Universidade de Aveiro Departamento de Química
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VALORIZAÇÃO DA SUBERINA DA CORTIÇA E DA CASCA EXTERNA DA BÉTULA

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Química, realizada sob a orientação científica do Doutor Carlos de Pascoal Neto, Professor Catedrático do Departamento de Química da Universidade de Aveiro e do Doutor Armando Jorge Domingues Silvestre, Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro.

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À minha família,
à memória dos meus Avós e
à CVX.

o júri

presidente

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palavras-chave

poliésteres, suberina, materiais de fontes renováveis, *Quercus suber* L., *Betula pendula* Roth, cortiça, bétula

resumo

O trabalho aqui apresentado teve como principal propósito o estudo do potencial da suberina como fonte de produtos de química fina e como precursor de novos materiais macromoleculares de origem renovável. O interesse na suberina reside, não só na sua ubiquidade e nas suas propriedades únicas em termos de composição química e hidrofobicidade, mas também no facto de ser um dos principais componentes macromoleculares dos subprodutos da indústria corticeira de *Quercus suber* L. no Sul da Europa, e da indústria de pasta de papel do Norte da Europa, que utiliza a *Betula pendula* Roth como matéria-prima.

A primeira parte do presente trabalho consistiu no estudo detalhado da composição química da cortiça de *Quercus suber* L. e respectivos resíduos industriais bem como da casca de *Betula pendula* Roth recorrendo a diferentes técnicas de caracterização, nomeadamente GC-MS, IV, RMN de ^{1}H e de ^{13}C, DSC, termomicroscopia, TGA e difracção de raios-X. Os resultados mostraram que os produtos de despolimerização da suberina representam tipicamente uma fracção substancial de todas as amostras. Para além da suberina, foram também identificados nas diversas amostras quantidades variáveis de compostos triterpénicos, lenhina, polissacarídeos e matéria inorgânica.

Os principais resultados da análise por GC-MS mostraram que todas as amostras de suberina despolimerizada são fontes abundantes de ω-hidroxiácidos e de ácidos dicarboxílicos, bem como dos correspondentes derivados epóxidados. No entanto, as quantidades relativas de cada componente identificado foram significativamente diferentes entre amostras. Por exemplo, em amostras de suberina da casca de *Quercus suber* L. isoladas por metanólise alcalina o composto maioritário encontrado foi o ácido 22-hidroxidocosanóico, enquanto que a suberina também proveniente da cortiça, mas isolada por hidrólise alcalina era composta maioritariamente pelo ácido 9,10-dihidroxioctadecanóico. Já no caso da amostra de suberina despolimerizada proveniente da casca externa da bétula o composto identificado como mais abundante foi o ácido 9,10-epoxi-18-hidroxioctadecanóico.

A caracterização das diversas amostras de suberina despolimerizada por FTIR e RMN de ^{1}H e de ^{13}C foram concordantes com os resultados de GC-MS, evidenciando a sua natureza predominantemente lipofílica. Foi ainda determinada a razão entre os grupos CO_2H/OH e CO_2CH_3/OH por RMN de ^{1}H das amostras convenientemente derivatizadas com isocianato de tricloroacetilo, verificando-se que a suberina despolimerizada possuía quantidades não-estequiométricas destes grupos funcionais.

A investigação do comportamento térmico das amostras de suberina despolimerizada, por DSC e termomicroscopia, bem como a análise por difracção de raios-X, permitiu concluir que algumas amostras de suberina despolimerizada possuíam importantes domínios cristalinos e pontos de fusão bem definidos, tipicamente próximos de 70 ^{o}C, enquanto outras amostras eram essencialmente amorfa. Factores como a fonte de suberina ou as condições de despolimerização estiveram na origem destas diferenças.

Neste trabalho estudaram-se também os extractáveis lipofílicos da cortiça e dos seus resíduos industriais, em particular os do pó industrial de cortiça e dos condensados negros, mostrando que os extractáveis lipofílicos são uma fonte abundante de compostos triterpénicos, em particular de ácido betulínico e de friedelina. Foram ainda identificadas fracções abundantes de ω-hidroxiácidos e de ácidos dicarboxílicos no condensado negro.

A segunda parte deste trabalho abordou a síntese e a caracterização de novos poliésteres alifáticos derivados de suberina. Estes materiais foram sintetizados utilizando, quer misturas de suberina despolimerizada, quer monómeros modelo estruturalmente análogos aos existentes na suberina. Recorreu-se para o efeito a duas aproximações distintas de polimerização por passos, a policondensação e a politransesterificação. Procurou-se em simultâneo maximizar a eficiência da polimerização em termos de peso molecular e de extensão da reacção e utilizar condições de reacção de química "verdes". Neste sentido, utilizou-se a policondensação em emulsão utilizando um tensioactivo como catalisador e a policondensação em massa utilizando a lipase B de *Candida antarctica*. Adicionalmente foram também testado os catalisadores trifluorometanosulfonato de bismuto(III) no caso da policondensação, e ainda os catalisadores clássicos óxido de antimónio(III) e o carbonato de potássio no caso da politransesterificação. Os poliésteres resultantes foram caracterizados através de várias técnicas, tais como IV, RMN (de ^{1}H e de ^{13}C), DSC, DMA, TGA, difracção de raios-X e medidas dos ângulos de contacto. Verificou-se que os catalisadores trifluorometanosulfonato de bismuto (III), óxido de antimónio(III) e carbonato de potássio conduziram aos rendimentos de isolamento dos polímeros resultantes mais elevados.

No caso dos poliésteres derivados da suberina os resultados em termos de rendimentos e pesos moleculares sofreram um incremento substancial quando a estequiometria da reacção de polimerização foi adequadamente balanceada (r=1) com a adição de uma quantidade extra de um comonómero. Verificou-se a predominância de diferentes estruturas consoante a amostra de suberina utilizada e as condições de síntese adoptada, predominando as cadeias lineares ou então quantidades substanciais de estruturas reticuladas.

Globalmente, este primeiro estudo sistemático da utilização de suberina como um precursor de novos poliésteres alifáticos confirmou o elevado potencial deste recurso abundante e renovável como precursor para preparar materiais macromoleculares.

keywords

polyesters, suberin, renewable resources, *Quercus suber* L., *Betula pendula* Roth, cork, birch

abstract

The purpose of this study was to evaluate the potential of suberin as a source of valuable fine chemicals and as a precursor to novel macromolecular materials based on renewable resources. Suberin is not only important because of its interesting properties (chemical composition, hydrophobic character, among others), but also because of its abundance in the by-products generated both by the *Quercus suber* L. cork industry activity present in the South of Europe, and the pulp and paper industry of the northern Europe using *Betula pendula* Roth as a raw material.

First, in order to approach that ultimate goal, the outer bark of two species, *Quercus suber* L. and the corresponding industrial residues, and *Betula pendula* Roth, were thoroughly characterised by GC-MS, FTIR, ^{1}H and ^{13}C NMR, DSC, thermomicroscopy, TGA and XRD. It was found that suberin-depolymerisation products represent a substantial fraction of these materials. Triterpenes were also detected in these samples in variable amounts. Lignin, polysaccharides and ashes were also identified.

The GC-MS analysis showed that all samples of suberin mixtures were abundant sources of ω-hydroxyalkanoic acids and α,ω-alkanedioic acids (including mid-chain epoxy and dihydroxy-derivatives). Their relative abundance was found to be considerably different among samples. In the case of cork methanolysis-depolymerised suberin, the dominant component identified by GC-MS was 22-hydroxydocosanoic acid, whereas in cork hydrolysis-depolymerised suberin, the most abundant compound was 9,10-dihydroxyoctadecanoic acid. In the case of suberin fragment mixtures from *Betula pendula* Roth, the most abundant component was 9,10-epoxy-18-hydroxyoctadecanoic acid.

FTIR, ^{1}H and ^{13}C NMR spectroscopy confirmed the GC-MS findings, showing clearly that all suberin fragment mixtures possess long non-polar chains terminated by hydroxy and carboxylic functional groups. The functional group ^{1}H NMR analyses of the trichloroacetyl isocyanate derivatised-suberin showed more specifically that carboxylic and hydroxy groups were not in equivalent amounts.

The study of the thermal behaviour of the depolymerised suberin samples by DSC and thermomicroscopy, as well as the XRD analysis, showed that suberin had either important crystalline domains, possessing well-defined melting points, typically near 70 ^{o}C, or, on the contrary, they could be essentially amorphous. Factors such as the suberin source, or the depolymerisation conditions adopted were responsible for these differences.

The detailed chemical composition of the lipophilic extractives of cork, and in particular of cork by-products generated during the industrial processing, was investigated by GC-MS. Industrial cork by-products along with cork can be considered as abundant sources of triterpenic compounds, and particularly of betulinic acid and friedeline, which are known to have promising applications, as such, or as precursors to bioactive components for biomedical applications. Significant fractions of ω-hydroxyalkanoic and α,ω-alkanedioic

acids were also detected in the cork industrial by-product denoted by black condensate.

In the second part of this study the synthesis of novel aliphatic suberin-based polyesters was studied. Both model long-chain aliphatic monomers, like those found in suberin, and the suberin reactive aliphatic fragments were polymerised by polycondensation and polytransesterification reactions. In these reactions, the emphasis was on the search for the conditions that on the one hand optimised the polymerisation reaction in terms of molecular weight and yield. On the other hand, an intensive search for greener synthetic pathways was performed, *e.g.* polycondensation of the suberin fragments under emulsion polymerisation conditions (*p*-dodecylbenzenesulfonic acid), or the use of *Candida antarctica* lipase B. Additionally, bismuth(III) trifluoromethanesulfonate was tested in the case of the polycondensations, whereas antimony(III) oxide and potassium carbonate were tested in the context of polytransesterification reactions. The ensuing polyesters were characterised by various techniques, namely FTIR, ^{1}H and ^{13}C NMR, SEC, DSC, DMA, TGA, XRD and contact angle measurements. The highest isolation yields were obtained when bismuth(III) trifluoromethanesulfonate, antimony(III) oxide or potassium carbonate were used as catalysts.

In the case of the polymerisation reactions of suberin depolymerisation-products improved results in terms of yields and molecular weights, were obtained when reactions were carried out under stoichiometrically balanced conditions by the addition of an appropriate amount of a compensating OH-bearing comonomer for cork suberin samples, and with a CO_2H-bearing comonomer for the birch suberin counterparts. Results showed that it was possible to synthesize completely renewable novel polyesters based with predominantly linear chains or instead with a substantial amount of cross-linked structures, just by using different suberin depolymerisation mixtures or different reaction conditions.

A final word is essential here to emphasise that this first systematic study about the exploitation of suberin as a precursor to novel aliphatic polyesters confirmed the huge potential of using this abundant renewable resource to prepare promising macromolecular materials.

Contents

1 Introduction

Why polymers from renewable resources? Are polyesters from renewable resources valuable alternatives to the petrochemical counterparts? What chemicals from plant renewable resources are available today? What were the main objectives of this study? What are the main subjects of each Chapter of this Thesis?

1.1 The context

Growing attention has been focused in recent years on polymers derived from renewable resources.[1-3] Behind this is the current technological, social and economic situation where issues like environment, waste disposal, depletion of non-renewable resources and also the unpredictable crude oil price fluctuations or its inevitable depletion are stimulating investigation aimed at developing macromolecular materials as alternatives to the current fossil-based polymers.[4] Other aspect, inevitably related with mankind, is the eternal aspiration of investigating and exploring novel materials with different and unique properties.

Although this renewed interest in polymers from renewable resources has gain wings recently, the advent of biopolymers is entangled with the birth of macromolecular science and technology towards the nineteenth century, or even earlier to the ancient times as evidenced by some artefacts.[5-6] There are several reports in history about the manipulation of polymers although still in a very rudimentary empirical fashion as in the case of the ancient Egypt where mummies were wrapped in cloth dipped in a solution of bitumen in oil of lavender. More recently, in 1839 Goodyear developed the vulcanization process that transformed the sticky latex of natural rubber to a useful elastomer for tire use. Later, in 1847, Schönbein synthesised celluloid, the first man-made thermoplastic, reacting cellulose with nitric acid. However, the relative importance of macromolecular materials based on renewable resources suffered thereafter a gradual setback due essentially to the petrochemical revolution of the last century. Many examples of petrochemical-based polymers could be cited as those produced by the pioneering work of Carothers at the Du Pont in the 1930s, namely Nylon (polyamides), Neoprene (poly(chloroprene), a synthetic rubber) or Teflon (poly(tetrafluoroethylene)).[6] These polymers found many useful applications until today; Nylon is generally used to produce fibres; Neoprene is suited to produce *e.g.* corrosion-resistant coatings; and Teflon is routinely used *e.g.* to coat non-stick frying pans. But many other examples of petrochemical-based polymers could be cited. In fact, the realm of polymers prepared from fossil resources is in everyday life, with huge quantities of plastics, elastomers, fibres, adhesives, paints, and packaging materials, associated to a variety of sophisticated macromolecular structures.[7]

In the beginning of the twenty first century the scenario just described is changing as the need for alternative petrochemical plastics becomes more urgent and science goes once more to an old alternative - polymers based on renewable resources. Concomitantly, many chemicals from forest and agriculture feedstocks are potentially available today in large amounts and ready to be used as building blocks of polymers (some are already in use). Many examples could be cited as sugars and their derivatives, vegetable oils, organic acids, glycerol, cutin, suberin, among many others.[1,7-8] The present work follows this pertinent interest in fine chemicals from renewable origin[9-10] (especially suberin biomass resources) and also their use in the preparation of several novel polyesters.[11-12]

1.2 Biomass and biorefineries

The use of renewable chemicals to synthesise polymers, including polyesters, is a simple concept that, as mentioned before has not the merit of being new, yet it encompasses the biorefinery concept so relevant in our days.[4]

At this point, before proceeding any further, it is important to clarify the definition of the term *renewable resources*. This is defined frequently as any vegetable or animal species which is exploited in a sustainable way, without its depletion, and renewed in a reasonable time scale (biological instead of geological time).[1] It is pertinent to emphasize that renewable resources are intrinsically valuable because they are ubiquitous, giving any society elements to sustainability, including with respect to polymeric materials.[7] Examples of renewable resources include both animal and plant sources, however only the latter are cited because animal sources are out of scope of this Thesis.

The most obvious source of plant renewable materials is plant biomass, including barks, fruits, seeds, foliage, stems, tubers, roots, and wood, among others. Biomass is composed essentially of carbohydrates, lignin, extractives, proteins, as well as other macromolecular components including oils.[4,13-14] Each of these biomass fractions can provide a vast portfolio of plant-derived chemicals as described briefly afterwards.

The *plant* carbohydrates comprise the world's most abundant biopolymers, cellulose, hemicelluloses and starch. They can provide a vast quantity of chemicals as those cited very recently by Boozell *et. al.* in a revised new "Top 10 + 4" list about biobased products opportunities from carbohydrates.[8] Among those are sugars derivatives like furans (*e.g.* 5-hydroxymethylfurfural, 2,5-furandicarboxylic acid), xylitol, or sorbitol.[2,8]

Other examples, are organic acids like, lactic acid, commercially produced from glucose fermentation.[8]

The lignin fraction composed essentially by three building blocks the *trans*-coumaryl alcohol, *trans*-coniferyl alcohol and *trans*-sinapyl alcohol is an interesting source macromolecular materials as well as of phenolic compounds like vanillin, gallic acid and ferulic acid.[13]

The extractives fraction of the biomass represents typically a minor fraction of the overall chemical composition. However, they constitute a valuable source of interesting chemicals. Extractives are low molecular weight compounds, comprising triterpenic derivatives, esters of fatty acids, alkanes, and even phenolic compounds. They can be isolated from its source by traditional liquid extraction techniques using either neutral organic solvents (*e.g. n*-hexane, dichloromethane, petroleum ether, methanol, etc) or water.[14] More recently techniques using supercritical fluids are also being applied.[15] The ensuing extractives are generally classified as lipophilic or hydrophilic, depending on the extraction technique used and of course in their polarity. One commercial example is the use of plant sterols (*e.g.* sitosterol, campesterol, and stigmasterol) in human nutrition in order to reduce cholesterol absortion.[16] The promising applications of some triterpenic compounds, directly or as precursors to bioactive components for biomedical applications,[17-20] has prompted our interest in studying its abundance in cork and by-products (Chapter 5).

Among the most interesting specific components that biomass can provide are noticeably oils, like castor and soya bean oil. Oils are triglycerides of fatty acids being palmitic, stearic, oleic, linoleic, linolenic, and ricinoleic acid some of the most commonly occurring.[7,21] Another, monomer which occurs naturally in triglycerides is glycerol.[8] Currently, it is available in high amounts mostly because of biodiesel production. One particularly interesting and ubiquitous source of polyfunctional oleochemicals is suberin.[9,22] It is composed mostly of long aliphatic chains with hydroxy and carboxylic acid functional groups (Chapters 2 and 4). Another source worth mentioning is cutin, also composed of long aliphatic chains with hydroxy and carboxylic acid functional groups (see reference 23 and references therein). Many other examples of plant biomass based compounds could be cited; actually the number of available biomass based chemicals is rising very quickly.[1,8,13] The exploitation of these renewable resources for the preparation

of materials and of all sorts of commodities, seems obvious and seems the next step forward.-

Other aspect to be highlighted is the *biorefinery concept*. Ragauskas *et al.*[4] in 2006 referred to it as a "concept for converting renewable biomass to valuable fuels and products".[4] Hence, a completely integrated biorefinery will transform multiple biomass feedstocks, to produce a portfolio of products, including fuels, energy, and chemicals.[24] Among these biorefineries of the future, the lignocellulosic feedstock (LCF) biorefineries, will probably be the most successful, on the one hand, because of the availability of feedstocks (*e.g.* agro-food wastes, or even paper wastes) at competitive prices and, on the other hand, because they do not compete with the supply of food.[25] The implementation of the biorefinery concepts in already existing agroforest-based activities and the concomitant need to upgrade the by-products generated in the processing of agricultural and forest products, represent a short-term response to this goal. In this context, the processing of the two species considered in this Thesis, cork oak and silver birch, are well established industrial activities that generate considerable amounts of cork and outer bark residues, which for the time being are under-exploited and could have considerable value as sources of lipophilic chemicals.[9]

The outer bark of *Quercus suber* L. is commonly known as cork. Cork production and its processing industry (mainly for the production of cork stoppers and thermal and/or acoustic insulation materials) exist mainly in the Mediterranean region.[26] In Portugal, about 157 000 ton of cork are produced per year, representing more than 50% of the world's production.[27] This industry generates substantial amounts of residues the so called "industrial cork powder", "black condensate", and "cooking wastewaters". The main residue is the "industrial cork powder" representing 34 000 ton in Portugal per year. It has an inadequate particle size to be suitable for current industrial uses.[28] Currently it finds no added value application rather than being burned to produce energy.[28] "Black condensate" is a residue of the production of black agglomerates, which involves the treatment of cork particles, without any adhesive, at temperatures in the range of 250-500 °C. During such thermal treatment of cork, vapours are formed and later condensed in autoclave pipes. Periodically, this by-product is removed (2 100 tons/year) and once more burned to produce energy.[28] The cooking of cork planks in boiling water is a key stage in wine stopper production, yielding "cooking wastewaters" as liquid effluent.

Betula pendula Roth commonly known as silver birch is an important species for pulp production in the Nordic countries. Birch logs contain about 3.4% outer bark.[29] Thus, considering a birch kraft pulp mill with an annual birch pulp production of 400 000 ton and the pulp yield roughly equal to 50 %,[30] this mill generates about 28 000 ton of outer bark,[31] which is, at the present, burnt in the biomass boilers of kraft pulp mills for energy production. Based on birch kraft pulp production figures, the total potential of birch outer bark in Finland can be estimated to be 135 000 ton in 2009.[32] Both cork and birch outer barks are sources of renewable chemicals, specially the interesting lipophilic suberin fragments,[22] but also triterpenic extractives, like friedeline, betulic acid, betulinol, among many others.[33]

1.3 Objectives of this study

The main objective of the present study was to develop novel suberin-based polyesters by simple and "greener" approaches. Concomitantly, this study also intended to develop new upgraded applications to cork (including cork residues) and birch outer bark.

To achieve these goals, the following steps were required:

- detailed understanding of the chemistry of suberin fragments from the outer barks of *Quercus suber* L. and *Betula pendula* Roth;
- detailed study of the lipophilic extractives of the poorly suberin enriched industrial cork residues;
- synthesis and characterisation of suberin model comonomers, assessing the reaction conditions and the properties of these suberin model polyesters;
- and finally, design of suberin-based polymers.

An overall objective of this study, doubtless an ambitious one, was to contribute to the development of this renewed area of polymer chemistry- polymers from renewable resources.

1.4 Outline of this Thesis

This manuscript consists of four parts and is divided in nine Chapters. In the first part (Part I) fundamental aspects concerning this study are briefly reviewed. Chapter 2 describes several aspects related to the occurrence, chemistry and physics of suberin. Chapter 3 reviews the fundamentals of polyester chemistry, presenting the Carothers equation and some of its extensions, the average molecular weights (distributions), and the Flory-Rhener equation related to the swelling of cross-linked polymers. This Chapter also gives an overview of polyesters from renewable resources and of green catalysts for polyesterification reactions.

The second part (Part II) presents the results, discussions, and conclusions of this study. In Chapter 4 a comparative study of the depolymerised suberin fragments from cork and birch outer bark is described, providing the results obtained by several chromatographic and spectroscopic techniques.

Chapter 5 gives a detailed description of the chemical characterisation of the lipophilic extracts of cork and some of its industrial by-products.

Chapter 6 describes the synthesis and characterisation of polyesters from suberin model comonomers.

In Chapter 7, fully biobased polyesters derived from suberin are revealed. The synthetic approaches used and the characterisations of the polyesters from cork and birch outer bark suberin are described. A comparison of their properties is also provided.

The third part (Part III) is composed of only one Chapter, the Chapter 8, describing the scientific approach adopted in this study. It lists the substrates and chemicals, the most important general procedures, and the techniques involved.

The last part (Part IV) and last Chapter (Chapter 9) of this study is an epilogue that highlights the most important achievements and conclusions of the research carried out (Chapter 4-7). More importantly, some perspectives and suggestions for future work are proposed.

1.5 References

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[PART I BIBLIOGRAPHIC REVIEW]

2 Suberin

Where is suberin encountered? What are its major sources? What is the importance of suberin to plants? How is the suberin macromolecular structure? How can suberin be spliced? What are the ensuing main chemical constituents? What are its relevant physical properties?

2.1 Introduction

As mentioned earlier in Chapter 1 there is an intense search for alternatives to petrochemical polymers and also obviously for new sources of building-blocks. Suberin emerges in this context as a valuable alternative, as a renewable source of lipophilic chemicals (Chapters 4), potential precursors to novel macromolecular materials (Chapter 7).[1-2]

Suberin is a widespread natural cross-linked biopolyester, regularly found in the cell walls of plants, where it plays a major role against physical, chemical, and biological aggressions.[2] Suberin is indeed referred to as being ubiquitous in plants,[2-3] present not just in normal, but also in wounded tissues.[4-5] However, only two trees produce barks which are sufficiently rich in suberin to justify its exploitation as renewable sources of chemicals and monomers, namely the *Quercus suber* L. (cork oak) and the *Betula pendula* Roth (silver birch).[2,6] Precisely the barks of these species were the suberin-biomass feedstocks adopted in this work.

Suberin is a naturally occurring aliphatic-aromatic cross-linked polyester composed of interesting and valuable polyfunctional monomers, especially the main components of the aliphatic domains. These are even-numbered ω-hydroxyalkanoic and α,ω-alkanedioic acids,[7-10] relatively rare in nature, restricted to the extracellular cutin and obviously present in high amounts in suberin. Suberin aliphatic fragment units can be isolated by alkaline hydrolysis or alcoholysis (most frequently methanolysis).[6-7]

The similarity between suberin and cutin is evident and it can not be ignered at this point. It is known that both are important lipophilic interfaces between the plant and the surronding medium, contributing to the regulation of water uptake into and out the plant.[4] However, some differences persist, in terms of their deposition in the cell walls of plants, either in the outer surface of the epidermal cells in cutinized tissues or in the inner surface of the cell wall as a layer or a multilayer in suberized tissues. Another important difference is related to their chemical composition, it is genarly believed that suberin is composed of both polyaliphatic and polyaromatic domains, opositly to cutin which is composed essencialy of polyhydroxylated fatty acids.[2,4]

The purpose of the present Chapter is to provide a concise assessment of the state of the art related to the suberin occurrence, its macromolecular structure, its

chemical splicing (depolymerisation through ester cleavage), and the qualitative and quantitative composition of the ensuing aliphatic fragments.

2.2 Occurrence

Suberin is mainly found in the periderm tissue of the outer barks of higher plants and in the tuber skins.[2,4,11] Some relevant examples include the outer bark of *Quercus suber* L. and of *Betula pendula* Roth (Figure 2.1), and the skin of *Solanum tuberosum* L. (potato). Suberin can also be found in the epidermis and hypodermis of roots of plants, like *Oryza sativa* L. (species of rice), *Zea may*s L. (corn) and *Ricinus communis* L. (castor oil plant), among others.[2,12] Additionally, the cicatricial tissues after wounding are also composed of suberin.[5] Suberin could be said to be almost ubiquitous in nature, albeit in very variable proportions.[2]

Figure 2.1 (Left) *Quercus suber* L. outer bark. (Right) *Betula pendula* Roth outer bark.

2.2.1 The cork and silver birch outer bark feedstocks

The cork of *Quercus suber* L. and the outer bark of *Betula pendula* Roth are important sources of suberin if not the most relevant ones, despite suberin ubiquity. However, both materials have today other applications rather than being used as sources of valuable suberin chemicals.

In the case of cork, this unique material finds a wide panoply of utilisations, such as in the traditional cork stoppers and insulating industries,[6] whereas birch is an important

species for pulp production, but its bark, to the best of our knowledge, finds no added-value application rather than being burnt for energy production.[13]

The cork oak (Figure 2.2) is an evergreen tree that grows in the western coastal areas around the Mediterranean Sea occupying an worldwide area of around 2 277 700 hectares.[14] Portugal concentrates around 33% of the world cork forest area spread throughout the country, but the largest forestland is in the south, especially in the Alentejo region. This forest has a very positive impact on Portugal, at both ecological and economic levels. Indeed, on the one hand, the *montado* (Figure 2.2), as it is known in Portugal, aids to preserve the soil, to regulate the water cycle and to preserve the biodiversity of flora and fauna.[15] On the other hand, the cork, (re)generated and harvested once every 9 to 12 years to preserve the tree, is used industrially, representing 159 000 tonnes of exported products in 2007, corresponding to around 854 M € of income.[14]

However, there is a long way to the first good quality cork planks. First, trees must be from 15 to 30 years old before the first harvesting can occur, and yet the ensuing cork, designated by virgin cork, has low quality. Second, the subsequent harvesting generates somewhat better-quality cork, but in general only the third and following cork oak striping generate good quality cork known as *amadia* cork. The average maximum biological age of the cork oak is approximately 200 years.

Figure 2.2 (Left) Cork oak forest (*montado*). (Right) Detail of a cork oak.

Cork is a plant tissue composed of tiny hollow cells of hexagonal prismatic shape stacked in base-to-base rows. The average cell thickness ranges from 1 to 1.5 μm and the hollow cells space is filled with air.[16] Due essentially to this peculiar structural features and

to the chemical composition of the cell walls, cork displays very interesting properties, such as a very low density (typically in the range of 0.150-0.160 g cm^{-3} for air-dried cork tissue),[16] hydrophobic character, elastic behaviour, and low conductivity to heat, sound or vibrations.[16] These have been the main characteristics that have led to its main uses throughout history. Cork has been used for manifold functions for at least 2000 years. In antiquity there are some reports about its use to float anchor ropes and fishing nets, for sealing vessels containing oil or wine, and in the soles for woman's shoes.[17-18] More recently, since the XVIII century, its prominent functions has been as cork stoppers for champagne and wine bottles, among many others, as for example for footwear, building, flooring, and furniture. However, at the beginning of the XXI century the traditional cork stoppers begun being challenged by synthetic substitutes, which is obviously a critical situation for the cork industry.

