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Research article

Plasticization of poly(3-hydroxybutyrate) with biobased terpenoid esters of geraniol

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Abstract. Poly(3-hydroxybutyrate) (P3HB) is, by far, one of the most promising bacterial polyesters at the commercial scale, but it is a brittle polymer due to physical aging occurring at room temperature, which promotes secondary crystallization. Plasticization is a cost-effective and technical approach to overcome or minimize, this drawback. In this work, the use of terpenoid-based organic compounds as plasticizers for P3HB is proposed. Geranyl esters with different chain length carboxylic acids, namely acetic (C2), propionic (C3), butyric (C4), and isovaleric (C5) acids, are used at a constant proportion with the main aim of improving the ductility of P3HB. In addition, thermal properties, morphology, and disintegration are also addressed. All geraniol-based plasticizers provided increased ductility. The elongation at break of neat P3HB (7.4%) increased up to 9.7%, which represents a percentage increase of 31.1%. A remarkable increase in toughness is also obtained by a change in the impact strength from 2.2 kJ·m^{-2} (neat P3HB) up to 3.4 kJ·m^{-2} with geranyl acetate. Thus, the proposed P3HB formulations widen the potential uses of P3HB since its ductile properties are improved. Other relevant results are related to the glass transition temperature reduction and an increase in the disintegration rate in controlled compost soil.

Keywords: *biodegradable polymers, poly(3-hydroxybutyrate), geraniol, terpenoids, plasticizer*

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1. Introduction

Currently, petroleum-derived polymers offer a wide application range in both single-use and long-lasting products [1]. This has led to exponential growth in the worldwide production of plastics, with subsequent environmental issues. As a matter of fact, it has been estimated that 8300 Mt of plastics have been produced between 1950 and 2015, and the number of plastic wastes reached 6300 Mt in this period. But the most worrying issue is that around 79% of these wastes have ended up in controlled landfills or directly in the natural environment [2]. To avoid plastic waste accumulation, biodegradable polymers have gained interest in the last decades [3].

In recent years, upgrading wastes as raw materials for new polymers has gained much interest. Polyhydroxyalkanoates (PHAs) includes a family of linear polyesters with interesting properties from an environmental standpoint. PHAs can be obtained through a bio-fermentation process by using specific bacteria such as *Cupriavidus necator*, *Bacillus*, *Streptomyches*, among others. These bacteria can digest a carbon source (*e.g.*, agro-food industry waste) through an anaerobic process [1, 4–6]. To achieve PHA synthesis,

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these microorganisms have to be subjected to some nutrient limitations in combination with an excess of carbon. In these conditions, bacteria can synthesize PHAs in the form of granules that act as energy storage [7].

Among all the existing PHAs, poly(3-hydroxybutyrate) (P3HB) is one of the most studied to date. It is a brittle polymer due to its high degree of crystallinity with large spherulites. After processing, it is quite ductile, but P3HB, like other PHAs, undergoes a physical aging process related to secondary crystallization that leads to a limited elongation [5, 8]. As reported by El-Hadi *et al.* [9], the embrittlement of P3HB is related to the following reasons. One is the secondary crystallization of the amorphous regions that takes place at room temperature since the T_g of P3HB is below room temperature and, additionally, the nucleation density of P3HB in the melt is very low, which plays a key role in appearing interspherulitic microcracks which have a negative effect on ductile properties. They also propose the use of different additives to overcome (or minimize) these effects, such as plasticizers, lubricants, nucleates, and other polymers to give physical blends. One interesting approach is blending P3HB with other biobased/biodegradable polymers, such as starch and cellulose-based polymers or poly(caprolactone) [10–12]. On the other hand, the use of plasticizers is, traditionally, more interesting from an industrial point of view since plasticizers also provide improved processability [13].

Plasticizers can be divided into two main groups depending on their molecular weight, namely polymeric and monomeric plasticizers. To avoid the potential toxicity of petroleum-based plasticizers, new green alternatives are being searched [14]. In the last decades, research has focused on the plasticization efficiency of bioderived plasticizers such as citrate esters (acetyl tributyl citrate, ATBC; triethyl citrate, TEC), glycerol and its esters (*e.g.*, triacetin, tributyrin), fumaric acid esters, isosorbide, and its esters, among others. Most of these plasticizer systems are very promising in terms of their plasticization efficiency on polyvinyl chloride (PVC) and poly(lactide) (PLA). Moreover, these alternative green plasticizers have been proposed as plasticizers in different polyhydroxyalkanoates [15–18]. Recently, terpenes and terpenoids have been proposed as building blocks for polymer synthesis and modification [19]. Terpenes include a wide range of organic compounds derived from isoprene. On the other hand, terpenoids, or isoprenoids, are terpene-based compounds with additional functional groups, usually oxygen-based functionalities. Terpenes and terpenoids include more than 80 000 chemical compounds that play a key role in flavor, fragrance, cosmetics, and the food industry [20, 21], and their use is increasing in pharmacology and medicine due to their exceptional bioactive properties [22–24]. Terpenoid esters have been proposed as biobased plasticizers, as reported by Mangeon *et al.* [25]. They obtained interesting plasticization properties of several terpenoids, namely linalool, geraniol, and geranyl acetate, on P3HB. Geranyl acetate gave the best results with an increase in elongation at the break on physically aged (10 days) samples from 2% (neat P3HB) up to 13%.

The present research aims to assess the potential of a series of terpenoid esters as biobased plasticizers for P3HB to be used in the conventional extrusion-injection molding process. As previous works have demonstrated the high plasticization efficiency of terpenoid esters, this research gives an in-depth study of the effect of the chain length of the carboxylic acid attached to the terpenoid by esterification. In particular, different esters of geraniol are proposed as environmentally friendly plasticizers for P3HB formulations, namely geranyl-acetate, -propionate, -butyrate, and -isovalerate. Their effect on mechanical properties is investigated since P3HB is a very brittle polymer and these green plasticizers can positively contribute to improving ductile properties and toughness. In addition, thermal properties, morphology, and the disintegration rate of P3HB plasticized formulations are studied. This research broadens the potential applications of terpenoid-based compounds in the polymer industry.

2. Experimental

2.1. Materials

A commercial grade of poly(3-hydroxybutyrate) (P3HB) P226 weight average molecular weight, (M_w = 426 000 Da) was supplied by Biomer (Krailling, Germany) in pellet form. Geranyl acetate (>97% purity), geranyl propionate (>95%), geranyl butyrate (>95%), and geranyl isovalerate (>95%) were supplied by Sigma Aldrich (Madrid, Spain). Table 1 gathers some physical properties of the geranyl esters, while Figure 1 shows the chemical structure of the base polymer, poly(3-hydroxybutyrate), and the different geranyl-based plasticizers.

Table 1. Physical properties and chemical structure of geranyl esters uses as plasticizers for P3HB.

