

*Long-chain polyhydroxyesters from natural occurring aleuritic acid as potential material for food packaging.*

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## **ABSTRACT**

Fatty polyhydroxyesters ( $C \geq 16$ ) are present in Nature as barrier polymers like cutin in some protective tissues of higher plants. The mimicry of these biopolymers is regarded as a strategy to design nontoxic and fully biodegradable food packaging films and coatings. To obtain cutin inspired materials we have used a natural occurring polyhydroxylated monomer like aleuritic (9,10,16-trihydroxypalmitic) acid and a direct and scalable synthesis route consisting in the

noncatalyzed melt-condensation polymerization in air. To reduce the number of hydroxyl groups and to increase hydrophobicity, palmitic acid has been used as a capping agent. Aleuritic-palmitic polyhydroxyesteres films have been obtained and characterized by Infrared Spectroscopy (IR), solid state  $^{13}\text{C}$  Magic Angle Spinning Nuclear Magnetic Resonance ( $^{13}\text{C}$  MAS-NMR), Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), and uniaxial tensile testing. Palmitic acid addition is found to have a direct effect on the water absorption capacity of samples but also on their structural weakening and tendency to side reactions like dehydration and oxidation. Blocking of chain propagation and branching points by palmitic units are proposed to be responsible for this behavior.

## **INTRODUCTION**

Most of the research on renewable and biodegradable food packaging material based on polyesters has been concentrated on polylactic acid (PLA) and short-chain polyhydroxyalcanoates (PHAs) (1). On the contrary, much less attention has been paid to mid-chain ( $\text{C}_6\text{-C}_{15}$ ) PHAs and, particularly, to long-chain polyhydroxyesters ( $\text{C}_{\geq 16}$ ) (2,3). One of the reasons is the lack of efficient routes to the obtaining of the bioplastic from bioreactors and genetically modified plants, as in the production of PLA and polyhydroxybutyrates (PHBs). However, long-chain polyhydroxyesters (mostly  $\text{C}_{16}$  and  $\text{C}_{18}$ ) are already abundantly present in Nature in the form of the plant biopolymer cutin. Cutin is an amorphous, nontoxic and fully biodegradable barrier polyester protecting higher plant tissues from massive water loss, pathogen and fungal infection and UV radiation (4). Based on such properties and on the analogy to other synthetic aliphatic polyesters, cutin-based materials could be used for biomedical applications like drug delivery systems, wound dressings and scaffold fabrication. Moreover, cutin's global abundance confers additional economical potential as a non-toxic, biodegradable and renewable food packing material. Consequently, the synthesis of such materials have been pursued by different ways (5,6,7).

However, cutin is an insoluble and infusible material which impedes its direct extraction from agricultural wastes and the further processing. Necessarily, the synthetic polyester reconstruction is via the polymerization of the hydroxyacid monomers obtained after the biopolyester degradation. Cutin monomeric composition varies among plant species and growing stage but it is dominated by C<sub>16</sub> and C<sub>18</sub> hydroxyacids (8,9) with an overall -OH/-COOH ratio above one. The properties of the resulting synthetic polyesters may be affected by such -OH/-COOH ratio as well as by other minor components accompanying the hydroxyacid extract. For that reason, we have selected pure aleuritic (9,10,16-trihydroxypalmitic) acid, the most hydroxylated member of the hydroxyacids present in cutins, as a reference monomer to conduct the synthesis of cutin-like films. To regulated the -OH/-COOH imbalance we have added palmitic acid as the non-hydroxylated C<sub>16</sub> counterpart. Conditioned by the insolubility and infusibility of the polyaleuritate obtained by a standard catalyzed polycondensation reaction in organic media (5), we have chosen a noncatalyzed melt-condensation polymerization in air as an alternative to directly prepare films and coatings. This method is envisaged as a low-cost and easily scalable method using no metal catalysts, aromatic hydrophilic agent or organic solvent compromising the innocuousness of the final product.

## **EXPERIMENTAL**

### **Materials**

Aleuritic (*DL-threo*-9,10,16-trihydroxypalmitic, C<sub>16</sub>H<sub>32</sub>O<sub>5</sub>) acid (93.8% by NaOH titration) and palmitic (C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>) acid (≥ 99%) were purchased from Fluka. Aleuritic acid was further purified by washing with cold water.