As a consequence, the development of other applications has been reinforced in the recent years, whether used in its pristine form or after specific physical or chemical modifications. Cork has been used as such in high-tech applications as in the automobile, military and also in the aeronautics and aerospace industries, as for example as a thermal protection material in NASA space shuttles.[19] Additionally, as mentioned by Turley[20] in a short article about the issue "cork farmers in Portugal, Spain and other Mediterranean countries might soon be able to rescue their threatened businesses by investing in a new revenue stream: polymers".

Cork is mainly composed of suberin (30-60%), but also contains lignin (19-22%), polysaccharides (12-20%) and extractives (9-20%).[7-9,16,21] The relative abundance of these families of compounds is quite variable, depending on factors such as the geographical origin and quality of the tree, and/or even the different parts of the tree from which the cork is harvested.[9,22-23]

The silver (*syn.* European white) birch (Figure 2.3) is a deciduous tree with a wide natural distribution area in Europe and in some regions of Asia, ranging from the Atlantic to eastern Siberia.[24] Although the species occurs throughout almost the whole Europe, the most abundant birch resources are in the temperate and boreal forests of northern Europe.[24]

It is a typical pioneer tree species, fast growing and a vitality of about 90 years. It can be found either in mixed forests with other birch species and dominated by coniferous species, or in pure stands.[24] These forests have a very relevant positive impact at both

ecological and economic levels in the countries where they are grown. It is known that as a pioneer tree, one of its functions is to improve soils, but also to preserve the biodiversity with a number of forest organisms associated with birch trees (*e.g.* flowers and fungi).

Figure 2.3 (Left) Silver birch forest. (Right) Detail of a birch tree bark.

In terms of economy, in northern Europe it is among the most commercially important trees. For example, in Finland data from 2005 indicated that birch is one of the most abundant tree, representing around 4% of the total volume of the growing stock.[25] Its wood is mainly used for pulp and fuelwood purposes, but it can also be grown for high quality saw timber.[13] As for the outer bark, it is only used as a low value energy source and is burned in large furnaces. It is however composed of high added-value compounds that can be classified into four major families: suberin (30-60%), lignin (15-30%), polysaccharides (~30%) and extractives (20-40%).[26]

2.3 Function and native structure of suberin

Suberin forms important lipophilic interfaces between the plant and its environment contributing significantly to the regulation of water, the transport of solutes in and out of the plant tubers, and also acting as a barrier to pathogenic attack.[4,12,27] Additionally, plant organs respond to wounding by suberization. Essentially, wounding causes suberization not only on tubers and other organs that are normally protected by suberin, but also on fruit and leaves that are normally protected by cutin.[5,27] The important function of suberin in plants is related with its location in the cell walls, apart of course from its native

structure. The native structure of suberin, so deeply related with its function in plants, has been a matter of several studies and an intense scientific debate.[7,10-11,28] It is well known today that suberin is composed of both polyaliphatic and polyaromatic domains (Figure 2.4), although some debate still persists about the suberin native structure concerning the spatial arrangement of these domains. However, the presence of both domains is generally recognised and is pointed out as a characteristic feature of suberin compared, for example, to cutin, where only an aliphatic domain is present.[12]

It is known that the polyaliphatic domains of suberin (Figure 2.4) are composed of crossed-linked polyester macromolecules, where the main compounds, identified by gas chromatography coupled with mass spectrometry (GC-MS), are ω- hydroxyalkanoic and α,ω-alkanedioic acids (even C_{16} to C_{26}).[2]

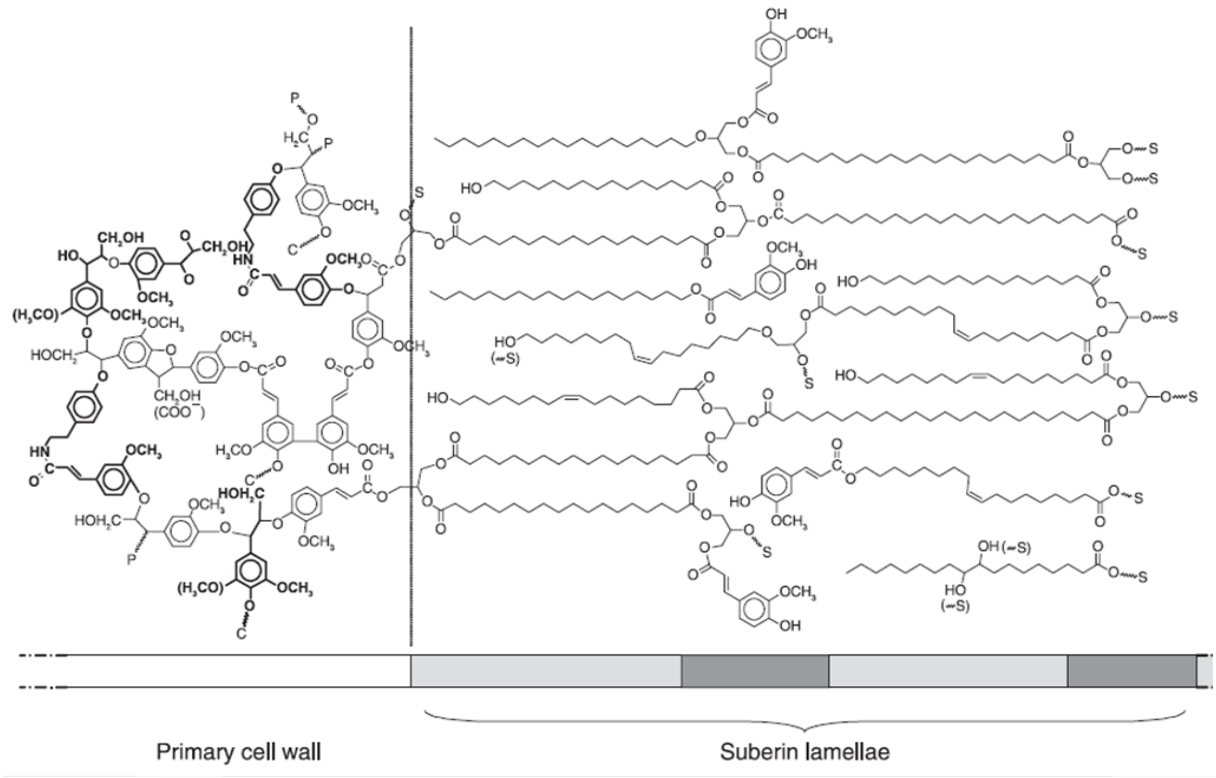

Figure 2.4 The suberin model of *Solanum tuberosum* L. proposed by Bernards in 2002.[28] C, P and S stand for carbohydrate, phenolic and suberin, respectively.

The complete nature of the polyaromatic domain (Figure 2.4) is still in debate today, but solid state nuclear magnetic resonance (NMR) studies and chemical analysis elucidated some aspects about this complex macromolecular structure (see *e.g.* reference

2 and references therein). These studies showed the existence of different populations within the polyaromatic domains,[2,11] *viz.,* one consisting mostly of hydroxycinnamates (*e.g.* ferulic acid) esterified with ω-hydroxyalkanoic acids, lying in the suberin lamellae, and the other located in the primary cell walls was identified as a lignin-like polymer of the guaiacyl type,[29] with coniferyl alcohol as the monomeric unit (Figure 2.4). Glycerol is a key element in the suberin structure (Figure 2.4), playing the role of a "bridge" among the aliphatic monomers and also between the polyaliphatic and the polyaromatic domains, thus being responsible for the three-dimensional structure of suberin.[30]

This current suberin model is consistent with the typical lamellar structure observed by transmission electron microscopy (TEM). These lamellae show alternate opaque and translucent bands attributed to successive layers of aliphatics and esterified phenolics, as depicted in Figure 2.4.[28]

2.4 Cork and birch outer bark suberin monomer composition through ester cleavage

2.4.1 Depolymerisation methods

The *in situ* suberin is a cross-linked insoluble polymer and its removal from suberized tissues is an essential step both for the detailed chemical characterization of this natural material, as well as for the development of some new applications. Typically, suberin removal is readily achieved through a simple depolymerisation reaction involving the chemical scission of the various ester moieties in the network, followed by the isolation of the ensuing aliphatic fragments. In birch and cork, the suberin isolation yield varies typically between 20 to 50% of the extractive-free bark.[2]

The most commonly used chemical depolymerisation procedure is alkaline methanolysis, although others alcoholysis or hydrolysis procedures have also been reported.[2]

The methanolysis approach is frequently performed using methanolic sodium methoxide.[3,7,9,31-32] If a very mild methanolysis is preferred, like in the chemical elucidation of suberin structure, calcium oxide or calcium hydroxide[33] can be used instead. It is observed that the first methanolic conditions mentioned give rise essentially to the total cleavage of the esters linkages resulting in the isolation of the monomeric suberin units, oppositely to the mild conditions where only partial cleavage[33] of the suberin network

occurs and oligomeric blocks were frequently identified by electrospray ionization coupled with tandem mass spectrometry (ESI MS/MS).[11,33]

The alkaline hydrolysis can be performed using sodium hydroxide in an ethanol/water solution, but once more if very mild hydrolysis was preferred potassium hydroxide[34] can be used instead in addition to shorter reaction times yet achieving full suberin depolymerisation. This latter hydrolysis approach is mostly used to avoid unwanted cleavage of the epoxy ring groups.[34] Figure 2.5 summarises the essential steps followed in cork suberin monomers isolation comprising a pre-extraction step.

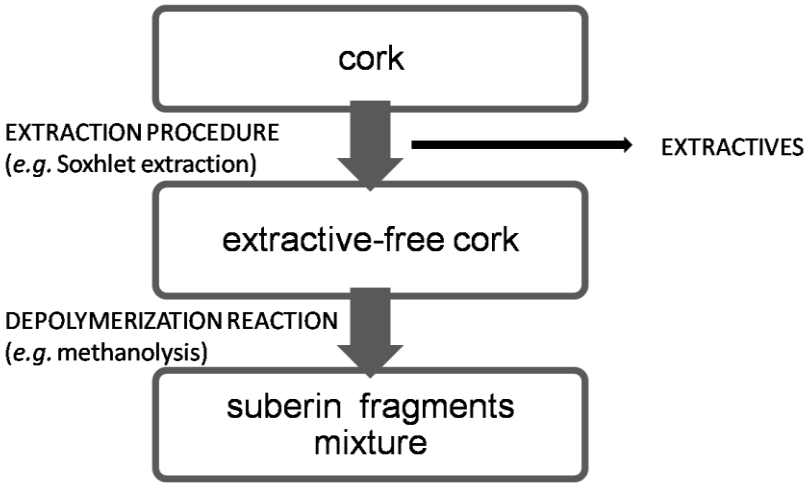

Figure 2.5 Schematic representation of suberin components mixture isolation from cork.

2.4.2 Monomer composition of cork and birch outer bark suberin

The aliphatic suberin depolymerisation monomers are routinely characterised by GC-MS analyses giving both a qualitative and a quantitative picture of suberin. The structures of the most representative elements of each family are depicted in Figure 2.6.

The main suberin components are α,ω-alkanedioic and ω-hydroxyalkanoic acids characterized by the presence of even-numbered aliphatic chains (C_{16} to C_{26}), but with a predominance of the C_{18} and C_{22} homologues.[2,6,11,28,35] Some of these acids are functionalised at mid-chain by insaturations, vicinal di-hydroxy or epoxide groups.

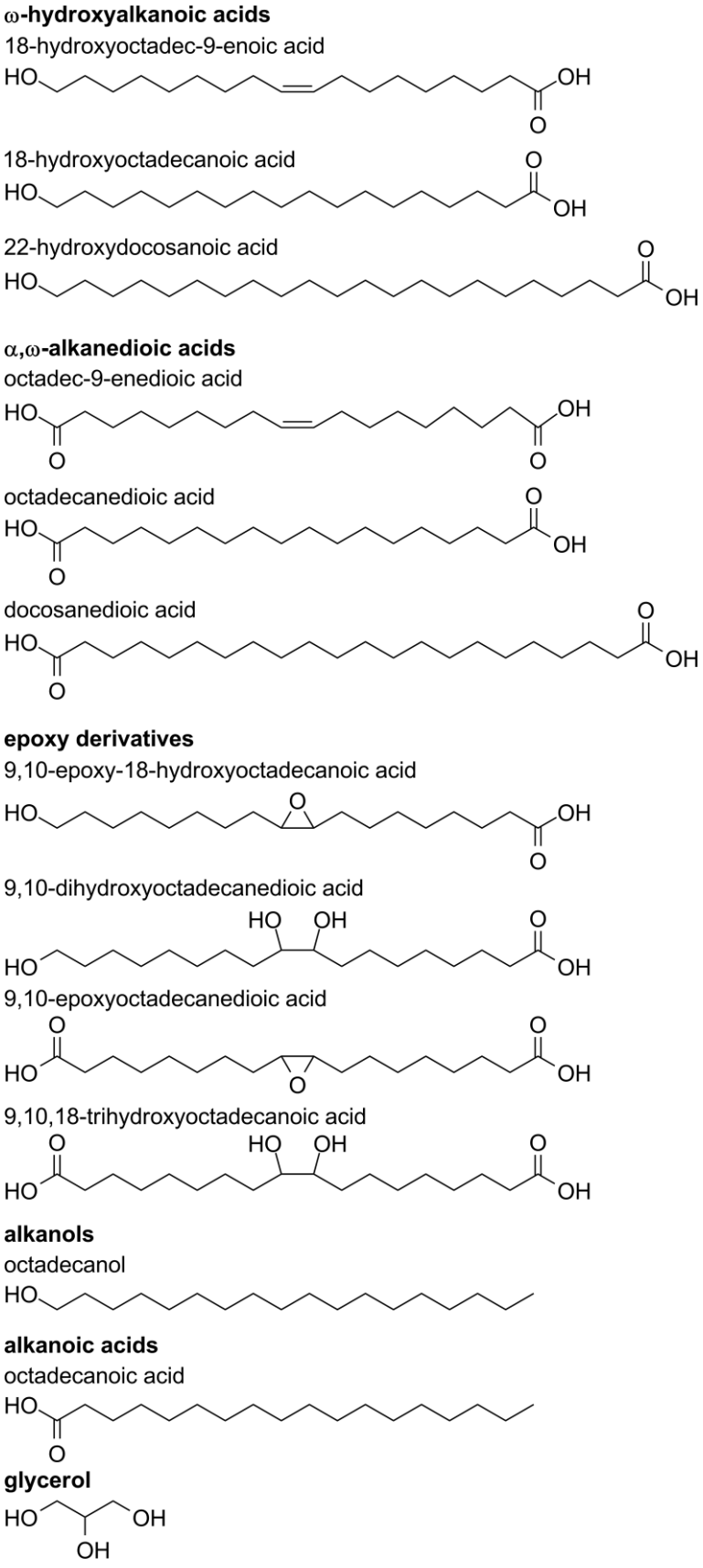

Figure 2.6 Representative structures of the aliphatic suberin monomers.

Other families of compounds present in smaller amounts in the suberin aliphatic extracts are alkanols and alkanoic acids. In these two families the compounds most commonly found are even C-numbered chains ranging from C_{16} to C_{26}, although some references to C_{12} and to C_{28}-C_{30} are also mentioned for alkanoic acids.[2] Some authors describe the presence of suberan, a higher molecular weight non-hydrolysable aliphatic fraction, yet not completely understood.[8,36]

The chemical composition just described is in its essence common to suberins from different sources. Nevertheless, there are subtle differences among samples, explained not just because of the experimental conditions used to characterise the suberin components, or even the depolymerisation conditions used to remove it from suberized tissues, but also because there are variability among species and even between samples of the same species. In what concerns the variability within cork oaks there are several studies addressing the subject in terms of geographical origin or quality (virgin or “amadia” grade).[22-23,37]

There are also several works about the chemical characterisation of suberin just from one species,[7-9,34] but comparative studies of the aliphatic suberins monomers of cork and silver birch outer bark isolated under similar conditions are scarce. Indeed, before the work presented in this Thesis in Chapter 4,[1] to our knowledge there was only one study due to Holloway, dating back from almost 30 years ago when even the suberin macrostructure was still a very diffuse concept.[3] This premier work gives detailed and valuable information, but results should be examined with care as there is no indication on the use of internal standards or reference to the detection yields of the GC-MS analyses of the suberin samples. Table 2.1 summarises the main published results rearranged in order to present them on percentage of each fragment in the identified mixture of compounds.[3]

Results indicated a predominance of epoxy derivatives for birch suberin, being 9,10,18-trihydroxyoctadecanoic acid (~29%) the most abundant compound identified. As for cork suberin both ω-hydroxyalkanoic acids and epoxy derivatives dominate, being 22-hydroxydocosanoic acid (~20%), 9,10-epoxy-octadecanedioic acid (~16%), and 9,10-epoxy-18-hydroxyoctadecanoic acid (~15%) the most abundant compounds found.

Spectroscopic studies on the aliphatic suberin depolymerisation products of cork, namely proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR), and FTIR revealed to be consistent with the GC-MS features indicating a predominance of aliphatic

chains which bore polar hydroxy and carboxylic groups, and also a small quantity of unsaturations.[8]

Table 2.1 Main results of the GC analyses of the cork and birch outer bark suberin depolymerisation products after their initial separation by preparative TLC on silica gel (cork and birch, respectively).[3] Results are expressed in percentage of each fragment in the identified mixture of compounds.

family	cork	birch
ω-hydroxyalkanoic acids	**41**	**21**
16-hydroxyhexadecanoic acid	1	Tr
18-hydroxyoctadec-9-enoic acid	11	3
18-hydroxyoctadecanoic acid	tr	Tr
20-hydroxyeicosanoic acid	2	4
22-hydroxydocosanoic acid	20	14
24-hydroxytetracosanoic acid	7	tr
26-hydroxyhexacosanoic acid	tr	-
α,ω-alkanedioic acids	**9**	**9**
hexadecanedioic acid	1	1
octadec-9-enedioic acid	3	1
octadecanedioic acid	tr	1
eicosanedioic acid	1	2
docosanedioic acid	3	4
tetracosanedioic acid	1	tr
hexacosanedioic acid	tr	tr
epoxy derivatives	**44**	**49**
dihydroxyhexadecanoic acid	**-**	4
9,10-epoxy-18-hydroxyoctadecanoic acid	15	16
9,10-epoxyoctadecanedioic acid	16	tr
9,10,18-trihydroxyoctadecanoic acid	5	29
9,10-dihydroxyoctadecanedioic acid	8	tr
1-alkanols	**2**	**[a]tr**
octadecanol	tr	-
eicosanol	tr	-
docosanol	1	-
tetracosanol	1	-
hexacosanol	tr	-
octacosanol	tr	-
alkanoic acids	**1**	**[a]tr**
hexadecanoic acid	tr	-
octadecanoic acid	tr	-
eicosanoic acid	tr	-
docosanoic acid	1	-

family	cork	birch
tetracosanoic acid	tr	-
hexacosanoic acid	tr	-
octacosanoic acid	tr	-
TOTAL	**97**	**79**

[a]tr, trace.

2.5 Physical properties of suberin

The physical and chemical physical characterization of suberin aliphatic fraction is an indispensible step for the detailed characterization of this remarkable material, though throughout neglected in many works. The physical properties of suberin methanolysis depolymerisation products from cork, has been however, extensively studied by Cordeiro *et al.* in the 1990's.[38-40] In what concerns other suberin samples literature is scarce or even inexistent. The cork suberin methanolysis depolymerisation products showed a high degree of crystallinity, as indicated by their differential scanning calorimetry (DSC) trace (Figure 2.7) and corroborated by observation of suberin by polarised-light microscopy with heating and cooling cycles.[38]

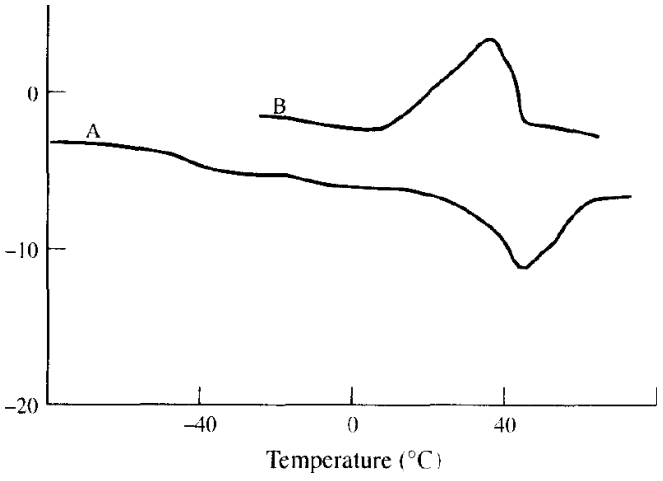

Figure 2.7 DSC thermogram of cork suberin methanolysis depolymerisation products: (A) heating and (B) cooling cycle. Adapted from reference 38.

The DSC trace showed a broad melting peak around 31 °C (Figure 2.7), typical of a wide distribution of molecular weights and probably also associated with the resolution of the equipment used at that time. The observation of the sample under polarised light showed consistently a substantial fraction of anisotropic material below 40 °C.

The thermal gravimetric analyses (TGA) of the same suberin sample in a nitrogen atmosphere showed the onset of decomposition around 300 ^{o}C. The density of this sample was determined with a water calibrated pycnometer. At 20 ^{o}C the density was estimated to be *c.a.* 1.08 which is a value relatively high compared to alkanes with the same molecular size *c.a.* 0.7.[38] Other properties such as the rheological behaviour or the water contact angles were also determined by the same authors.[39-40]

2.6 Conclusions

The most relevant aspects of suberin nature worth emphasising are their ubiquitous abundance in the vegetable realm, although with a remarkable abundance in the outer barks of cork oak and silver birch. Other aspect is that the aliphatic fraction of suberin mixture of monomers is highly enriched in valuable chemicals especially long chain ω-hydroxyalkanoic acids, hence being a natural source for this family of compounds. The relevant amount of crystalline domains in methanolysis depolymerisation products of cork can be considered an important property regarding its manipulation and potential applications.

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3 Polyesters chemistry

What are step-growth polymers? Polyesters, what are they? What are the polycondensation and polytransesterification reactions? How do the degree of polymerisation and its distribution vary with the extent of the reaction? How does a stoichiometric imbalance influence the degree of polymerisation? How are molecular weights distributed? What happens if polyfunctional monomers are used in polyesterification reactions? To swell or not to swell? What plant derived polyesters have been synthesised? How could polyesters be synthesised? Are there environmentally benign polyesterification reaction conditions?

3.1 Introduction. Overview of polyesters

Polymers, as it is well known, are large molecules constituted by smaller structural units designated as monomer units and linked by covalent bonds. They are so common to everyday life, as already mentioned in Chapter 1, that it could be stated without exaggerating that they are one of the pillars of modern life. Step-growth polymers were historically the first synthetic polymers to be investigated in the beginning of the last century by two well known scientists Carothers and Flory.[1-3] The former focused his work essentially on the experimental aspects of synthesis, like the preparation of Nylon, while the latter contributed to the theoretical bases of step-growth reactions.

The central feature of step-growth polymerisation is the slow building of chains in a systematic stepwise fashion.[1-3] As a result of this step-growth mechanism, typically high molecular weight materials result from a large number of steps.[2-3] These polymers encompass a wide family, including polyesters, polyamides and polyurethanes characterised by the presence of ester (-COO-), amide (-NHCO-) or urethane (-OCONH-) functional groups in their main chain, respectively. Polyesters are one of the most versatile families of compounds comprising widely different materials with a large spectrum of characteristics and applications.[4] Polyesters can be as diverse as fibres, liquid crystals and temperature-resistant high-performance polymers. Another aspect usually referred to as a main advantage of polyesters is their inherent degradability due to the hydrolytically labile ester bonds in the main chain. In practice however, only aliphatic polyesters will degrade over a reasonable time scale, unless very severe conditions are applied.

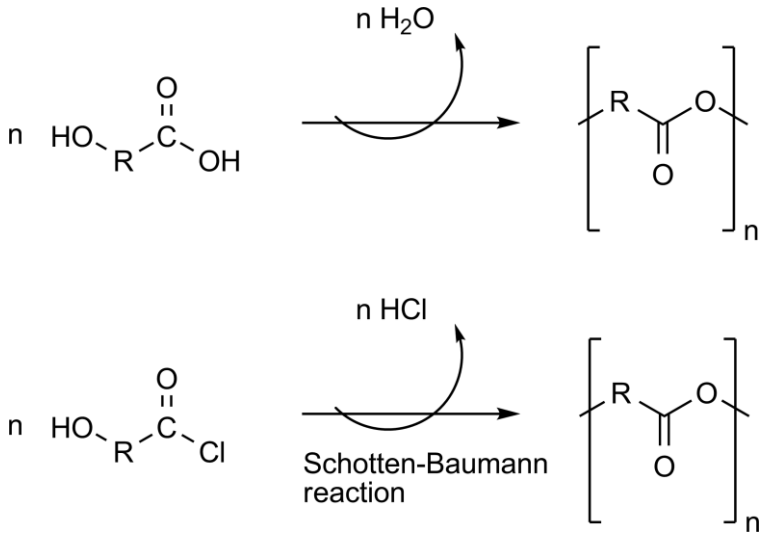
The purpose of the present Chapter is to provide a succinct assessment of the fundamental bases of polyester chemistry related with this work and some relevant examples of polyesters. It describes very briefly such concepts as degree of polymerisation, determination and control of the average molecular weight in linear and non-linear polyesterifications, and average molecular weight between cross-links. An overview of polyesters from renewable resources, including two commercial examples will also be given. Additionally, technical aspects of polyesterification processes and catalysts will be reviewed succinctly.

3.2 General polyesterification reactions

Linear polyesters are routinely synthesised by the stepwise polymerisation of difunctional monomers such as hydroxyacids (Scheme 3.1) or a combination of a diol and a diacid, or as in the case of the Schotten-Baumann reaction[3] an acid dichloride (Scheme 3.1). These polymerisation reactions are usually referred to as polycondensations because they involve the elimination, in each step, of a by-product like water or hydrochloric acid.

The *polycondensation* term was first proposed by Carothers and was used to classify all step-growth polymers, since at that time only the polyesters and polyamides syntheses involving the elimination of water or hydrochloric acid were known. However, soon several exceptions to this classification were noted, *e.g.* polyurethanes and epoxide resins.

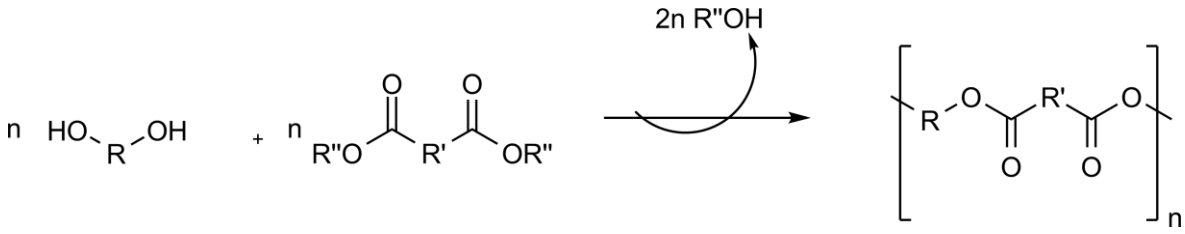
Today polymerisations are classified according to the chain growth mechanism, rather than based on the formation of a by-product or not, *i.e.,* step-growth and chain reactions.[1] Polycondensation is still used today, but it is restricted to those step-growth polymerisations, as described in the beginning of this paragraph, involving the formation of a small molecule as by-product (water or hydrochloric acid).



