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Additional Information

Effect of plasticizers on thermal and physical properties of compression-moulded poly[(3-hydroxybutyrate)-co-(3-hydroxyvalerate)] films

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Abstract

Poly[(3-hydroxybutyrate)-co-(3-hydroxyvalerate)] (PHBV) is a promising bio-based, biodegradable polymer for replacing synthetic polymers, but its brittleness limits its application range. With the aim of improving the mechanical properties of PHBV films, different plasticizers (polyethylene glycol (PEG 200, 1000 and 4000), lauric acid (LA) and stearic acid (SA)) were incorporated into the film formulation at 10 wt%. All plasticized films showed lower melting temperature and crystallization degree than pure PHBV films. All plasticizers, except SA, reduced film stiffness and resistance to break, and increased the films' water sorption capacity and solubility as well as their water vapour permeability, but only PEG1000 yielded more extensible films. PEG1000 and PEG4000 gave rise to the most heat-resistant plasticized films, while LA and SA highly promoted the heat-sensitivity of PHBV. PEG1000 was the most effective at plasticizing PHBV films, and it was the only plasticizer that partially mitigated the ageing effects. However, a greater ratio of plasticizer would be required to adapt PHBV mechanical properties to some packaging requirements.

Keywords: Tensile properties, ageing, PHBV, crystallization, thermal stability.

1. Introduction

In recent years, numerous research works have focused on the valorisation of biodegradable polymers obtained from organic waste. Materials derived from renewable sources containing polysaccharides, lipids and proteins can be consumed by microorganisms, especially by bacteria, in order to obtain monomers such as hydroxyalkanoic acids (with many structural variations) and D- and L-lactic acid [1]. These monomers have been used to produce polyhydroxyalkanoates (PHAs) and polylactic acid (PLA), respectively, two of the most important biodegradable polymers derived from renewable sources [2,3].

PHAs are a family of linear polyesters of 3, 4, 5 and 6-hydroxyacids synthesised by a wide variety of bacteria, including strains of *Pseudomonas*, *Bacillus*, *Ralstonia*, *Aeromonas* or *Rhodobacter* [4]. These polymers are present in the cells as cytoplasmic inclusions, which are used as energy reserves [5,6]. Depending on polymer composition, a wide range of PHAs with desirable properties can be obtained, from polymers which are stiff and crystalline to others that are flexible and rubbery [7].

Poly-hydroxybutyrate (PHB) is one the most widely studied PHAs [8;9]. The physical properties of PHB are often compared to those of isotactic polypropylene because they have similar melting points, degrees of crystallinity and glass transition temperatures [10, 11]. PHB presents good processability, and it yields materials with a high degree of transparency and stiffness. Even if PHB has many interesting properties, its inherent brittleness, relatively low thermal stability, ageing behaviour and high cost of production restrict its range of applications [12] and thus it is often combined with other polymers such as PLA to obtain films with improved properties [13; 14; 15;16].

PHB's great fragility is described as a result of different factors, which also contribute to its ageing behaviour: (1) secondary crystallization of the amorphous phase since its glass transition is close to room temperature; (2) low nucleation density, which leads to "large" spherulites or the appearance of cracks in the inter-spherulitic confinements that worsen the film's mechanical properties [17,18]. In order to reduce the brittleness and thermal instability of PHB, copolymers like hydroxyvalerate (known as PHBV) have been obtained. As reported by Savenkova, Gercberga, [19], these copolymers give rise to much less brittle materials, with higher extensibility, than the homopolymers; the higher the hydroxyvalerate content, the more flexible is the material.

Additionally, in order to reduce PHBV brittleness, different plasticizers have been used. These reduce the intermolecular forces along polymer chains, which improve the flexibility and chain mobility, at the same time that they provoke a decrease in glass transition temperature and changes in the crystallization behaviour. The effect of different kinds of plasticizers on the mechanical and thermal properties of PHBV matrices has been studied. Some of these are polyols, such as propylene glycol (PG) or glycerol (G), [20]; citrates, such as triethyl citrate (TEC) [21] and acetyl butyl citrate (ATBC) [12]; polyolefins, such as polyisobutylene [19]; dibutylphthalate (DBP) [21]; dibutyl sebacate [19]; oils and triglycerides, such as castor oil, soybean oil and epoxidised soybean oil (ESO) [20]; surfactants like sophorolipid [22] or poly-ethylene-glycol with medium molecular weight (PEG1000) [20]. In general, medium molecular weight substances with oxygen atoms (e.g. ethers or ketones), which are accessible for interactions with the polymer matrix, such as PEG1000, TEC or DBP, have effectively improved the films' stretchability [20]. The addition of some plasticizers hindered the PHB and PHBV crystallization in line with the interruption of interactions among the polymer chains

[12,17,22]. However, some plasticizers, such as PG, PEG1000 or ESO, promoted molecular mobility, thus favouring the crystallization process [20].

PEGs of different molecular weight have already been used as effective plasticizers in different polymers [23-25], and could be a good alternative for PHBV matrices. No previous studies into the effect of the molecular weight of this compound on its plasticizing effectiveness in PHBV films have been found. Likewise, although surfactants, such as fatty acids, have been used as plasticizers in different matrices [26-28] their potential for PHBV plasticization has not been analysed either.

In this study, the effect of the addition of PEG with different molecular weight and two fatty acids (lauric acid or stearic acid) on crystallization behaviour and thermal stability, tensile properties and water affinity of PHBV films was characterized. In addition, the films were analysed in terms of their microstructural and optical properties, and the impact of storage under controlled conditions (ageing process) on film properties was also evaluated.

2. Materials and methods

2.1. Materials

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) 8% (PHBV) was provided in pellet form by NaturePlast (Caen, France). Polyethyleneglycol 200 (PEG200), polyethyleneglycol 950-1050 (PEG1000), polyethyleneglycol 4000 (PEG4000) and lauric acid (LA) were supplied by Sigma-Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany). Stearic acid (SA) was obtained from Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain).

