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PAPER

Suberin isolation from cork using ionic liquids: characterisation of ensuing products†

Rui Ferreira,‡[a] Helga Garcia,‡[a] Andreia F. Sousa,[b] Marija Petkovic,[a] Pedro Lamosa,[a] Carmen S. R. Freire,[b] Armando J. D. Silvestre,[b] Luís Paulo N. Rebelo[a] and Cristina Silva Pereira*[ac]

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Cholinium alkanoates, a class of benign ionic liquids, were demonstrated to efficiently extract suberin domains from cork. A detailed characterisation of the extracted material has yet to be attained. In the present study the significance of the alkylic chain length of the anion and the ionic liquid's basicity was investigated. The results obtained emphasise cholinium hexanoate's selection; it proved to be a straightforward process, also ensuring the recyclability and reusability of the ionic liquid. The extracted suberinic material has been thoroughly characterised for the first time by ATR-FTIR, NMR, GC-MS and thermal analyses. Data showed that it is mainly composed of oligomeric or polymeric aliphatic esterified structures, resulting from suberin partial cleavage. More than 40 wt% of the extracted suberinic material was found to be cross-linked. Even though, the composing monomeric units were similar to those usually identified in suberin samples obtained by the conventional extraction processes. These data pave the way for advanced studies of suberin monomers/oligomers as building-blocks for the development of novel biopolymers and biomaterials.

Introduction

Biomass feedstocks constitute a source of numerous value-added compounds, such as biopolymers, biofuels, and building-block chemicals.[1] Cork, the outer bark of *Quercus suber* L., is a remarkable plant composite displaying a very specific combination of properties, such as elasticity, compressibility, low density, low permeability, and significant chemical and microbial resistance.[2] Historically, cork utility goes back to the ancient Romans, and since then has been used essentially to manufacture stoppers and thermal/sound insulation materials. Globally, $\geq$ 300 000 tonnes of cork are processed *per annum* by industry, generating large amounts of residues (*ca.* 22 wt%), especially cork of small grain size, which, despite its interesting chemical composition, is generally burned to produce energy.[3]

Cork is composed of suberin, lignin, polysaccharides, and extractives (approximately 50, 20, 20 and 10 wt%, respectively).[4–6] Suberin, an aromatic-aliphatic cross-linked bio-polyester, represents *per se* a source of property-enhancing additives.[7] It is a three-dimensional complex network occurring in the secondary plant cell wall. While its composition and native structural organisation are still controversial, the domains of suberin are generally thought to be arranged in a lamellar-type structure.[5,7–10] The aliphatic domain is composed mostly of even numbered units (C_{16}–C_{26}) of aliphatic alcohols, alkanoic acids, ω-hydroxyalkanoic acids (some with mid chain epoxy functionalities) and α,ω-alkanedioic acids. The aromatic domain shows a quite distinctive composition, with some similarities to lignin, predominantly composed of hydroxycinnamic acid units, with residual amounts of *p*-coumaryl, coniferyl, and sinapyl alcohols.[5,6,8,11–13] The suberin monomers are cross-linked *via* ester bonds involving glycerol units or aliphatic hydroxyl and carboxylic moieties.[12,14] However, the nature of the linking of suberin to the other cell wall domains remains uncertain.[15]

Depolymerisation of *in situ* suberin and its simultaneous isolation from the plant composite is traditionally a laborious process requiring harsh chemical processes.[7] These processes involve extensive ester bond cleavage, normally attained through alkaline methanolysis with sodium methoxide,[5,11,12,16,17] or alkaline hydrolysis.[18] Furthermore, suberin partial depolymerisation, leading to the formation of oligomeric structures, can be achieved using more gentle processes although with limited extraction yields, *e.g.* methanolysis catalysed by calcium oxide.[19]

[a] *Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apartado 127, 2781-901, Oeiras, Portugal. E-mail: spereira@itqb.unl.pt; Web: http://www.itqb.unl.pt*
[b] *CICECO and Department of Chemistry, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal*
[c] *Instituto de Biologia Experimental e Tecnológica (IBET), Apartado 12, 2781-901 Oeiras, Portugal*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2nj40433h
‡ Equally contributing authors.

The last few decades have witnessed an exponential growth of interest in ionic liquids – a disparate class of chemicals composed solely of ions that are liquid below a temperature conventionally defined as 100 °C.[20] Several hundred ionic liquids are already available and characterised,[21] and one can reasonably estimate that millions of cation/anion combinations are possible. Furthermore, fine-tuning of the cation and/or the anion might be used to address very specific thermophysical and chemical properties,[20] and even biological activity.[22] Ionic liquids usually exhibit a set of remarkable features, such as negligible vapour pressure,[23] bulk non-flammability, thermal stability, and high solvent ability.[20] The latter characteristic arises from the combination of the organic functionalities of the ions with the Coulombic environment created by them, resulting in a structural arrangement of charged and apolar micro-domains.[24,25] A combination of the aforementioned properties with the simplicity of their synthesis and potential recyclability has led to the use of ionic liquids in various extant industrial applications.[21]

One of the most elegant examples of the industrial potential of ionic liquids was the demonstration that some imidazolium-based systems could successfully solubilise *in situ* cellulose.[21,26–28] This has been suggested to involve disruption of the intermolecular hydrogen-bonding network of cellulose.[27,29–31] More recently, other imidazolium-based ionic liquids have been shown to solubilise suberin isolated enzymatically from potato[32] and to extract lignin from lignocellulosic materials.[33] However, the recalcitrance to biodegradation of the imidazolium moiety,[34] together with its toxicity,[22] may restrict the large-scale application of these interesting observations.

In this context, there is no doubt that one landmark was the demonstration, by our group, that some cholinium alkanoates can efficiently extract *in situ* suberin from cork.[35] These ionic liquids have also shown to be both benign to eukaryotic organisms and biodegradable.[35] However, the detailed chemical and structural characterisation of the extracted suberinic material is yet to be attained. This constitutes the main goal of the present study and a key aspect to understand and improve the efficiency of this process. The suberinic materials isolated with cholinium hexanoate were characterised in terms of chemical composition, morphology, and thermal behaviour. Aiming to better understand the extraction process some alkanoates not considered in the previous study, were also investigated. The extracted suberinic material was found to be mainly composed of oligomeric and polymeric ester type structures.

Materials and methods

Cork

Granulated cork was obtained from the cork producers Amorim & Irmãos SA (St[a] Maria de Lamas, Portugal). The samples were ground to a fine powder (60 mesh) using a centrifuge mill (Retsch) and the cork extractives removed by sequential Soxhlet extraction with solvents of increasing polarity (dichloromethane, ethanol and water) as previously described by Gil *et al.*[17] The extractive-free cork powder, hereinafter defined solely as cork, was further washed in an excess of deionised water for complete removal of low molecular weight compounds, and then dried prior to use.