Scheme 3.1 Synthesis of linear chain polyesters by step-growth polycondensation.

Polyesters can also be synthesised by polytransesterification (ester interchange) typically involving the reaction between alkyl ester moieties (*e.g.* methyl esters) and hydroxy groups with elimination of the corresponding aliphatic alcohol.[2] The monomers

used can be either an alkyl ester of a hydroxyacid, or, instead, both a diester of a dioic acid and a diol, as represented in Scheme 3.2.



Scheme 3.2 Synthesis of linear chain polyesters by step-growth polytransesterification.

Both polycondensations and polytransesterifications are equilibrium reactions and hence in order to shift the reactions towards the polymers formation, the by-generated secondary products must be continuously removed from the reaction medium using a convenient approach,[1] for example a stream of an inert gas, a vacuum system with a collecting trap, or even molecular sieves.[1,5] Other approaches exist, however, today in which there is no need to eliminate the secondary products, as will be discussed below in the Subsection 3.8.1 about green catalysts.

Crosslinked polyesters could be synthesised using similar reactions as those just described above, but using monomers with functionality greater than 2.

3.3 Carothers equation

The Carothers equation was put forward in the 1930s by the pioneer of step-growth polymerisation. It was established considering the syntheses of linear polyesters involving stoichiometric quantities of difunctional monomers and equal reactivity of functional groups (a simplifying assumption proposed by Flory). This important equation relates the number-average degree of polymerisation (DP_n) with the extent of the reaction (p) related to percentage of consumption of the reactive functions.[1]

$$DP_n = \frac{1}{1-p} \qquad \textbf{3.1}$$

The equation is particularly suggestive of the imperative need to have the reaction reaching as near completion as possible, in order to prepare high molecular-weight polymers. For example, when p = 0.95 only a DP_n of 20 is obtained, but when p = 0.99 the

DP_n increases drastically to 100. For simplicity, the average symbol has been omitted for DP_n and likewise with similar average representations, throughout this Thesis.[1]

Stoichiometric imbalance. An exact stoichiometric balance of monomers is of major importance for high *DP'*s, thus implying the use of quite pure monomers, *i.e.* the molar ratio between the functional groups must be as close to unity as materially possible. This is obviously more readily achieved using pure hydroxiacids, rather than in the case of, for example, diols and diacids. An extension of the Carothers equation[1] takes into consideration the actual ratio between the number of functional groups (*r*),

$$DP_n = \frac{1+r}{1+r-2rp} \qquad \textbf{3.2}$$

Thus for a reaction with p =0.99, when r = 0.95, a DP_n ~ 28 is only attained, rather than DP_n ~ 100 for r = 1.

3.4 Average molecular weights and polydispersity index

All natural and synthetic polymers, not just polyesters, are a mixture of chains of different length, *i.e.* a random distribution of molecules of different molecular weight defined as polydisperse.[1,3] Polymers are therefore characterised by average molecular weights like the number-average molecular weight (M_n) determined experimentally by colligative techniques, such as osmotic pressure. M_n follows the conventional definition for the arithmetic mean value of any statistical quantity,

$$M_{\mathrm{n}} = \frac{\sum N_i M_i}{\sum N_i} \qquad \textbf{3.3}$$

where N_i is the number of chains (or moles of chains) with degree of polymerisation equal to i, and molecular weight M_i. Thus, each chain in a polymer sample provides the same contribution to M_n, irrespective of their size.

Another parameter regularly used to characterise polymers molecular weights is the weight-average molecular weight (M_w) determined experimentally by techniques like light scattering. M_w is defined by the following equation,

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} \qquad \textbf{3.4}$$

This quantity is related to both the size of the polymer chains and their number. Thus, since M_w depends on the size of polymer molecules, the longer a chain, the higher its contribution to M_w.

In general M_n and M_w are the most used averages in a polymer characterisation, although there are others like the *z*-average molecular weight M_z (not used in this work) measured by the ultracentrifugation technique,

$$M_z = \frac{\sum N_i M_i^3}{\sum N_i M_i^3} \quad \textbf{3.5}$$

Other techniques, like size-exclusion chromatography (SEC), provide information about the entire distribution of molecular weights in a polymer sample.

These averages (equations 3.3-3.5) give relevant information about a polymer and the ratio between M_w and M_n, provides a clear picture about the breadth of its size distribution,

$$PDI = \frac{M_w}{M_n} \quad \textbf{3.6}$$

This quantity is designated by polydispersity index (*PDI*) and is always greater than one. In the case of linear step-growth polymers, and thus including linear polyesters, *PDI* can be determined having in consideration the Flory distribution by the equation,

$$PDI = {M_w}/{M_n} = 1 + p \quad \textbf{3.7}$$

This equation is particularly relevant, suggesting that when the reaction tends toward completion M_w/M_n tends to 2.

3.5 Non-linear systems

In earlier sections, only the simplest case, involving linear systems, was considered. However, this is not always the case, since polyesterifications can also be carried out using monomers bearing more than 2 functional groups, thus leading to the formation of highly branched structures and ultimately, in certain instances, of insoluble three-dimensional cross-linked structures.[1] In these cases, a more general Carothers equation can be derived using the average functionality factor (f_{av}),

$$f_{av} = \frac{\sum N_i f_i}{\sum N_i} \quad \textbf{3.8}$$

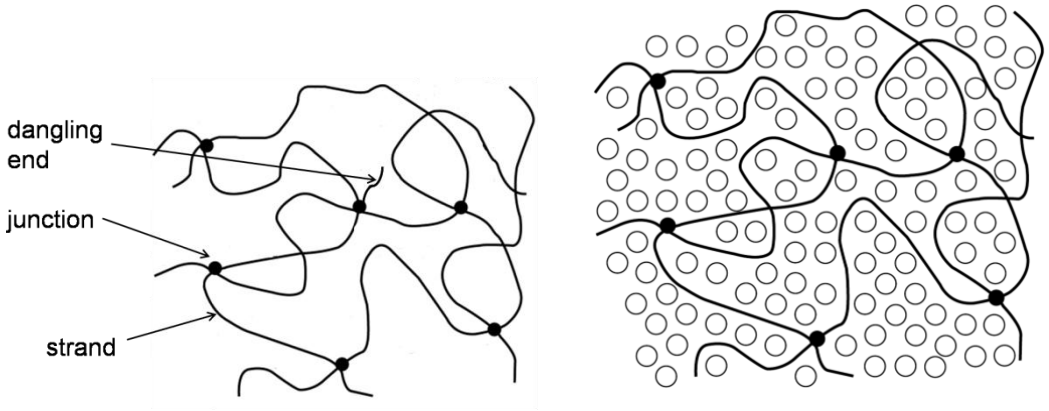
where N_i and f_i are the number or moles of molecules and the functionality of the *i*th component in the reaction mixture, respectively. Hence, DP_n becomes,

$$DP_n = \frac{2}{2 - p f_{av}} \quad \textbf{3.9}$$

It is apparent that this expression reduces to Equation 3.1 for the case of f_{av} = 2. It follows that with these non-linear polycondensations, DP_n increases drastically compared with linear counterparts. Thus, for example, for p = 0.95 when the reaction involves monomers with f_{av} = 2.1, DP_n becomes equal to 400, as compared with 20 when only bifunctional monomers are used (f_{av} = 2).

3.5.1 Swelling measurements

One of the characteristic features of cross-linked polymers, obviously including polyesters, is their ability to undergo swelling rather than dissolution, when thermodynamically compatible liquids penetrate into the polymer network (see illustration of Scheme 3.3). Due to this important feature, cross-linked polymers are frequently characterised using equilibrium swelling experiments.[3,6]



Scheme 3.3 Schematic illustration of a cross-linked polymer network (Left) before and (Right) after being swollen by a compatible liquid (adapted from reference 6).

The earliest theory of swelling equilibrium was established at the beginning of last century by Flory and Rehner. There are however several other theoretical models, since this issue is still a matter of debate within the scientific community.[6] The present Chapter

addresses only the essential aspects of the classical Flory-Rehner theory which will be applied in Chapter 7 and which is deemed quite adequate in the present context.

The Flory-Rhener equation[3,6] expresses the maximum swelling, *i.e.*, the equilibrium swelling of a lightly cross-linked polymer in terms of volume fraction of the polymer in the swollen gel (ϕ_e),

$$\ln(1-\Phi_e)+\Phi_e+\chi\Phi_e^2=\frac{\upsilon_e}{N_A}\frac{V_1}{V_0}\left(\frac{\Phi e}{2}-\Phi_e^{1/3}\right) \qquad \textbf{3.10}$$

where χ is the polymer-solvent interaction parameter, υ_e the number of strands, V_1 the solvent molar volume, V_0 the initial dry polymer volume, and N_A Avogadro's number. The cross-link density expressed as the average molecular weight between cross-links (M_c) is related to υ_e by the following equation,[6]

$$\frac{v_e}{N_A V_0}=\frac{\rho}{M_c}\left(1-\frac{2M_c}{M}\right) \qquad \textbf{3.11}$$

where ρ is the density of the dry polymer and M the molecular weight of the prenetwork chains. Thus, if χ is known, equations 3.10 and 3.11 can be applied and M_c calculated, using the measured volume of the swollen polymer at equilibrium ($V_e = V_0/\phi_e$).

3.6 Structure properties relations

One of the most important aspects of polymer chemistry, if not the most important, is the understanding of the polymer structure in order to assess polymer properties and behaviour. In this sense, the melting and the glass transition temperatures play an important role in determining the ultimate behaviour and applications of a polymer.

The glass transition and the melting temperatures of a polymer, usually designated by T_g and T_m, depend on several properties, namely the regularity of monomer units in the chain, the flexibility of its backbone, the intensity of intermolecular forces, the molecular weight, as well as the cross-link density (this associated with T_g).[1,3]

It is known that a highly flexible chain tends to have a lower T_g and T_m than a polymer with a highly rigid chain. One typical comparison is between poly(ethylene) and poly(ethyleneterephthalate), having glass transitions around -85 and 69 ^{o}C and melting temperatures around 27 and 265 ^{o}C, respectively.[1] Polymers with strong intermolecular forces tend to have higher values of T_g and T_m, oppositely to polymers with weaker

interactions. These situations are the general cases of polymers having strong dipole-dipole interactions (*e.g.* poly(vinyl alcohol)) and those having essentially dispersive interactions between polymer chains (*e.g.* polyoleofins). High molecular weights also tend to increase both T_g and T_m. The cross-link density of polymers is known to increase T_g.

It is important to recall that the occurrence of crystallisation in polymers depends on a number of basic factors related to the regularity of the macromolecular structure, including unit enchainment, tacticity and conformational organisation. Thus, many conventional polymers, like atactic vinyl structures, cannot crystallise, as is also the case of random copolymers. Obviously these materials will not display a melting feature, *i.e.* no T_m. Moreover, even those polymers which possess the structural aptitude to crystallise, very seldom achieve a very high degree of crystallinity, because of the intrinsically high relaxation times of their macromolecules. It is therefore more appropriate to speak about "semicrystalline polymers" instead of crystalline polymers.

3.7 Overview of polyesters from renewable resources

The preceding Sections dealt with the essential aspects related to the distribution of polymer sizes as a function of the extent of the reaction and the concentration of the reactants. Now the next paragraphs will focus on relevant polyesters from renewable resources.

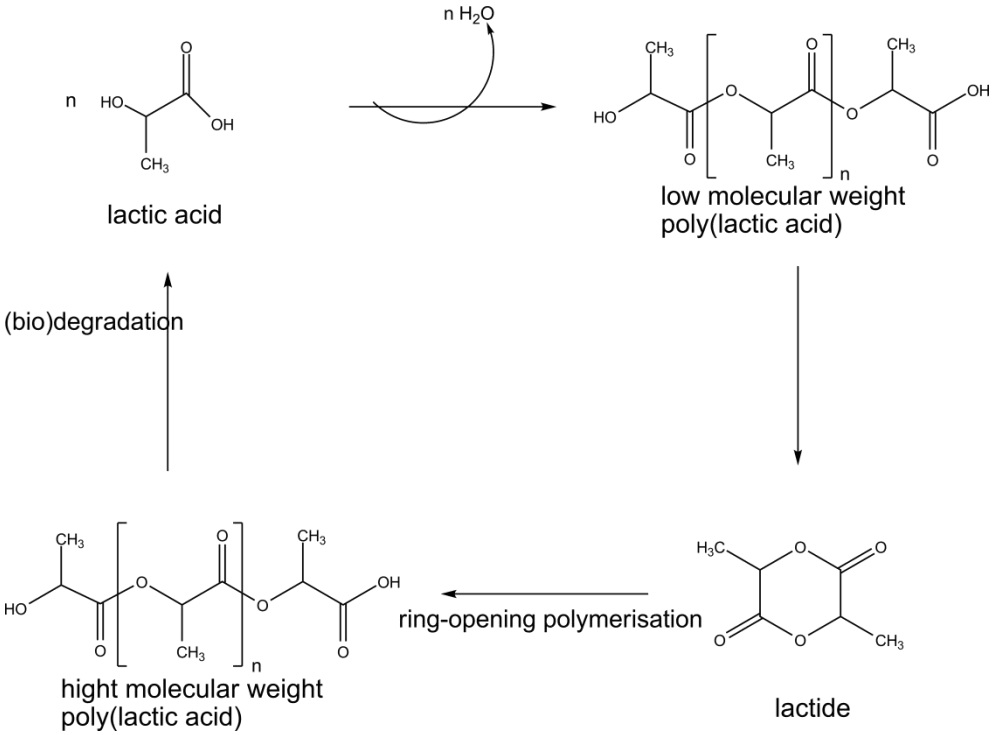
These polyester's materials have a forefront position in the plastics world thanks to their unique and vast array of properties, *e.g.* fibre forming ability, potential biodegradability, in some cases also biocompatibility, among many others.[1,4] There are already a wide number of studies concerning the use of renewable resources in the syntheses of polyesters, but there are only a few commercial examples of plant derived plastics mostly due to their relatively high cost *vs.* their petrochemical homologues.[7]

3.7.1 Plant derived polyesters: two commercial examples

Nevertheless, one particularly successful example worth focusing is poly(lactic acid). This polyester commonly designated by PLA is a highly versatile, biodegradable, biocompatible, aliphatic polyester commercially available in relatively large scale. Cargill Dow Polymers LLC started in 2004 the world's first full-scale PLA plant in Blair, Nebraska, capable of producing 140 000 tons per year.[8] The monomeric precursor of PLA, lactic acid is routinely produced by the bacterial fermentation of carbohydrates and PLA itself can be

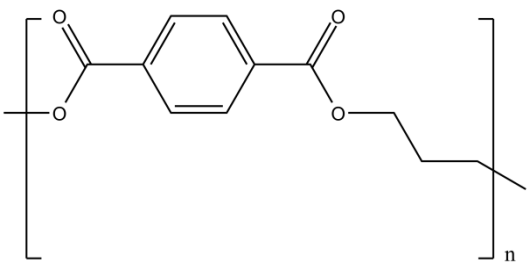
produced in high molecular weights by ring-opening polymerisation of the lactide (cyclic dimer of lactic acid) as described in Scheme 3.4.[9]

The ensuing polymer typically presented a glass transition temperature ranging from 50 to 80 ^{o}C, whereas its melting temperature ranged from 130 to 180 ^{o}C depending on the degree of crystallinity and molecular weight.[9] Its main applications are in short-term packaging due to its biodegradability, and also in biomedical applications such as implants, sutures, or drug encapsulations due to its biocompatibility in contact with living tissues.[9]



Scheme 3.4 Representation of a possible PLA lifecycle (adapted from reference 9).

Other polyesters are only partially renewable, as poly(trimethylene terephthalate), with the trade name Sorona (Scheme 3.5), produced by the giant of plastics DuPont.[10-11]



Scheme 3.5 Poly(trimethylene terephthalate).

This polyester is synthesised using 1,3-propanediol partially derived from corn starch and the petrochemist based terephthalic acid. DuPont claims that this polymer is already 37% renewable by weight.[11] It is a linear polymer with a glass transition temperature of approximately 50 ^{o}C and a melting temperature of *c.a.* 228 ^{o}C.[12] Its main use is as a fibre for clothing and textiles in general.

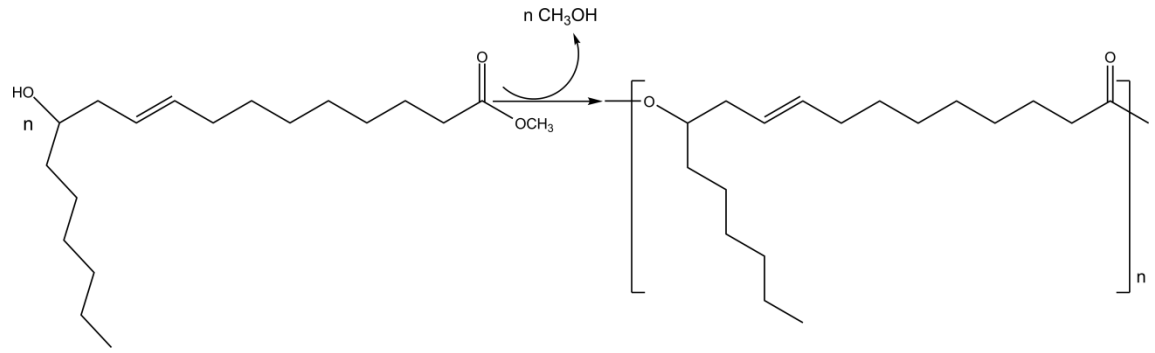
Although the number of other renewable-based polyesters produced and commercialised in large scale is still limited, there are countless initiatives in literature addressing the subject, searching for syntheses of polyesters from bioresources and in some cases also using greener reaction conditions. In the following Subsection, some relevant examples of aliphatic polyesters from renewable resources will be described briefly.

3.7.2 Aliphatic polyesters from renewable resources

One of the most relevant group of polyesters are aliphatic polyesters derived from aliphatic alkanediols and α,ω-alkanedioic acids (or in alternative derived from hydroxyalkanoic acids) ready available from inexpensive renewable resources. Some authors claimed they could “replace many conventional plastics soon” because of their biodegradability, acceptable mechanical and thermal properties comparable to low-density poly(ethylene) and poly(styrene).[13]

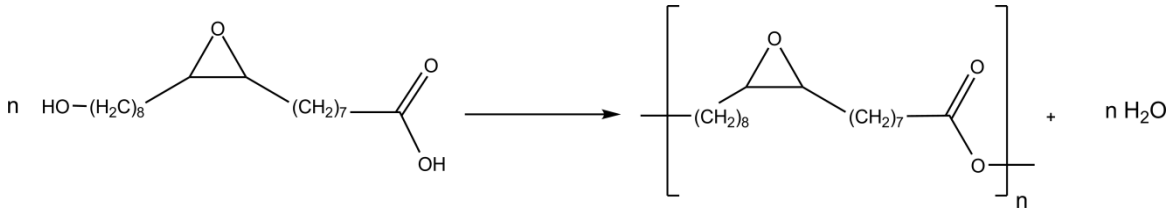
Among the most interesting vegetable oil-based monomers, ricinoleic acid occupies undoubtedly a privileged position due to its bifunctionality (both hydroxy and carboxylic acid groups are present).[7,14] Oppositely to most naturally occurring aliphatic acids having only one functional group. Slivniak *et al.*[15] focused their work on the syntheses of a ricinoleic acid-lactic acid copolymer using two different approaches *viz.* direct polycondensation and polytransesterification. The ensuing copolyesters were obtained in variable molecular weights depending on the syntheses approach used (number average molecular weight around 2-11 kDa). Another work due to Ebata *et al.*[16] also used ricinoleic acid (Scheme 3.6) to prepare the corresponding homopolymer and post-cross-linking by radical polymerisation. This work has the merit of having used both a renewable monomer and an ecofriendly syntheses approach *i.e.* bulk enzymatic polytransesterification using a lipase (*Peseudomonas cepacia*). The ensuing polyesters presented weight-average molecular weights between 2 and 100 kDa depending on the polymerisation conditions adopted (temperature, time, drying agent or pressure), and for the highest molecular weight polymers a glass transition temperature around -75 ^{o}C. After

cross-linking with dicumyl peroxide, at high temperature, the ensuing product had a gel fraction of approximately 98% and a glass transition temperature of ~ -65 ^{o}C.



Scheme 3.6 Synthesis of poly(ricinoleate).

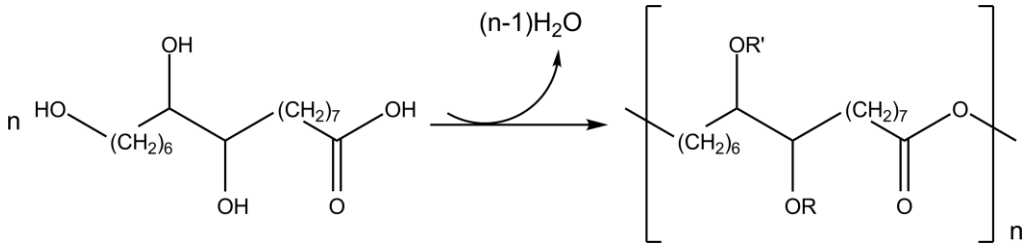
The use of suberin oleochemicals monomers to prepare polyesters is an interesting approach first reported by Olsson and coworkers.[17] They have used 18-hydroxy-9,10-epoxyoctadecanoic acid, isolated from birch outer bark suberin after laborious processing of suberin depolymerisation mixtures. Nevertheless, the polycondensation reaction was conducted under mild conditions using the *Candida antarctica* lipase B at moderate temperature (Scheme 3.7). The ensuing polyester presented interesting properties as the persistence of its epoxy function and high molecular weights (up to 20 kDa).



Scheme 3.7 Synthesis of poly(18-hydroxy-9,10-epoxyoctadecanoate) from 18-hydroxy-9,10-epoxyoctadecanoic acid.

A similar study from Heredia-Guerrero *et al.*[18] focusing the polycondensation reaction of a single cutin monomer was published very recently. The most abundant 9(10),16-dihydroxyhexadecanoic acid in cutin was successfully self-polymerised (without any catalyst), in spite of the intrinsic stoichiometric imbalance of 9(10),16-dihydroxyhexadecanoic acid (1CO_2H/2OH). The reaction occurrence was confirmed by attenuated total reflection Fourier transform infrared (ATR FTIR) spectroscopy. These same authors also reported a study on the emulsion polycondensation of a cutin mimetic polyester using a very similar monomer, 9,10,16-trihydroxyoctadecanoic acid (Scheme

3.8).[19] Once more the stoichiometric imbalance of the reaction was not considered but the polyester formation was confirmed by ATR FTIR and ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR). The detailed characterisation of this polyester showed that, like cutin counterpart, it was mostly an amorphous polymer displaying a broad halo around 2θ ~20^{o} in their X-ray diffraction (XRD) pattern. The DSC thermogram showed a glass transition around -2 ^{o}C and two small endothermic events around 46 and 73 ^{o}C, that could be related with the presence of larger short-range ordered domains. This differed from cutin which presented a lower glass transition (-47 ^{o}C) and no endothermic peaks.



Scheme 3.8 Synthesis of poly(9,10,16-trihydroxyhexadecanoate) from 9,10,16-trihydroxyhexadecanoic acid. R and R' stand for H or another cross-linked fragment as stated in reference 19.

In short, on one hand, there is an intense activity in the scientific community in what concerns the investigation of renewable-base polymers. Indeed, there are very interesting works dealing with the syntheses and characterisation of polyesters based on renewable resources some of them with already commercial products. It is clear, that a revitalised polymer chemistry is emerging very rapidly. In medium to long term, it is generally believed that switching over to biomass as the raw material for plastic production is inevitable, if oil is as finite resource in a reasonable time scale as it is believed to be.[10]

One of the major challenges dealt with in polymer syntheses is indeed to use renewable resources, but also concomitantly to approach convenient synthetic pathway avoiding toxic catalysts. This topic is discussed in the following section.

3.8 Polyesterification processes

Polyesterification processes can be single phase processes, like typically in the case of bulk, melt and solution polyesterifications, or multiphase processes, as in the emulsion or phase-transfer polymerisations.[2]

Bulk polymerisation is the most straightforward approach where only the reactants and an adequate catalyst are used. To prevent crystallisation these syntheses are usually performed at temperatures higher than the melting point of the ensuing polymer and are referred to as melt polymerisations. Polymerisations can also be carried out in solution, and in this case monomers and catalyst are dissolved in a non-monomeric liquid solvent at the beginning of the polymerisation reaction. The liquid is usually also a solvent for the resulting polymer or copolymer. Conducting polymerisation reactions in a solvent allows an effective dispersion of the heat liberated by the process; in addition, solutions are much easier to stir than bulk media. Emulsion polymerisation is usually carried out in water with the monomer or polymer dispersed in the form of an emulsion using an adequate surfactant. Continuous rapid stirring is needed and each micelle plays the role of a miniature reaction vessel, as will be described more in detail below. In phase-transfer polyesterifications the complementary monomers are dissolved respectively in water and a non-polar solvent and the system is vigorously stirred in the presence of a phase-transfer agent which facilitates the encounters of the monomers at the interface. A typical example of this technique is the reaction of a diol in a basic aqueous solution with a diacid chloride dissolved in a non-polar solvent. The condensation occurs at the interface and the released hydrochloric acid is neutralised by the basic medium.

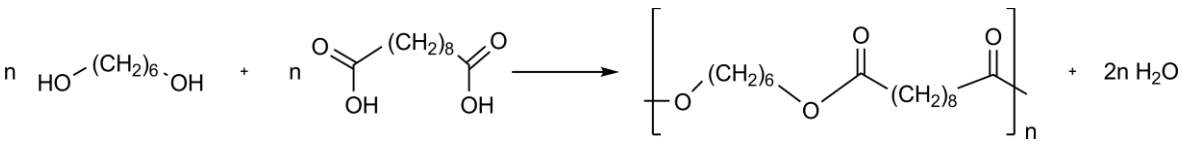
Polyesterification reactions are most conveniently carried out with the aid of classical catalysts like the Brønsted acids used in polycondensations. Some examples are sulphuric acid, hydrochloric acid, hydrobromic acid, phosphoric acid, and poly(phosphoric acid).[5,20] Other catalysts, frequently used in polycondensations are Lewis acids like tin and titanium compounds (*e.g*. dibutyl tin oxide, titanium tetrachloride).[20] In what concerns the transesterification polymers they can be synthesised using standard base catalysts like potassium carbonate, sodium hydroxide, potassium hydroxide, or 4-dimethylaminopyridine, or Lewis acids, *e.g.*, antimony(III) oxide (Sb_2O_3). Some of these catalysts are hazardous to the environment, indeed today science face an enormous challenge concerning the search for convenient, inexpensive synthetic processes avoiding toxic catalysts and still producing high conversion yields and molecular weights. This has

prompted several articles on the subject, in particular regarding direct polycondensation or polytransesterification reactions of aliphatic monomers.[13,17,21-24]

3.8.1 Green catalysts for polyesterification reactions

Metal trifluoromethanesulfonates (Lewis acid catalysts), usually designated by triflates, have reached a very important place in the arsenal of proposed polycondensation catalysts.[25-27] Thus, bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$)-mediated polycondensation reactions of aliphatic monomers, have attracted some interest throughout scientific communities due to their low toxicity, ease of handling, low cost, and stability.[26-27] It is thought that $Bi(OTf)_3$ acts as a precursor which, upon hydrolysis, releases enough triflic acid (the corresponding Brønsted acid)[5] to promote the esterification reaction.[28]

Takasu *et al.*[25] studied the chemoselective direct polycondensation of different dicarboxylic acids and alcohols including the renewable monomers succinic acid, glycerol, and sorbitol and explored a one-step procedure involving the selective reaction of primary hydroxy groups. The polycondensation was conducted under mild temperatures and using scandium trifluoromethanesulfonate ($Sc(OTf)_3$) as catalyst. The reaction proceeded to afford linear polyesters with pendant hydroxy groups in their backbone in excellent yields (η ~ 99%), and M_n between 4 and 26 kDa. More recently, Kricheldorf *et. al.*[26-27,29] published several papers on the use of metal trifluoromethanesulfonate catalysts in the syntheses of polyesters, also by direct polycondensation. They tested several triflate catalysts including sodium, magnesium, aluminium, zinc, tin, scandium, lanthanum, samarium, yttrium, and hafnium, and used different aliphatic monomers, namely C_3-C_{10} diols and dicarboxylic acids (see *e.g.* in Scheme 3.9 one of the system studied[27]).