2.2. Preparation of films

PHBV films were prepared by melt blending and compression-moulding. PHBV was mixed with a constant amount of PEG200, PEG1000, PEG4000, LA or SA (10 wt. % w/w with respect the total solids) on a two-roll mill (Model LRM-M-100, Labtech Engineering, Thailand) at 180 °C and 15 rpm for 10 min. Pure PHBV without plasticizer, used as control film, was processed under the same conditions. Films were obtained by pressing 3.5 g of each blend by using a hydraulic press (Model LP20, Labtech Engineering, Thailand). Steel sheets were pre-heated for 5 min and then compression was performed at 180 °C for 4 min at 100 bar, followed by a cooling cycle to 60°C for 3 min. The films obtained were conditioned at 25 °C and 53% RH for 1 or 5 weeks (initial and final times respectively) prior to testing. In this way, six types of sample were obtained: without plasticizer (PHBV) and with the different plasticizers (PHBV-PEG200, PHBV-PEG1000, PHBV-PEG4000; PHBV-LA and PHBV-SA).

2.3. Film characterization

2.3.1. Film thickness

Film thickness was measured to the nearest 0.0025 mm with a Palmer digital micrometer at six random positions.

2.3.2. Scanning electron microscopy

The cross-section of the samples was observed using a Scanning Electron Microscope (JEOL JSM-5410, Japan). Conditioned film samples were immersed in liquid N₂, cryofractured, fixed on copper stubs, gold coated and observed using an accelerating voltage of 10 kV.

2.3.3. Differential scanning calorimetry (DSC)

DSC analyses were carried out by using a differential scanning calorimeter (Star^c System, Mettler-Toledo, Inc., Switzerland). Film samples (~7 mg) were weighed in aluminium

pan and sealed. Samples were analysed using a double scan: a first heating step from -60 °C to 200 °C at 10 °C/min, then cooling to -60 °C at 50 °C/min and a second heating step at 10 °C/min to 200°C. An empty aluminium pan was used as reference. Measurements were taken in duplicate for each sample under a nitrogen stream of 20 mL/min. The crystallinity degree of the polymer in the films was estimated by using Eq. (11) from the melting enthalpy values (ΔH) of the samples (J/g PHBV) and the melting enthalpy of 100% crystalline PHB ($\Delta H^0_{PHB} = 132$ J/g polymer) [29], by assuming that only PHB crystals are formed in PHBV.

$$X_c(\%) = \frac{\Delta H}{\Delta H^0_{PHB}} \cdot 100 \quad (11)$$

Pure plasticizers were also analysed in terms of their melting properties. Thus, a cooling scan at 10°C/min, followed by 10 min at isothermal conditions (10°C below the melting temperature) and a subsequent heating scan at the same heating rate was applied.

2.3.4. Thermogravimetric analysis (TGA)

A thermogravimetric analyser (Star^e System, Mettler-Toledo, Inc., Switzerland) was used to measure the thermal weight loss of each type of film in duplicate in a temperature range between 25 °C and 600 °C at a heating speed of 10 °C/min under a nitrogen stream of 20 mL/min.

2.3.5. Tensile properties

Mechanical performance of the films was studied according to ASTM standard method D882 [30]. A Universal Testing Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to obtain the stress–strain curves of the samples. From these curves, tensile strength (TS), elastic modulus (EM) and elongation (E) at break of

the films were obtained. Film samples (2.5 x 10 cm) were placed in film-extension grips and stretched until breaking at 50 mm min⁻¹. Measurements (eight replicates per formulation) were considered after film conditioning.

2.3.6. Moisture content

Conditioned films (for 1 or 5 weeks) were dried for 24 h at 60 °C in a convection oven (J.P. Selecta, S.A. Barcelona, Spain) and finally placed in a vacuum oven to complete film drying. Measurements were taken in triplicate.

2.3.7. Film water solubility

Film solubility was determined in triplicate for each film sample by placing it in bi-distilled water at a film:water ratio of 1:100 for 72 h. Then, the film samples were dried in a convection oven (J.P. Selecta, S.A., Barcelona, Spain) for 24 h at 60 °C, and finally drying completed in a vacuum oven. Solubility was expressed as the percentage of dissolved solid mass with respect to the total film solids.

2.3.8. Water sorption isotherms

Film samples were placed in a desiccator with P₂O₅ to complete drying. Afterwards, film samples, in triplicate, were placed at 25 °C in hermetic chambers containing saturated salt solutions with different water activity (*a_w*): LiCl (0.11), CH₃COO₂K (0.23), MgCl₂(0.33), K₂CO₃ (0.43), Mg(NO₃)₂ (0.53), CuCl₂ (0.68), NaCl: (0.75), KCl (0.84). Samples were weighed periodically until constant weight ($\Delta m \approx 0.00001$ g), when the equilibrium was assumed [31]. Then, the equilibrium moisture content was determined by drying them in a vacuum oven at 60 °C and 6.7 KPa for 2 days. The GAB model (Eq. (10)) [32] was applied to fit the sorption data by using a non-linear procedure with the solver tool of Microsoft Excel 2013®.

$$W_e = \frac{W_0 \cdot C \cdot K \cdot a_w}{(1 - K \cdot a_w) \cdot (1 + (C - 1) \cdot K \cdot a_w)} \quad (10)$$

where W_e is the equilibrium moisture content on dry basis, a_w is water activity, W_0 is the monolayer moisture content, C and K are equation parameters, both being temperature dependent and related to the water sorption energy of the film.