### **Polymer Synthesis**

Polyhydroxyesters from nominal aleuritic:palmitic acid (mol:mol) mixtures (ALE:PAL = 10:0, 9:1, 8:2, 7:3, 6:4 and 5:5) were prepared by placing about 130 mg of the mixture in an open mould (30 mm x 10 mm and 0.5 mm deep) and heated in air inside an oven at 150 °C for 10 h and 20 h. Final products were light brown and rubbery solid films with thickness ranging from 370 µm for (10:0) to 245 µm for (5:5). Samples were infusible and insoluble in solvents such as chloroform, light alcohols, toluene, tetrahydrofuran, dimethylformamide and dimethyl sulfoxide.

## **Polymer Characterization**

### **Infrared Spectroscopy**

Transmission infrared spectra were collected in a FTIR spectrometer (FT/IR-6200, JASCO) using 10 mm diameter pellets containing 2 mg of sample and 150 mg of dry KBr. In each run, 32 scans were accumulated. A triglycine sulphate (TGS) detector operated at 4 cm<sup>-1</sup> resolution was used and, for quantitative analysis, spectra have been normalized to the area of the  $\nu_{C-H}$  band (2800-2900 cm<sup>-1</sup>).

### **Thermogravimetric Analysis (TGA)**

TGA experiments of aleuritic:palmitic acid mixtures were carried out with a TGA/DSC analyzer SDT Q600 (TA Instruments). Samples were heated from RT to 150 °C at 10 °C/min under dry N<sub>2</sub> flow (100 mL/min) and then left at 150 °C for 8 h. The DSC analysis show no thermal event but palmitic and aleuritic acid melting at 66 °C and 95 °C, respectively.

### **Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (MAS-NMR)**

<sup>13</sup>C Magic Angle Spinning Nuclear Magnetic Resonance (<sup>13</sup>C MAS-NMR) proton decoupling single-pulse spectra of solid polymers were obtained with a Bruker DRX400 spectrometer using a magnetic field of 9.36 T and equipped with a multinuclear probe. Specimen were cut into small as possible pieces using a razor blade knife and packed in 4 mm zirconia rotors.

Magic angle spinning was operated at 10 kHz. The spectra were acquired at a frequency of 100.61 MHz, using a  $\pi/6$  pulse width of 2.5  $\mu$ s and a pulse space of 10 s, which ensured nuclei full relaxation and allowed quantitative measurements. In each run, 8500 spectra were accumulated and quantification was done from peak areas using the “*dmfit*” program (10). For quantitative comparison of spectra, they are plotted after being normalized to the area of the aliphatic carbon signal and the chemical shifts are reported in ppm from tetramethylsilane.

### **X-ray Diffraction (XRD)**

XRD diffraction patterns were obtained with an X’Pert Pro (PANalytical) diffractometer using monochromated  $\text{CuK}_\alpha$  radiation. Sample films were placed on a zero background silicon single crystal plate adapting them to the goniometer in a  $\theta$ - $2\theta$  configuration.

Variable temperature XRD analysis was carried out using a Bruker D8 Advance instrument equipped with a  $\text{Cu K}_\alpha$  radiation source and an Anton Paar TTK 450 low-temperature chamber.

### **Differential Scanning Calorimetry (DSC)**

DSC thermograms of polymers were acquired with a DSC Q20 (TA Instruments) from  $-70^\circ\text{C}$  to  $150^\circ\text{C}$  under nitrogen flow (50 mL/min) at  $10^\circ\text{C}/\text{min}$  using non-hermetic aluminum pans. Square pieces of the films were first cooled to  $-70^\circ\text{C}$ , heated to  $150^\circ\text{C}$  and kept at this temperature for 2 minutes to eliminate residual moisture. Then, a cooling-heating cycle from  $-70^\circ\text{C}$  to  $150^\circ\text{C}$  was performed. The glass transition temperature ( $T_g$ ) was obtained from the second heating using the inflection method.