Scheme 3.9 Polycondensation reaction between 1,6-hexanediol and decanedioic acid.

The reactions were conducted in bulk, at a moderate temperature (~ 80 ^oC), and under reduced pressure. It was found that bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$) was one of the most convenient in terms of extent of reactions (around 95%), average molecular weights of the ensuing polymers (up to 35 kDa) and also because Bi^{3+} is the

least toxic among the heavy metals. One of the approaches selected in this work was precisely the above mentioned $Bi(OTf)_3$ catalytic system due to the excellent results found with similar systems and also because of the mild reaction conditions involved.

In the past decade, a few studies have been published on the polycondensation (or just condensation) of aliphatic monomers in water in the presence of a Brønsted acid surfactant catalyst, in which the (poly)condensation is attained at the interface of the emulsion.[13,22-23] In aqueous solution, a surfactant like *p*-dodecylbenzenesulfonic acid (DBSA) and the aliphatic monomers form micelles, which have a hydrophobic core, through hydrophobic interactions (Figure 3.8). It has been stated that the driving force for the polycondensation is associated with the fact that the water molecules generated during the polycondensation reaction are expelled from the micelles because of the hydrophobic nature of their core.[22-23] Consequently; the equilibrium position between monomers and their polyesters is shifted toward polyester formation. This approach is particularly interesting because of the use of water, which is obviously a safe, environmentally benign and very cheap solvent. One example[22] is the esterification of dodecanoic acid with 3-phenyl-1-propanol using DBSA (16.6 x 10^{-3} mol dm^{-3}) above its critical micelle concentration[30] (8.4 x 10^{-3} mol dm^{-3}). A conversion yield of η ~ 84 % was reached within a few hours reaction (only 2 h) at a low temperature (~40 ^{o}C).

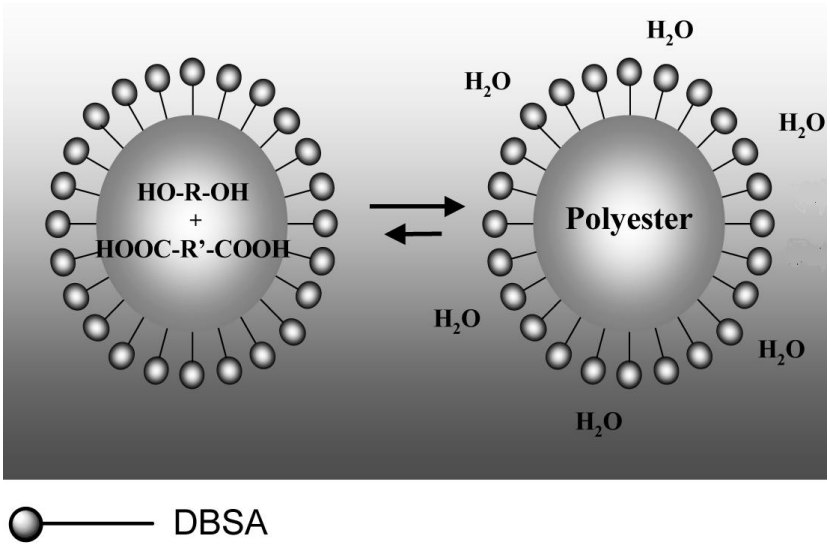


Figure 3.8 Illustration of direct polycondensation at the emulsion interface (adapted from reference 13).

In another study by Takasu *et al.*,[13] the polycondensation of 1,9-nonanediol with dodecanedioic acid, as well as of other homologous monomers, was studied. The reaction

was conducted in water at 80 ^{o}C using DBSA (0.98 mol dm^{-3}). The average molecular weights of these aliphatic polyesters ranged from 1.4 to 10.1 kDa, and their polydispersity indexes were close to 2. These investigations followed the pioneering work in emulsion polycondensation by Saam,[24] who adopted a reverse-micelle system in toluene.

Another approach successfully used by several authors is the mild enzymatic polycondensation or polytransesterification using lipase catalysis.[16-17,21,31-37] Lipases are serine hydrolases that catalyse *in vivo* the hydrolysis of lipids of fatty acids and glycerol in an aqueous emulsion environment (at the oil-water interface).[38] The general mechanism of lipase catalysed synthesis of (poly)esters is generally believed to rely on a conformation change at the oil-water interface, followed by formation of an enzyme-substrate complex involving the enzyme active site and the reactants. The pertinent discovery of lipases which catalyse esterification reactions *in vitro* at relatively high temperatures and in organic media made biocatalysis a valuable tool in polymer chemistry.[38] Lipases can be isolated from a variety of sources, like for example porcine pancreas, and many microorganisms. In particular, the commercially available *Candida antarctica* lipase B (CALB) is produced by a genetically modified *Aspergillus oryzae* microorganism and immobilised on a macroporous acrylic resin. This enzyme is active for the polycondensation (or polytransesterification), both in bulk or organic media, leading to the formation of polyesters in reasonably high yields.[35]

Olsson *et. al.,*[17] has already mentioned above, studied the polycondensation of 18-hydroxy-9,10-epoxyoctadecanoic acid from birch outer bark suberin using the CALB lipase. These reactions were carried out either in bulk or in the presence of an organic solvent. Interestingly bulk polycondensations gave, at a much shorter reaction time, molecular weights comparable to those obtained with toluene (15 and 20 kDa, respectively). The reactions were conducted at moderate temperatures (75 or 85 ^{o}C), but during a reasonable period of time (48 h), and using a relatively high amount of lipase (~25 % w/w).

3.9 Conclusions

This Chapter dealt with the main aspects related to average degree of polymerisation and its distribution, and properties of both linear and non-linear polymers. Additionally, two examples of polyesters from renewable resources, already commercialised, and several polyesters also from renewable resources and developed in recent years were cited.

The vast array of polyesterification catalysts was briefly reviewed with an emphasis on environmentally benign strategies. These general considerations are of utmost importance for the full understanding of the reactions described in the next part of this Thesis – Part II Results & Discussion.

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[PART II RESULTS & DISCUSSION]

4 Cork and birch outer bark as renewable sources of lipophilic chemicals: a comparative study

"To suberin or not to suberin"? Which was the suberin content in cork and in silver birch outer bark? Was suberin an interesting source of aliphatic chemicals? Which were the most abundant compounds found in depolymerised suberin mixtures from cork and from silver birch outer bark? Were there any differences among suberin samples from different sources or isolated by distinct procedures? Birch outer bark suberin or cork suberin? Which were the main physical properties of suberin mixtures? Were there any differences? Were suberin fragments mixtures adequate monomers for polyesters synthesis?

4.1 Introduction

Suberin is an interesting material with unique potential applications, as have been emphasised throughout this Thesis and in particular in Chapter 2. This is mainly due to its natural abundance in the outer bark of species like cork oak and silver birch, but also because suberin constitutes an abundant source of valuable compounds, such as ω-hydroxyalkanoic acids and α,ω-alkanedioic acids and the homologous mid-chain dihydroxy or epoxy derivatives,[1-2] that are otherwise quite rare in nature. Indeed, hydroxyalkanoic acids are only additionally found in exploitable amounts in the seed oils of *Ricinus communis* L. (castor oil) and *Lesquerella* spp., and, of course, in cutin.[2-3]

The suberin intrinsic value has been recognised by several authors,[1-4] who explored its use in several different applications. One of the most interesting applications of suberin fragments involves their use as a source of monomers for the synthesis of polymeric materials, as extensively reviewed by Gandini *et. al,*[2] notably polyurethanes synthesis through the polycondensation of depolymerised suberin mixtures,[5-6] polyesters through the polycondensation of one specific suberin compound,[7] or the whole depolymerised suberin substrate. One example, already cited in Chapter 1, is the polycondensation of 9,10-epoxy-18-hydroxyoctadecanoic acid monomer, after isolation from depolymerised suberin.[7] However, suberin mixtures of monomers, and not just one monomer, could also be applied. Their chemical composition can be controlled to tailor copolyesters with different properties (*e.g.* linear or cross-linked), under appropriately optimised reaction conditions.

In the present study, the chemical composition of suberin depolymerisation products was manipulated by varying the species of suberin source (cork oak or silver birch), using different depolymerisation procedures (hydrolysis or methanolysis), and/or by a fractionation procedure where solvents of different polarity were used. Additionally, the suberin fragments obtained from “industrial cork powder” (ICP), an important residue generated during the production of cork agglomerates, was also studied.[8] The ensuing suberin mixtures from cork, industrial cork powder and birch outer bark were thoroughly characterised, particularly in terms of their monomer composition, through GC-MS analysis (Subsection 4.3.1),[9] the molar ratio between carboxylic and hydroxy groups by ^{1}H NMR spectroscopy (Subsection 4.3.3.3),[10] but also of the suberin physical properties using thermal and diffraction techniques (Section 4.4). This Chapter also deals with a

comparative study of the chemical composition of cork oak (*Quercus suber* L.) and birch (*Betula pendula* Roth) outer bark. This study aims at contributing significantly to the cutting-edge knowledge of the two species, [9] since the last comparative study about suberin chemical composition from cork and birch outer bark was published thirty years ago.[11] All the experimental details related to the present Chapter are summarised in Chapter 8 (PART III Experimental).

4.2 Overall chemical composition

Table 4.2 summarises the overall composition of cork, industrial cork powder and birch outer bark.

Table 4.2 Group composition (w/w %) of cork, industrial cork powder (ICP) and silver birch outer bark (birch).

	cork	ICP	birch
suberin			
[a]HDS	22	11	35
[b]MDS	[c]52, [d]26	11	-
[e]extractives	9	6	40
ash	4	6	1
Klason lignin	33	38	9
carbohydrates	23	27	6

[a] Hydrolysis-depolymerised suberin. [b] Methanolysis-depolymerised suberin [c] Suberin extracted with dichloromethane. [d] Suberin extracted with dichloromethane followed by fractionation with *n*-hexane. [e] Total extraction yields (dichloromethane, methanol and water).

The suberin depolymerisation yields of both cork and birch outer bark were within the typical ranges reported in the literature (22-35% and 26-52% for hydrolysis and methanolysis depolymerisation, respectively).[2] However, both cork and birch outer bark suberin hydrolysis yields were at the lower limit of the mentioned ranges, probably because the depolymerisation reactions were conducted under mild conditions. Conversely, the methanolysis procedure gave relatively high suberin yields (~52%), except, obviously, when the suberin was extracted with dichloromethane, followed by fractionation with *n*-hexane (26%). The methanolysis procedure promoted the isolation of both suberin fragments and, most probably, of other fractions, which accounts for the 52% yield, except, again, when the fractionation with *n*-hexane was adopted. ICP yielded considerably lower amounts of suberin, around 11%, when compared with the native cork, which could be an obstacle for future applications, where high suberin yields would be

mandatory. Hence, ICP was not used as a source of suberin compounds. In this study, the three samples also gave considerably different yields for the other components. While birch outer bark gave a high content of extractives, around 40%, and low contents of other macromolecular components (*viz.* 9% of Klason lignin and 6% of carbohydrates), cork and ICP gave lower contents of extractives (9 and 6%, respectively) and higher amounts of Klason lignin (33 and 38%, respectively) and carbohydrates (23 and 27%, respectively). The Klason lignin content in cork and ICP was unusually high, compared with previous published results.[12-13]

Besides the expected differences between birch outer bark and cork samples, several other differences between cork and ICP samples were observed. ICP had a higher content of lignin, with smaller amounts of suberin, probably it was rich in the inner and outer surface fractions of cork planks, rejected during cork stopper manufacture. In particular, the outer surface (the major fraction of ICP) is more lignified than the “bulk”, and certainly enriched in complex mixtures of photodegraded extractives, polysaccharides, lignin and suberin, due to environmental exposure. Additionally, the fact that only about 90% of the mass of ICP was accounted for should also be related to the particular nature of this fraction, the remaining 10% being water-soluble polar organic compounds released during the depolymerisation reactions.

The extractive contents of cork were within the typical values found for mature (“Amadia” grade) cork.[12,14] The major components of the dichloromethane and methanol extracts of cork and ICP were triterpenoids, with cerine and friedeline as the major compounds in cork, and betulinic acid in ICP extracts, as reported in detail in Chapter 5. The ethanol extract of birch outer bark was only briefly studied by GC-MS and the results showed that triterpenoids were its main constituents, with betulinol representing about 80% of the total.

These results had necessarily implications in the process adopted in this work to obtain the depolymerised suberin mixtures. Indeed, the predominance of extractives and low contents of lignin in birch outer bark, when compared with cork or ICP, were important aspects of suberin isolation. Therefore, in the case of birch outer bark, a pre-extraction step before the suberin isolation was the procedure adopted and indeed, after extractive removal, birch outer bark residues were considerably enriched in suberin and, upon alkaline hydrolysis, the ensuing mixtures were particularly rich in suberin fragment units (preserving the epoxy ring moieties).

In the case of cork, and in particular of ICP, the depolymerisation reactions were conducted under mild alkaline hydrolysis conditions in order to ensure that the ensuing products had negligible quantities of residual lignin and polysaccharides contaminants. Hence, the hydrolysis depolymerisation of suberin from cork and ICP was conducted for a relatively short period of time, using a KOH ethanol/water solution, as described in Chapter 8 (PART III Experimental).

4.3 Monomer composition of depolymerised suberin

The wide selection of depolymerised suberin mixtures were characterised in detail and their possible use in the preparation of novel biopolyesters evaluated. These mixtures were hydrolysis-depolymerised suberins from cork and its industrial cork powder, and from birch outer bark, referred hereafter as HDS_{cork}, HDS_{ICP}, and HDS_{birch}, respectively. Also used were cork and ICP methanolysis-depolymerised suberins extracted with dichloromethane or with dichloromethane followed by fractionation with *n*-hexane, referred hereafter as DCM-MDS_{cork}, DCM-MDS_{ICP}, and HEX-MDS_{cork}, respectively. All suberin depolymerisation mixtures were characterised by several chemical and physical techniques, namely GC-MS, ATR FTIR spectroscopy, ^{1}H and ^{13}C NMR, DSC, dynamic mechanical analysis (DMA), TGA, polarised light thermal microscopy (also known as thermomicroscopy), and XRD.

4.3.1 Gas chromatography-mass spectrometry analysis

The GC-MS results confirmed, as expected, that all samples were abundant sources of interesting monomers, namely, ω-hydroxyalkanoic and α,ω-alkanedioic acids, and the corresponding epoxy derivatives.[9] Several long-chain alkanols and alkanoic acids, glycerol and ferulic acid were also identified, but in smaller amounts, in accordance with previously reported results.[2]

A typical chromatogram of an HDS_{birch} sample is shown in Figure 4.9. The identification of the chromatographic peaks was based on the equipment's mass spectral library coupled with comparisons with previously published data (references 14-17 and references therein).

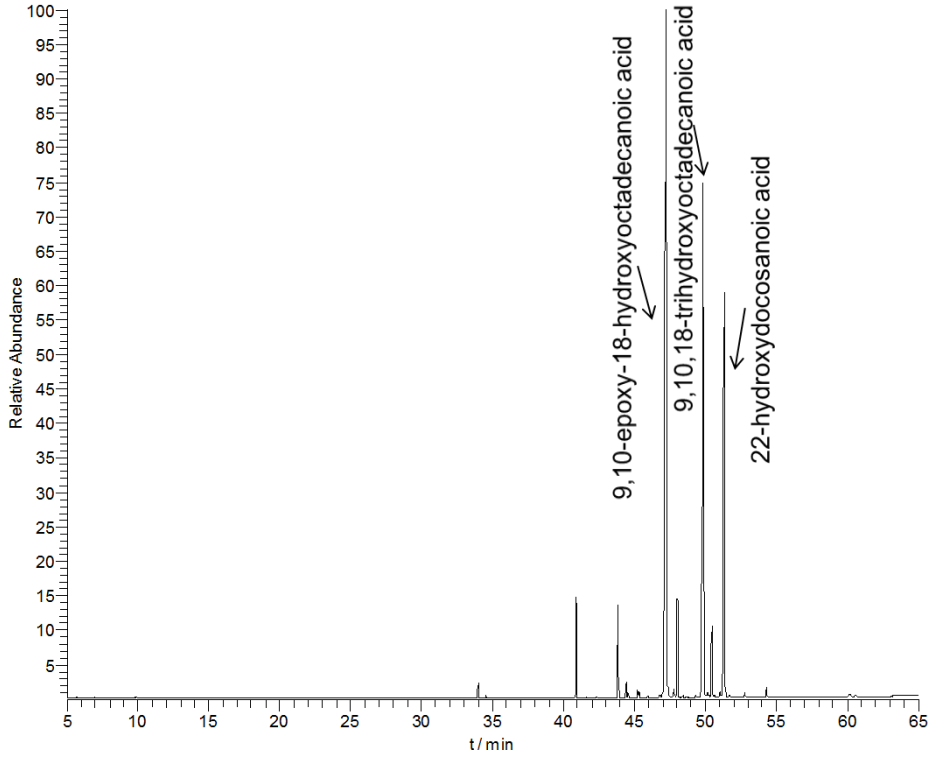


Figure 4.9 Typical GC chromatogram of HDS_{birch}.

Table 4.3 summarises the compounds identified in the various suberin mixtures and the corresponding quantification, both obtained by GC-MS.

Although, as expected, the chemical compositions of all depolymerised suberin samples were qualitatively similar, there were relevant quantitative differences among them, which could be used afterwards for the preparation of different polyesters. One relevant difference, clearly highlighted by the GC-MS analysis (Table 4.3), was the predominance of the polyfunctional epoxy derivatives in HDS_{birch}, with 9,10-epoxy-18-hydroxyoctadecanoic acid (~156.47 mg g^{-1}) as the most abundant component of this group (Figure 4.10), accounting for around 53% of all compounds identified by GC-MS. The second most abundant component found in this same HDS_{birch} sample was 9,10,18-trihydroxyoctadecanoic acid (~74.37 mg g^{-1}), resulting from the cleavage of the epoxy ring of its homologue. The most abundant ω-hydroxyalkanoic acid found in birch outer bark was 22-hydroxydocosanoic acid (~28.36 mg g^{-1}). Results also showed that HDS_{birch} was the only sample with a very low proportion of monofunctional alkanoic acids and 1-alkanols, thus less prone to suffer chain growth interruption during the course of a polycondensation reaction.

Table 4.3 GC-MS contents of the main compounds identified in the depolymerised suberin mixtures from cork, ICP and birch outer bark (mg of compound per g of depolymerised suberin).

compound	HDS_{cork}	HDS_{ICP}	HDS_{birch}	[a]$DCM\text{-}MDS_{cork}$	[a]$HEX\text{-}MDS_{cork}$	[a]$DCM\text{-}MDS_{ICP}$
ω-hydroxyalkanoic acids	**27.15**	**37.78**	**53.68**	**107.11**	**127.41**	**117.79**
10-hydroxydecanoic acid	-	0.70	-	-	-	-
14-hydroxytetradecenoic acid	4.42	-	-	-	-	-
16-hydroxyhexadecanoic acid	8.67	-	-	2.81	2.19	5.41
18-hydroxyoctadecanoic acid	-	-	11.80	0.37	-	0.75
18-hydroxyoctadec-9-enoic acid	9.03	18.50	-	18.16	34.28	30.97
20-hydroxyeicosanoic acid	-	4.18	12.93	3.31	4.65	3.82
20-hydroxyeicos-10-enoic acid	-	-	-	1.97	-	1.99
22-hydroxydocosanoic acid	5.03	14.40	28.36	70.45	86.29	61.05
24-hydroxytetracosanoic acid	-	-	0.59	8.80	-	13.07
other ω-hydroxyalkanoic acids	-	-	-	1.24	-	0.73
α,ω-alkanedioic acids	**13.49**	**54.22**	**1.67**	**9.16**	**38.97**	**6.77**
tetradecanedioic acid	6.48	-	-	-	-	-
hexadecanedioic acid	-	11.70	0.27	0.18	4.82	0.32
hexadecenedioic acid	5.90	-	-	-	-	-
8-hydroxyhexadecanedioic acid	-	2.65	-	3.72	-	1.48
octadecanedioic acid		3.03	0.38	-	-	-
octadec-9-enedioic acid	1.11	25.12	0.77	1.77	8.40	1.74
eicosanedioic acid	-	3.20	0.25	-	-	-
eicos-9-enedioic acid	-	1.88	-	-	-	-
docosanedioic acid	-	6.64	-	3.49	25.75	3.23
epoxy derivatives	**98.95**	**86.19**	**241.36**	**97.34**	**114.80**	**88.77**
6,16-dihydroxyhexadecanoic acid	-	-	1.95	-	-	-
7,8-dihdroxytetradecanedioic acid	-	-	-	0.34	-	9.34
9,10-epoxy-18-hydroxyoctadecanoic acid	-	-	156.47	-	-	12.94
9-hydroxy-10-methoxyoctadecanedioic acid	-	2.38	-	11.82	15.52	14.52
9,10,18-trihydroxyoctadecanoic acid	-	14.43	74.37	10.10	47.98	9.06
9,18-dihydroxyoctadecanoic acid	-	-	1.18	-	-	-
9,18-dihydroxy-10-methoxyoctadecanoic acid	-	-	-	14.05	9.05	2.06
10,18-dihydroxy-9-ethoxyoctadecanoic acid	6.03	-	-	-	-	-
9,10-dihydroxyoctadecanedioic acid	34.26	27.37	-	8.80	14.62	40.83

compound	HDS_{cork}	HDS_{ICP}	HDS_{birch}	[a]DCM-MDS_{cork}	[a]HEX-MDS_{cork}	[a]DCM-MDS_{ICP}
other epoxy derivatives	58.66	42.01	7.39	52.23	27.63	0.02
1-alkanols and alkanoic acid	**12.10**	**8.41**	**0.44**	**19.93**	**25.39**	**20.41**
hexadecanol	-	-	-	-	-	1.13
octadecanol	-	-	-	0.07	-	7.82
eicosanol	-	0.13	-	0.75	1.82	3.71
docosanol	-	-	-	9.41	8.97	1.05
tetracosanol	1.14	1.39	-	3.03	-	0.02
hexacosanol	-	-	-	1.17	-	-
tetradecanoic acid	1.47	-	-	-	-	-
hexadecanoic acid	-	3.39	-	-	-	5.56
octadecanoic acid	-	1.70	-	0.18	-	1.12
octadec-11-enoic acid	-	1.19	-	-	-	-
eicosanoic acid	-	0.61	0.19	-	-	-
docosanoic acid	9.49	-	0.25	4.40	11.77	-
tetracosanoic acid	-	-	-	0.92	2.83	-
ferulic acid	**34.28**	**10.00**	**1.35**	**6.91**	**-**	**7.16**
glycerol	**1.01**	**2.96**	**-**	**0.12**	**-**	**0.14**
TOTAL	**186.98**	**199.56**	**298.50**	**240.57**	**306.57**	**241.04**

[a] In the form of methyl esters.

HDS_{cork} and HDS_{ICP} also contained relevant quantities of epoxy derivatives (98.95 and 86.20 mg g^{-1}, respectively), although not in such high amounts as in the birch outer bark sample. The most abundant compound of this group was 9,10-dihydroxyoctadecanedioic acid (34.36 and 27.37 mg g^{-1}, respectively). Also present were the ω-hydroxyalkanoic acids, 18-hydroxyoctadec-9-enoic acid (9.03 and 18.50 mg g^{-1}, respectively) and 22-hydroxydocosanoic acid (~5.03 and 14.40 mg g^{-1}, respectively). Most importantly, HDS_{cork} was the only sample bearing non-negligible quantities of ferulic acid (34.28 mg g^{-1}).

Another relevant difference among samples was the pronounced predominance in HEX-MDS_{cork} of both difunctional ω-hydroxyalkanoic and α,ω-alkanedioic acids, with 22-hydroxydocosanoic acid (~86.29 mg g^{-1}) and docosanedioic acid (~25.75 mg g^{-1}) as the most abundant component of each group, respectively (Figure 4.10). Even for DCM-MDS_{cork} where ω-hydroxyalkanoic acids were identified in quantities comparable with the HEX homologue, there were relatively poor amounts of α,ω-alkanedioic acids. The MDS_{ICP} sample also had high contents of ω-hydroxyalkanoic acids, being 22-hydroxydocosanoic acid (~61.05 mg g^{-1}) the most abundant compound identified, and smaller amounts of α,ω-alkanedioic acids.

This GC-MS analysis shows that only part of the isolated depolymerised suberins (Table 4.3) correspond to suberin monomeric components (less than 30%, in agreement with previously reported results for *Quercus suber* cork).[2,14] The high percentage of undetected components was probably related to the presence of a non-volatile fraction, not detectable by GC-MS. A possible explanation is that this fraction could be composed of suberan-type high molecular-mass aliphatic moieties.[15]

The structures of the main components identified by GC-MS are shown in Figure 4.10.

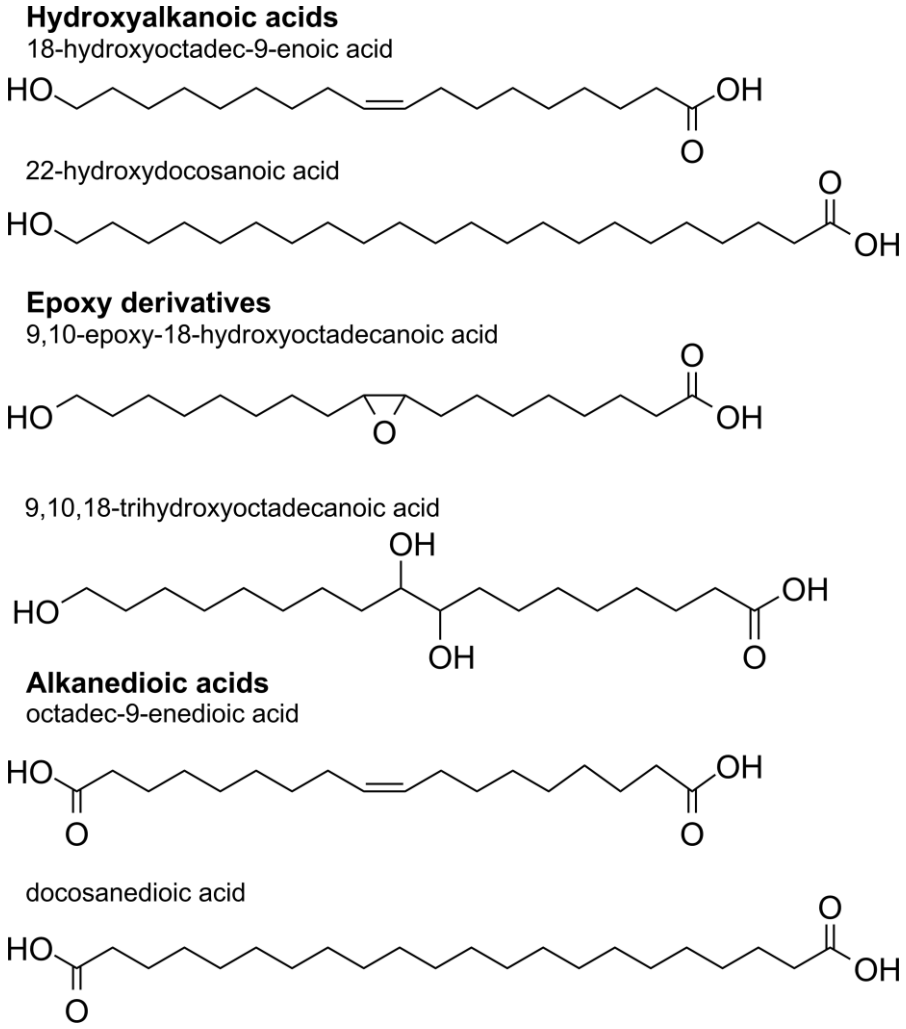


Figure 4.10 Structures of the most abundant compounds identified by GC-MS in depolymerised suberin mixtures.