2.3.9. Water vapour permeability

Water vapour permeability (WVP) was determined at 25 °C, according to the ASTM E-96-95 [33] gravimetric method, as described by Vargas, Albors [34]. Distilled water was placed in Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium) to obtain 100% RH on one side of the film, while an oversaturated magnesium nitrate solution was used to control the relative humidity on the other side of the film. In order to reduce resistance to water vapour transport, a fan was placed on the top of the cup. The slopes of the steady state period of the curves of weight loss as a function of time were determined by linear regression in order to calculate WVTR, Eq. (8). WVP measurements Eq. (9) were taken in quadruplicate for each type of film.

$$WVTR = \frac{J}{A} \quad (8)$$

$$WVP = \frac{WVTR}{P_{w1} - P_{w2}} \cdot L \quad (9)$$

Where:

WVTR water vapour transmission rate, $\text{g h}^{-1} \text{m}^{-2}$

J slope of the plotting of weight loss vs. time, g h^{-1} .

A area of the film, m^2

P_{w1} partial pressure of water vapour on the film's underside, Pa

P_{w2} partial pressure of water on the film's upper surface, Pa

L film thickness, m.

2.3.10. Optical properties

The transparency of films was studied by applying the Kubelka–Munk theory for multiple scattering to the reflection spectra [35]. The surface reflectance spectra of the films were obtained from 400 to 700 nm using a spectrophotometer CM-3600d (Minolta Co., Tokyo, Japan) on both white and black backgrounds. The internal transmittance (T_i) was obtained by applying Eq. (1), where R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated by Eqs. (2) and (3), where R is the reflectance of the sample layer backed by a known reflectance (R_g). Measurements were taken in triplicate for each sample.

$$T_i = \sqrt{(a - R_0)^2 - b^2} \quad (1)$$

$$a = \frac{1}{2} \cdot \left(R + \frac{R_0 - R + R_g}{R_0 \cdot R_g} \right) \quad (2)$$

$$b = (a^2 - 1)^{\frac{1}{2}} \quad (3)$$

The reflectance of an infinitely thick layer of the material (R_∞) was also determined by means of Eq. (4) in order to obtain the CIE $L^*a^*b^*$ colour coordinates: Lightness (L^*), Chroma (C_{ab}^*) (Eq. (5)) and Hue (h_{ab}^*) (Eq. (6)), using D65 illuminant/10° observer, as reference. The colour difference (Eq. (7)) between the different films and the control film was determined.

$$R_\infty = a - b \quad (4)$$

$$C_{ab}^* = \sqrt{(a^{*2} + b^{*2})} \quad (5)$$

$$h_{ab}^* = \arctg\left(\frac{b^*}{a^*}\right) \quad (6)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (7)$$

2.4. Statistical analysis

Statgraphics Centurion XVI (Manugistics Corp., Rockville, MD) was used to carry out statistical analyses of data through analysis of variance (ANOVA). Fisher's least significant difference (LSD) was used at the 95% confidence level.

3. Results

3.1. Microstructure and thermal behaviour of plasticized and non-plasticized films.

Figure 1 shows the SEM micrographs of the cross-sections of the different films. Non-plasticized PHBV films showed quite a homogeneous structure, exhibiting typical brittle fracture in most of the observed areas, as previously reported by other authors [36]. Nevertheless, plastic deformation and a few threads of a deformed material are discernible on the fracture surface of plasticized films between the brittle zones, as has been described for semicrystalline polymers [37], which was promoted by the plasticizing effect of the amorphous regions in the films. Whereas no notable microstructural effect was observed when PEGs were incorporated to the films, the addition of fatty acids (LA and SA) enhanced the roughness of the surface fracture, thus indicating a different rearrangement of the polymer molecules due to the specific interactions with these less polar plasticizers. In all cases, brittle and plastic deformation regions were observed in the micrographs, with different frequency, depending on the observation area. This is typical of semicrystalline structures such as PHBV films.

To quantify the crystallinity degree in the different films, as affected by plasticizers, DSC analysis was carried out. Table 1 shows melting temperature (T_m) and melting enthalpy (ΔH_m) of PHBV obtained from both first and second heating scans. The values of the first scan revealed the actual crystalline state of PHBV in the thermo-compressed films, whereas for values of the second scan revealed that the thermal history was deleted. Crystallinity degree (X_c) was determined from the melting enthalpy values of first heating step and are also shown in Table 1. As it has been previously observed for PHBV films plasticized with ATBC [12], plasticizers reduced the crystallization degree of the polymers while decreased melting temperature, without notable differences among the different compounds. Crystallization temperature (T_c) and crystallization enthalpy (ΔH_c) of the polymer were also obtained from the cooling scan (Table 1). ΔH_c values were lower than the corresponding ΔH_m values, according to the supercooling effects. These values reflect the crystallization inhibition provoked by plasticizers (lower ΔH_c values in plasticized films). Plasticisers also decreased T_c with respect to the pure polymer, although supercooling effects were similar in all cases; difference between T_c and T_m was 67°C for net PHVB vs. 72-75°C for most of the plasticized films, except for LA (66°C). This also agrees with the PHBV crystallization inhibition induced by plasticizers. In general, the melting temperature reflected the lamella thickness [21]. The obtained results suggested that in all plasticized films thinner lamellae were obtained as compared to net PHBV.

Glass transition temperature (T_g) of the amorphous phase of the polymer was also analysed in the first heating scan. All film formulations showed glass transition at 7°C, without significant differences between samples, which is not coherent with the expected effect of plasticizers in the amorphous phase [12]. This suggests a lack of integration of these compounds within the polymer amorphous region. In this sense, phase separation

of plasticizers could lead to their crystallization in the films. In order to know their melting properties, DSC analysis was carried out and Table 2 shows the melting temperature and enthalpy obtained for pure plasticizers. In all cases, melting temperature was within the temperature range considered for the DSC analyses, except PEG200 which is liquid in the whole interval. Nevertheless, there was no PEG or LA crystallization in PHBV films, as deduced from DSC curves (Fig. 2), where the corresponding melting peaks were not observed. Therefore, no clear phase separation of these compounds in the films can be assumed. On the contrary, there was no complete integration of SA in the polymer matrix, since the DCS thermograms (Fig. 2) exhibited a melting endotherm at 69 °C (ΔH_m : 9.7 J/g film) attributed to SA melting. According to the enthalpy value, and taking into account both the melting enthalpy of totally crystallized SA (ΔH_m : 230J/g) and its mass fraction in the film, it was estimated that 42% of added SA was in crystalline form in the films, thus evidencing phase separation of this compound. After ageing for 5 weeks, a progress in SA crystallization was observed ($\Delta H_m=14.2$ J/g film), which means 62% degree of crystallization for SA in the films. This suggests that SA was not compatible with the PHBV matrix and that it is progressively separated, crystallizing in a different phase.