### **Tensile Tests**

Uniaxial tensile tests of films were measured using an MTS Criterion 42 machine equipped with a 10 N load cell and applying a 0.02 N preload. Rectangular uniform pieces (7 mm x 20

mm) were cut and brought to rupture at a constant deformation rate of 0.2 mm/min at room environmental conditions. Stress values were calculated using the specimen cross-section under no applied load. The Young's modulus was obtained from the initial slope of the stress-strain curve. Experiments were repeated at least for six specimen per sample and parameters averaged to ensure reproducibility.

### **Water absorption**

Water absorption capacity of polymers was measured gravimetrically by exposing about 30 mg of sample to a 95% relative humidity atmosphere for 24h inside a closed chamber.

## **RESULTS AND DISCUSSION**

### **Chemical characterization of polyesters**

The thermal noncatalyzed melt-condensation polymerization of pure aleuritic acid in air at 150°C has been previously studied and two reaction stages have been detected (6). Thus, the ester formation (1<sup>st</sup> stage) is followed by an oxidative vicinal diol cleavage and oxidation of surplus hydroxyl groups (2<sup>nd</sup> stage). Accordingly, the ALE:PAL polyesters have been prepared at two reaction times: 10 h and 20 h; conditions at which full esterification and diol cleavage were respectively observed for polyaleuritate.

IR spectra, Fig. 1, confirm the formation of the esters as stated by the  $\nu_{C=O}$  around 1735  $\text{cm}^{-1}$  and the (OC-O-C) stretchings at 1177  $\text{cm}^{-1}$  and 1247  $\text{cm}^{-1}$  (11). Also by the weak contribution of the free acid IR peak around 1700  $\text{cm}^{-1}$ . At longer reaction time, weak bands at 1803  $\text{cm}^{-1}$  and 1778  $\text{cm}^{-1}$ , as well as the  $\nu_{C=C}$  around 1630-1635  $\text{cm}^{-1}$  are caused by minor side oxidations (formation of peroxides, acyl peroxides and peroxyesters) and to hydroxyl dehydration reactions, respectively (12,13). While peroxidation seems to be favored by the

addition of palmitic acid and by the increment of the reaction time, there is no clear trend for the dehydration process.

To further characterize the polyesters, solid state single pulse  $^{13}\text{C}$  MAS NMR spectra have been acquired, Fig. 2. The main carbonyl peak at 174.2 ppm evidences the formation of the corresponding esters (3,14), which is corroborated by the weak acid  $^1\text{H}$  MAS NMR signals around 8 ppm (Supporting Information Fig. S1). The ester peak is accompanied by a growing component of free -COOH groups at ~177 ppm. The contribution of generated residual peroxygenated carbonyl species to this 177 ppm peak is discarded because their lower  $^{13}\text{C}$  chemical shifts (13,15,16,17). However, the presence of an acid anhydride cannot be ruled out (15).

Solid state single pulse  $^{13}\text{C}$  MAS NMR spectra in the C-O region also show that most of primary hydroxyls are esterified (intense peak at 65.0 ppm,  $-\text{CH}_2\text{-CO-O-}\underline{\text{C}}\text{H}_2-$ , vs the weak contribution at 63 ppm,  $\text{HO-}\underline{\text{C}}\text{H}_2-$ ) (3,18). Consequently, the esterification of secondary hydroxyls has to be a minority because of the COOH/OH imbalance in the monomer mixtures. The peak at 75.0 ppm is then due to the excess of free secondary hydroxyl groups (14) and weak signals around 73 ppm and 82 ppm reveal the minor formation of esters with only one of the two vicinal secondary hydroxyls.

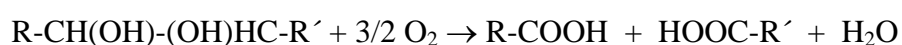
### **Palmitic incorporation**

Despite the considerable load of palmitic acid in the preparation monomeric mixture, the incorporation of PAL units to the polyester network causes mild IR spectra modifications like the redshift of ester ( $1179\text{ cm}^{-1}$  to  $1175\text{ cm}^{-1}$ ) and methylene ( $\nu_{\text{as(-CH}_2\text{-)}}$  from  $2932\text{ cm}^{-1}$  to  $2927\text{ cm}^{-1}$ ) bands. However, the main observation is the little evidence of the  $\nu_{\text{as}}$  of the methyl (- $\text{CH}_3$ ) fingerprint at  $2954\text{ cm}^{-1}$ ; all of which suggest a low palmitic retention within the prepared polyesters. This finding is confirmed by the thermogravimetric analysis of the

ALE:PAL mixtures, Table 1, which indicate that about 2/3 of the added PAL is thermally eliminated before undergoing esterification. Meanwhile the aleuritic fraction remains intact.