It is possible to anticipate that if the aim was to prepare predominantly linear polyesters, HEX-MDS$_{cork}$ should be used preferentially (highly rich in 22-hydroxydocosanoic acid). Conversely, if branched or cross-linked counterparts were

preferred, then HDS_{birch} should be the choice precursor, because of its higher content in epoxy derivatives, *e.g.* 9,10-epoxy-18-hydroxyoctadecanoic acid.

4.3.2 Attenuated total reflection Fourier transform infrared analysis

The ATR FTIR analysis of suberin depolymerisation products were consistent with the GC-MS data described above, and obviously with the aliphatic nature of suberin, bearing hydroxy and carboxylic functional groups.

ATR FTIR analysis of HDS samples. Two typical ATR FTIR spectra of HDS suberin samples are shown in Figure 4.11, which displayed two strong bands near 2918 and 2851 cm^{-1} arising from the anti-symmetrical and symmetrical stretching modes of the C-H bond (νCH asym and νCH sym, respectively) of the methylene group, respectively. Additionally, they exhibited a very intense band near 1703 cm^{-1} arising from the C=O stretching vibration (νCO), typical of carboxylic acids and a very broad band around 3463 cm^{-1} arising from the O-H stretching mode (νOH) of alcohols and carboxylic acids, overlapping the C-H stretching bands (3100-2800 cm^{-1}), corroborating the presence of COOH moieties. Its sharper features in the HDS_{birch} spectrum (Figure 4.11) was probably due to the high degree of crystallinity of the birch outer bark samples.[18]

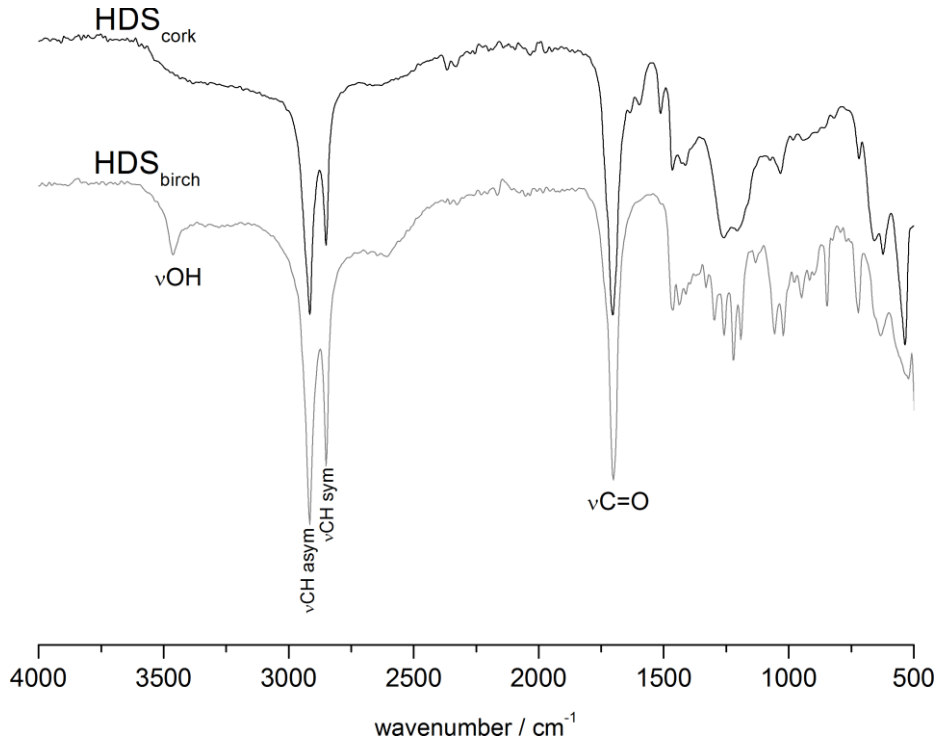


Figure 4.11 ATR FTIR spectra of HDS_{cork} and HDS_{birch} suberin mixtures.

ATR FTIR analysis of MDS samples from cork. The ATR FTIR spectra of MDS suberin samples (Figure 4.12), in accordance with the HDS counterparts, showed two very strong bands νCH asym and νCH sym (2916 and 2850 cm^{-1}, respectively) and a broad νOH band (3456 cm^{-1}). Their spectra also displayed a new band near 1737 cm^{-1} assigned to the C=O stretching of an ester moiety, and no νC=O band of carboxylic acids.

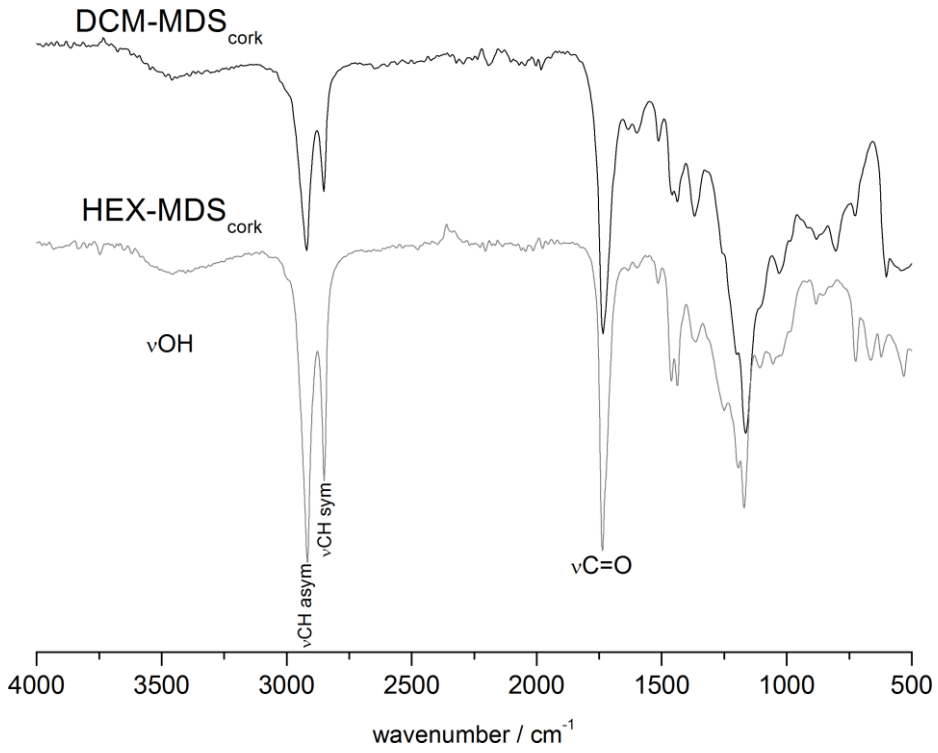


Figure 4.12 ATR FTIR spectra of MDS_{cork} and HEX-MDS_{cork} suberin mixtures.

4.3.3 Nuclear magnetic resonance analysis

Suberin mixtures were characterised by ^{1}H and ^{13}C NMR spectroscopy. Additionally, the ^{1}H NMR analysis was also exploited to assess the relative contents of both carboxylic and both primary and secondary hydroxyl groups in suberin depolymerisation mixtures, after derivatisation with trichloroacetyl isocyanate (TAI).[10]

4.3.3.1 ^{1}H nuclear magnetic resonance analysis of MDS samples

A typical ^{1}H NMR spectrum of MDS_{cork} suberin is shown in Figure 4.13, and the characteristic chemical shifts and integrations of both underivatised HEX- and DCM-MDS_{cork} are given in Table 4.4.

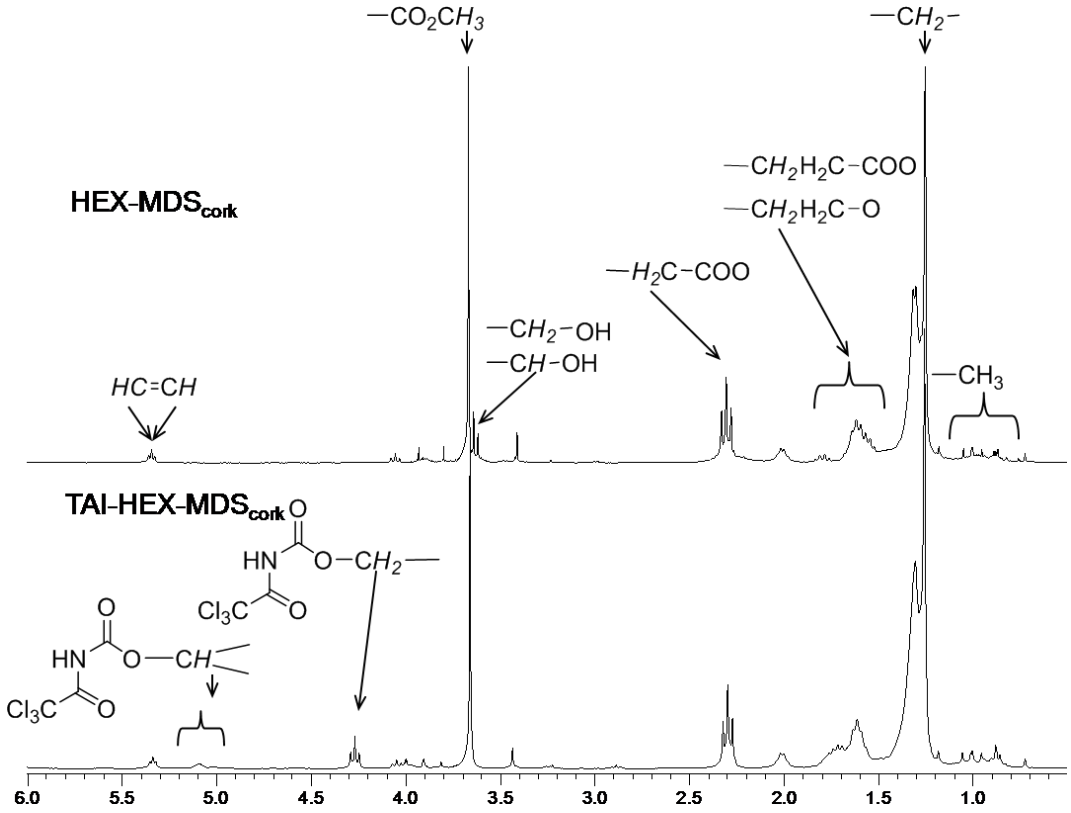


Figure 4.13 ^{1}H NMR spectra of MDS_{cork} before (HEX-MDS_{cork}) and after derivatisation with TAI (TAI-HEX-MDS_{cork}).

These spectra showed, weak resonances at δ 0.72-1.05 ppm, as the most relevant signals, assigned to the C*H*$_3$ protons; an intense multiplet around δ 1.25-1.31 ppm, attributed to C*H*$_2$ protons of the alkylic chains; a multiplet at δ 1.61-1.71 ppm, also typical of C*H*$_2$ protons in the β position to the hydroxy and ester groups (C*H*$_2$CH$_2$O and C*H*$_2$CH$_2$CO$_2$CH$_3$); a weak multiplet at 2.01 ppm, assigned to the allylic C*H*$_2$ protons adjacent to the CH=CH groups; a triplet at δ 2.30 ppm, assigned to the protons of C*H*$_2$CO$_2$CH$_3$ groups; a weak triplet at δ 2.34 ppm, assigned to the C*H*$_2$ protons adjacent to free CO_2H groups, resulting from residual hydrolysis reactions (only present in DCM-MDS_{cork}); and a multiplet at δ ~3.64 ppm, assigned to terminal C*H*$_2$OH protons overlapped with the resonances of mid-chain C*H*OH proton resonance and an intense and sharp singlet at δ 3.66 ppm, assigned to the protons of the CO_2CH_3 groups. Finally, a low intensity triplet at δ 5.34 ppm, corresponding to the protons of the C*H*=C*H* groups was also detected.

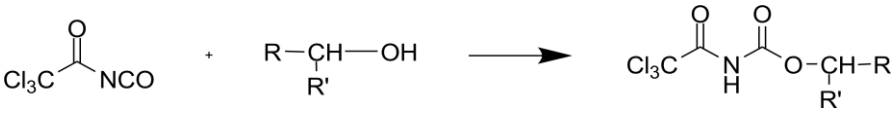
In general, the ^{1}H NMR spectra of MDS were consistent with previously published data[15] and obviously with the aliphatic nature of suberin, dominated by the signals arising

from the aliphatic methylene protons, in the region between 1.25 and 2.01 ppm, typically representing approximately 70% of all protons. The resonances directly related to the OH and $COOCH_3$ functional groups (δ 3.64 and 3.66 ppm, respectively) represented a smaller percentage of all protons, *viz.*, ~10%.

These spectroscopic evidences clearly reflected the formation of depolymerisation products, as already suggested by the GC-MS results (Subsection 4.3.1).

The use of the MDS_{cork} ^1H NMR spectra for the quantitative determination of the reactive functional groups was hampered by the overlapping between CH_2OH and $CHOH$ resonances (δ 3.64 ppm) and also by the partial overlapping between those two peaks and CO_2CH_3 resonances, at 3.64 and 3.66 ppm, respectively (Figure 4.13). This limitation was easily overcome in the ^1H NMR of the TAI derivatised samples.

^1H NMR analysis of the TAI-MDS_{cork} samples. Since the methanolysis depolymerisation mixtures were essentially in the form of methyl esters, only the hydroxy groups reacted with TAI (Scheme 4.10). This condensation was almost instantaneous and occurred with both primary and secondary groups, resulting in the formation of urethane derivatives. In order to ensure complete derivatisation of the hydroxy groups, three different volumes of TAI were tested, 45, 100, and 200 µL for ~15 mg of MDS. It was observed that there was no significant difference among the ensuing TAI-MDS ^1H NMR spectra, which indicated that typically ~45 µL of TAI were sufficient to derivatise all the free OH groups. Given that the TAI molecule is aprotic, when an excess of this reagent is used, no additional resonance appeared in the spectrum.



Scheme 4.10 Reaction between trichloroacetyl isocyanate (TAI) and primary (R' = H) or secondary (R' = alkyl chain) hydroxy groups.

The ^1H NMR spectra of TAI-MDS samples showed essentially the same resonances as their MDS counterparts (illustrated for TAI-HEX-MDS_{cork} in Figure 4.13), except for the resonance assigned to the CH_2OH and $CHOH$ protons, which shifted from δ 3.64 ppm to 4.27 ppm and 5.00-5.09 ppm, respectively, together with a new resonance observed at δ 8.44-10.44 ppm, assigned to NH protons (not shown), in tune with the expected reactions[19] (Scheme 4.10). Hence, the derivatisation procedure allowed the correct integration of CO_2CH_3, CH_2O, and CHO resonances to be assessed, since they

were no longer overlapping. In this way, the ratio between carboxylic and hydroxy groups was determined with accuracy, together with the relative amount of primary and secondary hydroxy groups. Once more, both TAI-HEX and TAI-DCM-MDS_{cork} showed similar ^{1}H NMR profiles, differing only in the resonance integrations (Table 4.4).

Table 4.4 Relevant ^{1}H NMR resonances of MDS samples before (HEX-MDS_{cork} and DCM-MDS_{cork}) and after TAI derivatisation (TAI-HEX-MDS_{cork} and TAI-DCM-MDS_{cork}).

δ / ppm	**assignment**	**[a]multiplicity**	**[b]integration**			
			DCM-MDS_{cork}	**TAI-DCM-MDS_{cork}**	**HEX-MDS_{cork}**	**TAI-HEX-MDS_{cork}**
0.72-1.05	C*H*$_3$	m	21.0	16.6	5.6	6.7
1.25, 1.31	C*H*$_2$	m	87.6	75.0	55.7	59.2
1.61-1.71	C*H*$_2$CH$_2$OH, C*H*$_2$CH$_2$CO$_2$CH$_3$	m	26.6	23.1	14.0	15.1
2.01	C*H*$_2$CH=CH	s	3.3	3.0	2.6	2.5
2.30	C*H*$_2$CO$_2$CH$_3$	t	8.3	6.9	7.8	6.7
3.64	C*H*$_2$OH C*H*OH	m	4.1	-	2.2	-
3.66	CO$_2$C*H*$_3$	s	9.3	7.6	8.6	8.7
4.27	C*H*$_2$O-TAI	t	-	1.5	-	1.9
5.00-5.09	C*H*O-TAI	m	-	1.1	-	0.8
5.34	C*H*=C*H*	t	1.0	1.0	1.0	1.0
8.44-10.44	N*H*	s	-	tr	-	tr

[a] s = singlet; t = triplet; m = multiplet. [b] All values of areas of integration are the average from the spectra of three MDS samples; tr= trace.

4.3.3.2 ^{1}H nuclear magnetic resonance analysis of the HDS samples

The ^{1}H NMR spectra of the HDS samples (Table 4.5) showed the following characteristic signals: weak resonances at *δ* 0.70-1.03 ppm, assigned to the C*H*$_3$ protons; a strong multiplet at *δ* 1.23-1.30 ppm, ascribed to C*H*$_2$ protons of the alkyl chains; a multiplet at *δ* 1.53-1.82 ppm, also typical of C*H*$_2$ protons, but in the β position to the hydroxyl and free carboxylic groups (C*H*$_2$CH$_2$OH and C*H*$_2$CH$_2$CO$_2$H); a weak multiplet at *δ* 1.98 ppm, assigned to the C*H*$_2$ protons adjacent to the CH=CH groups; a weak triplet at *δ* 2.26 ppm, assigned to the protons of the C*H*$_2$CO groups; a triplet at *δ* 2.35 ppm, assigned to the C*H*$_2$CO$_2$H protons; a multiplet at *δ* 3.65 ppm, assigned to the C*H*$_2$ protons of C*H*$_2$OH, overlapped with the resonances of mid-chain C*H*OH proton; and a low intensity triplet at *δ* 5.31 ppm, attributed to the protons of the C*H*=C*H* groups. Additionally, only the HDS_{birch} spectrum displayed a resonance at *δ* 2.90 ppm, attributed to the C*H* protons of the epoxy ring.

Table 4.5 Relevant ^{1}H NMR resonances of HDS samples from cork and birch outer bark before (HDS_{cork} and HDS_{birch}) and after TAI derivatisation (TAI-HDS_{cork} and TAI-HDS_{birch}).

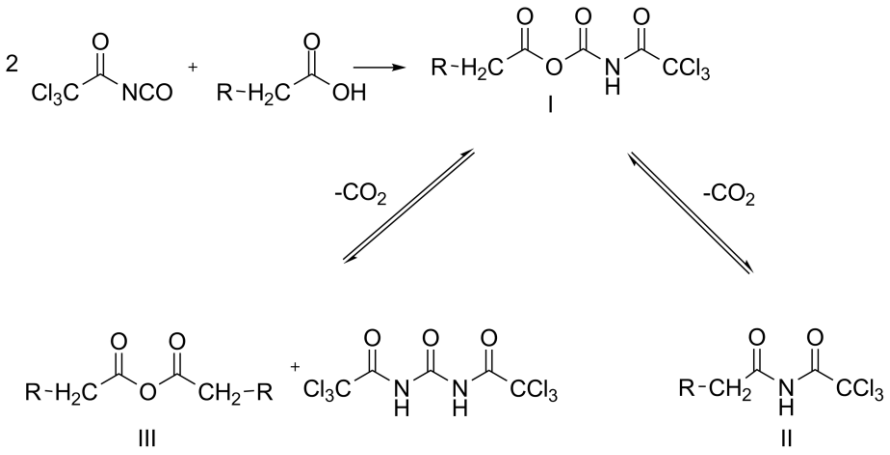
δ / ppm	**assignment**	[a]**multiplicity**	[b]**integration**			
			HDS_{cork}	**TAI-HDS_{cork}**	**HDS_{birch}**	**TAI-HDS_{birch}**
0.70-1.03	**C*H*$_3$**	m	7.6	11.3	1.3	3.7
1.23, 1.30	**C*H*$_2$**	m	48.8	64.2	46.7	67.2
1.53-1.82	**C*H*$_2$CH$_2$OH C*H*$_2$CH$_2$CO$_2$H**	m	12.6	19.4	19.7	26.0
1.98	**C*H*$_2$CH=CH**	s	2.4	3.1	1.7	2.2
2.26	**C*H*$_2$CO**	t	1.3	1.4	tr	tr
2.35	**C*H*$_2$CO$_2$H**	t	5.0	-	5.0	-
2.42	**C*H*$_2$CO$_2$-TAI**	t	-	0.4	-	1.8
2.56	**C*H*$_2$CO$_2$-TAI**	t	-	3.7	-	2.1
2.86	**C*H*$_2$CO$_2$-TAI**	t	-	0.7	-	-
2.90	**C*H* (epoxy)**	m	-	-	2.3	4.8
3.65	**C*H*$_2$OH, C*H*OH**	m	2.3	-	4.6	-
4.24	**C*H*$_2$O-TAI**	t	-	1.8	-	5.6
5.07	**C*H*O-TAI**	m	-	1.0	-	0.7
5.31	**C*H*=C*H***	t	1.0	1.0	1.0	1.0
8.38-10.43	**N*H***	s	-	tr	-	5.4

[a] s = singlet; t = triplet; m = multiplet. [b] All values of areas of integration are the average from the spectra of three MDS samples.

The ^{1}H NMR spectra of HDS samples, like those of their MDS counterparts, were dominated by the signals arising from the aliphatic methylene protons (~80% of all protons), whereas the resonances directly related to the OH and CO_2H functional groups played a modest role (~10% of all protons). The signal related to the epoxy rings also played a very modest role (3% of all protons) and was only detected in the HDS_{birch} sample. All these results agree with the previous GC-MS findings, which indicated that depolymerisation mixtures are composed of structures in which aliphatic chains dominate, but which bear polar groups, mainly constituted of hydroxy and carboxylic moieties. In addition, epoxy groups are regular moieties found in suberin, especially, in accordance with GC-MS results, in HDS_{birch}.

The partial overlapping of C*H*$_2$OH and C*H*OH resonances at 3.65 ppm and C*H*$_2$CO$_2$H and C*H*$_2$CO resonances at 2.26-2.35 ppm in HDS samples again hampered the direct quantitative determination of the ratios CO_2H/OH and between primary and secondary OH's. This limitation was again overcome in the corresponding TAI-HDS spectra.

^{1}H NMR analysis of the TAI-HDS samples. With the HDS samples, TAI reacted with both the hydroxy and the free carboxylic groups. As shown in Scheme 4.11, the reaction between CO_2H and TAI gives derivative **I** as the main product. However, its partial decarboxylation results in the formation of amide **II** and anhydride **III**, with the elimination of a biuret.[19]



Scheme 4.11 Reaction between TAI and a carboxylic acid function.

The ^{1}H NMR spectra of TAI-HDS were similar to those of their HDS counterparts (Table 4.5), differing only in the resonances associated with the functional groups. These new resonances were a weak triplet at *δ* 2.42 ppm, assigned to the C*H*$_2$ protons adjacent to the amide group (**II**); a triplet at δ 2.56 ppm, attributed to the protons of the C*H*$_2$ groups adjacent to the urethane (**I**) moiety; a weaker triplet at *δ* 2.86 ppm, attributed to the C*H*$_2$ protons adjacent to the anhydride group (**III**); a triplet at *δ* 4.24 ppm, assigned to the protons of the C*H*$_2$O-TAI groups; a multiplet at *δ* 5.07 ppm, attributed to the proton of the mid-chain C*H*O-TAI groups; and weak resonances at *δ* 8.38-10.43 ppm, assigned to the N*H* proton. The resonances at *δ* 2.42, 2.56, and 2.86 ppm, related to protons adjacent to the derivatised COOH groups, and the resonances at *δ* 4.24 and 5.07 ppm, from protons adjacent the derivatised OH groups, were used for their quantitative determinations.

4.3.3.3 Determination of the carboxylic and hydroxy functional group ratios

The ratio between the number of carboxylic and hydroxy groups (*r*) present in the different suberin fragments was determined using the ^{1}H NMR results of the TAI-derivatised samples. In the TAI-MDS$_{cork}$ samples, *r* was simply calculated from the ratio between the integration area of the resonances of the OC*H*$_3$ protons ($A_{\mathrm{CO_2}CH_3}$) at δ 3.66 ppm and those of the C*H*$_2$ and C*H* protons adjacent to the derivatised hydroxy groups ($A_{CH_2\mathrm{O-TAI}}$, $A_{CH\mathrm{O-TAI}}$, respectively) at *δ* 4.27 ppm and *δ* 5.00–5.09 ppm, respectively, *viz.*,

$$r \approx \frac{\left[A_{\mathrm{CO_2CH_3}}/3\right]}{\left[A_{\mathrm{CH_2O-TAI}}/2+A_{\mathrm{CHO-TAI}}\right]} \quad \textbf{4.12}$$

In the TAI-HDS samples, the equation

$$r \approx \frac{\left[A_{\mathrm{CH_2CO_2-TAI}}/2\right]}{\left[A_{\mathrm{CH_2O-TAI}}/2+A_{\mathrm{CHO-TAI}}\right]} \quad \textbf{4.13}$$

was used instead, where $A_{\mathrm{CH_2CO_2-TAI}}$ is the sum of the integration areas of the resonances of the CH_2 protons adjacent to the derivatised COOH at δ 2.42, 2.56, and 2.86 ppm, $A_{\mathrm{CH_2O-TAI}}$ is the integration area of the resonances of CH_2 protons adjacent to the derivatised primary hydroxy groups, at δ 4.24 ppm, $A_{\mathrm{CHO-TAI}}$ is the integration area of the resonances of C*H* protons adjacent to the derivatised secondary hydroxy groups, at δ 5.07 ppm. If ring opening of the epoxy moieties present in HDS_{birch} takes place, the *r* value should be recalculated by the following equation,

$$r \approx \frac{\left[A_{\mathrm{CH_2CO_2-TAI}}/2\right]}{\left[A_{\mathrm{CH_2O-TAI}}/2+A_{\mathrm{CHO-TAI}}+A_{\mathrm{CH}}\right]} \quad \textbf{4.14}$$

where A_{CH} is the integration area of the resonance of C*H* protons of the epoxy ring at δ 2.90 ppm.

The average values of *r* for each suberin sample are given in Table 4.6.

Table 4.6 Results of ^{1}H NMR analysis of TAI-derivatized suberin: depolymerised suberin; ratio between the number of carboxylic and hydroxy groups (r); and standard deviation (σ).

	[a] ***r***	***σ***
DCM-MDS$_{cork}$	1.43	0.17
HEX-MDS$_{cork}$	1.67	0.04
HDS$_{cork}$	1.27	0.12
HDS$_{birch}$	[b]0.56 or 0.33	0.04

[a] Each *r* value was calculated as an average of the ^{1}H NMR spectra of three aliquots of each suberin sample.
[b] *r* = 0.56 or 0.33, correspond to the *r* value calculated if ring opening of the epoxy moieties has not occurred or the opposite.