Terpenoid ester	Molecular weight [g·mol^{-1}]	Density [g·mol^{-1}]	Boiling point at 760 mmHg [°C]	Molar volume [cm^3·mol^{-1}]
Geranyl acetate	196.29	0.907	243	216.4
Geranyl propionate	210.32	0.899	253	233.9
Geranyl butyrate	224.34	0.896	257	250.4
Geranyl isovalerate	238.37	0.889	277	268.1

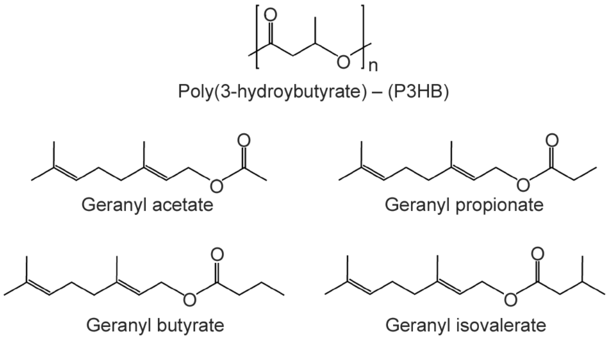

Figure 1. Chemical structure of poly(3-hydroxybutyrate) and different geranyl-based plasticizers.

2.2. Calculation of theoretical solubility parameters

Miscibility between the base polymer and the proposed plasticizer is a key factor in obtaining good plasticization properties. This can be theoretically predicted by calculating the solubility parameters (δ). The group contribution method proposed by Hoftyzer and van Krevelen [26] takes into account different contributions to the total solubility parameter (δ), namely, the dispersive forces contribution (δ_d), the polar forces contribution (δ_p), and the hydrogen bonding contribution (δ_h). If the solubility parameter coordinates of the proposed plasticizer fall into this region, good miscibility could be expected. Otherwise, poor miscibility will be obtained. A simple 2D model simplification allows determining the solubility region by plotting δ_h versus δ_v, where $\delta_v = (\delta_d^2 + \delta_p^2)^{1/2}$. In this plot, the solubility region is represented by a circle with the center on the corresponding δ_v and δ_h values of P3HB and the characteristic radius (R_0) of P3HB, which is 8.5 (MJ·m^{-3})$^{1/2}$ [27]. Moreover, it is possible to calculate the distance (R_a) between the three solubility parameter coordinates of P3HB and the different geranyl esters (GEX), as shown in Equation (1):

$$R_a = \sqrt{4(\delta_{dGEX} - \delta_{dP3HB})^2 + (\delta_{pGEX} - \delta_{pP3HB})^2 (\delta_{hGEX} - \delta_{hP3HB})^2} \quad (1)$$

The relative energy difference (*RED*) obtained by dividing the calculated distance (R_a) and the solubility radius (R_0) (Equation (2)) is a good indicator of potential miscibility: good miscibility ($RED < 1$), poor miscibility ($RED > 1$), threshold ($RED = 1$) [28]:

$$RED = \frac{R_a}{R_0} \quad (2)$$

2.3. Processing of P3HB formulations plasticized with geranyl esters

Prior to sample processing, the P3HB pellets were dried in an air-circulating oven for 24 h at 60 °C in order to remove moisture. The P3HB pellets were then manually mixed with 20 phr (20 parts by weight of plasticizer with respect to one hundred weight parts of P3HB) of each of the different geranyl-ester terpenoids in a zipper bag. Compounding of plasticized P3HB formulations was carried out in a twin-screw co-rotating extruder ($L/D = 24$, $D = 25$ mm) from DUPRA S.L. (Castalla, Spain) with a rotating speed of 40 rpm and a temperature profile of the extrusion barrel from the feeder to the nozzle of 170 , 170, 175, and 180 °C. After cooling down to room temperature, the compounded strands were ground into pellets in a mill. Finally, the plasticized P3HB pellets were processed by injection molding in a Meteor 270/75 from Mateu & Solé (Barcelona, Spain) in order to obtain standard samples. The temperature profile used in the injection molding machine was 170, 175, 175, and 180 °C from the hopper to the injection nozzle with a cooling time of the material inside the mold of 10 s. The labels of the plasticized formulations are denoted as P3HB/GE**X**, where **X** is **A** for geranyl acetate, **P** for geranyl propionate, **B** for geranyl butyrate, and **I** for geranyl isovalerate. As PHA undergo physical aging above their characteristic glass transition temperature (T_g), after processing, all samples were stored in a vacuum desiccator and aged at room temperature for 21 days before testing [29].

2.4. Characterization techniques

2.4.1. Mechanical properties

Tensile properties were measured using a universal test machine Ibertest ELIB 30 from SAE Ibertest (Madrid, Spain) equipped with a 5 kN load cell following ISO 527. Dumbbell-shaped specimens with a total length of 150 mm and a cross-section of 10×4 mm, were tested at 23±2 °C and a relative humidity of 50±10% at a crosshead speed was 5 mm/min. Prior to testing, samples were stored in a vacuum desiccator to ensure all specimens had the same conditions. Five different specimens were tested, and average values of the main tensile parameters were calculated, namely the maximum tensile strength (σ_{max}) and the elongation at break (ε_b). In addition, the elastic modulus (E_t) was accurately determined using an axial extensometer IB/MFQ-R2 from Ibertest (Madrid, Spain) coupled to the universal testing machine.

The impact strength of P3HB plasticized with geranyl esters was obtained using a Charpy impact pendulum (1 J) from Metrotec S.A. (San Sebastian, Spain), following ISO 179. Unnotched rectangular samples with a cross-section of 10×4 mm, were tested at 23±2 °C and a relative humidity of 50±10%. At least 5 different unnotched specimens were tested, and the corresponding values of impact strength were averaged.

The hardness of the different formulations was determined with a Shore D durometer model 673-D from J. Bot S.A. (Barcelona, Spain) following ISO 868. The hardness of each plasticized P3HB formulation was determined by averaging 10 values taken at different points of 5 specimens.

2.4.2. Differential scanning calorimetry (DSC)

Characterization by differential scanning calorimetry (DSC) was carried out in triplicate using a DSC 821 calorimeter from Mettler-Toledo Inc. (Schwerzenbach, Switzerland) in nitrogen atmosphere (66 ml·min^{-1}). Samples, weighing between 7–9 mg, were subjected to a dynamic program with three stages: a first heating cycle from –50 to 180 °C was followed by cooling to –50 °C, and finally, subjected to a second heating cycle from –50 to 250 °C. The heating/cooling rate for all the stages was 10 °C·min^{-1}. The melt peak temperatures (T_m) and their corresponding melting enthalpies (ΔH_m) were obtained from the second heating cycle. The degree of crystallinity of P3HB (χ_c) was determined according to the Equation (3):

$$\chi_c\,[\%] = \frac{\Delta H_m}{\Delta H_m^0 \cdot w_{PHB}} \cdot 100 \tag{3}$$

where ΔH_m [J·g^{-1}] is the measured melting enthalpy of the P3HB, ΔH_m^0 is the melting enthalpy of a theoretical fully crystalline P3HB and was taken as 146 J·g^{-1}, as reported in the literature [8], and w_{P3HB} is the weight fraction of P3HB in the corresponding plasticized formulation.