Ionic liquids

The complete list of ionic liquids (Fig. 1) used in this study is as follows: 1-ethyl-3-methylimidazolium hexanoate ($[C_2mim][O_2CC_5H_{11}]$); cholinium hexanoate ($[N_{111}C_2H_4OH][O_2CC_5H_{11}]$); cholinium octanoate ($[N_{111}C_2H_4OH][O_2CC_7H_{15}]$) and cholinium decanoate ($[N_{111}C_2H_4OH][O_2CC_9H_{19}]$). The cholinium alkanoate

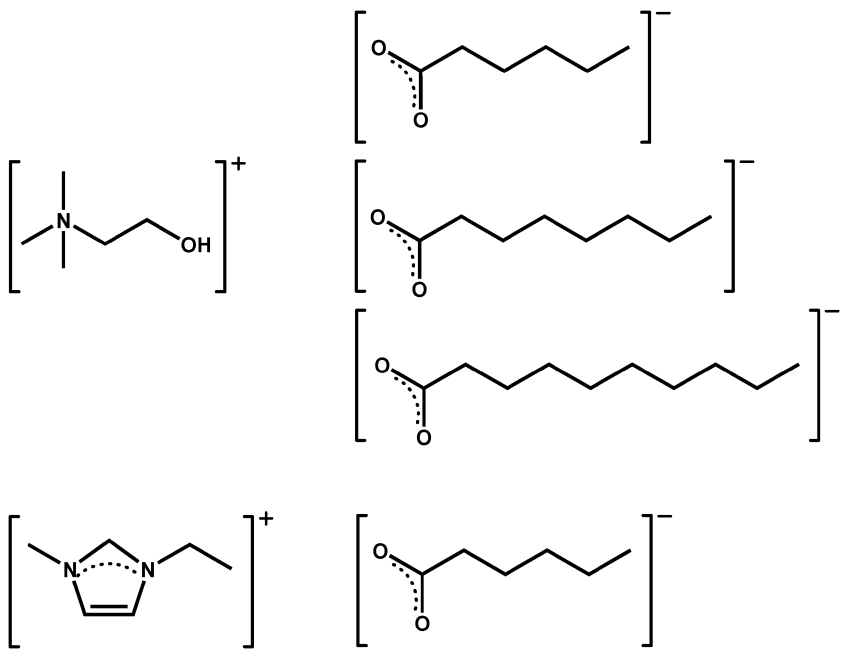


Fig. 1 Chemical structures of the tested ionic liquids. From the top: cholinium hexanoate, octanoate and decanoate, and 1-ethyl-3-methylimidazolium hexanoate.

salts were synthesised by dropwise addition of the corresponding acid to aqueous cholinium hydrogencarbonate (Sigma ~80% in water) in equimolar quantities, as described by Petkovic *et al.*[36] The 1-ethyl-3-methylimidazolium hexanoate was prepared using a similar method. First the chloride anion in 1-ethyl-3-methylimidazolium chloride was exchanged by hydroxide using an ion-exchange column (Amberlite® IRN-78). The resulting 1-ethyl-3-methylimidazolium hydroxide was then neutralised by an equimolar quantity of hexanoic acid. 1-Ethyl-3-methylimidazolium chloride of high purity was purchased from Sigma.

Ionic liquids purity was verified by ^{1}H and ^{13}C NMR spectroscopy at 25 °C, on a Brüker Avance III 400 spectrometer (Brüker BioSpin, Rheinstetten, Germany), and further confirmed by CHNS elemental analysis and electrospray ionisation mass spectrometry (ESI-MS) (Waters LCT Premier fitted with electrospray). The ionic liquids were dried prior to use by stir-heating *in vacuo* (40–70 °C, 24–48 h, *ca.* 0.01 mbar). The water contents, determined by Karl-Fischer titration, were below 0.5 wt%. The obtained salts fulfilled the requirements of the present study.

Other chemicals

Ammonium nitrate ($[NH_4][NO_3]$), lithium nitrate ($Li[NO_3]$), dimethyl sulfoxide (DMSO), sodium hydroxide (≥ 97%), dichloromethane (99%), *n*-hexadecane (99%), decanedioic acid (99%), 12-hydroxydodecanoic acid (≥ 97%) and deuterated trichloromethane (99.8%) were purchased from Sigma.

Suberinic material extraction

The experiments followed the protocol previously described by Garcia *et al.*,[35] with some modifications (Fig. 2). These aimed exclusively at facilitating and speeding-up the filtration step due to the large amount of suberinic materials being processed. Briefly, the ionic liquid was mixed with powdered cork (ionic liquid : cork ≈ 9 : 1 wt/wt) and kept at 100 °C during 4 h, with stirring (each in triplicate). At the end of the extraction process, DMSO was added to reduce the viscosity of the mixture,[27] facilitating its filtration through a 0.45 μm nylon membrane (Millipore, MA, USA). The cork insoluble residue was then washed thoroughly with an excess of water at 80 °C, and dried at 50 °C under a nitrogen purge until constant weight was attained. The ensuing filtrate, *i.e.* ionic liquid, the extracted material, DMSO and the water added to wash the cork insoluble residue, was kept at 4 °C for 1 h. This led to the precipitation of the extracted suberinic material, which was then recovered by centrifugation (30 min at 4 °C and 2450 *g*), washed twice with an excess of water to remove any remaining ionic liquid, and dried under a nitrogen flux, at 50 °C until constant weight was attained.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra were collected on a Bruker IFS66/S FTIR spectrometer (Bruker Daltonics, MA, USA) using a single reflection ATR cell (DuraDisk, equipped with a diamond crystal). Data were recorded at room temperature, in the range of 4000–600 cm^{-1}, by accumulating 258 scans with a resolution of 8 cm^{-1}. Five replica spectra were collected for each sample in order to evaluate reproducibility (OPUS v5.0).