All cork-derived samples showed more carboxylic than hydroxy groups, with the higher values obtained for MDS_{cork}, *viz.* between 1.43 and 1.67, depending on the solvent used in the extraction step. On the contrary, the birch outer bark HDS displayed more hydroxy than carboxylic acid groups ($r \approx 0.56$). The presence of epoxy rings in HDS_{birch} represents a substantial additional source of OH groups. Thus, if ring opening of the

epoxy moieties occurs, a substantial increment in OH functionality arises and the *r* value decreases from 0.56 to 0.33.

The *r* value for cork disagrees considerably with those from GC-MS analysis of suberin fragments, which gave systematically *r* < 1 (Subsection 4.3.1 and reference 14). This discrepancy can be rationalised by the fact that the GC-MS results bore an intrinsic limitation associated with the fact that only about 30% of fragments were in fact identified, as opposed to the present spectroscopic analysis of the TAI-derivatised samples, in which the whole mixture was inspected. Therefore, it is possible to conclude that all the cork-derived suberin extracts studied in this work bore a higher content of CO_2H (or CO_2CH_3) groups compared with the OH counterparts.

The ^{1}H NMR analysis of the TAI-derivatized suberin samples were also used to determine the relative abundance of primary and secondary hydroxy groups. These proportions were calculated by the ratio $[A_{CH_2O-TAI}/2]/[A_{CHO-TAI}]$. However, if ring opening of the epoxy moieties of HDS_{birch} takes place, this ratio should be calculated instead by the ratio $[A_{CH_2O-TAI}/2]/[A_{CHO-TAI}+A_{CH}]$. Results summarised in Table 4.7 showed that HDS_{birch} displayed the highest relative amount of primary OH groups (3.83), followed by HEX-MDS_{cork}, HDS_{cork}, and DCM-MDS_{cork} (1.23, 0.86, and 0.66, respectively). The use of the fractionation step with *n*-hexane thus led to an increase in the $A_{CH_2O-TAI}/A_{CHO-TAI}$ ratio. The ratio corresponding to the HDS_{birch} decreases drastically, if the epoxy ring opening takes place, from 3.83 to 0.89.

Table 4.7 Results of ^{1}H NMR analysis of TAI-derivatised suberin: depolymerised suberin; ratio between the number of primary and secondary hydroxy groups $[A_{CH_2O}/A_{CHO}]$; and standard deviation (σ).

	[a] A_{CH_2O}/A_{CHO}	σ
DCM-MDS_{cork}	0.66	0.04
HEX-MDS_{cork}	1.23	0.12
HDS_{cork}	0.86	0.05
HDS_{birch}	[b] 3.83 or 0.89	0.32

[a] Each ratio was calculated as an average of the ^{1}H NMR spectra of three aliquots of each suberin sample.
[b] Ratios correspond to values calculated if ring opening of the epoxy moieties has not occurred or the opposite.

The knowledge of the precise quantity of functional groups present in suberin fragments is essential, not just for the present detailed characterisation, but also, and especially, in the context of their use as monomers in the preparation of polyesters, where

the relative content of primary and secondary alcohols could have an important influence on the properties of the ensuing materials. In fact, the alternative use of predominantly ω-hydroxy fatty acids or mid-chain hydroxy fatty acids (primary or secondary OH groups, respectively) as monomers for the synthesis of polyesters will necessarily have a significantly influence in the physical properties of the ensuing polymers, namely their crystallinity (through the packing ability) and melting temperature, among other properties.

Moreover, as mentioned in Chapter 3, an *r* value of unity is essential to ensure optimum yields and the highest molecular weights of the resulting polymers. Hence, in this work the various depolymerised suberin mixtures need to be balanced through the addition of an appropriate OH-bearing comonomer for cork suberin samples, and of a CO_2H-bearing comonomer for the birch outer bark suberin counterparts.

These results clearly showed the potentiality of this method to access the functionality ratios in depolymerisation mixtures of suberins from different species, or of other natural sources of hydroxyacids (*e.g.*, lesquerella oil or castor oil).

4.3.3.4 ^{13}C nuclear magnetic resonance analysis of the suberin samples

The ^{13}C NMR spectra of suberin mixtures confirmed the main features of the ^{1}H NMR counterpart. Table 4.8 summarises the characteristic chemical shifts of all MDS suberin samples.

Table 4.8 Main peaks from ^{13}C NMR spectra of methanolysis-depolymerised suberin from cork.

assignment	δ / ppm	
	MDS_{cork}	$HEX\text{-}MDS_{cork}$
$\boldsymbol{C}H_3$	14	14
$\boldsymbol{C}H_2$	23-30	25-30
$\boldsymbol{C}H_2COCH_3$	34	34
$O\boldsymbol{C}H_3$	51	51
$\boldsymbol{C}H_2OH$	64	64
$\boldsymbol{C}HOCH_3$	-	69
$\boldsymbol{C}HOH$	74	74
$\boldsymbol{C}=\boldsymbol{C}$	130	130
$\boldsymbol{C}O_2CH_3$	174	174

The most relevant signals included several resonances around $\delta \approx$ 23-34 ppm, assigned to the CH_2 carbons in different chemical environments; a resonance around δ 51 ppm, attributed to the carbon of OCH_3 group, a resonance around δ 64 ppm, attributed to

the carbon of CH_2OH group, a signal at δ ~130 ppm corresponding to the carbons of the *C*=*C* group, and a resonance around δ 174 ppm, assigned to the carbon of the carbonyl group of a CO_2CH_3.

The main difference between the spectra of MDS (Table 4.8) and HDS's (Table 4.9 and Figure 4.14) suberins was related to the fact that the former samples were isolated as methyl esters of carboxylic acids, instead of free carboxylic acids.

Table 4.9 Main peaks from the ^{13}C NMR spectra of hydrolysis-depolymerised suberin from cork, and birch outer bark.

assignment	**δ / ppm**	
	HDS_{cork}	**HDS_{birch}**
CH_3	14	-
CH_2	23-32	25-30
CH_2CH (epoxy)	-	33
CH_2CO_2H	34	34
CH (epoxy)	-	57
CH_2OH	63	63
C*=*C	130	130
CO_2H	180	180

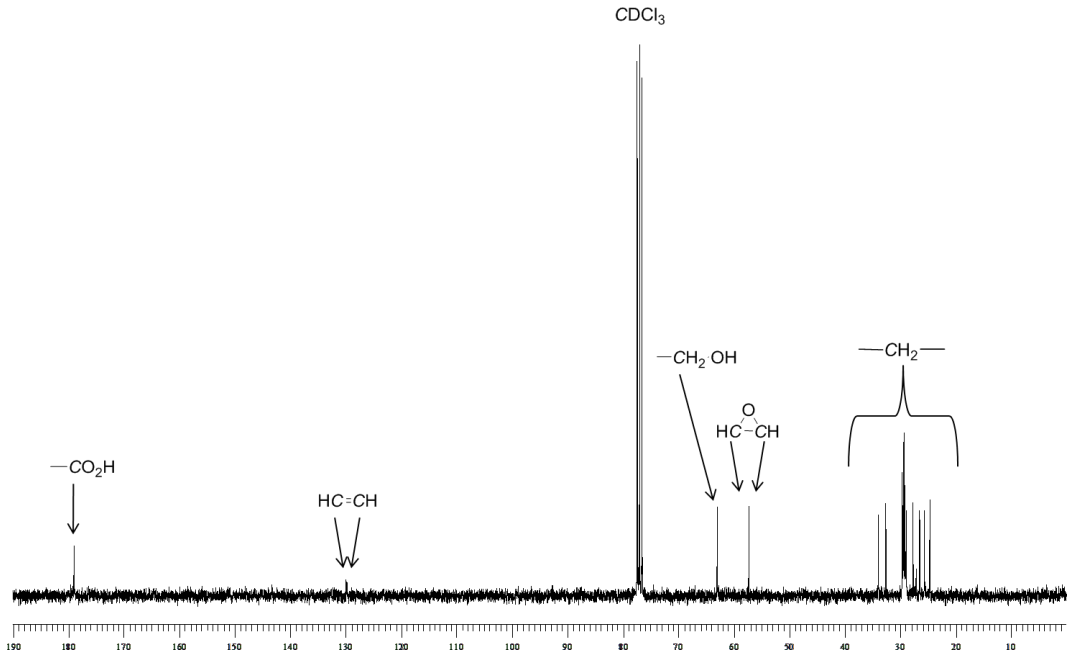


Figure 4.14 Typical ^{13}C NMR spectrum of HDS_{birch}.

Indeed, the HDS spectra showed a new resonance around δ 180 ppm, assigned to the carbon of the carbonyl group of a carboxylic acid. Accordingly, the resonance around

δ 174 ppm, attributed to the CO_2CH_3 carbon, was not observed. Additionally, the HDS_{birch} spectrum displayed the typical resonances of the epoxy ring at δ 33 and 57 ppm (Figure 4.14), assigned to the carbon of a CH_2 group adjacent to an epoxy ring and to the *C*H carbon of an epoxy group, respectively.

Although suberin is a very complex material, its detail characterisation showed which monomers were the most abundant in each suberin mixture anticipating the most probable ensuing polymer structures.

4.4 Physical properties of depolymerised suberin

The GC-MS, ATR FTIR and NMR analyses of suberin fragments were enlightening about the chemical composition of this complex material, and most importantly they pointed out relevant differences between samples. Several other techniques were used to complement this study, including thermal analysis and X-ray diffraction. Hence, a more detailed chemical and physical picture of suberin emerged, allowing a sound assessment about its use in the preparation of biopolyesters and even about the most probable properties of the ensuing materials.

4.4.1 Differential scanning calorimetry and dynamical mechanical analysis

Typical DSC thermograms of depolymerised suberin are shown in Figures 4.15 and 4.16, whereas the glass transition temperature (T_g), crystallisation temperature (T_c), and the melting temperature (T_m) values are summarised in Table 4.10.

Both HEX-MDS_{cork} and HDS_{birch} suberins exhibited important crystalline domains, as suggested by well-defined melting peaks in their DSC thermograms. HEX-MDS_{cork} thermogram (Figure 4.15) displayed, on heating, two broad peaks around 24 and 43 ^oC and a sharper peak around 69 ^oC together with a very subtle transition around -50 ^oC most probably a glass transition associated with the amorphous phases (see below). In the case of HDS_{birch}, the corresponding thermogram (Figure 4.16) displayed, in the heating trace, a well defined crystallisation peak around 27 ^oC, followed by a sharp melting peak around 70 ^oC. These results compared favourably with those obtained with the pure 9,10-epoxy-18-octadecanoic acid (T_m = 77.6 ^oC), the most abundant fragment identified by GC-MS in HDS_{birch} suberin.

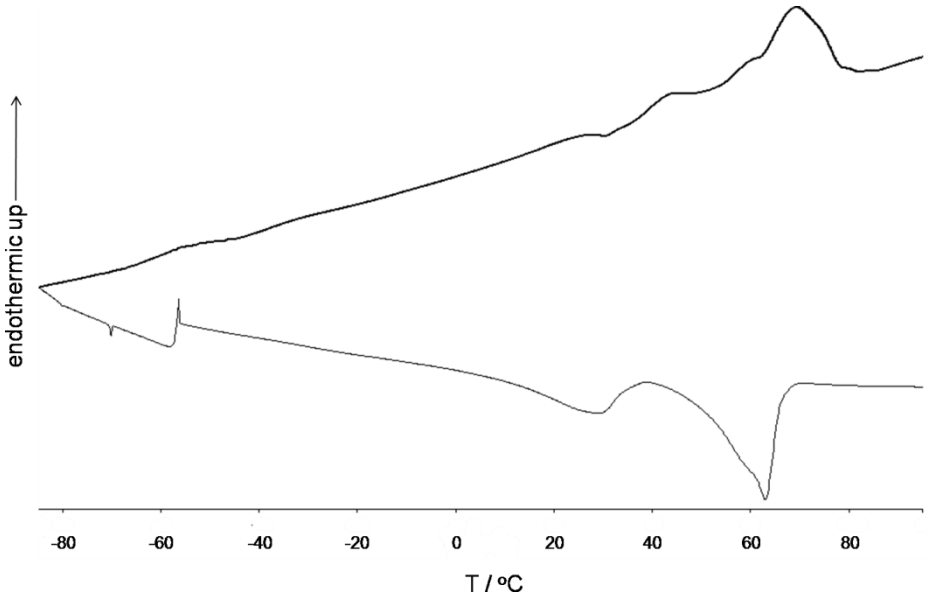


Figure 4.15 DSC thermograms of the first heating (black trace) and cooling (grey trace) scans of HEX-MDS_{cork}.

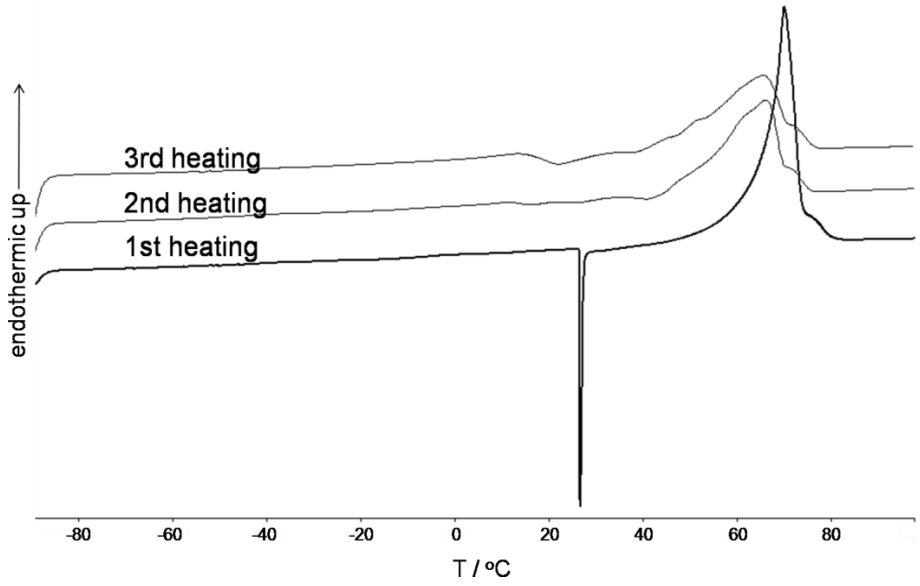


Figure 4.16 DSC thermograms of the first (black trace), second and third (grey traces) heating scans of HDS_{birch}.

The other suberin mixtures, HDS_{cork} and DCM-MDS_{cork}, on the contrary, exhibited a low degree of crystallinity, as indicated by their DSC thermograms and corroborated by their X-ray diffraction patterns (Subsection 4.4.4). The DSC thermograms exhibited extremely broad melting peaks, over a large range of temperatures (roughly 20 to 80 ^{o}C). This behaviour is in tune with the HDS_{cork} and DCM-MDS_{cork} complex composition, already suggested by their GC-MS analysis, making their crystallisation more difficult. The main

features of the DSC cooling traces of all suberin samples were, in general, in agreement with their heating counterparts (except for a slightly better resolution), as can be observed in Figure 4.15, a fact that corroborated the main attributions described below.

The thermal features of all suberin samples were quite reproducible after several heating and cooling cycles. Indeed, the second cycle was always very similar to the subsequent cycles, as shown in Figure 4.16 for HDS_{birch}. Furthermore, for any given sample, after a resting period of 13 days the heating and cooling traces were equivalent to those observed in the first series of scans.

Table 4.10 The glass transition temperature (T_g), crystallisation (T_c) and melting (T_m) temperatures of the suberin samples.

	[a] T_g / °C	[b] T_c / °C	[b] T_m / °C	[c] T_c / °C
HDS_{cork}	-39.4	-	25.7, 36.9, 61.7, 78.2	31.84, 59.04, 66.79
HDS_{birch}	-30.7	26.8	70.1	23.00, 63.42
[d] $DCM\text{-}MDS_{cork}$				
$HEX\text{-}MDS_{cork}$	-46.3	-	23.50, 43.14, 69.11	32.14, 66.42

[a] *Tg* was determined by DMA at 1 Hz using the tan δ maximum. [b] T_c and T_m were determined by DSC at 10 °C min^{-1} (1st heating trace). [c] T_c was determined by DSC at 10 °C min^{-1} (1st cooling trace). [d] Not determined.

The glass transition temperatures of all suberins were determined by DMA because this technique (although requiring a substantial amount of sample) has a higher sensitivity to the glass transition process compared with DSC measurements. A typical DMA thermogram is shown in Figure 4.17.

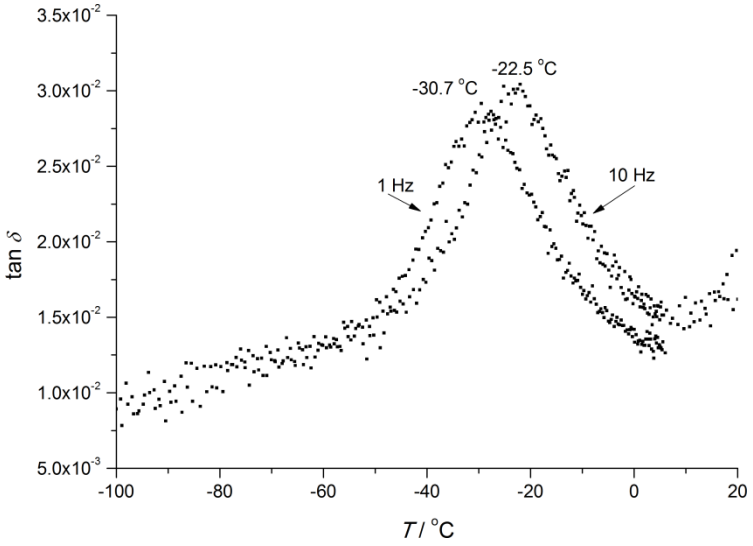


Figure 4.17 HDS_{birch} DMA thermogram showing tan δ *vs. T* and at an applied frequency of 1 and 10 Hz.

Additionally, DMA measurements were carried out at two frequencies (1 and 10 Hz), in order to confirm the nature of the relaxation process. Since shifts were in the range of 8 ^{o}C per decade of frequency, they were most certainly glass transitions. All suberins exhibited T_g values below room temperature (between -46.3 and -30.7 ^{o}C).

4.4.2 Polarised light thermal microscopy analysis

The thermal behaviour of HEX-MDS$_{cork}$ and HDS$_{birch}$ samples were also followed by polarised light thermal microscopy, a valuable tool to study phase transitions, since the visual observation gave important additional information.[20-21] This was especially true because the DSC curves of the depolymerised suberins were very complex. The association of thermomicroscopy results with the information obtained by DSC, allowed a deeper insight into the multiple thermal transitions that took place when the samples were subjected to thermal cycles.

The direct observation of HEX-MDS$_{cork}$ samples, at room temperature, under polarised light, highlighted different regions, as illustrated in Figure 4.18. HEX-MDS$_{cork}$ displayed important crystalline domains (anisotropic material) corresponding to the brighter regions, with several different colours (birefringence of the polarised light); together with amorphous domains (isotropic material) corresponding to the blue background (Figure 4.18). Dark brown areas also appeared, probably corresponding to a non-homogeneous dispersion of the sample in the quartz holder.

As the temperature was raised from ~20 to 100 ^{o}C, several distinct phenomena were observed. The first alteration was a very slow fusion process of part of the material, beginning slightly above 40 ^{o}C (see picture of HEX-MDS$_{cork}$ at $T \approx 40.3$ ^{o}C in Figure 4.18. From around 60 ^{o}C to 85 ^{o}C, another fusion of the brighter domains was clearly observed ($T \approx 72.6$ ^{o}C, Figure 4.18). It began very slowly and only part of the material melted until approximately 66 ^{o}C. Thereafter, a very quick fusion process of a different microcrystalline domain and the fluidisation of the blue matrix occurred, with a visible movement of the melted material. Observation of the system above 85 ^{o}C consisted only in a continuous uniform blue liquid phase, corresponding to the background ($T \approx 88.2$ ^{o}C, Figure 4.18). Interestingly, the DSC trace had also showed endothermic transitions around 43 and 69 ^{o}C.

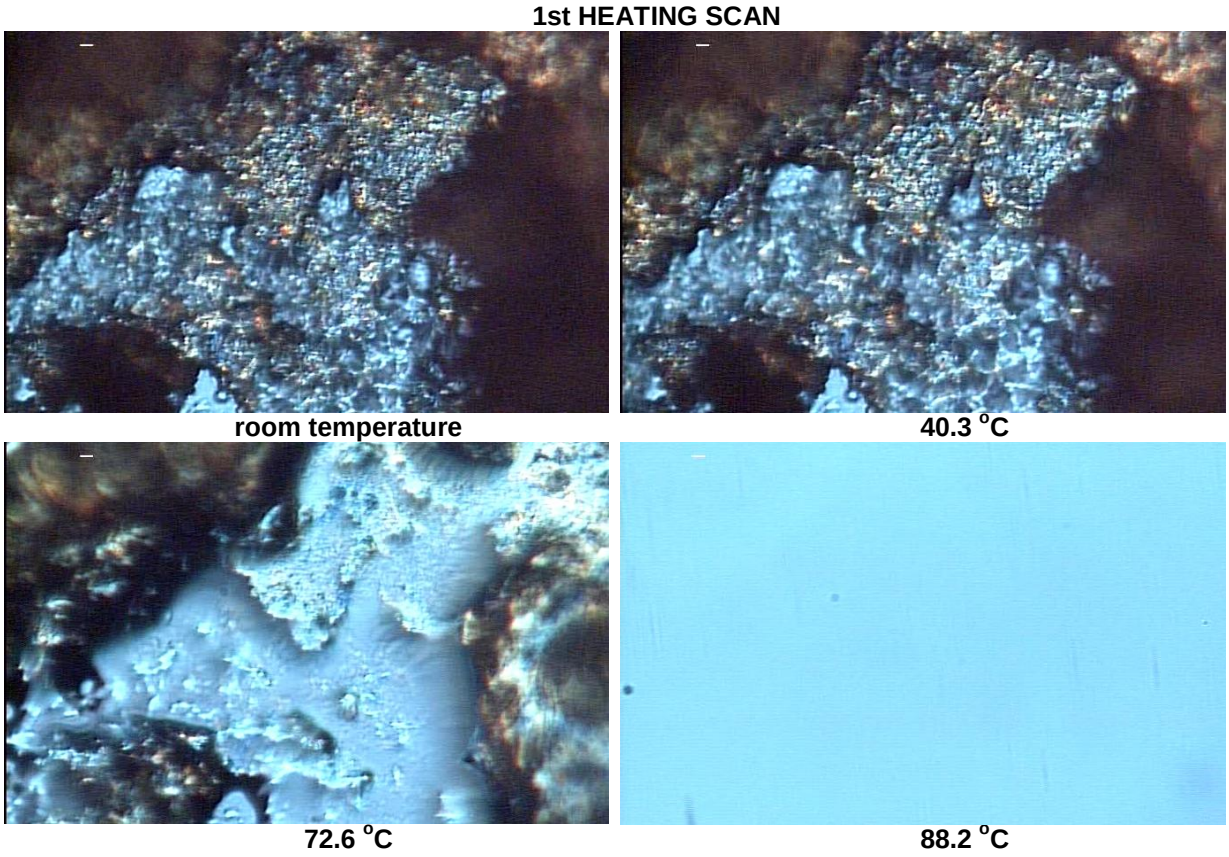


Figure 4.18 HEX-MDS$_{cork}$ suberin observed under polarised light thermal microscopy, during the 1st heating scan, at the indicated temperatures.

Then the sample was subjected to a cooling cycle from 100 to -100 °C (Figure 4.19). At around 70 °C the first crystals began to form (T ≈ 70.2 °C, Figure 4.19), and the number of isotropic crystals increased very rapidly as the temperature was lowered to ~62 °C (T ≈ 62.3 °C, Figure 4.19). Below 60 °C the crystallization continued through a slow formation of crystalline aggregates (T ≈ 40.5 °C in Figure 4.19) down to room temperature. The ensuing texture of the sample at room temperature was heterogeneous, having coloured crystals dispersed in a deep blue matrix as shown in Figure 4.19. Clearly, the sample did not retain the original appearance at room temperature before the heating and cooling cycle (Figure 4.18 and Figure 4.19).

From around 20 °C down to -67 °C, no visible alteration of the system was noted (pictures not shown). Around -67 °C a fracture pattern appeared probably related to the glass transition associated with the amorphous domains. Consistently, the DSC thermogram displayed two endothermic events, *i.e.,* a well-defined peak around 66°C and a broader peak at about 32 °C. Also, present was a small peak close to -53 °C, probably due to a mechanical event, like the fracture observed by thermomicroscopy.

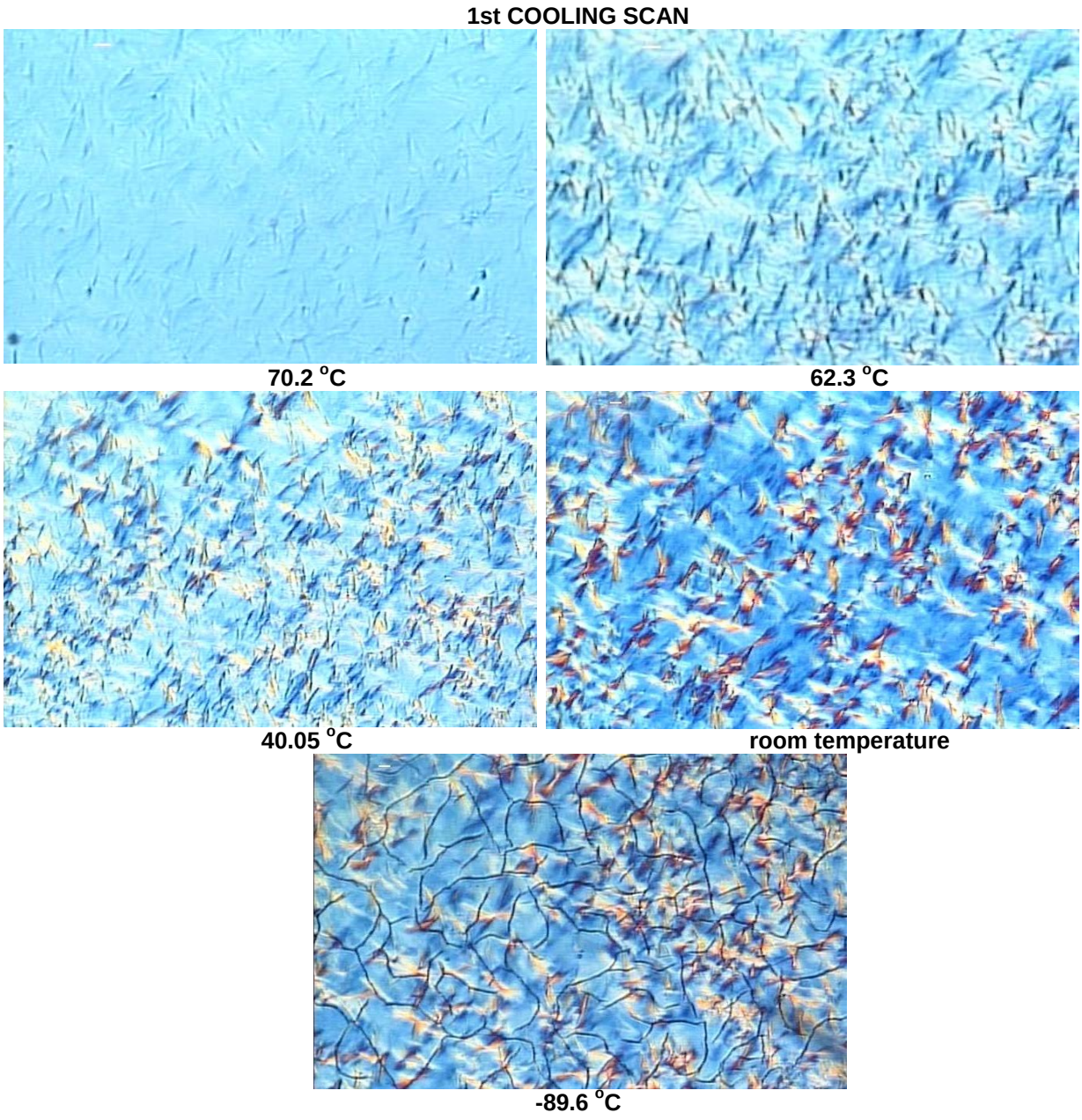
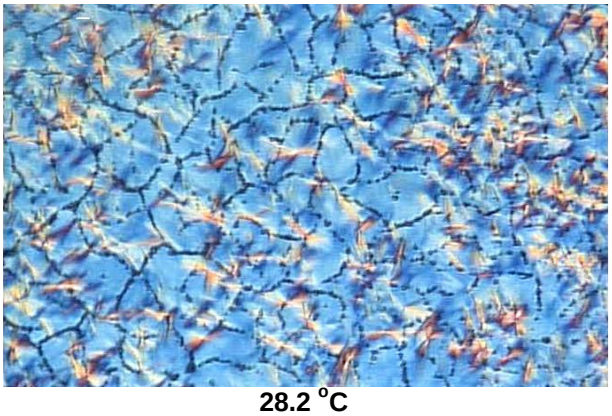


Figure 4.19 HEX-MDS$_{cork}$ suberin observed under polarised light thermal microscopy, during the 1st cooling scan, at the indicated temperatures.