2.4.3. Dynamic mechanical thermal analysis (DMTA)

Dynamic-mechanical thermal analysis of the neat P3HB and plasticized P3HB formulations with different geranyl esters was carried out in a Mettler-Toledo DMA1 (Columbus, OH, USA) using a single cantilever configuration. DMTA runs were conducted at a constant frequency of 1 Hz, a force of 0.02 N, and a maximum deflection of 10 µm. Rectangular samples with a size of 20×6×3 mm were subjected to a temperature sweep from –100 to 100 °C at a constant heating rate of 2 °C·min^{-1}. Plots of the evolution of the storage modulus (E') and the dynamic damping factor ($\tan\delta$) as a function of temperature were collected. The glass transition temperatures (T_g) of neat P3HB and plasticized P3HB formulations were obtained from the peak temperature $\tan\delta$ curves.

2.4.4. Field emission scanning electron microscopy (FESEM)

The effect of geranyl esters on surface microstructure/topography of plasticized P3HB formulations was analyzed in a field emission scanning electron microscope (FESEM) ZEISS ULTRA55 from Oxford instruments (Abingdon, UK) operated at a voltage of 2 kV and an average working distance of 4 mm. Fractured samples from impact tests were firstly coated with a platinum alloy in high vacuum sputter coater EM MED20 from Leica Microsystem (Milton Keynes, UK).

2.4.5. Disintegration test in compost soil

Disintegration test in compost soil of neat P3HB and plasticized P3HB formulations with different geranyl esters was carried out following ISO 20200:2015 – '*Determination of the degree of disintegration of* *plastic materials under simulated composting conditions in a laboratory-scale test*' at 58 °C and 55% relative humidity. To this, rectangular samples with dimensions 25×25×1 mm were previously obtained by a hot press process at 160 °C. The weight loss was monitored from the weekly extraction of samples and calculating the weight loss following Equation (4). At least 3 replicates were carried out to obtain the average results.

$$\text{Weight loss [\%]} \quad = \frac{W_0 - W_\text{f}}{W_0} \cdot 100 \qquad (4)$$

where W_0 is the inital weight of the sample and W_f is the weight of the sample after the extration.
Since ISO 20200:2015 does not give a threshold a material must reach to be considered compostable, the disintegration time has been taken from EN 13432 '*Requirements for packaging recoverable through composting and biodegradation – Test scheme and evaluation criteria for the final acceptance of packaging*' which defines the evaluation criteria for the final acceptance of compostable packaging. According to this standard, a material can be considered compostable if the percentage weight loss is equal to or higher than 90% after a maximum compost time of 12 weeks.

3. Results and discussion

3.1. Theoretical approach to the solubility of P3HB and geranyl esters as potential plasticizers

Solubility parameter coordinates corresponding to dispersive forces (δ_d), polar forces (δ_p), and hydrogen bonding (δ_h) of P3HB and the proposed terpenoid-based plasticizers. The volar volume of the geranyl esters is shown in Table 1, while the molar volume of P3HB was taken as 73.2 $\text{cm}^3\cdot\text{mol}^{-1}$ as indicated in the literature [27]. P3HB has a solubility parameter of 19.79 $(\text{MJ}\cdot\text{m}^{-3})^{1/2}$. Therefore, it is expectable good miscibility between P3HB and all four geranyl esters used due to their δ parameters ranging from 16.55 to 16.94 $(\text{MJ}\cdot\text{m}^{-3})^{1/2}$ [30]. There is a clear decreasing tendency on δ parameter of geranyl esters as the number of carbon atoms of the carboxylic acid used to esterify geraniol increases. This is also evidenced by the distance R_a values between the δ parameters of geranyl esters and P3HB, with values ranging from 6.03 (geranyl acetate) to 6.75 (geranyl isovalerate). Since the solubility sphere of P3HB has a radius of 8.5 $(\text{MJ}\cdot\text{m}^{-3})^{1/2}$, the *RED* values are lower than 1 (Figure 2) for all geranyl esters considered in this work, with the lowest value for geranyl acetate (0.71) and the highest value for geranyl isovalerate (0.79). As indicated previously, as the *RED* value becomes lower, better solubility will be expected. Brüster *et al.* [28] have reported *RED* values of 0.29 for poly(ethylene glycol) (PEG) which is a well-known plasticizer for poly(lactide), while very good plasticization properties have been obtained with myrcene and limonene as plasticizers for PLA with *RED* values of 0.99 and 0.93, respectively. Hence, the low *RED* values of geranyl esters suggest good plasticization on P3HB.

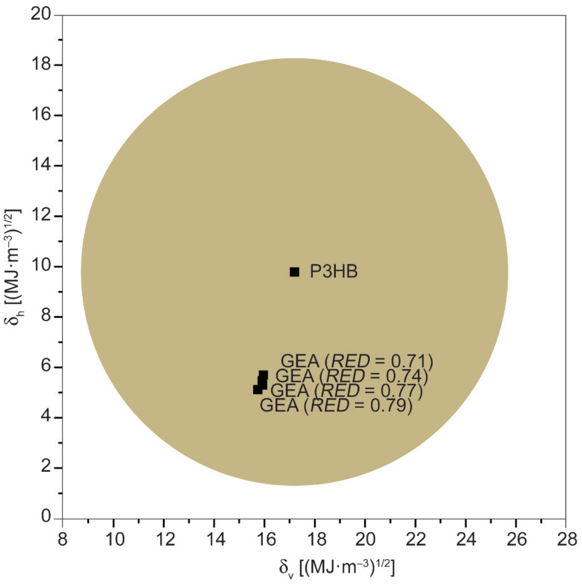

Figure 2. Bagley's solubility diagram for poly(lactide) – (PLA) and dibutyl itaconate (DBI) where the $\delta_\text{v} = (\delta_\text{d}^2 + \delta_\text{p}^2)^{0.5}$.

3.2. Mechanical properties of plasticized P3HB.

Figure 3 gathers the main mechanical properties of neat P3HB and plasticized P3HB formulations with geranyl esters after the physical aging at room temperature. Neat P3HB has a tensile strength of 26.5 MPa and a tensile modulus of 1640 MPa with a very low elongation at a break of 7.4%, which are typical of this PHA, which is, by far, one of the main drawbacks of PHA in general [31, 32]. This low elongation ability is due to its high degree of crystallinity after physical aging at room temperature. Smith *et al.* [33] have reported an even lower elongation at break 1.5% on P3HB obtained from sugar beet molasses. This P3HB polymer was characterized by a high degree of crystallinity of 60%. In fact, most commercial grades of P3HB include some additives to avoid excessive crystallization as it is the commercial