The values of all PHVB crystallization/melting parameters after 5 weeks of storage were not included in Table 1 since there were no significant differences with respect to the initial values. Thus, no progress in crystallization of polymer throughout storage can be deduced neither in non-plasticized nor in plasticized PHBV. Fabra, López-Rubio [38] also observed that the post-processing time did not alter the T_m or X_c , in PHBV-PEG1000 multilayer films. Branciforti, Corrêa [12], also reported similar behaviour for PHBV (18% V) plasticized with ATBC, although changes in the crystalline forms occurred, as deduced from the changes in the WAXD patterns. After 15 storage days, the mean size

of the crystalline domains of the spherulites increased in plasticized films (recrystallization), although it was not associated with an increase in the crystalline volume fraction of the copolymer.

Incorporation of plasticizers slightly affected thermal degradation behaviour of PHVB films, as can be observed in Figure 3. The temperature values corresponding to the onset of decomposition (T_{onset}) and to the maximum degradation rate (T_{peak}) for the different film formulations are shown in Table 3. A significant decrease in both T_{onset} and T_{peak} of the films occurred for samples plasticized with PEG 200, LA and SA, whereas films plasticized with PEG 1000 and 4000 exhibited similar temperature values to pure PHVB. A second degradation step at higher temperatures was observed in films with PEG 1000 and 4000, as reported for commercial PHA matrices containing additives and plasticizers, which could be associated with the formation of degradation products involving both polymer and plasticizer, with different thermal behaviour.

3.2. Effect of plasticizers on tensile properties of the films

Table 4 shows elastic modulus (EM), tensile strength (TS) and elongation at break (E%) of pure PHBV films and the PHBV films containing plasticizers. Both EM and TS significantly decreased when LA and PEG of different molecular weights were added to the matrix, as previously observed for other plasticizers in PHBV matrices [12,20,21]. Nevertheless, SA enhanced the film stiffness without notable reduction of its resistance to break. In terms of the elongation at break, PEG (especially PEG1000) was the only plasticizer that yielded more extensible films (32% increment). The observed behaviour suggests that, although inter-chain forces were weakened by the incorporation of PEG or LA in the film, as deduced by the reported decrease in the film stiffness, not enough promotion of the film extensibility was obtained to extend the polymer applications to determined packaging requirements. The particular effect of SA could be explained by its

crystallization in the films, which could contribute to the observed hardening effect; the films become more brittle, harder and less flexible.

After 5 weeks of storage, practically all samples became harder and more brittle; EM increased while the elongation at break decreased, as observed by other authors in PHBV films [2,12,17,19,36,39]. These changes have been attributed to the secondary crystallization of the spherulites, although no notable increase in the crystallization degree has been observed [12], as previously commented on. The lack of adequate solubility of plasticizers in the polymer matrix could also contribute to the development of tensile properties throughout storage (progressive separation) as well as their relative low plasticization effect in terms of the promotion of film extension [20]. A greater solubility of plasticizer in PHBV would increase the film extensibility [21], whereas phase separation, due to low solubility, could promote film brittleness in line with the reduction of overall cohesion forces of the polymer network due to the matrix interruption.

3.3. Effect of plasticizers on water affinity of the films

Fig. 4 shows the sorption isotherms at 25 °C for pure PHBV films and for plasticized PHBV films. The sorption isotherm of pure PHBV films exhibited the typical shape of type III isotherms, where the equilibrium water content remained constant until water activity values of 0.7, from which the water content sharply increased. The addition of the plasticizers, except for SA, increased the water sorption capacity of the PHBV matrices especially at a_w values above 0.4. This behaviour is coherent with the higher hydrophilic character of PEG and LA, which enhanced the overall water affinity of the film matrix.

GAB model fits the experimental data accurately, as can be seen in Fig. 4. The monolayer moisture contents (W_0) of the plasticized films were significantly higher than that of pure

PHBV films, especially for the different PEGs, although no changes occurred with SA, which did not notably modify the water affinity of the matrix.

Coherently with these results, the water solubility of the plasticized films increased with respect to the non-plasticized PHBV (Table 5). All of this revealed the different hydrophilic-hydrophobic balance of polymer and plasticizers, which also pointed to possible phase separation. No significant changes in the film solubility occurred after ageing for 5 weeks (values not included).

Likewise, the water vapour permeability (WVP) values of PHBV films (Table 5) notably increased in plasticized films, except for SA where no differences were observed. Pure PHBV films showed WVP values similar to those obtained by Corre, Bruzard [2] for PHBV with 8 % hydroxivalerate. The increase in the free volume of the polymeric structure, provoked by plasticisation, enhances diffusion rate of water molecules and then permeation rate [40]. Similarly, water solubility is also promoted in the more polar plasticized regions, which also contributes to the increase in permeability values. This effect was not expected for SA, according to its water affinity, hence its addition did not induce changes in the WVP of the films. In PEG-containing films, WVP increased with the decrease in the molecular weight of the plasticizer, according to the greatest hydrophilic character of low molecular weight PEG. In general, the WVP of the films did not significantly change during film ageing, although some tendency to decrease was observed in plasticized films, which could be attributed to the rearrangement of the matrix components previously described, such as secondary crystallization or possible phase separation.