The second and third heating and cooling cycles showed events similar to those observed in the first, but the fracture pattern (not present in the 1st cycle) in the second heating began to disappear around ~ 20 °C (Figure 4.20), and only vanished completely at about 74 °C in simultaneous with other events, as the crystals melting and the matrix fluidisation.

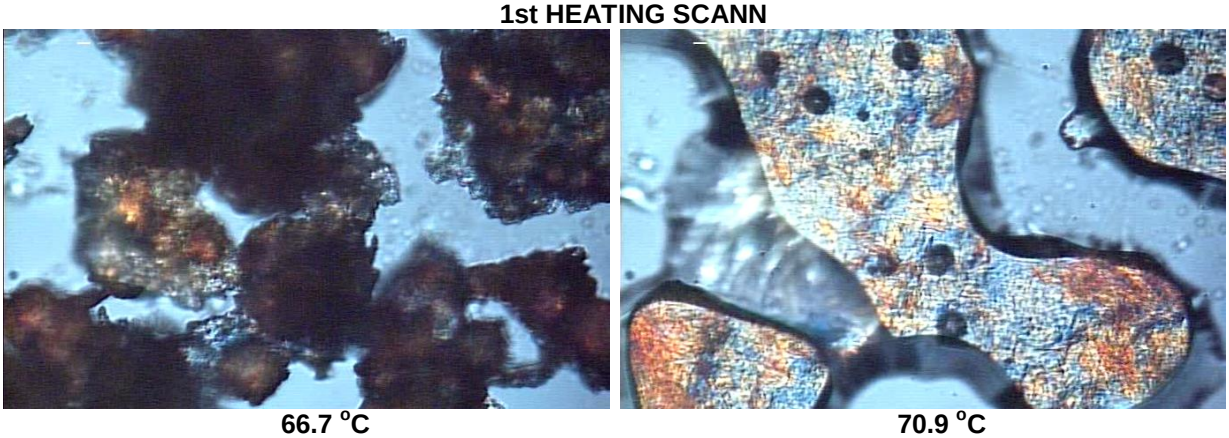


28.2 °C

Figure 4.20 HEX-MDS_{cork} suberin observed under polarised light thermal microscopy during the 2nd heating scan, at 28.2 °C.

The pristine HDS_{birch} suberin displayed at room temperature an overall opaque appearance (pictures not shown), preventing the observation of any clear-cut features. This was most probably because the sample was a solid powder at this temperature. When it was heated up to 100 °C, a sluggish fusion process of part of the material was the first observed feature, between 64 and 67 °C (T ≈ 66.7°C, Figure 4.21). At about 70 °C, another melting event occurred very rapidly, with movement of the liquid matrix carrying some residual crystals (T ≈ 70.9 °C, Figure 4.21). Above 84 °C, only a continuous uniform blue liquid phase was observed (not shown).

1st HEATING SCANN



66.7 °C **70.9 °C**

Figure 4.21 HDS_{birch} suberin observed under polarised light thermal microscopy during the 1st heating scan, at the indicated temperatures. The black circles at the right of the picture are solvent residues trapped in the bulk of the suberin.

During the cooling scan from 100 to -100 °C, the first visible alteration was the appearance of the first crystals near 65 °C (T ≈ 65.0 °C, Figure 4.22). The number of isotropic crystals then increased and grew in size until about 57 °C (T ≈ 57.0 °C, Figure

4.22). When the sample approached 20 °C, a very subtle event occurred, where the texture of the blue matrix became less rough and brighter domains appeared ($T \approx 19.2$ °C, Figure 4.22). The DSC trace of HDS_{birch} showed consistently two exothermic peaks at ~63 and 23 °C.

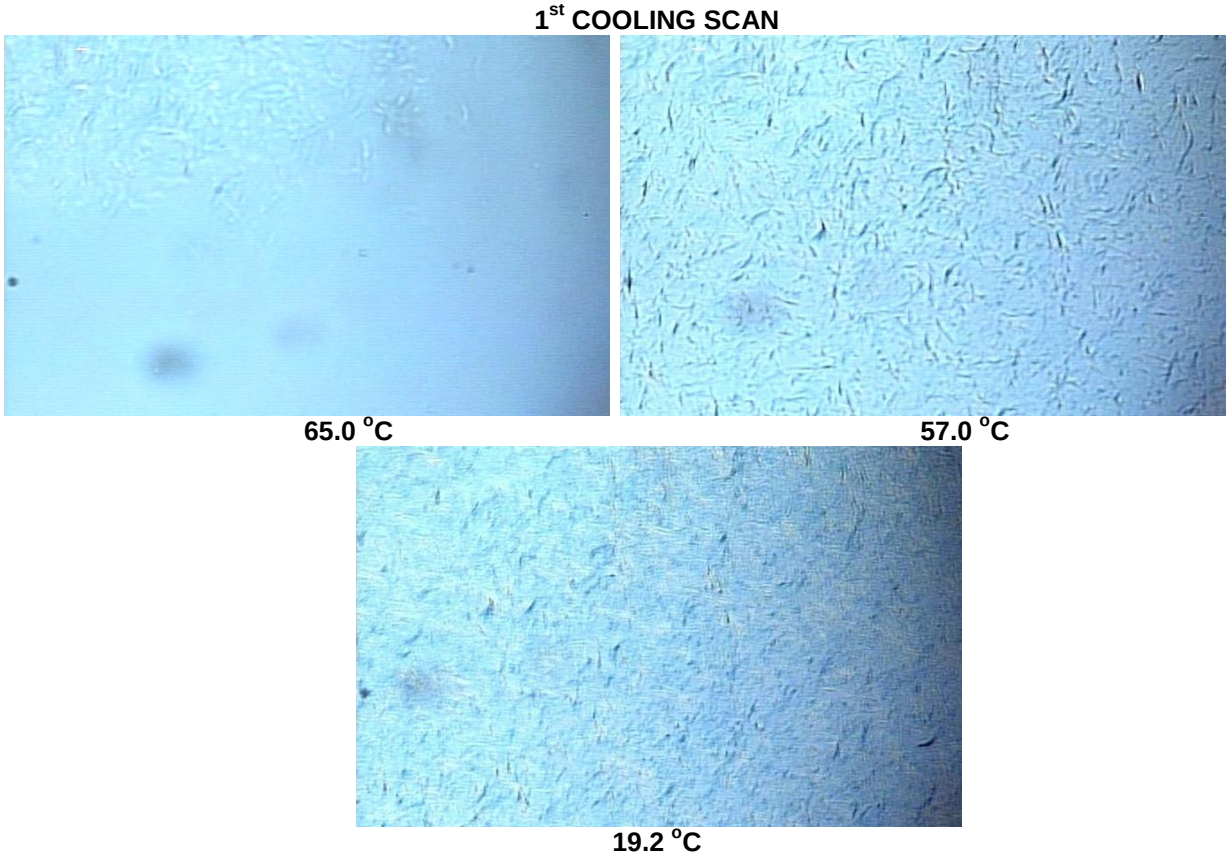
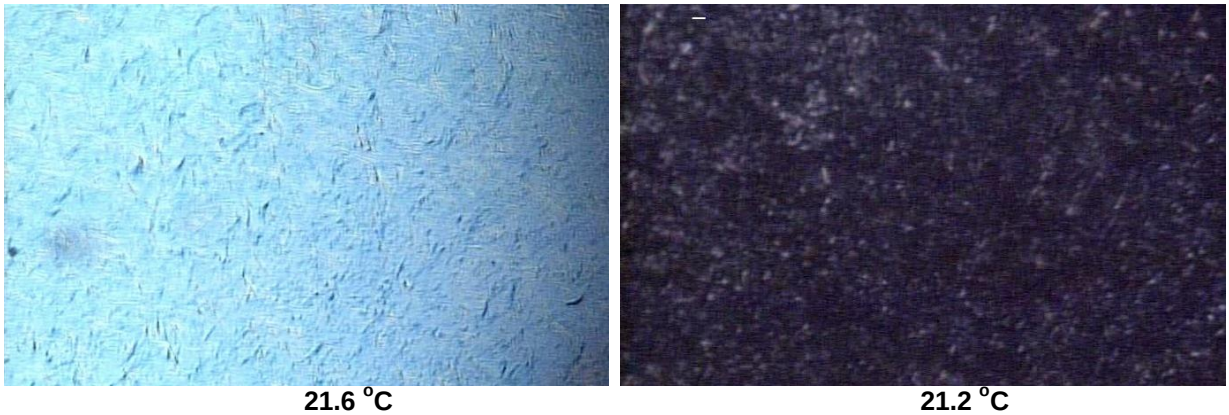


Figure 4.22 HDS_{birch} suberin observed under polarised light thermal microscopy during the 1st cooling scan, at the indicated temperatures.

The cooling scan highlighted the different domains of HDS_{birch} suberin at room temperature, *viz.* a mixture of coloured crystalline domains and an amorphous/isotropic blue phase ($T \approx 21.6$ °C, Figure 4.23). When cross-polarised light was applied, a black amorphous matrix became visible together with small bright spots corresponding to the crystalline material ($T \approx 21.2$ °C, Figure 4.23).



21.6 °C **21.2 °C**

Figure 4.23 HDS_{birch} suberin observed under polarised light thermal microscopy during the first cooling scan (Left) without or (Right) with the use of cross-polarisers, at the indicated temperatures.

The samples with a lower degree of crystallinity, namely HDS_{cork} and MDS_{cork}, were not investigated in detail, because preliminary results had demonstrated that it was quite difficult to study the thermal behaviour of these samples by this technique, since the phenomena were obscured by their strong brown colour.

4.4.3 Thermogravimetric analysis

TGA was used to investigate the thermal decomposition behaviour of the suberin samples under a nitrogen atmosphere. All appeared to be thermally stable up to 187 °C (weight loss lower than 2%) and thereafter degraded essentially in three distinct steps (Table 4.11 and Figure 4.24). The former threshold temperature provided an indication of the upper limiting temperature at which the polyesterification reactions could be carried out, without promoting any thermal degradation (Chapters 7 and 8).

Table 4.11 Degradation temperatures (T_d) of suberins.

	T_d / °C
HDS_{cork}	384, 438, 572
HDS_{birch}	265, 427, 456
MDS_{cork}	379, 437, 605
HEX-MDS_{cork}	314, 420, 458

Figure 4.24 shows a typical TGA thermogram of HDS_{cork}, with the three characteristic features, beginning at about 384 °C, followed by another weight loss around 438 °C, and a third weight loss at ~572 °C. These TGA measurements revealed a total weight loss of 93% between 188 and 587 °C.

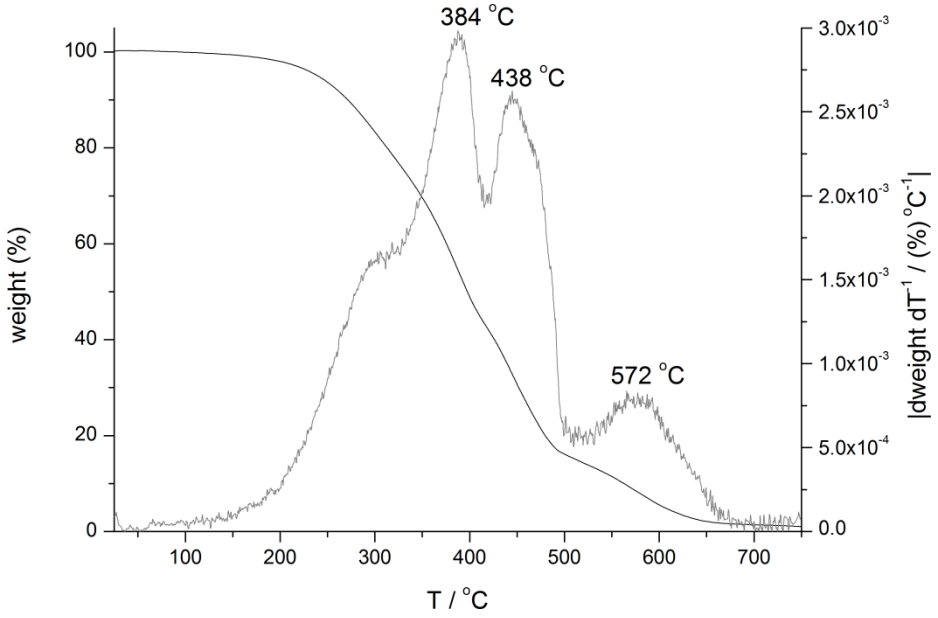


Figure 4.24 TGA and derivative TGA thermograms of HDS_{cork}.

4.4.4 X-ray diffraction analysis

The HDS_{birch} and $HEX\text{-}MDS_{cork}$ samples displayed a high degree of crystallinity, as indicated by their thermal analysis (Subsections 4.4.1 and 4.4.2) and corroborated by their XRD diffractions. Both gave rise to patterns with sharp peaks, as depicted in Figure 4.25 (a) and (b).

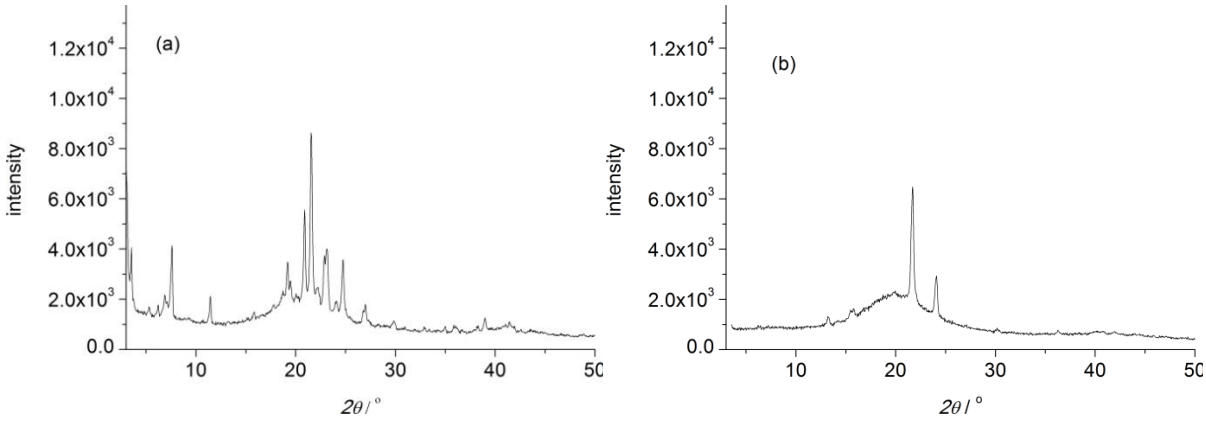


Figure 4.25 XRD patterns of (a) HDS_{birch} and (b) $HEX\text{-}MDS_{cork}$.

In the case of HDS_{birch}, the diffractogram (Figure 4.25 (a)) bore several signals between $2\theta \approx 3\text{-}43^{o}$, showing a complex pattern that could be considered as a combination of XRD patterns of several suberin compounds. Indeed, the combination of patterns of suberin model compounds, *viz.* decanedioic acid, 1,12-dodecanediol, 12-hydroxydodecanoic acid, 12-hydroxyoctadecanoic acid, and 9,10-epoxy-18-octadecanoic

acid, resembled the original HDS_{birch} pattern (Figure 4.26). This strongly suggested the presence of several different microcrystalline domains. Nevertheless, the single pattern which most resembled the HDS_{birch} counterpart was that of the most abundant compound found in this sample by GC-MS, i.e. the 9,10-epoxy-18-octadecanoic acid. Additionally, the ICDD database[22] gave the epoxy derivative *cis*-9,10-epoxystearic acid as the closest similarity.

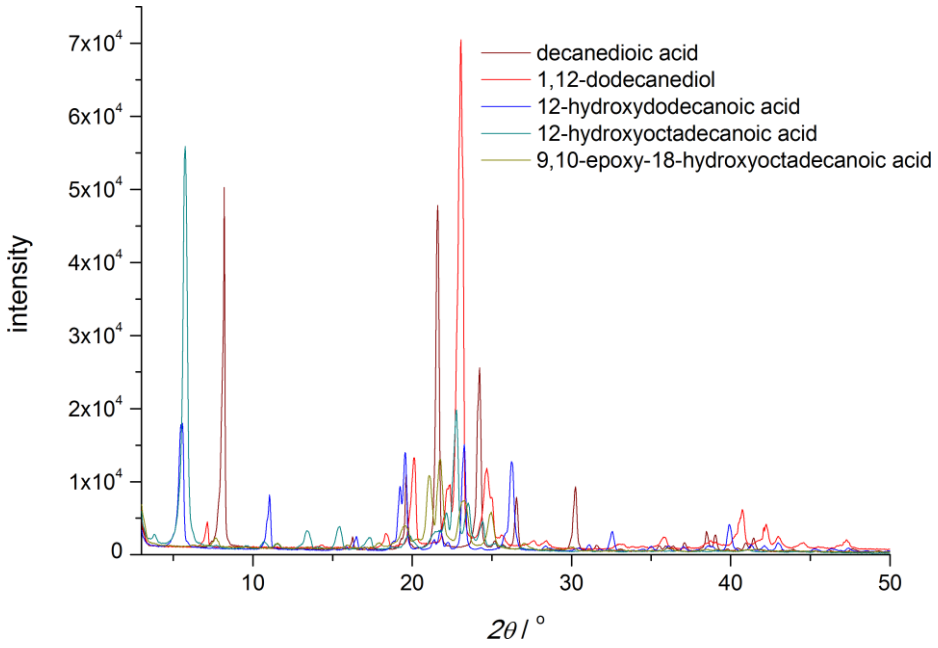


Figure 4.26 XRD patterns of suberin model systems.

HEX-MDS_{cork} showed a simpler pattern (Figure 4.25 (b)) with well-resolved peaks, the most intense of which appeared at 2θ ~ 22 [110], 24 [200], 30 [210], and 36° [020], assigned to the orthorhombic system, with a smoothly amorphous halo centred at $2\theta \approx 20^{o}$. This pattern compared favourably with literature results for long chain hydrocarbon mixtures.[23]

The essentially amorphous character of HDS_{cork} and MDS_{cork} samples was confirmed by XRD, with a pronounced amorphous halo centred on $2\theta \approx 21^{o}$, and poorly resolved peaks at 2θ near 19, 21, 23 and 24° (see *e.g.* Figure 4.27). This behaviour was probably associated with the more complex chemical composition of these samples, as already emphasised.

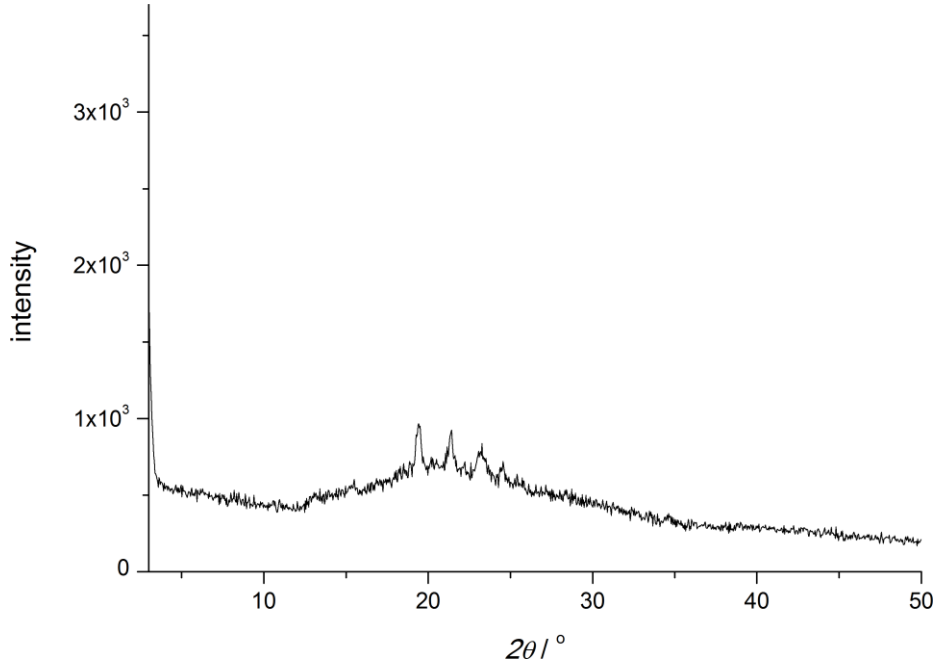


Figure 4.27 XRD pattern of HDS_{cork}.

4.5 Conclusions

The aliphatic fragments of suberin arising from the cleavage of its ester groups were characterised in detail by GC-MS. The results showed that cork suberin was an abundant source of 22-hydroxydocosanoic acid (up to 86 mg g^{-1}), whereas birch outer bark suberin was rich in 9,10-epoxy-18-hydroxyoctadecanoic acid (156 mg g^{-1}).

FTIR, ^{1}H and ^{13}C NMR spectroscopy confirmed the GC-MS findings, showing clearly that all suberin fragment mixtures possess long non-polar chains terminated by hydroxy and carboxylic functional groups. The functional group ^{1}H NMR analyses of the trichloroacetyl isocyanate derivatised-suberin showed more specifically that carboxylic and hydroxy groups were not in equivalent amounts (cork suberin had an excess of CO_2H or CO_2CH_3 over OH groups, while, on the contrary, in birch OH groups were predominant over CO_2H ones).

The study of the thermal behaviour of the depolymerised suberin samples by DSC and thermomicroscopy, as well as the XRD analyses, showed that suberin had either important crystalline domains, possessing well-defined melting points, typically near 70 ^{o}C, or, on the contrary, they could be essentially amorphous. Factors such as the suberin

source, or the depolymerisation conditions adopted were responsible for these differences.

Given the composition of these suberin fragments, polyesters seemed an obvious application for this renewable resource, as studied in Chapter 7. However, only cork and birch outer bark had reasonably high quantities of suberin (22-56%) and hence only these sources were used to synthesise completely renewable-based polyesters (Chapter 7). ICP was instead too poor in suberin (only 11%) and its exploitation for polyesters synthesis was not considered viable. However, its valorisation was considered alternatively in terms of the detailed chemical composition of its lipophilic fraction and, therefore, its potential as source of chemicals, given the reasonable quantity of extractives (6%) it contains (Chapter 5).

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5 Low molecular weight lipophilic extractives from cork and industrial cork by-products

What were the extractives content in cork and in its by-products samples? What were the most relevant differences between samples? Why? What were the main triterpenes found by GC-MS in cork and by-product extracts? Could the lipophilic extracts of cork by-products be used in higher value applications, rather than the squalid burning fate?

5.1 Introduction

Cork is an interesting source of valuable chemicals, especially suberin-derived compounds as highlighted in the preceding Chapter, but also of triterpenic compounds, present essentially in its lipophilic extractives. The triterpenic fraction of cork extractives contains essentially friedeline, cerine, betuline, betulinic acid, smaller amounts of sterols such as β-sitosterol,[1-7] and a minor fraction of other friedelane- and lupane-type components.[8-9] Although friedeline and cerine are in general identified as the main triterpenic compounds, the abundance and composition of this fraction is also highly variable (see, *e.g.*, refs 2, 4 and 10). Other cork-related samples, such as the industrial cork powder or the black condensate, are also potential sources of these valuable chemicals, but still not studied. Although there is a significant amount of work focused on cork composition,[2,10-13] including a few studies of the composition of cork plank (triterpenic and phenolic) extractives throughout the industrial processing,[3-4,14] there was a gap in what concerns the cork by-products.

Both industrial cork powder and black condensate samples are generated during the cork industry activity. Very briefly, as was already described in Chapter 1, in the cork stopper industry, after harvesting and a resting period in the field and/or in the mill, the cork planks are immersed in boiling water for around one hour, yielding cooking wastewaters as a liquid effluent.[15] Afterwards, the cork stoppers are produced and, once more, cork residues enriched in the outer and the inner surfaces of cork planks, are rejected and used as input in the agglomerate industry.

The agglomerate industry during the production of granulated cork, rejects cork powder particles with a size distribution (very modest dimensions) inadequate to be used in the manufacture of agglomerates.[15] This by-product is designated by industrial cork powder and today finds no added-value application, since it is burnt to produce energy.[16] The black agglomerates are produced by treatment of cork particles with adequate size, without any adhesive, at temperatures in the range of 250-500 ^{o}C. During such thermal treatment of cork, vapours are generated, which are condensed in the autoclave pipes. Periodically, this by-product is removed and also burnt.[16]

On the one hand, the search for new applications of cork by-products is particularly attractive within the scope of the biorefinery concept applied to forest-based industries, where the concomitant upgrading of all the by-products generated during wood

and forest products processing represents logical issues within this situation.[17] On the other hand, the abundance of some of triterpenes in cork, together with their promising applications, directly, or as precursors to bioactive components for biomedical applications,[18-21] prompted the present study of the detailed characterisation of the lipophilic extractives of cork by-products by GC-MS.

First, the extractive contents in cork, boiled cork, industrial cork powder and black condensate were determined gravimetrically (Section 5.2). Thereafter, the GC-MS chemical composition of all extracts was determined (Sections 5.3 and 5.4). All the experimental details concerning these issues are summarised in Chapter 8 (PART III Experimental).

5.2 Extractives content in cork and by-products

The extractives of cork and some of its residues, generated by the stopper and agglomerate industries, were characterised in detail by GC-MS. These samples were extractives from cork, boiled cork (obtained after boiling the cork planks in water), industrial cork powder, and black condensate, referred hereafter as EXT_{cork}, EXT_{Bcork}, EXT_{ICP} and EXT_{Bcond}, respectively. Additionally, the inner and outer fractions of cork and boiled cork were isolated and their extractive contents also studied. Table 5.12 summarises the dichloromethane, methanol and water extraction yields of all samples studied.

Table 5.12 Dichloromethane, methanol and water extraction yields (w/w %) of cork, boiled cork, including their inner and outer fractions counterparts, industrial cork powder and black condensate.