P3HB Biomer P226, used in this study that contains low-molecular-weight softeners and nucleating agents to minimize the effects of secondary crystallization or physical aging. Plasticization of P3HB leads to lower tensile strength and tensile modulus since plasticizers contribute to weakening polymer-to-polymer interactions, which in turn, have a positive effect on elongation [34, 35]. The tensile modulus of P3HB-plasticized materials with geranyl esters is remarkably reduced to values in the 600–700 MPa range, which represents a percentage decrease of about 60% with regard to neat P3HB. A similar tendency can be observed for tensile strength that is reduced to values ranging from 13.5 to 14.6 MPa, which is a typical effect of plasticization. Panaitescu *et al.* [36] have reported similar results in plasticized P3HB formulations with epoxidized soybean oil (ESO), polyethylene glycol (PEG), and acetyl tributyl citrate (ATBC). They observed a decreasing tendency on both tensile modulus and tensile strength as the plasticizer amount increased. With regard to the elongation at break, interesting results are obtained with the different geranyl esters. Neat P3HB shows an elongation at a break value of 7.4%, while the plasticized P3HB formulation containing 20 phr geranyl isovalerate noticeably improved elongation at break up to 9.7%, which represents a percentage increase of about 31%. Similar results were reported by Panaitescu *et al.* [36] regarding elongation at break. They concluded that plasticized P3HB formulations containing 5 wt% ESO or ATBC, led to a percentage increase in elongation at break of 45 and 37%, respectively, with regard to neat P3HB. Despite the plasticization effects of all four geranyl esters being corroborated by a clear decrease in both tensile strength and modulus, the effect on elongation at break is not as noticeable as in other aliphatic polyesters such as poly(lactide) – PLA. Plasticization of PLA with 5–30 wt% plasticizers such as triethyl citrate (TEC), acetyl tributyl citrate (ATBC), polyethylene glycol (PEG), epoxidized vegetable oils (EVO) leads to a clear decrease in tensile strength and modulus, but the elongation at break is remarkably improved from 5–10% (neat PLA) up to 15–300% with these plasticizers [37–39]. As the crystallization rate of P3HB is low and the nucleation density is low, too, large spherulites are formed during cooling. Subsequent physical aging promotes secondary crystallization which, in turn, will promote some internal shrinkage which leads to microcrack formation. This phenomenon has been well explained by El-Hadi *et al.* [9]. They revealed that the embrittlement of PHB was related to the secondary crystallization process taking place at room temperature due to a glass transition temperature below room temperature. In addition to this phenomenon, P3HB shows a very low nucleation density which results in large spherulites. Secondary crystallization is responsible for the appearance of small spherulites that act as bridges between the crystallites thus constraining the mobility of the P3HB chains in the amorphous regions, as reported by Crétois *et al.* [40]. All these phenomena lead to poor ductile properties and, subsequently, the elongation at break is so low and, even by the addition of miscible plasticizers, the elongation at break is not improved in a remarkable way [41]. As observed by Bibers *et al.* [42], in general, plasticization of P3HB gives exceptional results for a plasticizer content above 30 wt%, while moderate increases in elongation at break are obtained with lower plasticizer contents. They also concluded this threshold for high plasticization effects highly depends on the chemical structure of a proposed plasticizer. On the other hand, Mangeon *et al.* [43] reported high deformative characteristics on P3HB with more complex plasticizers derived from interpenetrating networks based on sunflower oil (SO) and trimethylolpropane tris(3-mercaptopropionate) (TriSH).

With regard to the impact strength, all geranyl-based esters provide an improvement. Neat P3HB has an impact strength of 2.2 kJ·m^{-2}, while the plasticized P3HB formulation with geranyl acetate gives an impact strength of 3.4 kJ·m^{-2}, which represents a percentage increase of about 54.5%. These results agree with those reported by Nosal *et al.* [44], in plasticized P(3HB-*co*-3HV) with fatty acid esters with different hydrocarbon chain lengths. They concluded that fatty acid-based plasticizers with shorter chain lengths provide the best impact strength properties on notched samples, by increasing from 1.4 kJ·m^{-2} P(3HB-*co*-3HV) to 4.0–4.1 kJ·m^{-2} for plasticized formulations with oleic acid methyl ester, palmitic acid methyl ester or lauric acid ethylene glycol monoester. By using geranyl esters as plasticizers for P3HB, the obtained results suggest that the shorter the chain length of the esterifying acid of geraniol, the higher the impact resistance. Umemura *et al.* [45], have also reported improved impact resistance in plasticized P3HB with triethyl citrate (TEC) at weight fractions ranging from 0 to 0.3. They observed an

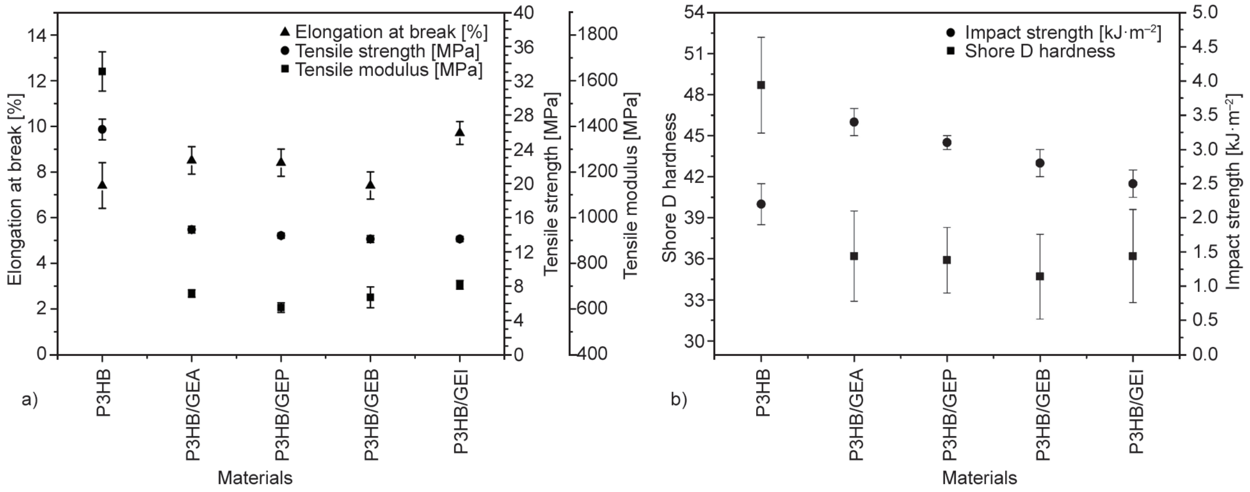

Figure 3. Summary of the main mechanical properties in terms of a) tensile test and b) hardness and impact strength.

increase in the impact resistance (Izod impact test) on 28 days aged samples from 49 $J\cdot m^{-1}$ (neat P3HB) up to 253, 301, and 371 $J\cdot m^{-1}$ for TEC weight fractions of 0.1, 0.2, and 0.3, respectively.

Regarding Shore D hardness, as expected, all four geranyl esters show a similar decrease. The Shore D hardness of neat P3HB is 48.7 while all plasticized P3HB formulations with geranyl esters show a Shore D hardness in the 34.7–36.2 range. This softening effect is directly linked with the increased free volume after plasticization and the subsequent weakening of the polymer-to-polymer chain interactions. This phenomenon has also been reported by Bouit *et al.* [46] in plasticized PLA with different amounts of epoxidized sunflower oil. Garcia-Garcia *et al.* [47] reported a similar decrease in Shore D hardness in P3HB plasticized with several epoxidized vegetable oils. In particular, they obtained a decrease in Shore D hardness from 57.3 (neat P3HB) to 53.5 (20 wt% ELO).