Scanning electron microscopy (SEM)

Samples were dried prior to use and coated with a thin layer of gold using a sputter coater (Polaron E-5100). Electron micrographs were recorded using an analytical field emission gun-scanning electron microscope (FEG-SEM: JEOL 7001F with

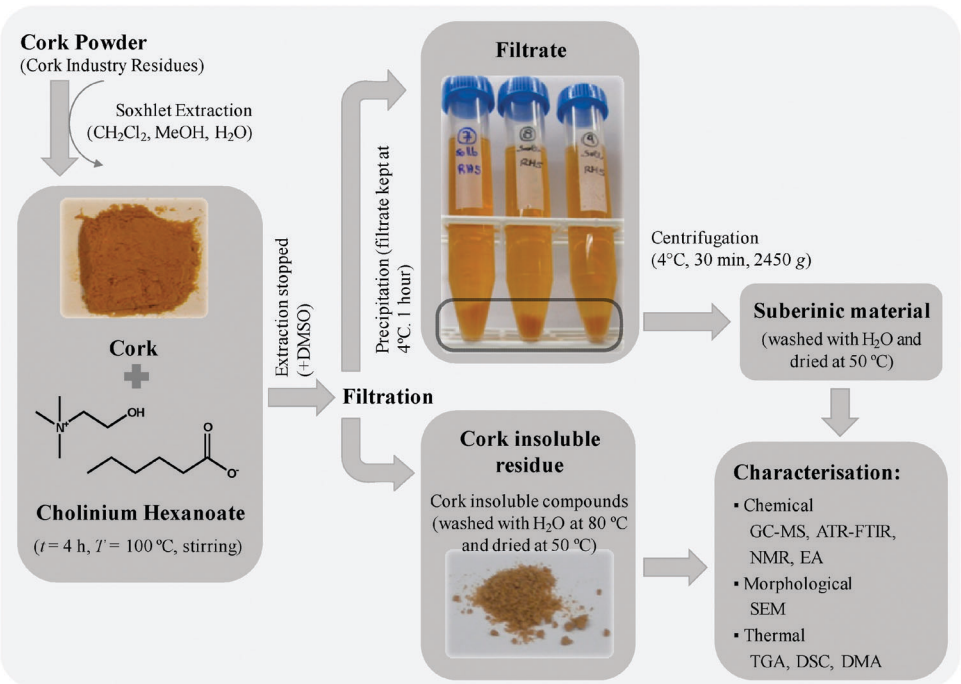


Fig. 2 Schematic view of the scientific plan used in this study, where cholinium hexanoate is taken as an example.

Oxford light elements EDS detector) operated at 5–10 kV. The micrographs presented here were carefully selected, and are regarded to be representative of the different fractions.

Elemental analysis (EA)

Elemental composition (C, H and N) was determined using a Leco TruSpec® Series elemental analyser. The oxygen (O) content was assumed to be the remaining amount of the sample and was calculated from the C, H and N composition.

Nuclear magnetic resonance spectroscopy (NMR)

1D (^{1}H, ^{13}C) and 2D homo- and heteronuclear solution NMR spectra of suberinic materials were acquired on a Avance III 800 spectrometer (Brüker, Rheinstetten, Germany) working at a proton operating frequency of 800.33 MHz, equipped with a three channel 5 mm inverse detection probe head with pulse-field gradients along the *Z*-axis. Spectra were run at 25 °C using standard Brüker pulse programs. ^{1}H and ^{13}C chemical shifts are referenced to trichloromethane. ^{13}C spectra were recorded at 201.24 MHz using the APT (attached proton test) sequence. ^{13}C Cross Polarization Magic/Angle Spinning NMR (CP/MAS NMR) spectra were recorded at 9.4 T on a Brüker 400 spectrometer using 9 kHz spinning rate and MAS with proton 90° pulses of 4 μs. Chemical shifts are given in ppm from glycine. All NMR spectra were processed and analysed with MestreNova v. 6.0 (MestreLab Research S.L.).

Gas chromatography–mass spectrometry (GC-MS)

A Trace GC 2000 Series gas chromatograph equipped with a Thermo Scientific DSQ II mass spectrometer was used. The GC-MS was first calibrated with pure reference compounds (12-hydroxydodecanoic acid and decanedioic acid), representative of the major classes of suberinic compounds, relative to *n*-hexadecane (internal standard). Compounds identification was based on the equipment spectral library (Wiley-Nist) and on previously published data based on their EI-MS fragmentation patterns and/or retention times.[11,13,18,37] Each sample was analysed by two complementary methods:

- Method 1, suberinic material was converted to the corresponding trimethylsilyl derivatives and analysed as previously described,[11] allowing identification of monomeric structures present in the mixture;

- Method 2, in order to analyse the composition of the oligomeric/polymeric fraction of suberinic material, samples were submitted to an alkaline hydrolysis step in order to release their monomeric constituents. Briefly, the samples were treated with a solution of 0.5 M NaOH in methanol/water (1 : 1, v/v), at 95 °C, during 4 h.[38] The mixture was cooled to room temperature, acidified to pH 3–3.5 with 1 M HCl, extracted three times with dichloromethane, and dried in a rotary evaporator. Finally, samples were trimethylsilylated as mentioned above, prior to GC-MS analysis.

Thermogravimetric analysis (TGA)

TGA data were obtained using a TGA-Q50 TA Instrument. All samples were run in crimped aluminium pans with pin-hole under a nitrogen atmosphere (100 cm^3 min^{-1}). Samples were dried *in situ* at 100 °C for 30 min and heated up to 600 °C, at a heating rate of 1 °C min^{-1}. Universal Analysis version 4.4A software was used to determine the degradation temperature ($T_{x\%,\mathrm{deg}}$), onset temperature (T_{onset}), the weight of water adsorbed by the sample in equilibrium with atmosphere ($\mathrm{wt}_{\mathrm{H_2O}}$), the weight of the solid residue remaining at 600 °C ($\mathrm{wt}_{600\ ^\circ\mathrm{C}}$) and the derivative thermograms. $T_{x\%,\mathrm{deg}}$ and T_{onset} are, respectively, defined as the temperature of a specific weight loss after the drying step, and as the intersection of the baseline weight after the drying step with the tangent of the weight *vs*. temperature curve as decomposition occurs. $\mathrm{wt}_{\mathrm{H_2O}}$ is defined as the weight loss occurring since the beginning of the experiment until the end of the *in situ* drying step.

Differential scanning calorimetry (DSC)

DSC analyses were carried out with a DSC – Q200 TA Instrument. The DSC was calibrated for temperature and heat flow with indium samples and operated under constant purging of nitrogen (50 cm^3 min^{-1}). Samples were hermetically sealed in aluminium pans and heated/cooled up to 120/−80 °C at a constant rate of 5 °C min^{-1}, followed by a 5 min isotherm at 120/−80 °C. Three heating/cooling cycles were repeated. The first cycle was used to clear the sample thermal history. When the second and the third cycles were identical, the latter was used for data collection. The characteristic peaks were analysed using Universal Analysis, version 4.4A software. Melting temperature (T_{m}) was determined as the minimum of the melting endothermic peak during the heating cycle.