3.4. Optical properties of plasticized and non-plasticized films

Internal transmittance (T_i) of the films and colour parameters are shown in Table 6. High values of T_i are related with highly homogeneous and transparent films. The T_i values at

550 nm (the wavelength at which the highest differences were observed) evidenced that while films containing PEG or LA were more transparent than control films, and that the addition of SA significantly reduced the film's transparency. This agrees with the above mentioned crystallization of SA in the film, which promotes light dispersion with the corresponding increase in opacity. In agreement with the changes in the film's light transmission properties when plasticizers were incorporated, the colour parameters, lightness (L^*), chroma (C_{ab}^*) and hue (h_{ab}^*), also changed. Nevertheless, the total colour differences (ΔE) were below 2.4 units, which is below the limit of the human eye perception [41]. Thus, these differences are not relevant to develop packaging materials. Optical parameters after 5 weeks of storage did not show significant differences with respect to the initial values, and thus the values were not included.

4. Conclusions

The plasticizers used led to apparently homogeneous PHBV-based matrices, as supported by DSC analyses, but crystallization of SA in the films occurred. Although the addition of PEG and LA significantly decreased the stiffness and the resistance to break of PHBV films, only PEG1000 yielded more extensible films. PEG1000 was also the only plasticizer that partially mitigated ageing effects. All plasticizers, except SA, increased the water affinity of the films, promoting an increase in WVP and water solubility. All plasticizers slightly decreased the thermal stability of the films. PEG1000 and PEG4000 gave rise to the most heat-resistant plasticized films while LA and SA highly promoted the heat-sensitivity of PHBV. Among the compounds studied, PEG1000 was the most effective at plasticizing PHBV films, although a greater ratio would be required to adapt PHBV mechanical properties to certain packaging requirements.

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Table 1. Melting point (T_m), normalized melting enthalpy (ΔH_m), degree of crystallinity (X_c), crystallization temperature (T_c) and crystallization enthalpy (ΔH_c) of the films.

Mean values \pm standard deviation.

Film	1st heating scan		X_c	Cooling		2nd heating scan	
	T_m (°C)	ΔH_m (J/g)		T_c (°C)	ΔH_c (J/g)	T_m (°C)	ΔH_m (J/g)
PHBV	171.2 \pm 1.2 ^a	97.4 \pm 0.7 ^a	73.8 \pm 0.5 ^a	103.66 \pm 0.02 ^a	75 \pm 1 ^a	168.13 \pm 0.04 ^a	99 \pm 3 ^a
PHBV-PEG 200	166.9 \pm 0.4 ^b	81.4 \pm 1.4 ^{bc}	61.66 \pm 1.06 ^{bc}	98.19 \pm 0.12 ^{ab}	63.4 \pm 0.9 ^b	162.8 \pm 0.4 ^b	85 \pm 2 ^{bcd}
PHBV-PEG 1000	169.12 \pm 0.02 ^c	79 \pm 2 ^b	59.06 \pm 1.5 ^b	95 \pm 3 ^b	60.6 \pm 0.2 ^c	165.71 \pm 0.02 ^{ac}	81 \pm 1 ^{bc}
PHBV-PEG 4000	169.7 \pm 0.4 ^c	81.8 \pm 0.4 ^{bc}	62.0 \pm 0.3 ^{bc}	95 \pm 6 ^b	58.6 \pm 0.5 ^d	166.2 \pm 0.2 ^{ac}	80 \pm 4 ^c
PHBV-LA	166.26 \pm 0.15 ^b	86 \pm 3 ^b	65 \pm 3 ^c	100 \pm 2 ^{ab}	69.9 \pm 0.6 ^e	165 \pm 2 ^{bc}	89 \pm 2 ^d
PHBV-SA	163.6 \pm 0.2 ^d	84 \pm 4 ^{bc}	64 \pm 3 ^{bc}	92.9 \pm 0.2 ^b	68 \pm 0.8 ^a	159.04 \pm 0.06 ^d	86 \pm 1 ^{bd}

a-d: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).

Table 2. Melting point (T_m), melting enthalpy (ΔH_m) of the pure plasticizers. Mean values \pm standard deviation.

Plasticizer	T_m ($^{\circ}\text{C}$)	ΔH_m (J/g)
PEG200	-83.9 ± 0.2	21 ± 2
PEG1000	35.4 ± 0.3	173 ± 4
PEG4000	59.2 ± 0.2	206 ± 5
LA	43.8 ± 0.4	193 ± 3
SA	69.2 ± 0.2	230 ± 3

Table 3. Thermal degradation parameters of the PHBV films, non-stored (initial) and stored for 5 weeks (final).

Mean values \pm standard deviation.

Film	T _{onset} (°C)		T _{Peak} (°C)		% Weight residue at 600°C	
	Initial	Final	Initial	Final	Initial	Final
PHBV	261.42 \pm 0.04 ^{a1}	259.7 \pm 0.2 ^{a2}	269.7 \pm 0.2 ^{a1}	267.8 \pm 0.5 ^{a2}	0.6 \pm 0.1 ^{ab1}	0.6 \pm 0.2 ^{a1}
PHBV-PEG 200	248 \pm 2 ^{bc1}	243 \pm 4 ^{b1}	258 \pm 1 ^{bc1}	257.5 \pm 1.2 ^{b1}	0.4 \pm 0.3 ^{ab1}	0.6 \pm 0.3 ^{a1}
PHBV-PEG 1000	255 \pm 2 ^{ab1}	243 \pm 5 ^{b1}	265 \pm 1 ^{ab1}	255 \pm 3 ^{b2}	0.46 \pm 0.12 ^{ab1}	0.69 \pm 0.02 ^{a1}
PHBV-PEG 4000	253 \pm 2 ^{ab1}	243 \pm 2 ^{b2}	264 \pm 3 ^{ab1}	255.2 \pm 1.5 ^{b1}	0.48 \pm 0.08 ^{ab1}	0.6 \pm 0.2 ^{a1}
PHBV-LA	242 \pm 6 ^{c1}	242.37 \pm 0.06 ^{b1}	253 \pm 6 ^{cd1}	256 \pm 2 ^{b1}	0.7 \pm 0.2 ^{a1}	0.869 \pm 0.003 ^{a1}
PHBV-SA	228 \pm 6 ^{d1}	240.4 \pm 0.5 ^{b1}	246 \pm 6 ^{d1}	254.8 \pm 0.4 ^{b1}	0.18 \pm 0.01 ^{b1}	0.59 \pm 0.02 ^{a2}

a-d: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).