3.3. Thermal properties of plasticized P3HB

The main thermal parameters of P3HB and its plasticized formulations with geranyl esters obtained by differential scanning calorimetry (DSC) are gathered in Table 2. The first heating cycle in Figure 4 shows the thermal behavior after 21 days of aging. The second stage corresponds to a cooling process in which the crystallization can be observed, and, finally, the third stage (second heating) contains thermal information after a controlled cooling stage without additional aging. First, it is worth noting the presence of two peaks, a small peak at about 50 °C and a second peak at 170 °C. The first peak, at about 50 °C, corresponds to the additives used in commercial PHB grades, as reported by Panaitescu *et al.* [36] and Garcia-Garcia *et al.* [48]. The second peak, located at 170 °C corresponds to the melting of the crystalline fraction of P3HB. In the first heating cycle, it is possible to see that the addition of geranyl-based plasticizers leads to a decrease in the characteristic melting peak temperature of P3HB by 10 °C. This phenomenon has been attributed to the weakening effect of the polymer-to-polymer chain interactions due to the plasticizers, as observed by Kurusu *et al.* [49]. A slight decrease of 7–10 °C can also be observed with regard to the small peak related to low molecular weight polyesters. Another interesting phenomenon provided by plasticizers is an increase in the degree of crystallinity (χ_c [%]). The higher mobility of the polymer chains allows them to pack into a crystalline structure, and therefore, the degree of crystallinity of the plasticized P3HB formulations is higher. This phenomenon has been reported by Chaos *et al.* [50] in plasticized PLA and PHB with tributyl citrate (TbC). Despite having a higher degree of crystallinity, the plasticized P3HB offered increased ductile properties, as mentioned in the previous section, this being a key issue after aging at room temperature. Additionally, it is also worth noting that the shape of the melting peak of the geranyl-plasticized P3HB samples (especially with geranyl isovalerate and geranyl propionate) shows two melting peaks which can be attributed to the formation of different crystals [36]. It is important to bear in mind that PH3B can crystallize into two different crystal structures, the thermally stable α-form, which is obtained during the crystallization from the melt, and the metastable β-form that can appear as a consequence of the orientation of the free chains in the

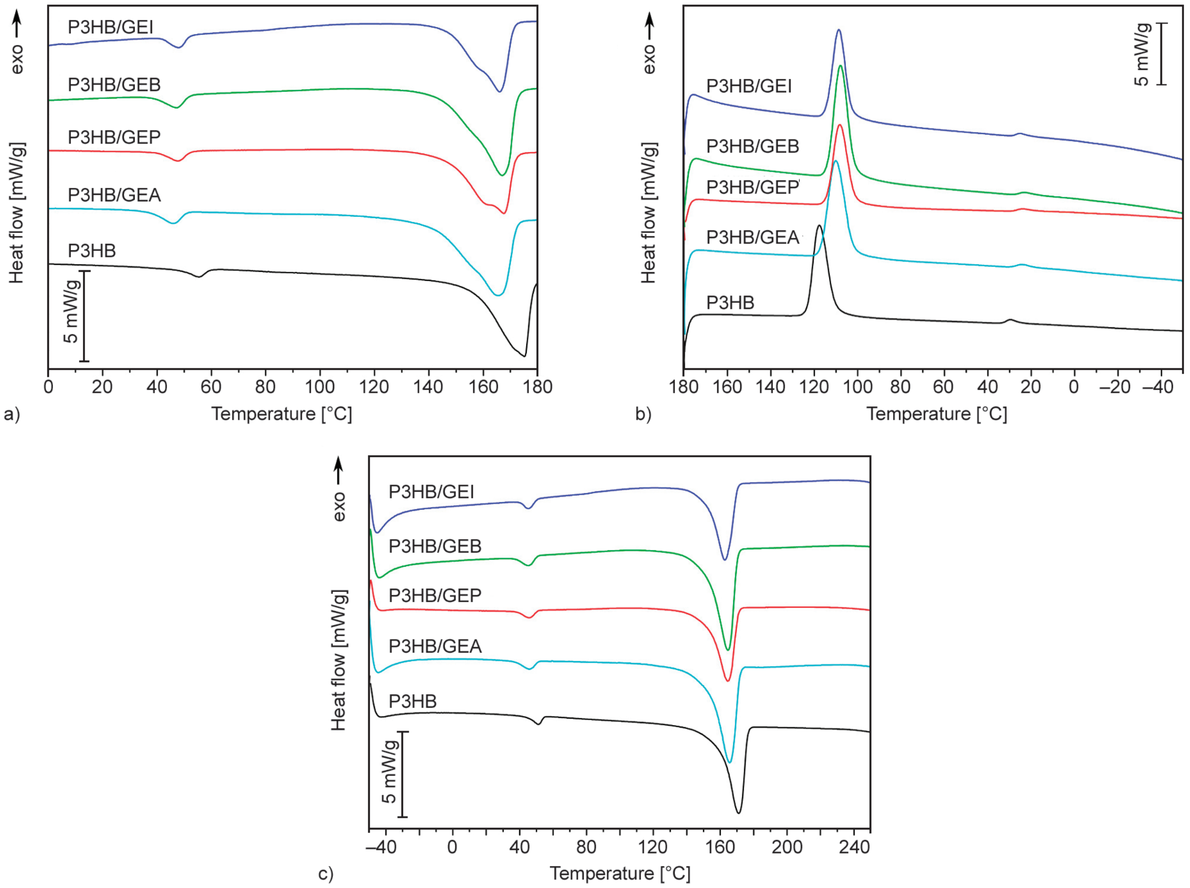

Figure 4. Differential scanning calorimetry (DSC) analysis of neat P3HB and plasticized P3HB formulations with different geranyl esters, a) first heating scan, b) cooling, and c) second heating scan.

amorphous regions. While the amount of β-form crystals remains almost constant during storage or aging, the amount of α-form crystals increases during the aging at room temperature [51].

The crystallization can be observed in the cooling stage (see Figure 4b). One interesting phenomenon that stands out is that the crystallization peak temperature is moved to lower temperatures in the geranyl-plasticized P3HB formulations. Kurusu *et al.* [49] proposed this phenomenon is due to the presence of a plasticizer that allows polymer chains to rearrange in a packed way since the plasticizers contribute to increased polymer chain mobility.

With regard to the enthalpies, once they are normalized by taking into account the actual P3HB content, allowed to calculate the degree of crystallinity by using Equation (3). Table 2 includes the degree of crystallinity (χ_c [%]) for all three stages scheduled in the DSC. The effect of the plasticizers can be clearly observed in the first heating cycle applied on samples subjected to 21 day aging. The degree of crystallinity of neat P3HB is 36.5%, while this is increased to values ranging from 42.3% for geranyl propinate-plasticized P3HB to 45.9% for geranyl acetate-plasticized P3HB, thus suggesting the shorter the chain length of the carboxylic acid esterified with geraniol, the higher the crystallinity since polymer chain mobility is less restricted with lower molecular weight compounds. With regard to the second heating cycle, a slight decrease in the characteristic melting temperatures can be observed. This is due to a controlled cooling cycle which is, by far, slower than the fast cooling down after injection molding. The degree of crystallinity of all P3GB-based formulations does not show any relevant change. This effect has been reported by Chen *et al.* [52]. They revealed the effect of the cooling rate on the degree of crystallinity in a P(3HB-*co*-3HHx) copolymer. It is also worthy to remark on the work by Crétois *et al.* [40]. They concluded that the secondary crystallization takes place at room temperature and is responsible for the appearance of small crystallites that can act as a bridge between large crystallites formed during the primary crystallization from the melt state. This bridging effect constrains the chain mobility of P3HB chains in the amorphous regions, and consequently,

Table 2. Main thermal properties of neat P3HB and plasticized P3HB formulations with different geranyl esters in terms of melting/crystallization temperatures (T_m, T_c), melting/crystallization enthalpies (ΔH_m, ΔH_c), and the degree of crystallinity (χ_c).