Dynamic mechanical analysis (DMA)

DMA measurements were carried out with Tritec 2000 DMA Triton equipment operating in the bending (single cantilever) mode. Tests were performed at 1 and 10 Hz and the temperature was varied from −100 to 150 °C at 2 °C min^{-1}. A small amount of the powdered sample was dispersed in a foldable stainless steel sheet from Materials Pocket of Triton technology.

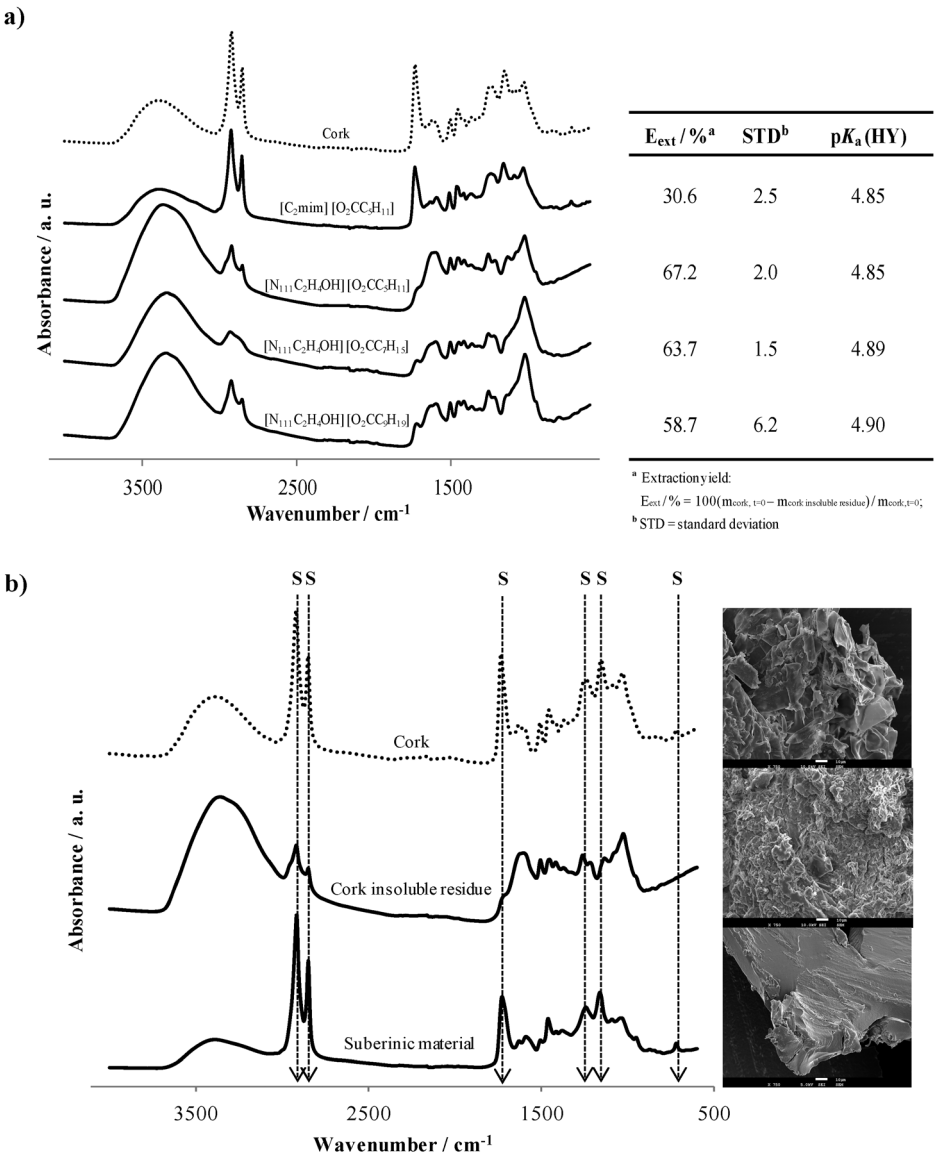
Results and discussion

Cholinium hexanoate, a biocompatible and biodegradable ionic liquid, was demonstrated to promote a highly efficient extraction of the suberin domains from cork.[35] These findings were based on analyses of the IR absorption peaks of cork insoluble residues (which can be attributed to its specific constituents without significant error, ESI 1†).[5,6,12,39] The efficiency ranking of the previously tested anions (*viz*. ethanoate < DL-lactate < butanoate ≈ *iso*-butanoate < hexanoate) suggested that the extraction process was controlled by the length of the anion alkylic chain and increases progressively with its basicity.[35] The alkaline requirement of the ionic liquid based process for the extraction of suberinic materials from cork resembles conventional approaches where this is taken as a critical factor.[5,11,12,16–19,37] In fact, hexanoic acid alone was observed to be unable to extract suberin from cork.[35] In order to mimic the Coulombic component of an ionic liquid environment, eutectic mixtures of inorganic salts ([NH_4][NO_3] + Li[NO_3]) were tested. These mixtures also failed to extract cork components (extraction yields < 5 wt%).

Extraction of suberinic materials from cork with alkanoate based ionic liquids

Inspired by these initial findings, the extraction of suberinic materials by cholinium alkanoates carrying a long alkylic chain anion and high basicity was tested in the present study. These included the previously tested cholinium hexanoate,[35] and also cholinium octanoate or decanoate, here tested for the first time. Their extraction ability was initially determined comparing untreated cork with cork treated with each of the ionic liquids, *i.e.* cork insoluble residue (Fig. 3). Even though the basicity increases slightly with the length of the anion alkylic chain (hexanoate < octanoate < decanoate), cork mass losses and ATR-FTIR spectral profiles of the cork insoluble residues were comparable (Fig. 3a). The ATR-FTIR spectra showed a remarkable reduction of the peak intensities attributed to suberin (2921, 2852, 1737, 1242, 1158 and 724 cm^{-1}). In addition, the polysaccharide and lignin domains in the cork insoluble residues remained apparently unaltered. Data make apparent that the tested cholinium alkanoates led to an extensive extraction of suberin domains from cork. In view of cork chemical variability, one can assume that the extraction yields obtained in this study are comparable to the maximum yields reported for alkaline methanolysis of cork (~55 wt%).[12]

In the present study, it also became apparent that the cholinium cation *per se* plays an important role for suberin extraction. In fact, 1-ethyl-3-methylimidazolium hexanoate was unable to efficiently extract suberin from cork (extraction yields of 30.6 wt%, Fig. 3a). The superior performance of the cholinium hexanoate, relative to the 1-ethyl-3-methylimidazolium



E_{ext} / %[a]	STD[b]	pK_a (HY)
30.6	2.5	4.85
67.2	2.0	4.85
63.7	1.5	4.89
58.7	6.2	4.90

[a] Extraction yield: $E_{ext}/\% = 100(m_{cork,\ t=0} - m_{cork\ insoluble\ residue})/m_{cork,\ t=0}$;
[b] STD = standard deviation

Fig. 3 Analysis of the extraction of suberin domains from cork with selected ionic liquids, namely 1-ethyl-3-methylimidazolium hexanoate, cholinium hexanoate, cholinium octanoate and cholinium decanoate. (a) ATR-FTIR spectra of cork insoluble residue. Side table shows suberin extraction yield after ionic liquid treatment and the pK_a of the conjugate acid (HY) of the corresponding anion. (b) ATR-FTIR spectra of the cork insoluble residue and the suberinic material after extraction with cholinium hexanoate. Side figures show the corresponding SEM images (magnification 750×). Vertical lines stand for major peaks assigned for suberin (S).