To quantify the actual palmitic content in the polyesters, the methyl ( $-\underline{\text{C}}\text{H}_3$  at 15 ppm)  $^{13}\text{C}$  MAS NMR peak has been integrated and referred to the total aliphatic signal. Calculated palmitic molar fractions ( $X_p$ ) are included in Table 1 and confirm the considerable palmitic loss indicated by the thermogravimetric analysis.

The C-O  $^{13}\text{C}$  MAS NMR region is more sensitive to the introduction of palmitic units into the polyester framework, Fig. 2. Thus, the population of free hydroxyls (mostly secondary at 75 ppm) is drastically reduced, but such loss is not directly reflected in the formation of the corresponding C-O linkages in esters. Indeed, the analysis of the area of  $^{13}\text{C}$  MAS NMR peaks, Fig. 3, reveals a net loss of C-O bonds, either in the form of free or esterified hydroxyls, and a simultaneous increment of the population of C=O species. Both, the free hydroxyl loss and the formation of additional carbonyl groups can also be observed from other experimental results like the increment of the intensities of the  $\nu_{\text{C=O}}$  IR bands in Fig. 1 and the magnification of the 2.3 ppm and 4.2 ppm  $^1\text{H}$  MAS NMR peaks associated to methylene contiguous to  $-\text{COO}-$  in esters (3,14,19,20) (Supporting Information Fig. S1). To account for these observations, we propose the occurrence of a series of oxidation reactions consuming  $-\text{OH}$  and leading to the formation of  $-\text{COOH}$  that may stay as acid or undergo additional esterification. Particularly, a vicinal diol cleavage and further oxidation to acid process:



Interestingly,  $^{13}\text{C}$  MAS NMR data indicate that the vicinal diol cleavage mechanism is potentiated as the PAL content is increased, despite the lower concentration of ALE units bearing the vicinal diol group. Other side oxidation reactions like the formation of peroxides



and peroxyesters follow the same trend, as deduced from the IR spectra and the experimental oxygen content, Table 1.

Also, oxidative reactions are more extended as the synthesis time is increased, as observed when results obtained after 10 h and 20 h reaction are compared. In any case, the transformation into carbonyl species is not enough to account for the calculated hydroxyl losses, Fig. 3. Additional processes should be considered, among them alcohol dehydration. Unsaturation is actually detected by IR (1630-1635  $\text{cm}^{-1}$ ) and  $^{13}\text{C}$  MAS NMR ( $\sim 130$  ppm).

PAL incorporation also have an effect on the water absorption capacity of the ALE:PAL polyhydroxyesters, Table 1. At the full esterification regime (10 h), the hydroxyl reduction derived from both the ALE:PAL ratio and the reaction with palmitic acid units, is consistently translated into a progressive reduction of the water absorption capacity. However, after the diol cleavage oxidation is extensive (20 h), such decreasing trend disappears and becomes constant. Though IR spectra of this series show a reduction of hydroxyl groups,  $^{13}\text{C}$  MAS NMR data reveals the formation of new free carboxylic acid groups that may act as hydrophilic centers compensating for the hydroxyl loss.

### **Structural characterization**

The lack of a series of characteristic bands in the 1400-700  $\text{cm}^{-1}$  region of the IR spectra already suggests that polyesters prepared from ALE:PAL mixtures are quite amorphous (21). This is confirmed by the characteristic broad peak at  $2\theta \approx 19.8^\circ$  (spacing 0.448 nm) in the XRD diagrams (Supporting Information Fig. S2).