Sample	First heading			Cooling			Second heading		
	T_m [°C]	ΔH_m [$\mathrm{J\cdot g^{-1}}$]	χ_c [%]	T_c [°C]	ΔH_c [$\mathrm{J\cdot g^{-1}}$]	χ_c [%]	T_m [°C]	ΔH_m [$\mathrm{J\cdot g^{-1}}$]	χ_c [%]
P3HB	174.9±0.3	53.3±0.4	36.5±0.7	117.7±0.2	59.3±0.5	40.6±0.8	171.0±0.3	69.2±0.4	47.4±0.7
P3HB/GEA	165.5±0.2	67.0±0.6	45.9±0.5	110.0±0.3	63.1±0.4	43.2±0.8	164.6±0.3	68.6±0.5	47.0±0.6
P3HB/GEP	166.0±0.2	61.7±0.5	42.3±0.7	114.9±0.2	58.8±0.6	40.3±0.9	163.9±0.3	64.4±0.4	44.1±0.5
P3HB/GEB	166.7±0.3	65.3±0.7	44.7±0.6	108.3±0.4	66.5±0.3	45.5±1.2	164.4±0.2	69.0±0.3	47.3±0.5
P3HB/GEI	166.2±0.3	62.0±0.4	42.5±0.6	114.4±0.2	56.3±0.5	38.6±1.4	162.6±0.2	66.0±0.5	45.2±0.6

plasticization does provide the typical increase in the degree of crystallinity.

3.4. Dynamic mechanical thermal analysis (DMTA) of plasticized P3HB

The effects of plasticizer addition on dynamic mechanical thermal properties of neat P3HB and its plasticized formulations with geranyl esters are summarized in Figure 5. In general, the storage modulus, E' decreases with increasing temperature. The first phenomenon to be highlighted is the lower E' values obtained in all plasticized P3HB formulations with geranyl esters, compared to neat P3HB above –100 °C. As previously indicated, plasticizer addition leads to increased free volume, so that, the polymer chain mobility is allowed and, subsequently, the rigidity decreases. The storage modulus of neat P3HB at –20 °C is 1756 MPa, and this is reduced to values in the 893–1013 MPa range for all four geranyl esters used in this work. This behavior was observed by Barbosa *et al.* [53], in plasticized P(3HB-*co*-3HV) with oligomeric polyester.

With regard to the dynamic damping factor, tan δ, which represents the dissipated-to-stored energy ratio (E''/E'), the peak maximum around 0 °C could be taken as the glass transition temperature (T_g). For neat P3HB, the T_g is close to 4.3 °C; this thermal transition is related to an α-relaxation process in which the polymer changes from a glassy to a rubbery state. The effect of plasticizer addition on T_g can be clearly detected by a decrease in the peak maximum temperature of tan δ plots [54, 55]. It is worth noting that the T_g for the plasticized P3HB formulation with geranyl isovalerate is the lowest of all four geranyl esters used in this study, with a value of –13.6 °C, which is responsible for the highest elongation at break (9.7%) observed in a tensile test. As proposed by Greco *et al.* [56], an indicator of plasticization efficiency is the decrease in T_g a plasticizer can provide to a polymer. In this case, all four geranyl esters provide a decrease in T_g, which suggests geranyl esters are suitable as environmentally friendly plasticizers for P3HB.

3.5. Morphology study of plasticized P3HB

The surface morphology or topography of neat P3HB and plasticized P3HB formulations with geranyl esters have been obtained by field emission scanning electron microscopy (FESEM) (see Figure 6). The morphology of the fracture surfaces is directly related

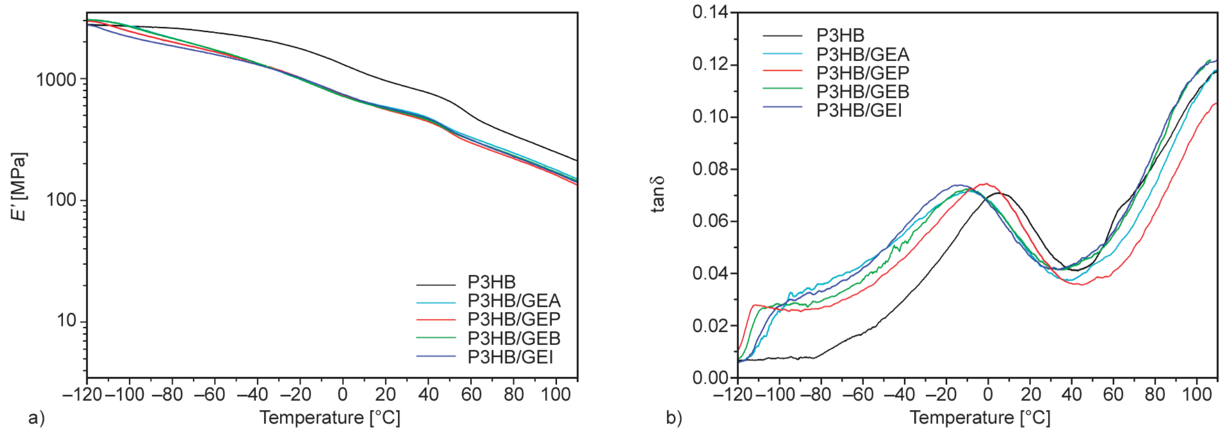

Figure 5. Dynamic-mechanical thermal analysis (DMTA) curves of neat P3HB and plasticized P3HB formulations with different geranyl esters in terms of a) storage modulus, E' *vs*. temperature, and b) dynamic damping factor, tan δ *vs*. temperature.

to the macroscopic mechanical behavior mentioned above since clear changes in surface roughness can be observed. Neat P3HB shows the typical brittle fracture characterized by a flat fracture surface (see the inset image at 2500×), indicating the low plastic deformation capacity that took place during fracture. Melendes-Rodriguez *et al.* [57] also obtained similar fracture morphologies for different PHA Blends. With the introduction of geranyl esters, there is an increase in the energy absorption capacity, as mentioned above, which results in a change in the fracture surface which is less flat as a consequence of the plastic deformation, and, subsequently, the surface roughness is increased. Moreover, a phenomenon that arises in plasticized P3HB formulations is the presence of spherical shapes associated with phase separation since plasticizers preferentially locate in the amorphous regions and, in less extent into