Table 1 Elemental analysis of cork, cork insoluble residue, and suberinic material

	C/wt%	H/wt%	O/wt%	N/wt%
Cork	61.90	7.41	30.14	0.55
Cork insoluble residue	55.20	6.37	36.17	2.26
Suberinic material	67.40	9.09	22.35	1.16
Suberin[13]	68.00	9.76	20.66	n.d.

n.d.—not determined.

hexanoate, is probably related with the strength of interaction between cation and anion. In fact, the carboxylate moiety of the anion might strongly interact with the protic hydrogen in the imidazolium ring, partially blocking the extraction of suberin from cork.

Petkovic *et al.* demonstrated that the minimal inhibitory concentration of cholinium hexanoate against fungi was significantly higher (by one order of magnitude) than those of cholinium octanoate or decanoate.[36] It is therefore irrefutable that cholinium hexanoate raises the greenness of this novel suberin extraction process.

Chemical characterisation of the extracted suberinic material

The superior efficiency of cholinium hexanoate, together with its high biocompatibility,[36] makes ultimate its selection for a deeper characterisation of the extracted material, *i.e.* suberinic material.

Samples of cork, suberinic material and cork insoluble residue were analysed by SEM (Fig. 3b). This provided information on morphological alterations introduced in cork after extraction with cholinium hexanoate. The SEM images show that the cholinium hexanoate extraction process has substantially affected the morphology of powdered cork. A drastic destruction of the cork cell walls can be noticed in the cork insoluble residue. In addition, the suberinic material displayed a very homogenous morphology typical of non-structured material.

Elemental analysis showed that the C, H, O and N relative abundance in the suberinic material (Table 1) was similar to that of a suberin sample extracted through alkaline methanolysis.[13] As expected, due to the presence of long aliphatic chains, the suberinic material was more enriched in C and H when compared to the cork insoluble residue. Likewise, enrichment in hydroxycinnamic acid derivatives, *e.g.* feruloyltyramine,[40] might justify the increment in N relative content detected in the suberinic material. However, vestigial amounts of cholinium hexanoate might have contributed to the N relative content detected in the suberinic material, as well as in the cork insoluble residue. The increment in the O relative content detected in the cork insoluble residue is most probably due to its enrichment in polysaccharides and lignin.

The ATR-FTIR spectrum of the suberinic material (Fig. 3b) is dominated by major peaks at 2921, 2852 cm^{-1}, normally attributed to the long aliphatic chains of suberin.[5,12,13,39] In addition, the high intensity of the band at 1730 cm^{-1}, which is usually assigned to the vibration of carbonyl groups typical of esters, suggests that this material was extracted mainly in the esterified form.

The ^{13}C solid state NMR spectrum clearly demonstrates that the suberinic material (Fig. 4) owns an essential aliphatic and esterified nature. In fact, the two major resonances at δ 30 and 33 ppm are attributed to methylenic carbons of typical long aliphatic carbon chains; and the resonance at around δ 173 ppm is assigned to carbonyl carbons of ester groups (Fig. 4). The resonance at δ 148 ppm is usually assigned to quaternary carbons present in lignin-type structures.[6,17] However, it is difficult to discriminate if these quaternary carbons are the typical aromatic domains of suberin, lignin or both. Other resonances at about δ 54, 64, 73 ppm and δ 130 ppm were also detected and are assigned to carbons nearby hydroxyl or ester groups and to vinylic carbons, respectively. Though these resonances are typical of suberin, one cannot disregard that they might also be associated with the presence of polysaccharides and lignin, respectively.

As expected, the ^{13}C solid state NMR spectrum of the cork insoluble residue showed major resonances typical of polysaccharides and lignin at δ 54, 64, 73, 83, 105 ppm and δ 131–133, 148 ppm, respectively (Fig. 4). Although of low intensity, some typical suberin resonances (at δ 30, 33, 173 ppm) were also detected (in accordance with the corresponding ATR-FTIR spectrum, Fig. 3b). This seems to imply that suberin extraction from cork by cholinium hexanoate, though extremely efficient, was not complete.

The ^{13}C solid state NMR data further validate the initial interpretation of elemental and ATR-FTIR analyses. The extracted material, which shows an essential aliphatic and esterified nature typical of suberin, could not be completely solubilised in organic solvents. The dichloromethane insoluble cross-linked fraction of the suberinic material represents 42 ± 2 wt%. This observation reinforces that despite the high extraction efficiency of cholinium hexanoate, this process took place by partial depolymerisation of suberin.

Chemical characterisation of the organic soluble fraction of suberinic materials

In order to complete the chemical characterisation of the suberinic material, the organic soluble fraction was further characterised by ^{1}H and ^{13}C NMR spectroscopy. These methodologies were combined with 2D COSY, HSQC and HMBC studies[41] for refining the spectral attributions (ESI 2†). The data depicted in Table 2 include the list of the functional groups identified and their NMR assignment. The obtained ^{1}H NMR spectrum (Fig. 5) is characterised by the presence of a major group of resonances, in the range δ 1.25–2.38 ppm, associated with suberin methylenic groups, in different chemical environments, namely in the long aliphatic chains and nearby ester groups. Two additional resonances at δ 4.05 and 4.82 ppm, assigned to methylenic and methinic protons directly linked to an ester group, were also observed. These features were confirmed by ^{13}C NMR analysis (Table 2) which showed dominant aliphatic carbon resonances at δ 25–35 ppm and at 173–190 ppm assigned to –COO– groups. Other minor resonances were also detected, namely those assigned to vinylic groups (^{1}H δ 5.34 ppm; ^{13}C δ 130 ppm), aliphatic methyl groups (^{1}H: δ 0.72–1.05 ppm; ^{13}C: δ 12 ppm) and aromatic domains (^{1}H δ 5.92–8.09 ppm; ^{13}C δ 100–150 ppm). The presence of aromatic protons (highlighted in the magnified section of Fig. 5), confirms also the data observed in the