On the other side, the DSC thermograms show clear glass transitions ( $T_g$ ) and no melting events at low PAL content. However, the progressive incorporation of palmitic units has two effects: (i) the continuous reduction of  $T_g$  values and (ii) the development of an endothermic peak in the 2-8 $^\circ\text{C}$  range. In a first approach, both can be assigned to occluded water acting as

a plasticizer. However, and as calculated from the energy involved in such endothermic peak (~35 mJ in the 5:5 sample at 20 h), the amount of water should be about 3.5% (w/w), but diffraction patterns obtained at -20°C show no evidences of ice, Fig. 4. Nevertheless, the new weak XRD peak at  $2\theta \approx 21.2^\circ$  observed at -20°C correlates with such endothermic event and with the development of the 177 ppm  $^{13}\text{C}$  MAS NMR contribution, Fig. 2. Consequently, we assign the endothermic peak to the melting of the acid phase created by the oxidation of hydroxyls groups upon extended heating in air.

$^{13}\text{C}$  MAS-NMR data reveals that in poly ALE:PAL 10:0 in the full esterification stage, condensation is mostly with primary alcohols. The structure of these polyhydroxyesters can then be described as linear with some degree of branching via secondary hydroxyl esterification (6). Thus, if palmitic units are incorporated by reaction with primary hydroxyls, chain propagation will be terminated. On the other side, if secondary hydroxyls are esterified by PAL, branching will be blocked. In both cases the number-average molecular weight of the polyester will decrease and such reduction is proposed to be responsible for the progressive  $T_g$  diminishment observed upon palmitic acid addition, Table 1. Unfortunately, the insolubility of these polyesters prevents the experimental determination of molecular weights to support this hypothesis.

Schematically, the structure of these polyhydroxyesters can also be depicted as a primary framework made of ester bonds completed with a secondary network of hydrogen bonded hydroxyl groups. Thus, the effect of palmitic monomers in capping chain propagation and branching, as well as in reducing the population of hydroxyl groups, presumably leads to a weaker structure. This is confirmed by uniaxial tensile tests, Fig. 5. Thus, in the absence of significant oxidation (open circles), the incorporation of palmitic units causes the loss of rigidity and the rupture under lower stress. However, strain at break displays an anomalous trend, instead of growing, as expected from the diminishment of the Young's modulus caused

by palmitic addition, it decreases. We have associated this behavior to a non isotropic chemical composition of the films and to the formation of more altered layer at the air exposed side of the films where fracture may be better initiated and propagated.

Under oxidative cleavage conditions (closed circles), mechanical parameters become less dependent on the palmitic content but elastic modulus and stress at break values increase, indicating a reinforced structure. Our explanation is based on the occurrence of the vicinal diol cleavage reaction in such synthetic conditions. The formation of two additional –COOH groups and their esterification creates two new ester bonds compensating for the C-C breaking and gives rise to chain crosslinking, Scheme I. Mechanical collapsing at the highest  $X_p$  is due to excessive –COOH formation and hydroxyl depletion in their proximity preventing esterification and crosslinking.

IR and  $^{13}\text{C}$  MAS NMR data indicate that poly ALE:PAL susceptibility to the diol cleavage oxidation is enhanced by the increasing PAL content despite the lower secondary hydroxyl availability. Our explanation is that structural modifications induced by PAL incorporation favors oxygen diffusion through the polymer bulk. To check for this hypothesis, the side of the films prepared for 10 h and contacting the mould (with virtually no contact with air) has been analyzed by attenuated total reflectance infrared spectroscopy (ATR-FTIR) (Supporting Information Fig. S3). The growing presence of oxygenated species in this side of the film is an experimental evidence supporting the hypothesis of a better permeation to oxygen as a consequence of PAL addition.

## CONCLUSIONS

Polymeric films have been prepared from mixtures of aleuritic and palmitic acids by direct noncatalyzed melt-condensation in air. Though a noticeable amount of palmitic acid is thermally eliminated before undergoing esterification, its incorporation to the polyester

network causes an important reduction of the water absorption capability and leads to more hydrophobic materials. On the contrary, the mechanical performance of the films is significantly reduced as well as oxygen permeation and side oxidation reactions are potentiated. Mechanical properties can be improved by extending the reaction time to favor the occurrence of a vicinal diol cleavage side reaction leading to chain crosslinking, but water affinity is regained due to excessive –COOH formation.

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