1-2: Different superscripts within the same row indicate significant differences among non-stored and stored films ($p < 0.05$).

T_{onset}: Temperature at which the degradation process begins.

T_{Peak}: Temperature that corresponds to the maximum degradation rate.

Table 4. Elastic modulus (EM), tensile strength (TS) and elongation at break (E%) of the PHBV films, non-stored (initial) and stored for 5 weeks (final). Mean values \pm standard deviation.

Film	EM (MPa)		TS (MPa)		E (%)	
	Initial	Final	Initial	Final	Initial	Final
PHBV	1688 \pm 97 ^{a1}	1756 \pm 42 ^{a1}	37 \pm 3 ^{a1}	35 \pm 2 ^{a1}	3.4 \pm 0.3 ^{a1}	2.5 \pm 0.2 ^{a2}
PHBV-PEG 200	1317 \pm 59 ^{b1}	1504 \pm 61 ^{b2}	27 \pm 2 ^{b1}	27 \pm 2 ^{b1}	3.7 \pm 0.6 ^{a1}	2.9 \pm 0.2 ^{b2}
PHBV-PEG 1000	1260 \pm 71 ^{b1}	1219 \pm 90 ^{c1}	26 \pm 2 ^{b1}	22 \pm 3 ^{c1}	4.5 \pm 0.6 ^{b1}	3.3 \pm 0.4 ^{c2}
PHBV-PEG 4000	1339 \pm 88 ^{b1}	1228 \pm 88 ^{c2}	26 \pm 2 ^{b1}	21 \pm 2 ^{c2}	3.7 \pm 0.2 ^{a1}	2.8 \pm 0.2 ^{ab2}
PHBV-LA	1460 \pm 67 ^{d1}	1562 \pm 49 ^{b2}	28 \pm 2 ^{b1}	29 \pm 2 ^{b1}	2.8 \pm 0.3 ^{c1}	2.6 \pm 0.2 ^{a1}
PHBV-SA	1827 \pm 58 ^{c1}	1377 \pm 54 ^{d2}	33 \pm 2 ^{c1}	22 \pm 2 ^{c2}	2.5 \pm 0.3 ^{c1}	1.83 \pm 0.03 ^{d2}

a-d: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).

1-2: Different superscripts within the same row indicate significant differences among non-stored and stored films ($p < 0.05$).

Table 5. Water vapour permeability (WVP) and solubility (mg solubilized film/g initial dried film) of the films, non-stored (initial) and stored for 5 weeks (final). Mean values \pm standard deviation.

Film	WVP 10^{12} (g/m \cdot s \cdot Pa)		Solubility (mg/g)
	Initial	Final	Initial
PHBV	5.7 ± 0.4^{a1}	4.4 ± 1.4^{a1}	$<0.001^a$
PHBV-PEG 200	17 ± 2^{b1}	14.7 ± 1.2^{b1}	21.2 ± 1.7^b
PHBV-PEG 1000	11.7 ± 1.1^{c1}	13 ± 3^{b1}	34.2 ± 1.4^c
PHBV-PEG 4000	9.7 ± 0.2^{d1}	8.2 ± 0.3^{c2}	25 ± 2^d
PHBV-LA	8.6 ± 0.6^{d1}	5.7 ± 0.3^{a2}	3.2 ± 0.9^e
PHBV-SA	5 ± 0.8^{a1}	6.1 ± 0.8^{a1}	$<0.001^a$

a-e: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).

1-2: Different superscripts within the same row indicate significant differences among non-stored and stored films ($p < 0.05$).

Table 6. Internal transmittance at 550 nm (Ti), Lightness (L*), Chroma (C_{ab}*), hue (h_{ab}*) and Colour difference (ΔE) of the plasticized films with respect to pure PHBV films. Mean values ± standard deviation.

Film	Ti 550 nm	L*	C*	h*	ΔE
PHBV	65.2 ± 2.4 ^a	77.2 ± 0.3 ^a	19.2 ± 0.4 ^a	82.7 ± 0.2 ^a	-
PHBV-PEG 200	68.4 ± 0.9 ^b	75.1 ± 0.2 ^b	20.4 ± 0.2 ^b	80.7 ± 0.13 ^b	2.5
PHBV-PEG 1000	67.9 ± 0.5 ^b	76.3 ± 0.2 ^c	20.3 ± 0.2 ^b	80.9 ± 0.14 ^{bc}	1.5
PHBV-PEG 4000	66.7 ± 0.6 ^{ab}	76,1 ± 0.2 ^c	20.1 ± 0.2 ^b	81.1 ± 0.2 ^c	1.6
PHBV-LA	66.6 ± 0.9 ^{ab}	77.02 ± 0.07 ^a	18.9 ± 0.11 ^a	82.8 ± 0.11 ^a	0.3
PHBV-SA	63 ± 0.8 ^c	78.6 ± 0.4 ^d	18.1 ± 0.3 ^c	83.7 ± 0.3 ^d	1.8

a-d: Different superscripts within the same column indicate significant differences among formulations (p < 0.05).