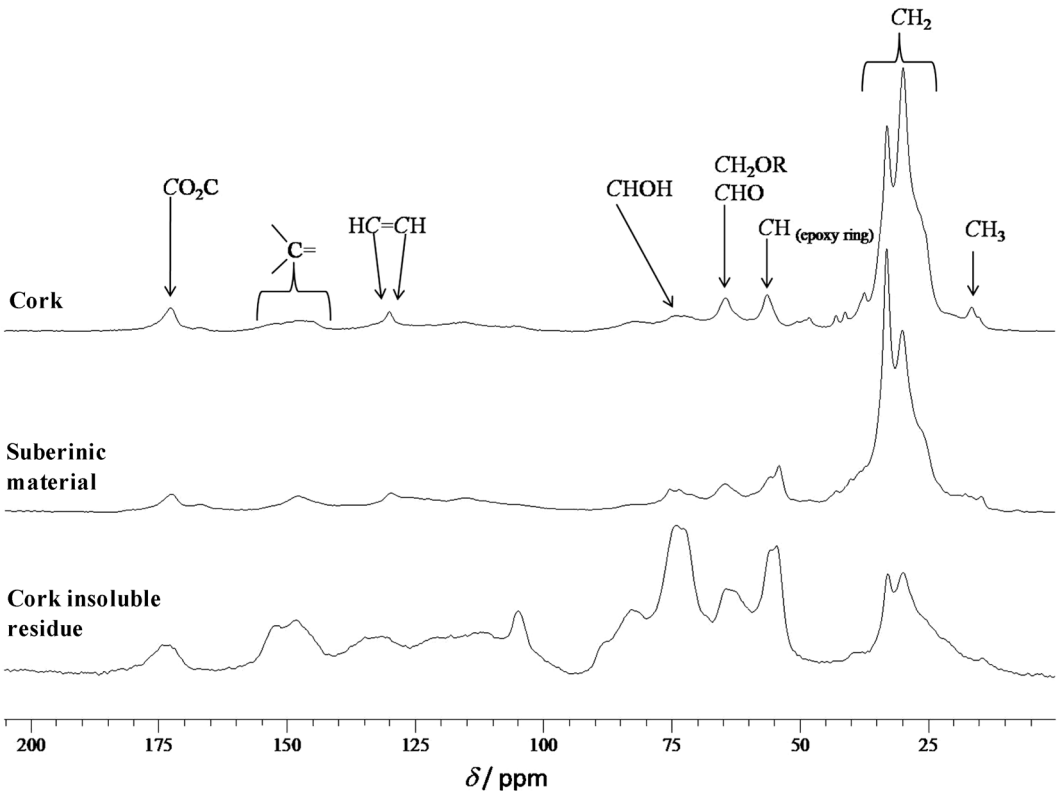


Fig. 4 ^{13}C CP/MAS NMR spectra of cork, and both suberinic material and cork insoluble residue after extraction with cholinium hexanoate. R stands for H or ester group.

Table 2 ^{13}C and ^{1}H NMR analysis assignments of the functional groups identified in the suberinic material

^{13}C δ/ ppm	^{1}H δ/ppm	Functional group	Assignment[5,11,13,16,17,42]
12	0.72–1.05	CH_3	Aliphatic methylic groups
25–35	1.25, 1.29	CH_2	Aliphatic methylenic groups
—	1.53–1.67	CH_2CH_2CO; CH_2CH_2O	Methylenes in the β position to hydroxylic, ester and carboxylic groups
—	2.00	CH_2=CH–CH	Allylic protons
—	2.25–2.38	CH_2COO; CH_2COOH	Methylenes linked to carboxylic moieties
35–41	3.00	CH	Epoxy ring
62	3.64	CH_2OH; $CHOH$	Primary and secondary alcohol
52	3.66	OCH_3	Methoxy groups
54	4.05	OCH_2	Methylenes adjacent to ester groups
n.i.	4.82	OCH	Methyne adjacent to ester groups
130	5.34	CH=CH	Vinylic groups
100–150	5.92–8.09	Ar	Aromatic signals
173–190	—	COO; $COOH$	Ester and carboxylic acid groups

n.i—not identified.

^{13}C solid state NMR. Overall, the NMR data clearly suggest that the structural features of the suberinic material extracted by cholinium hexanoate are highly consistent with those previously reported for suberin extracted by conventional methods from *Q. suber* cork.[5,11,13,16,17,42] Importantly, using conventional methods only the organic soluble suberinic monomers and oligomers released during hydrolysis are extracted.[7]

Chemical characterisation of the suberinic materials monomers

GC-MS analysis of the monomeric composition of the suberinic material (Method 1) led to the identification of only 3.9 wt% (Table 3). The low identification yield is certainly due to the fact that the suberinic material is mainly in the form of high molecular weight components, *i.e.* oligomeric or polymeric fractions of suberin-type structures. This reinforces the idea of its esterified nature, which is also compatible with the presence of insoluble cross-linked polyester type structures. The main families of monomeric compounds detected were extractives (not covalently bonded to cork), alkanoic acids and monoacylglycerols derivatives, even if each accounted for $\leq$1–2 wt%. Minor amounts of alkan-1-ols, hydroxyacids, alkanedioic acids and aromatic compounds were also identified (<0.2 wt%).

As reported above, analysis of the suberinic material by GC-MS led to a very low identification yield of monomers. In order to circumvent this, the GC-MS analysis was repeated in a sample hydrolysed by alkaline hydrolysis (η = 62 wt%) prior to the silylation (Method 2, Table 3). After hydrolysis, the amounts of detected compounds in the suberinic material were considerably higher, accounting for 36 wt% ($\sim$13.5 wt% of cork). These yields are close to those previously reported.[7,13] In the hydrolysed suberinic material, a considerable increment on the content of typical suberin hydroxyacids (*e.g.* 22-hydroxydocosanoic and 9,10,18-trihydroxyoctadecanoic) and alkanedioic acids (*e.g.* 9,10-dihydroxyoctadecanedioic), and α,ω-docosanedioic acids, as well as aromatic compounds and particularly