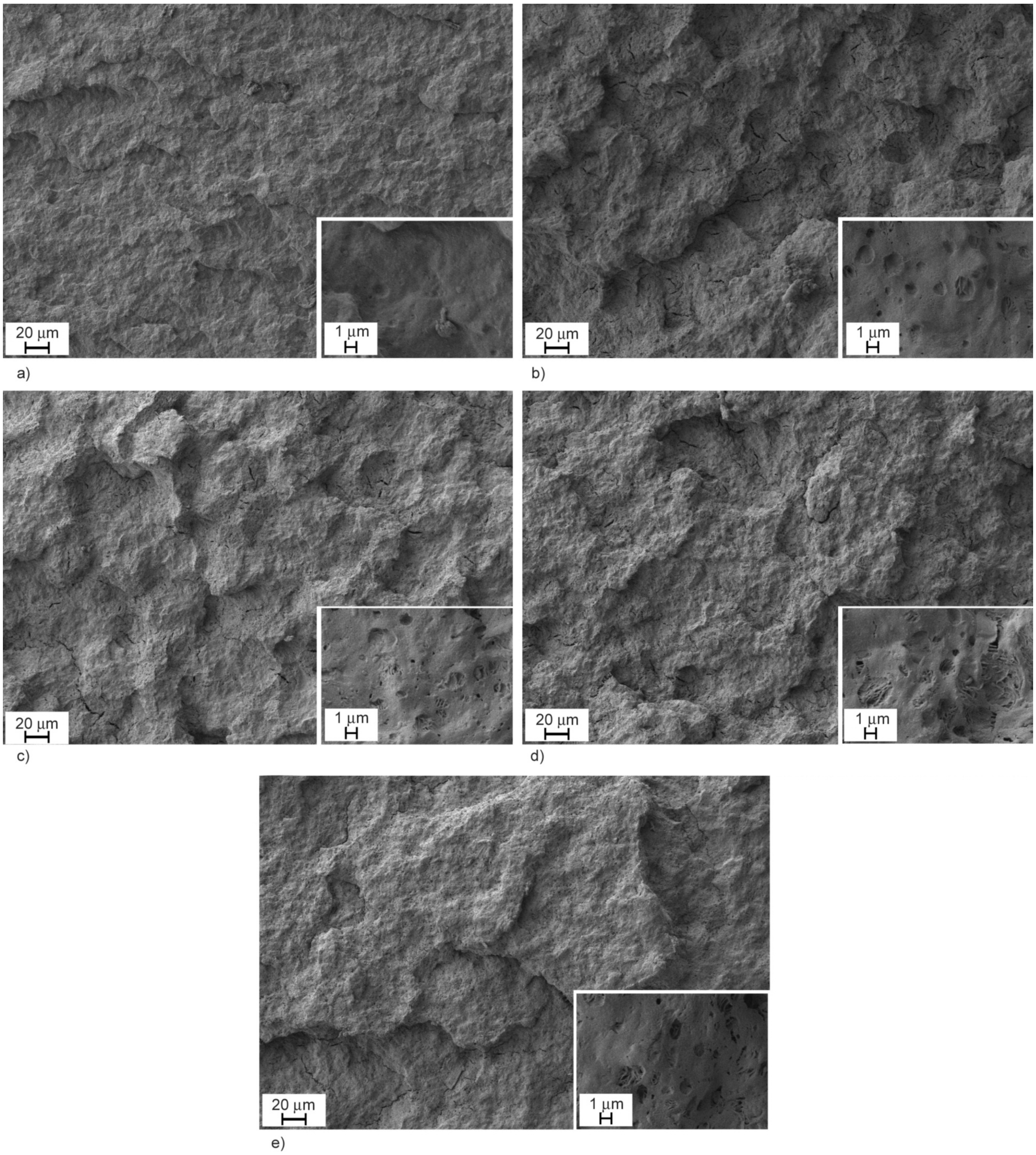

Figure 6. Field-emission scanning electron microscopy (FESEM) images of impact fractured samples (250× and insets at 2500×) of neat P3HB and plasticized P3HB formulations with different geranyl esters: a) P3HB, b) P3HB/GEA, c) P3HB/GEP, d) P3HB/GEB, and e) P3HB/GEI.

the crystalline lamellae. This phenomenon can be easily observed in detailed images taken at 2500×. Jeong *et al.* [58] attributed this phenomenon to partial miscibility of the plasticizer that leads to the phase separation that results in the formation of this morphology. Another interesting phenomenon is observable in plasticized P3HB formulations is the presence of microcracks which are more evident in plasticized P3HB with geranyl acetate. Kurusu *et al.* [49] observed this phenomenon and attributed it to plasticizer migration. Even with the presence of these microcracks, almost all plasticized P3HB formulations with geranyl esters show improved impact strength. As can be seen in Figure 6d (inset image at 2500×), there is clear evidence of plastic deformation by the appearance of filament-like structures resulting from plastic deformation before fracture. These morphologies can be observed in all four plasticized P3HB formulations with geranyl esters.

3.6. Effect of geranyl esters on the disintegration of plasticized P3HB formulations

One of the great advantages of PHA, such as P3HB is their ability to biodegrade in different environments, such as composting under controlled conditions. During the disintegration process, chains scission occurs, and, subsequently, low molecular weight chains are obtained, allowing some microorganisms such as fungi and bacteria to metabolize them, releasing CO_2, H_2O, and humus [59]. Weng *et al.* [60] studied the disintegration process of PHA from the surface to the bulk material. They observed a noticeable degradation in low-thickness samples after 10 days in compost. As reported by Arrieta [61], it is possible to obtain a full disintegration in a polyester such as poly(lactide), PLA, in controlled compost soil on samples with a thickness comprised between 200–500 μm in just 4 weeks, while the disintegration time takes 6–8 weeks for samples with a thickness of 4 mm. In this work, the disintegration test has been carried out on sample sheets with a thickness of 1 mm. Thus, higher degradation times could be expected with regard to films with lower thicknesses. Figure 7 shows the visual appearance of the specimens along the disintegration process in compost soil. As one can see, some slight evidence of degradation is obtained after 35 days incubation period while important signs of disintegration are seen after 49 days.

It is important to note the effect of plasticizers on the disintegration rate. The incorporation of geranyl-based plasticizers into P3HB matrix leads to an increased disintegration rate in compost soil. Despite ISO 20200 does not give any disintegration threshold, the EN 13432 standard establishes that material is biodegradable under compost conditions if the disintegrated weight fraction is equal to or superior to 90% after a maximum composting time of 12 weeks. The disintegration time (number of days/weeks needed to reach 90 wt% loss) is close to 60 days for neat P3HB while this time is reduced to 50 days in all four plasticized P3HB formulations with geranyl esters (see Figure 8). Arrieta [61] concluded that the incorporation of plasticizers accelerates the disintegration process, even considering that plasticizers also promote increased crystallinity. This is due to the increase in the free volume plasticizers provide, thus exposing hydroxyl groups with high affinity to water, which plays a key role in the first disintegration stages. In general, the increase in crystallinity has a negative effect on the disintegration rate since the highly packed crystalline phase is much more resistant to chain scission than polymer chains in the amorphous regions [62]. Nevertheless, the plasticizer counteracts this phenomenon, and the overall disintegration rate is improved with plasticizers.