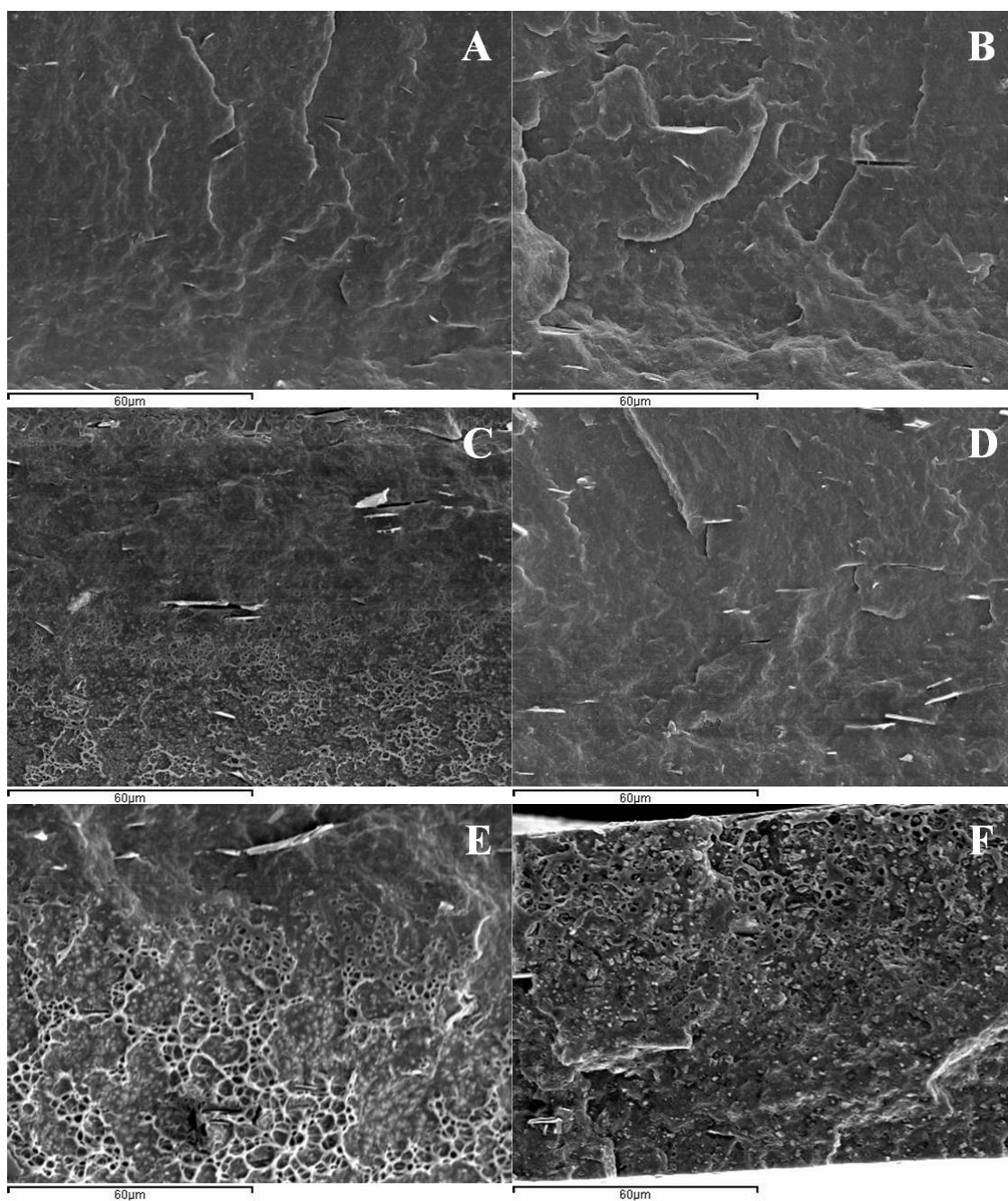


Figure 1. SEM images of the cross-sections of PHBV based films. A: pure PHBV, B: PHBV-PEG200, C: PHBV-PEG1000, D: PHBV-PEG4000, E: PHBV-LA, F: PHBV-SA.

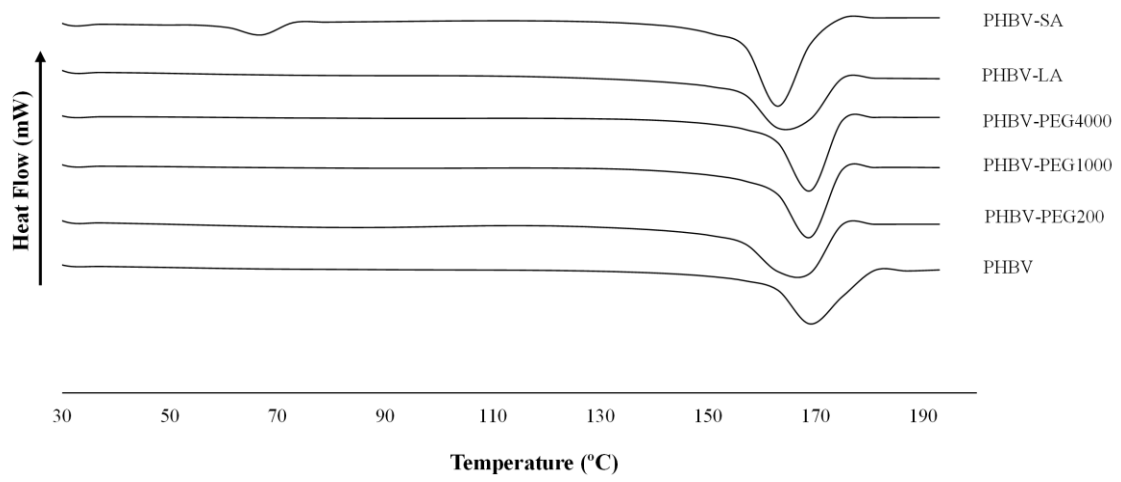


Figure 2. Thermograms for pure PHBV films and films containing 10% of plasticizer: PEG200, PEG1000, PEG4000, lauric acid (LA) and stearic acid (SA).