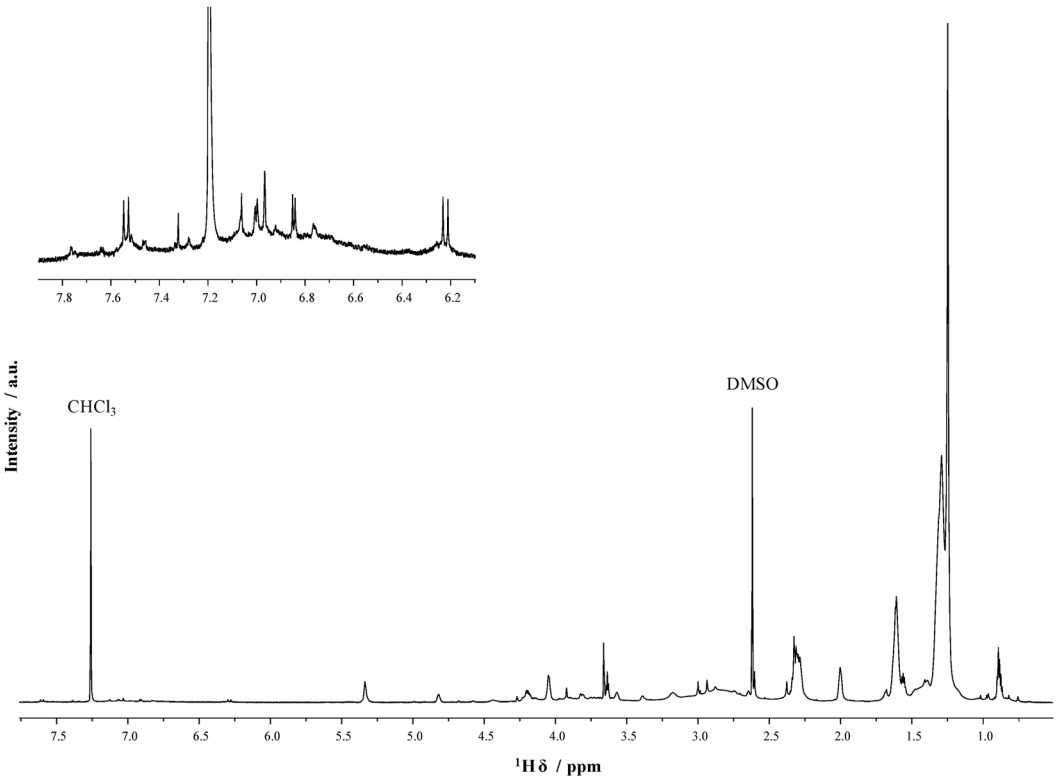


Fig. 5 ^{1}H NMR spectrum (800 MHz, 25 °C) of the suberinic material fraction soluble in deuterated trichloromethane. The magnified section of the spectrum corresponds to its aromatic domain.

ferulic acid, was observed.[7,11,37] Most monomeric compounds with ≥ 3 OH and/or COOH functionalities were only detected after hydrolysis of the suberinic material. Once more this is in accordance with its cross-linked and therefore insoluble nature.

Thermal characterisation of the extracted suberinic material

The thermal characterisation of the suberinic material was performed by TGA (Table 4, Fig. 6a) and DSC analyses (Fig. 6b). During the TGA analysis the samples were dried *in situ* showing weight losses (wt_{H_2O}) of 3.02, 5.76 and 2.01 wt% for cork, cork insoluble residue and suberinic material, respectively. Previous studies reported similar values for cork wt_{H_2O}.[43,44]

All the samples were observed to be thermally stable up to approximately 200 °C (Fig. 6a). The suberinic material showed the lowest $T_{5\%,\ deg}$. This suggests the presence of a small fraction of volatile molecules, certainly including the suberin free monomeric units detected by GC-MS analysis. The thermal resistance of the suberinic material (T_{onset} = 310.7 °C) was similar to that of cork (T_{onset} = 301.8 °C), and much higher than that of the cork insoluble residue (T_{onset} = 229.6 °C). This agrees with previous reports on similar materials.[45] The close similarity of their decomposition profiles, together with their comparable T_{onset} values, underlines the key role of suberin in cork's high thermal resistance. Accordingly, the cork insoluble residue presented the lowest thermal resistance, certainly owing to its high polysaccharides content, which normally displays $T_{deg} < 200$ °C.[2,39,46] Above 200 °C and up to 450 °C all samples showed a gradual multi-step weight loss, typical of complex biomass based samples. The solid residue remaining at 600 °C accounted for 20.5, 36.1 and 16.5 wt% for cork, cork insoluble residue and suberinic material, respectively. Even though lower $wt_{600\ °C}$ would be expected for suberinic materials,[13] similar values have been observed in chemically re-polymerised suberin monomers.[47] Hence the high thermal stability of the extracted suberinic material is most probably due to its esterified nature and the presence of cross-linked polyester type structures. In addition, the high value of $wt_{600\ °C}$ for the cork insoluble residue is apparently due to its high content in lignin.[48]

The DSC thermograms of cork and suberinic material, displayed one broad melting transition that spans for several tens of degrees (Fig. 6b), ranging approximately from 30 to 70 °C. This behaviour is probably a consequence of the complex composition of these samples.

A true glass transition for suberin was not observed by DSC, even upon testing different heating/cooling rates (data not shown). Nevertheless, the glass transition for suberin, herein estimated by DMA (which reports higher sensitivity), was −51.0 °C that agrees with previous reports.[45]

Environmental sustainability and ionic liquid recyclability

The sustainability of this extraction process can be ensured by optimal design of the filtration step in order to avoid the use of DMSO. Preliminary scale-up tests using cholinium hexanoate were performed (4 h at 100 °C with stirring). At the end of the extraction process, the mixture was immediately filtrated in a

Table 3 Main suberin monomers identified by GC-MS analysis of the suberinic material by Method 1 and Method 2, *i.e.* non-hydrolysed and hydrolysed samples respectively. Results are given in mg of compound *per* gram of suberinic material