It is important to bear in mind that P3HB is a highly crystalline polymer after the aging process due to secondary crystallization and aging of the amorphous regions. Therefore, the addition of a geranyl-based plasticizer does not lead to an increase in the overall crystallinity but a slight decrease has been observed in thermal characterization by DSC. Hence, the free volume increases, and subsequently, this allows moisture/water to enter during the disintegration process, leading to a faster hydrolysis process. Despite this is not the main feature sought by the addition of geranyl-based plasticizers into P3HB, it is an interesting feature since it is possible to tailor its disintegration rate. This increase could be interesting in managing the disintegration of PHA used in the packaging industry in shorter times.

4. Conclusions

Geranyl esters have been proposed as green plasticizers for poly(3-hydroxybutyrate) (P3HB). Geranyl esters were incorporated into P3HB by melt compounding and subsequent injection molding, thus

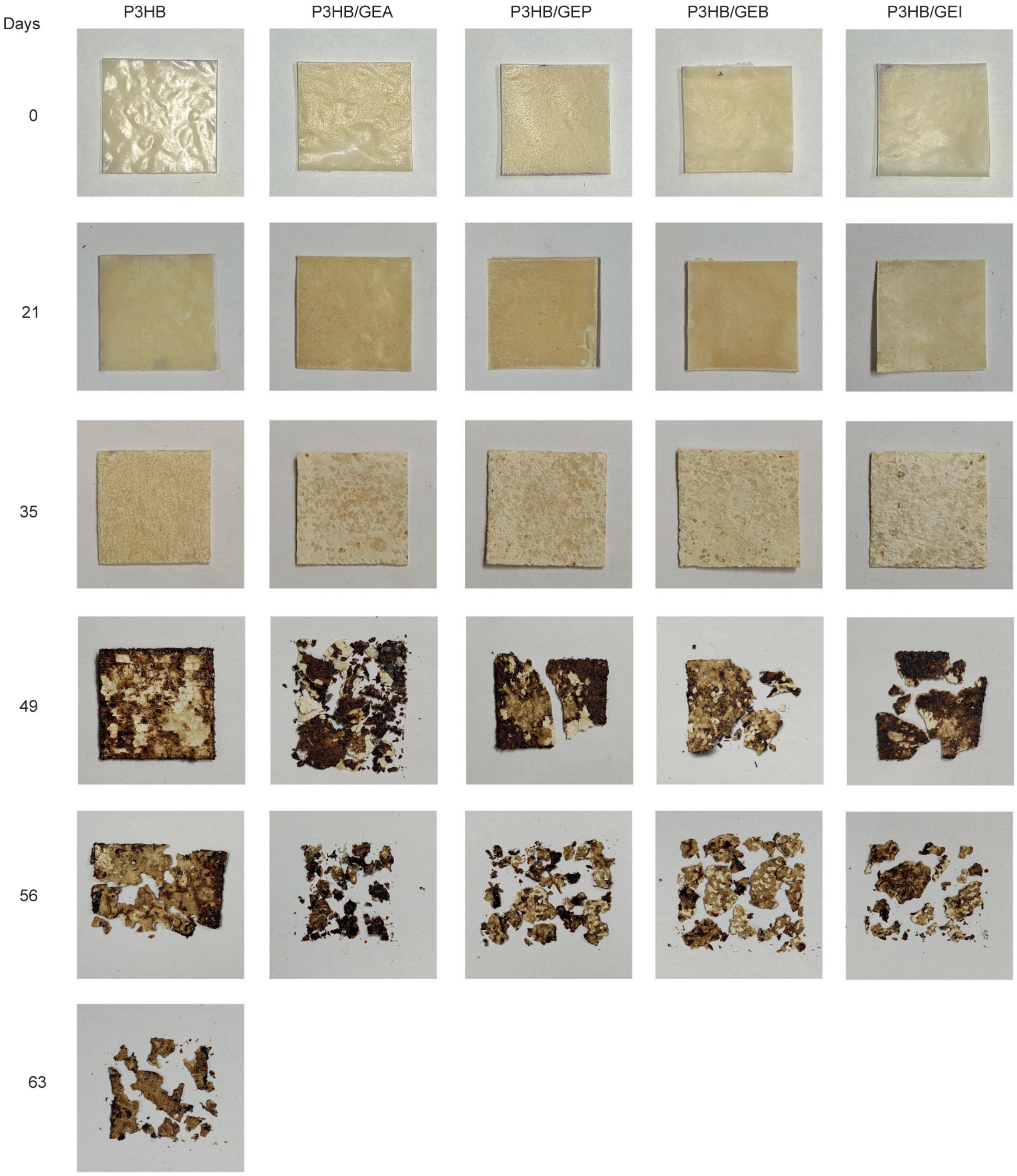

Figure 7. Visual aspect of neat P3HB and plasticized P3HB formulations (samples with dimensions 25×25×1 mm) with different geranyl esters at different incubation times during the disintegration test in controlled compost soil.

showing the feasibility of these plasticized formulations for industrial processing since the boiling point of these terpenoids is above the typical processing window of P3HB. A constant plasticizer content of 16.67 wt% was used with four different geranyl esters with varying the chain length of the esterifying carboxylic acid, namely geranyl acetate, geranyl propionate, geranyl butyrate, and geranyl isovalerate. The obtained results suggest these geranyl-based plasticizers provide an increase in ductile properties such as elongation at break and impact strength and overcome or at least, minimize, the intrinsic brittleness of P3HB. The elongation at break of the plasticized P3HB with geranyl isovalerate increases by 31%, while the best impact strength properties are obtained in formulations with geranyl acetate, with a percentage increase in impact strength of 54.5% on unnotched samples. In general, the shorter the chain length of the esterifying acid (acetic-C2, propionic-C3, butyric-C4, or isovaleric-C5), the better

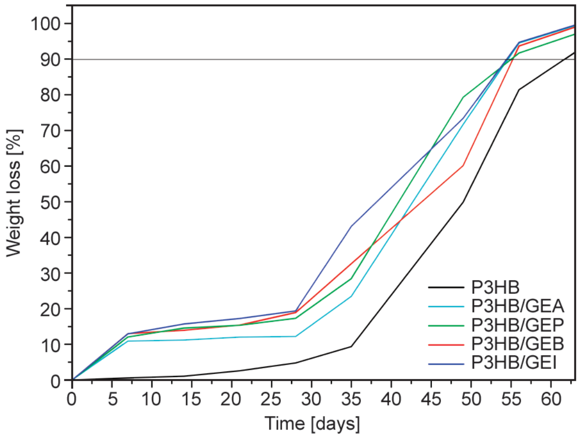

Figure 8. Weight loss evolution over time at the disintegration test in controlled compost soil of neat P3HB and plasticized P3HB formulations with different geranyl esters.

ductile properties are obtained. The plasticization efficiency has been assessed by a decrease in the glass transition temperature (T_g), changing from 4.3 °C for neat P3HB down to values of –13.6 °C for the plasticized formulation with geranyl isovalerate. These plasticizers do not compromise the biodegradation/disintegration in compost soil; in fact, these green plasticizers increase the disintegration rate by reducing the disintegration time (90% mass loss) from 60 days for neat P3HB to 50 days for all geranyl-plasticized formulations. These plasticizers represent an environmentally friendly alternative to petroleum-based plasticizers and additives for P3HB with improved ductile behavior.

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