromol 61:17–25

Kale G, Kijchavengkul T, Auras R, Rubino M, et al. (2007) Compostability of bioplastic packaging materials: an overview. Macromol Biosci 8:255–277

Knechtel RJ (1978) A more economical method for the determination of chemical oxygen demand. Water Pollut Control:25–29

Li D, Xia Y (2004) Electrospinning of nanofibers: reinventing the wheel. Adv Mater 16:1151–1170

Lourdin, N, Della Valle, G, & Golonna, P (1999) Polymères biodégradables: mise en œuvre et propriétés de l'amidon, Caoutchoucs et plastiques

Oksman K, Skrifvars M, Selin JF (2003) Natural fibres as reinforcement in polylactic acid (PLA) composites. Compos Sci Technol 63:1317–1324

Orhan Y, Buyukgungor H (2000) Enhancement of biodegradability of disposable polyethylene in controlled biological soil. Int Biodeterior Biodegrad 45:49–55

Mano JF, Sousa RA, Boesel LF, Neves NM, Reis RL (2004) Bioinert, biodegrabadle and injectable polymeric matrix composites for hard tissue replacement: state of the art and recent development. J Comput Sci Technol 64:789–817

Pêgo AP, Poot AA, Grijpma DW, Feijen J (2013) Biodegradable elastomeric scaffolds for soft tissue engineering. J Control Release 87:69–79

Perez S, Baldwin PM, Gallant DJ (2009) Structural features of starch granules. Academic Press, San Diego

Rasato D (2009) The emerging world of bioplastics: an industry 'father' looks forward

Reneker DH, Yarin AL, Zussman E, Xu H (2007) Adv Appl Mech 41: 43–195

Rudnik E (2013) Biodegradability testing of compostable polymer materials. In: Handbook of Biopolymers and Biodegradable Plastics: Properties, Processing and Applications. p 213–263

Sharma SK, Mudhoo AA (2011) Handbook of applied biopolymer technology: synthesis, degradation and applications. RSC Publishing, Cambridge

Shirai MA, Grossmann MVE, Mali S, Yamashita F et al (2013) Development of biodegradable flexible films of starch and poly(lactic acid) plasticized with adipate or citrate esters. Carbohydr Polym :19–22

Sweetlove C, Chenèble J, Barthel Y, et al. (2016) Evaluating the ready biodegradability of two poorly water-soluble substances: comparative approach of bioavailability improvement methods (BIMs). Environ Sci Pollut Res. doi:10.1007/s11356-016-6899-3

Tadasa K, Takeda K (1986) Anaerobic digestion of raw starch by bacillus species. J Ferment Technol 64:81–85

The Phan D, Debeaufort F, Voilley A, Luu D (2009) Biopolymer interactions affect the functional properties of edible films based on agar, cassava starch and arabinoxylan blends. J Food Eng 90:548–558

Thouand G (2014) Biodegradability assessments of organic substances and polymers. Environ Sci Pollut Res 16:9443–9444

Urtuvia V, Villegas P, González M, Seeger M (2014) Bacterial production of the biodegradable plastics polyhydroxyalkanoates. Int J Biol Macromol 70:208–213

Wang H, Gao XD, Zhou GC, Cai L, et al. (2008) In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit. Food Chem 106:888–895

Xie F, Halley PJ, Averous L (2012) Rheology to understand and optimize processibility, structures and properties of starch polymeric materials. Polym Sci 37:595–623

Yang H, Shen J (2006) Effect of ferrous iron concentration on anaerobic bio-hydrogen production from soluble starch. Int J Hydrog Energy 31:2137–2146

Zhang QX, Yu ZZ, Xie XL, Naito K, et al. (2007) Nanocomposites. Polymer 48:7193–7200