Identification	Method 1 $m_x/m_{suberin}$ mg/g	Method 2 $m_x/m_{suberin}$ mg/g
Alkan-1-ols	1.04	7.44
Octadecanol	0.03	0.11
Eicosanol	0.08	0.43
Docosanol	0.64	5.06
Tetracosanol	0.30	1.84
Alkanoic acids	5.70	10.76
Hexanoic Acid[a]	4.70	1.43
Tetradecanoic acid	0.03	0.24
Hexadecanoic acid	0.24	2.35
Octadeca-9,12-dienoic acid (linoleic acid)	—	0.18
Octadec-9-enoic acid (oleic acid)	0.03	0.21
Octadecanoic acid	0.38	3.16
Eicosanoic acid	0.04	0.12
Docosanoic acid	0.27	3.07
Hydroxyacids	1.75	191.28
10-Hydroxydecanoic acid	0.05	0.41
16-Hydroxyhexadecanoic acid	—	1.72
18-Hydroxyoctadec-9-enoic acid	0.04	33.83
18-Hydroxyoctadecanoic acid	—	0.79
20-Hydroxyeicos-9-enoic acid	—	1.29
20-Hydroxyeicosanoic acid	0.03	4.11
22-Hydroxydocosanoic acid	1.43	68.27
24-Hydroxytetracosanoic acid	0.20	7.94
9,18-Dihydroxy-10-methoxy-octadecanoic acid[b]	—	8.87
9,10,18-Trihydroxyoctadecanoic acid	—	53.00
cis-Mid-chain,18-dihydroxy-octadec-9-enoic acid	—	3.25
trans-Mid-chain,18-dihydroxy-octadec-9-enoic acid	—	2.55
Mid-chain,18-trihydroxy-eicosanoic acid	—	5.23
Alkanedioic acids	0.43	49.71
Hexadecanedioic acid	—	2.48
Octadecanedioic acid	Tr	0.64
Octadec-9-enedioic acid	—	6.32
9,10-Dihydroxyoctadecanedioic acid[b]	—	26.52
Eicosanedioic acid	0.22	1.83
9,10-Dihydroxyeicosanedioic acid	—	3.12
Docosanedioic acid	0.21	8.81
Aromatics	0.35	30.48
4-Hydroxy-3-methoxy-benzaldehyde (vanillin)	tr	0.65
4-Hydroxy-3-methoxybenzoic acid (vanillic acid)	0.20	1.31
3.4-Dihydroxybenzoic acid	0.08	—
4-Hydroxy-3-methoxy-cinnamic acid (*cis*-ferulic acid)	—	0.84
4-Hydroxy-3-methoxy-cinnamic acid (*trans*-ferulic acid)	0.06	27.68
Extractives	20.50	44.29
β-Sitosterol	1.47	2.63
Friedelin	9.76	10.14
Betulin	6.95	27.24
Betulinic acid	2.31	4.29
Monoacylglycerol derivatives	8.57	0.00
1-Monohexadecanoylglycerol	1.30	—
1-Monooctadecanoylglycerol	0.41	—
1-Monodocosanoylglycerol	1.18	—
1-Monotetracosanoylglycerol	1.20	—
1-Mono[docosanedi-22-oic acid-1-oyl]glycerol	4.48	—
Glycerol	1.92	0.43
Others	3.18	24.90
Other epoxy derivatives	—	24.90
n.i.	3.18	—
Total identified sample (wt%)	3.87	35.79

tr—trace amounts; n.i.—not identified. [a] Not accounted for compounds quantification. [b] Methoxyhidrin artefact from 9,10-epoxy-18-hydroxyoctadecanoic acid.[13]

Table 4 Degradation temperature ($T_{x\%,\ deg}$) and onset temperature (T_{onset}). Weight of water adsorbed by the samples in equilibrium with atmosphere (wt_{H_2O}) and weight of the solid residue remaining at 600 °C ($wt_{600\ °C}$)

	Cork	Cork insoluble residue	Suberinic material
$T_{5\%,\ deg}$/°C	231.4	213.0	205.2
$T_{10\%,\ deg}$/°C	262.6	239.8	257.4
T_{onset}/°C	301.8	229.6	310.7
$wt_{600\ °C}$/%	20.46	36.12	16.48
wt_{H_2O}/%	3.02	5.76	2.01

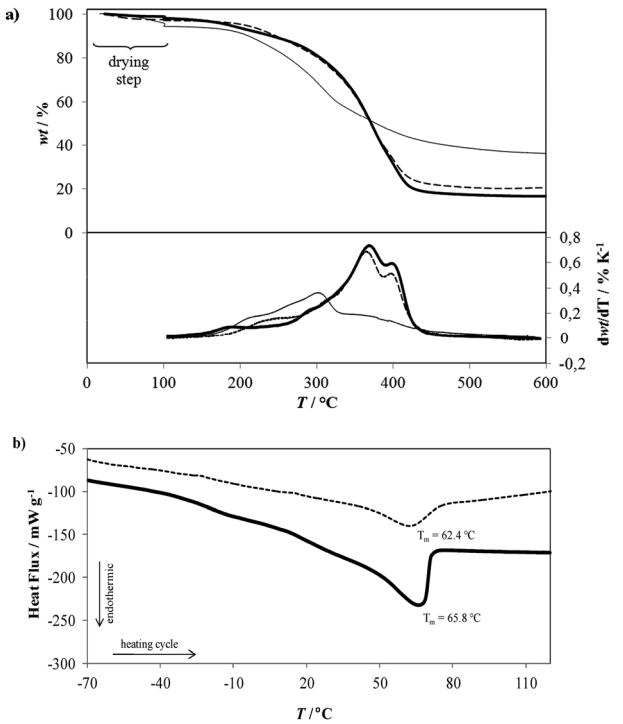


Fig. 6 Thermal analyses: (a) TGA thermogram (top) and the first derivative of weight loss as a function of temperature (bottom). (b) DSC thermogram. (—) cork insoluble residue, (—) suberinic material, (---) cork.

pressurised tank at *ca.* 80 °C in order to remove the cork insoluble residue. The ensuing filtrate was then diluted with water and cooled down to 4 °C, leading to precipitation of the extracted suberinic material. The extraction yield and composition (as issued from ATR-FTIR analysis of the materials) were similar to those reported above. The ionic liquid in the aqueous supernatant was recovered by eliminating the water

under high vacuum conditions (*ca.* 0.01 mbar). The purity of the recovered ionic liquid was verified by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. The yield of cholinium hexanoate recovered by this method was greater than 99%. When reused, and accounting for cork chemical variability, no significant loss of efficiency was observed, leading to suberin extraction yield of 58.3 ± 2.3 wt%.

Conclusions

The high potential of some cholinium alkanoates, having a long alkylic chain in the anion and high basicity, for extracting suberin from cork was investigated. Cholinium hexanoate showed excellent extraction efficiency and selectivity towards suberin, and high biocompatibility and biodegradability potential. Moreover, it could be easily recycled without loss of extraction efficiency. The chemical and thermal characterisation of the material extracted by cholinium hexanoate, *i.e.* suberinic material, was herein attained for the first time. The extracted material showed suberin typical features, with an aliphatic and esterified nature, and a high thermal resistance. The chemical analysis before and after alkaline hydrolysis reveals that the isolated suberinic material was mainly composed of cross-linked aliphatic polyester type structures derived from suberin. It seems reasonable to assume that a better understanding of the suberin extraction from cork by cholinium hexanoate, and the detailed study of the structural features of the resulting materials, might hold the solution of suberin *in situ* structural organisation.

Importantly, this process can be easily applied to other suberin enriched sources, such as birch outer bark. Numerous applications for these new suberinic materials could be envisaged, *e.g.* macromonomers for the synthesis of novel materials.

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