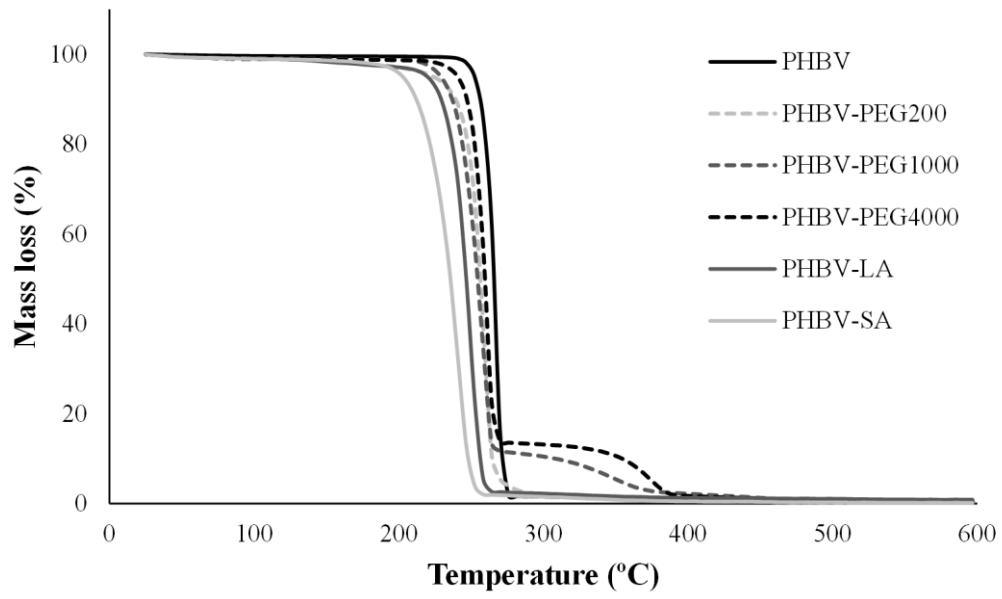


Figure 3. Thermogravimetric curves for pure PHBV films and containing 10% of plasticizer: PEG200, PEG1000, PEG4000, lauric acid (LA) and stearic acid (SA).

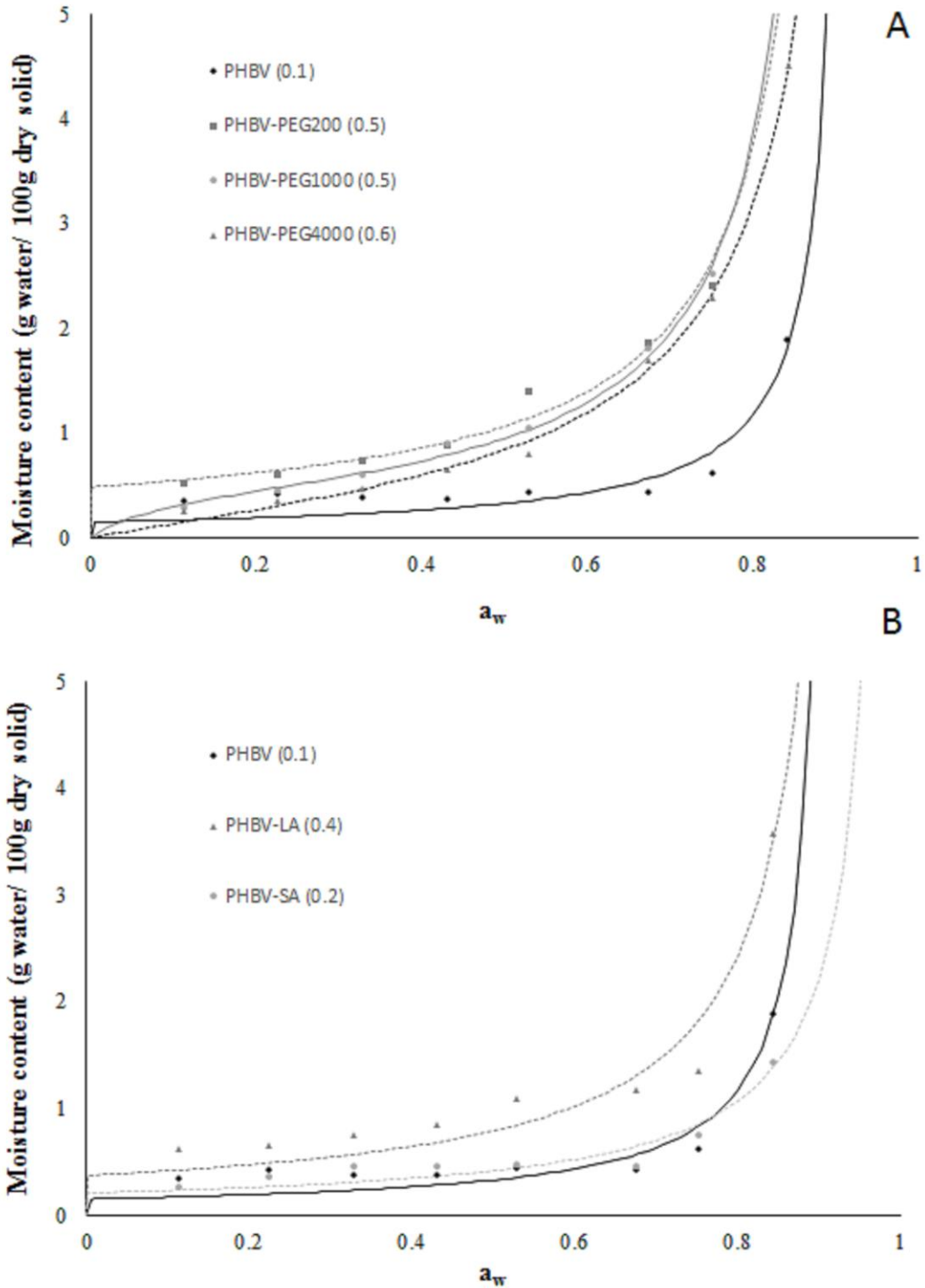


Figure 4. Water sorption isotherms of (A) pure PHBV film and PHBV films containing 10% of PEG200, PEG1000, PEG4000 and (B) control film and PHBV films containing 10% lauric acid (LA) and stearic acid (SA). Experimental points and GAB fitted model (lines). Values of the monolayer moisture content (W_0 , g/100 g solids) are shown for each film in brackets.