	EXT_{cork}			**EXT_{Bcork}**			**EXT_{ICP}**	**EXT_{Bcond}**
	whole	inner	outer	whole	inner	outer		
DCM	3.6	4.2	1.9	4.7	5.0	2.7	2.6	91.9
MeOH	1.7	2.8	1.5	2.2	3.6	1.8	1.9	5.5
water	3.7	2.1	1.4	1.1	2.6	1.1	1.4	0.3
TOTAL	**9.0**	**9.1**	**4.8**	**8.0**	**11.2**	**5.6**	**5.9**	**97.7**

The Soxhlet extraction yields of cork sample, using dichloromethane, methanol and water as solvents (DCM-$EXT_{cork,whole}$, MeOH-$EXT_{cork,whole}$, water-$EXT_{cork,whole}$, respectively), are within the typical values found for other samples of “amadia”-grade cork

(see, *e.g.*, reference 22), being the total amount of extractives around 9.0% of the dry cork samples. ICP contained, in general, a lower amount of extractives than those observed for cork and boiled cork samples (EXT_{ICP} ≈ 5.9%). Hence, a better understanding of this observation, and also of the differences found in the chemical composition between ICP and the other samples (as discussed below in Section 5.3), was pertinent at this point. Therefore, the inspection of the extractive contents in the inner and outer fractions of cork and boiled cork, in which ICP is highly enriched, were carried out.

The extraction yields of all the outer fraction of both $EXT_{cork,outer}$ and $EXT_{Bcork,outer}$ (but, especially the boiled samples) were very similar to those obtained for ICP (4.8, 5.6 and 5.9%, respectively) and consequently lower than those found for cork (9.0%). Oppositely, the inner fraction of both $EXT_{cork,inner}$ and $EXT_{Bcork,inner}$ showed higher extractive amounts than those found in ICP and consequently similar amounts as those found in cork (9.1, 11.2, 5.9 and 9.0%, respectively). These results can be related to the fact that, on the one hand, the outer fraction of cork suffered high environmental exposure which, most certainly, promoted some extent of degradation of the outer surface, thus explaining the lower amount of extractives. On the other hand, the inner cork fractions ($EXT_{cork,inner}$ and $EXT_{Bcork,inner}$) were obviously not significantly exposed to the environment and their extraction yields were slightly higher than that for cork sample counterparts, suggesting that in the cork planks the extractives were preferentially located in higher concentration in this fraction.

The DCM and MeOH extraction yields of the boiled cork (including fractions) were apparently higher than those of cork counterpart; because the loss of the water soluble extractives during the boiling stage was not taken into consideration in the determination of yields.

The black condensate sample displayed the highest amount of extractives of all studied samples (~ 97.7%), being most of these extractives obtained in the DCM extract (~ 91.9%). These high extraction yields are consistent with the volatile nature of this fraction.

5.3 Chemical composition of the lipophilic extractives of cork and by-products

Table 5.13 summarises the main GC-MS results for cork and its by-products.

Table 5.13 GC-MS contents of the main compounds identified in the dichloromethane, and in the total (DCM + MeOH) extracts of cork, boiled cork and industrial cork powder (mg of compound per kg of dry cork, Bcork or ICP sample).[a]

	EXT_{cork}						EXT_{Bcork}						EXT_{ICP}	
	whole		Inner		outer		whole		inner		outer			
	DCM	total	DCM	Total	DCM	total	DCM	total	DCM	total	DCM	total	DCM	total
betulinic acid	2196	2234	5037	5203	1571	1621	1177	1230	1457	1496	1884	1936	9860	11719
cerine	4635	4648	7328	7542	2468	2703	6083	6144	7809	7942	1919	1938	1886	2060
friedeline	2308	2323	3554	3829	1100	1370	2684	2745	4592	4959	1732	1778	1792	2009
betuline	324	335	446	481	464	492	331	343	417	435	358	371	764	875
β-sitosterol	539	539	648	648	247	247	778	778	916	916	244	244	254	254
ursolic acid	130	130	tr	25	tr	35	tr	17	tr	tr	tr	20		104
lupeol		71	tr	64	tr	tr	tr	67	tr	43	tr	20		60
TOTAL TRITERPENES	**10132**	**10280**	**17013**	**17792**	**5850**	**6466**	**11053**	**11324**	**15191**	**15791**	**6137**	**6307**	**14556**	**17081**
ellagic acid		1222		2784		1211		1513		5852		1110		1347
TOTAL	**13658**	**15065**	**18278**	**21841**	**6298**	**8173**	**12358**	**14122**	**16054**	**22587**	**7146**	**8441**	**15065**	**19254**

[a] Each value is the average of four injections with variation coefficients within 1.1-5.0%.

From a qualitative point of view, the composition of all cork, cork fractions, and ICP extracts were found to be quite similar and mainly constituted of triterpenic compounds, followed by lower amounts of alkanoic acids, alkanols, and phenolics. In addition to these families, considerable amounts of long-chain ω-hydroxyalkanoic and α,ω-alkanedioic acids and phenolics, in esterified forms, were also detected in EXT_{Bcond} (as described in Section 5.4). Cerine, friedeline, and betulinic acid (Figure 5.28) were the dominant components identified. Smaller amounts of other triterpenoids, such as betuline, β-sitosterol, ursolic acid, and lupeol, were also found.

Triterpenic compounds were identified by the systematic interpretation of their mass spectra, their elution order, and by comparison with previously published data.[7,23-27] All of the identified triterpenes were previously reported as cork components.[4,6-7]

The quantitative analysis of the extracts revealed that, regardless of the extraction time, the extraction of triterpenes with DCM was incomplete, particularly in the case of ICP samples, in which significant amounts of these components were still detected in the MeOH extract. Therefore, Table 5.13 presents both the abundances of compounds in DCM extract and in DCM plus MeOH (total abundance).

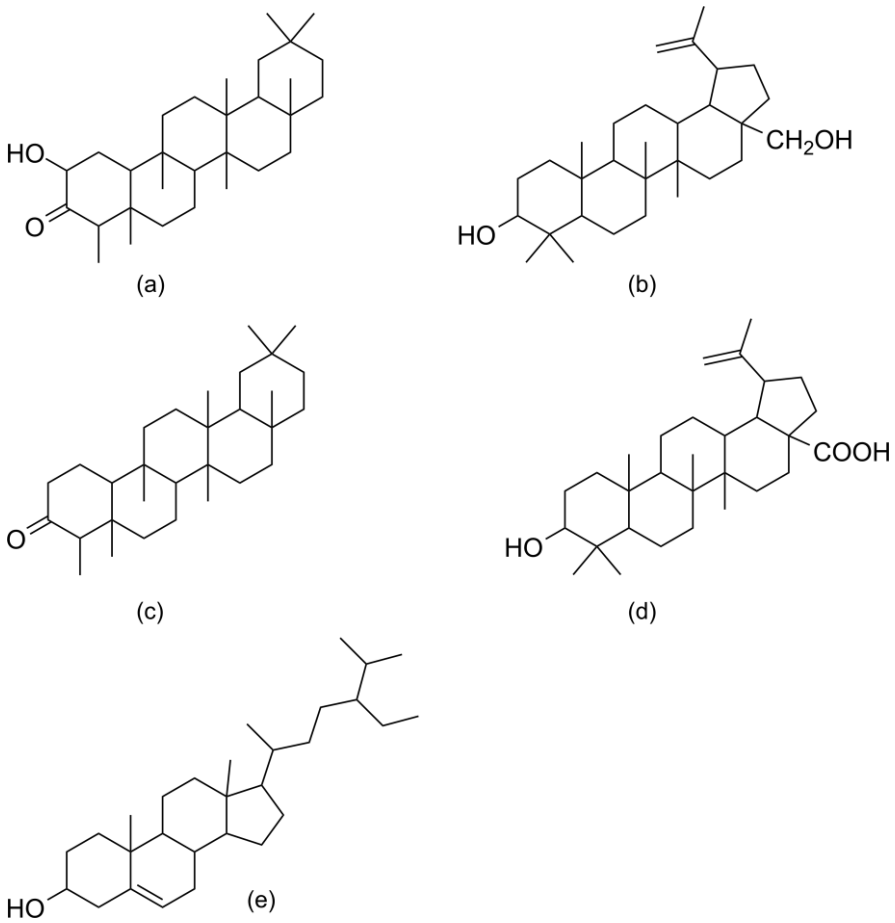

Figure 5.28 Structures of the most abundant triterpenes identified in the cork samples by GC-MS: (a) cerine, (b) betuline, (c) friedeline, (d) betulinic acid, and (e) β-sitosterol.

The GC-MS analysis of the DCM extracts of cork and ICP showed that they contained the same major compounds, although in significantly different amounts. Indeed, while in cork and boiled cork samples, cerine (1.9-7.9 g kg^{-1}) was generally the major component, followed by friedeline (1.4-5.0 g kg^{-1}) and betulinic acid (1.2-5.2 g kg^{-1}), in the case of ICP, betulinic acid was instead the major component (11.7 g kg^{-1}), representing more than 68% of the triterpenic fraction.

Results showed in Table 5.13 demonstrated that cork and boiled cork samples ($EXT_{cork,whole}$ and $EXT_{Bcork,whole}$), together with the inner fractions ($EXT_{cork,inner}$ and $EXT_{Bcork,inner}$), contained higher amounts of triterpenes than the outer fraction counterparts ($EXT_{cork,outer}$ and $EXT_{Bcork,outer}$), a result that is in agreement with the extraction yields given in Table 5.12.

Boiled cork samples including the inner and outer fractions had similar (or slightly higher) amounts of triterpenes as the corresponding cork samples. However, once again,

if adequately corrected for the loss of water-soluble extractives, an actual decrease in the amount of extractives would be generally observed.

Noteworthy, the amounts of triterpenic compounds estimated by GC-MS analysis in EXT_{ICP} (~ 17.08 g kg^{-1}) and in $EXT_{Bcork,outer}$ (~6.31 g kg^{-1}) or in $EXT_{cork,outer}$ (~6.47 g kg^{-1}) were not similar, as one could in principle have anticipated considering the similar extraction yields determined gravimetrically (Table 5.12). Although the abundances of most triterpenes (cerine, friedeline, and betuline) were comparable, the high concentration of betulinic acid in EXT_{ICP} cannot be related to the composition of $EXT_{Bcork,outer}$, $EXT_{cork,outer}$ or directly to any other cork fraction. The significant differences between the compositions of these samples suggested that they were not directly related, but must instead have originated from the variability of cork composition and, consequently, of that of ICP referred to above.

Apart from the major components reported above, small amounts of fatty acids (~0.3 g kg^{-1}) and aliphatic alcohols (~0.3 g kg^{-1}) were also identified in the DCM extracts. The most abundant alkanoic acids identified were docosanoic and tetracosanoic acid, followed by hexadecanoic, octadeca-9,12-dienoic, and octadecanoic acid. The main aliphatic alcohols identified were 16-hexadecanol, 18-octadecanol, 22-docosanol, and 24-tetracosanol.

The GC-MS analysis of the MeOH extracts (Table 5.13) also revealed small amounts of ursolic acid and lupeol in several samples and, more remarkably, considerable amounts of ellagic acid (1.11-5.85 g kg^{-1}). The analysis of the DCM and MeOH extracts after alkaline hydrolysis did not reveal any substantial increase in the amounts of identified compounds.

5.4 Chemical composition of the lipophilic extractives of black condensate

Table 5.14 summarises the GC-MS results of the analysis of black condensate extractives. These results revealed that EXT_{Bcond} was mainly composed of triterpenes (*c.a.* 11.0%), followed by smaller amounts of alkanols, alkanoic acids, and phenolic compounds, accounting for more than 18% of the mass of the black condensate (Table 5.14).

Table 5.14 GC-MS contents of the compounds identified in the dichloromethane extract of the black condensate, before and after alkaline hydrolysis (g of compound per kg of dry black condensate).

compound	before hydrolysis	after hydrolysis
1-alkanols	**8.09**	**94.79**
octadecanol	-	0.80
eicosanol	0.09	3.25
docosanol	2.78	30.56
tetracosanol	4.18	41.55
pentacosanol		3.45
hexacosanol	1.04	15.18
alkanoic acids	**7.31**	**88.44**
nonanoic acid	-	1.61
hexanoic acid	0.07	1.85
octadecanoic acid	-	0.79
octadec-11-enoic acid	-	2.69
octadeca-9,12-dienoic acid	-	1.10
eicosanoic acid	tr	1.86
docosanoic acid	2.50	25.55
tricosanoic acid	-	1.61
tetracosanoic acid	4.74	39.34
hexacosanoic acid	-	12.04
ω-hydroxyalkanoic acids	**-**	**114.31**
16-hydroxyhexadecanoic acid	-	1.63
18-hydroxyoctadec-9-enoic acid	-	6.69
20-hydroxeicosanoic acid	-	2.37
22-hydroxydocosanoic acid	-	63.94
24-hydroxytetracosanoic acid	-	35.99
26-hydroxyhexacosanoic acid	-	3.69
α,ω-alkanedioic acids	**-**	**21.18**
hexadecanedioic acid	-	2.38
octadecanedioic acid	-	3.70
octadec-9-enedioic acid	-	6.75
eicosanedioic acid	-	1.10
docosanedioic acid	-	7.25
phenolic compounds	**1.58**	**28.73**
ferulic acid	-	10.96
vanillic acid	0.11	0.73
3-vanillylpropanol	0.07	3.81
vanillylpropanoic acid	-	5.36
benzoic acid	-	3.48
cathecol	1.16	1.23
phenolic compounds derivatives	0.24	3.16
triterpenes	**109.59**	**185.62**
friedeline	79.46	95.29

compound	before hydrolysis	after hydrolysis
friedeline derivatives	9.11	8.73
betuline	1.32	13.13
betulinic acid	3.88	12.08
ergostene	-	8.49
hydroxy-Δ^{12}-dehydrolupen-3-one	3.10	17.23
β-sitostrol	8.96	20.93
stigmastan-3,4-diene	2.56	2.93
triterpenes derivatives	1.20	6.81
[b]others/ni	35.39	79.57
TOTAL	**154.66**	**612.64**

[a] Each value is the average of four concordant injections with variation coefficients within 1.1-5.0%. [b] ni = not identified.

As already explained in the introduction (Section 5.1), the black condensate is a highly volatile fraction of cork; which made the initial identification of only 16% of the sample mass unexpectedly low. The explanation of this fact relates to the presence of esterified lipophilic structures, hydrolysable by conventional hydrolysis reactions. These structures were most probably formed by condensation reactions during the thermal treatment of cork granulates, because in the cork DCM extracts the alkaline hydrolysis did not account for an increase in the compounds detected by GC-MS (results not shown). Indeed, a considerable increase in the compounds detected by GC-MS was observed after hydrolysis, accounting for approximately 61% of the mass of dry black condensate (Table 5.14 and Figure 5.29).

The triterpenes amount increased substantial after alkaline hydrolysis, *viz.*, from 110.2 to 185.6 g kg^{-1}, indicating that they were involved in ester linkages with other components. The triterpene friedeline (95.3 g kg^{-1}) continued to be the most abundant of this family, followed by smaller amounts of betuline, betulinic acid, and β-sitosterol. However, the increase in the amount of detected compounds (Table 5.14 and Figure 5.29) was mainly due to the increase in the amounts of alkanols (from 8.1 to 94.8 g kg^{-1}), alkanoic acids (from 7.3 to 88.4 g kg^{-1}), and phenolics (1.6-28.7 g kg^{-1}). Additionally, ω-hydroxyalkanoic and α,ω-alkanedioic acids were only detected in considerable amounts after alkaline hydrolysis (114.3 and 21.2 g kg^{-1}, respectively). These latter families of compounds are quite abundant in suberin,[11-12,28] and their presence in the black condensate by-product might have resulted from the cleavage of more labile ester functionalities of the suberin macromolecular structure during the thermal treatment, releasing oligomeric ester type structures not volatile enough to be detected by GC-MS

analysis. However, during the alkaline hydrolysis, full depolymerisation occurred, allowing their detection.

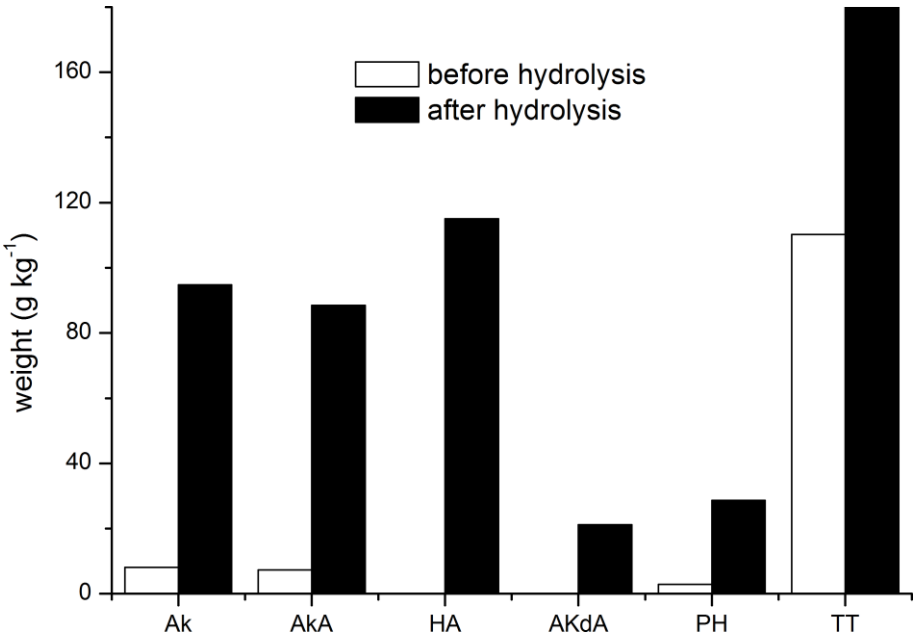

Figure 5.29 Contents of the major families of compounds identified by GC-MS in the black condensate sample. Ak, AkA, HA, AKdA, PH and TT stand for alkanol, alkanoic acid, ω-hydroxyalkanoic acid, α,ω-alkanedioic acid, phenolic and triterpene compounds.

5.5 Conclusions

This investigation provided valuable information on the compositions of the triterpenic fractions of cork and cork by-products, which were similar from a qualitative standpoint; but quantitatively very variable. Whereas in cork and boiled cork samples, cerine and friedeline were the major compounds, followed by betulinic acid, the latter was the major component of industrial cork powder, whereas friedeline was the major triterpene in the black condensates.

Industrial cork by-products can therefore be considered as abundant sources of triterpenic compounds (around 17 and 185 g kg^{-1}, respectively, for ICP and Bcond) and particularly of betulinic acid and friedeline, which are known to have promising applications, directly, or as precursors to bioactive components for biomedical applications.[18,21] Black condensates can additionally be a valuable source of other

aliphatic components, particularly of ω-hydroxyalkanoic (114 g Kg^{-1}) and α,ω-alkanedioic acids (21 g Kg^{-1}), after hydrolysis of the starting material.

The development of methodologies to isolate and adequately purify these promising compounds/fractions, instead of simply burning the cork residues, constitutes a stimulating challenge for the valorisation of cork as a renewable resource.

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6 Synthesis and characterisation of polyesters from suberin model comonomers

Why study suberin model systems? Which were the suberin model compounds selected? What were the optimum experimental conditions for the polymerisation reactions? What were the properties of the suberin model polyesters? Were there relevant differences among suberin model polyesters?

6.1 Introduction

As it was previously referred, suberin is a remarkable renewable resource, with a unique chemical composition, and, most importantly, suberin has an enormous potential as a source of interesting monomers for polyester synthesis (Chapter 4). However, suberin is a highly complex material, especially with regard to its chemical composition, as was shown in that same Chapter. Hence, the preparation of suberin polyesters and especially the understanding of the properties of the ensuing polymers constituted complex tasks. In this sense, prior to the suberin polymerisation reactions, to gain insight into the reactivity of these complex mixtures in polyesterification reactions, the behaviour of several suberin model systems of growing complexity was first studied, using monomers that resembled the most important suberin depolymerisation fragments (Chapter 4).

These model systems included first the simplest combinations involving homopolymers such the reactions of one hydroxyalkanoic acid, namely 12-hydroxydodecanoic acid or 12-hydroxyoctadecanoic acid, or a mixture of an alkanediol and α,ω-alkanedioic acid, namely 1,12-dodecanediol and decanedioic acid or 1,12-dodecanediol and dodecanedioic acid. The syntheses of copolyesters were then tackled. These more complex systems were studied with the aim of mimicking the natural complexity of suberin mixtures, in which several families of compounds are usually detected in their depolymerised mixtures, such as ω- and mid chain-hydroxyalkanoic acids and α,ω-alkanedioic acids (Chapter 4). The following combinations were thus chosen: 12-hydroxydodecanoic acid and 12-hydroxyoctadecanoic acid; 12-hydroxyoctadecanoic acid and 1,12-dodecanediol and dodecanedioic acid; and finally the more complex system involving all types of monomers 12-hydroxydodecanoic acid and 12-hydroxyoctadecanoic acid and 1,12-dodecanediol and dodecanedioic acid.

The polymerisations of the model comonomers were performed by polycondensation or polytransesterification reactions, which called upon monomers with free carboxylic acid groups or their methyl esters, respectively. Both these approaches seemed to be logical synthetic strategies to be tested, since the suberin fragments were isolated by hydrolysis or methanolysis giving therefore rise to monomers with either free carboxylic acid groups or their methyl esters.

Several polyesterification conditions were tested to prepare the suberin model polyesters, always attempting to follow the growing demand for greener synthetic

pathways;[1-6] namely emulsion polycondensation using *p*-dodecylbenzenesulfonic acid as catalyst, and bulk polycondensation using *Candida antarctica* lipase B or bismuth(III) trifluoromethanesulfonate as catalysts (Section 6.2).[7] Polytransesterifications using antimony(III) oxide were also carried out (Section 6.3). The structure and average molecular weight of the ensuing polyesters were characterised by FTIR, ^{1}H NMR, ^{13}C NMR, SEC and VPO (vapour-pressure osmometry) and their thermal properties by DSC. All the experimental details related to the present Chapter are provided in Chapter 8 (PART III Experimental).

Preliminary note. The term yield refers throughout this Chapter and the following one (Chapter 7) to the percentage of polymer recovered after precipitation of the corresponding polyester into an excess of methanol (see PART III Experimental, Chapter 8). This thus not implies that the unrecovered product was made up of monomeric species since in any polycondensation the monomers are rapidly consumed, as opposed to polyaddition reactions. It follows that the fraction that remained dissolved in methanol reflects the presence of oligomeric species. This was indeed verified on a number of systems by evaporating all volatiles from the filtered medium and taking infrared spectra of the viscous residue. In all instances, the oligomeric character of these materials was clearly corroborated. It is therefore important to emphasise that all these polycondensations behaved accordingly to a classical mechanism and that the decision to concentrate on methanol insoluble polyesters was applied throughout this Thesis in order to privilege their higher molecular weight fractions. In other words the formal yields of all these reactions were close to completion as expected and the present use of the term yield is therefore constrain by the empirical definition.

6.2 Polycondensation of suberin model comonomers

Several emulsion polycondensation conditions were tested using *p*-dodecylbenzenesulfonic acid and mixtures of variable complexity of different model monomers (Table 6.15). Two typical reactions are depicted in Scheme 6.12.

Scheme 6.12 Synthesis of (Top) poly(dodecamethylene decanedioate) and (Bottom) poly(12-hydroxyoctadecanoate).

The polyesters from both the simplest systems involving the homopolymers (runs 1-6) and the more complex ones producing copolymers (runs 7-10) were isolated in reasonable yields, varying between 23 and 58%, and number-average molecular weights ranging from 700 to 5000 (with agreement between values from SEC and VPO), with a polydispersity index always close to 2, based on SEC determinations, as expected for linear polycondensation polymers (Chapter 3).

Table 6.15 Experimental data for the emulsion polycondensation of suberin model compounds catalysed by *p*-dodecylbenzenesulfonic acid.[a]

run		t / h	[b] *r*	[c] *η* (%)	[d] M_n	[e] M_w/M_n	[f] M_n
1	1,12-dodecanediol + decanedioic acid	48	1.01	26	3 000	1.8	3 200
2		48	1.05	23	700	2.2	-
3	1,12-dodecanediol + dodecanedioic acid	120	0.99	36	2000	1.7	2100
4		144	1.06	45	1 800	1.7	-
5	12-hydroxydodecanoic acid	48	1.00	57	1 600	1.3	2 200
6	12-hydroxyoctadecanoic acid	48	1.00	32	-	-	-
7	12-hydroxydodecanoic acid + 12-hydroxyoctadecanoic acid	48	1.00	24	3 500	1.8	3 500
8	12-hydroxydodecanoic acid + dodecanedioic acid + 1,12-dodecanediol	48	0.99	37	5 000	1.7	-
9	12-hydroxyoctadecanoic acid + 1,12-dodecanediol + dodecanedioic acid	48	1.00	58	-	-	-
10	12-hydroxydodecanoic acid + 12-hydroxyoctadecanoic acid + 1,12-dodecanediol + dodecanedioic acid	48	0.99	43	1 700	1.7	2300

[a] Reactions were carried out as described in Section 8.10 of Chapter 8. [b] Ratio between the number of carboxylic and hydroxy groups of monomers. [c] Yield of precipitated polymer. [d] M_n were determined by SEC in $CHCl_3$. [e] Polydispersity index were determined by SEC in $CHCl_3$. [f] M_n were determined by VPO in $CHCl_3$.

The syntheses of these polyesters were carried in emulsion, typically during 48 h, but longer reaction times were also tested. If the reaction time was raised from the typical 48 to 120 hours the yield and the number-average molecular weight tended to increase

somewhat; as for example in the case of poly(dodecanemethylene dodecanoate), from 23 to 36% and 700 to 2000 Da (runs 2 and 3), respectively.

These results, as well as those presented below in Subsections 6.2.1 and 6.2.2, related to the detailed characterisation of the model polyesters, showed that it was possible to synthesise aliphatic polyesters from complex mixtures of monomers through a very simple experimental procedure based on an emulsifying acidic catalyst, with no need to remove the ensuing by-generated water from the reaction media. This was encouraging in terms of the extension of this process to suberin-based systems.

Next, bulk polycondensation of suberin model comonomers, catalysed by either the *Candida antarctica* lipase B or $Bi(OTf)_3$ (also known as Bi-triflate), were carried out (Table 6.16). The yield of the polyesters increased significantly, especially in the case of $Bi(OTf)_3$-catalysed polymerisations, *viz.* from 57% (run 5, Table 6.15) to 93 (run 12, Table 6.16).

The yield of the lipase-catalysed polymerisation of decanedioic acid and 1,12-dodecanediol was also higher than that obtained with the DBSA counterpart, *viz.*, 44% (run 11, Table 6.16) and 26% (run 1, Table 6.15), respectively. However, the molecular weight followed the opposite trend, decreasing from 3000 to 1000.

Table 6.16 Experimental data related to the bulk polycondensation of suberin model compounds.[a]

run		catalyst	*r*	*η* (%)	M_n	M_w/M_n
11	1,12-dodecanediol + decanedioic acid	CALB	0.99	44	1000	1.1
12	12-hydroxydodecanoic acid	$Bi(OTf)_3$	1.00	93	-	-
13	12-hydroxyoctadecanoic acid	$Bi(OTf)_3$	1.00	65	-	-

[a] Reactions were carried out as described in Section 8.10 of Chapter 8.

6.2.1 Spectroscopic characterisation

The typical FTIR spectra of Figure 6.30 illustrate the differences between a homopolymer and its precursor, with a new band at 1731 cm^{-1} arising from the C=O stretching vibration (νCO), typical of ester groups, and the absence of a detectable band at 1678 cm^{-1}, arising from the C=O stretching vibration (νCO), typical of carboxylic acid groups. Also, the bands near 3235 and 2546 cm^{-1}, assigned to the O-H stretching mode (νOH) of alcohols and carboxylic acids forming strong hydrogen-bonds, were absent.[8] All the other spectra of polyesters and copolyesters displayed the same clear-cut features. The FTIR spectra of all polyesters studied confirmed the success of the polymerisations.

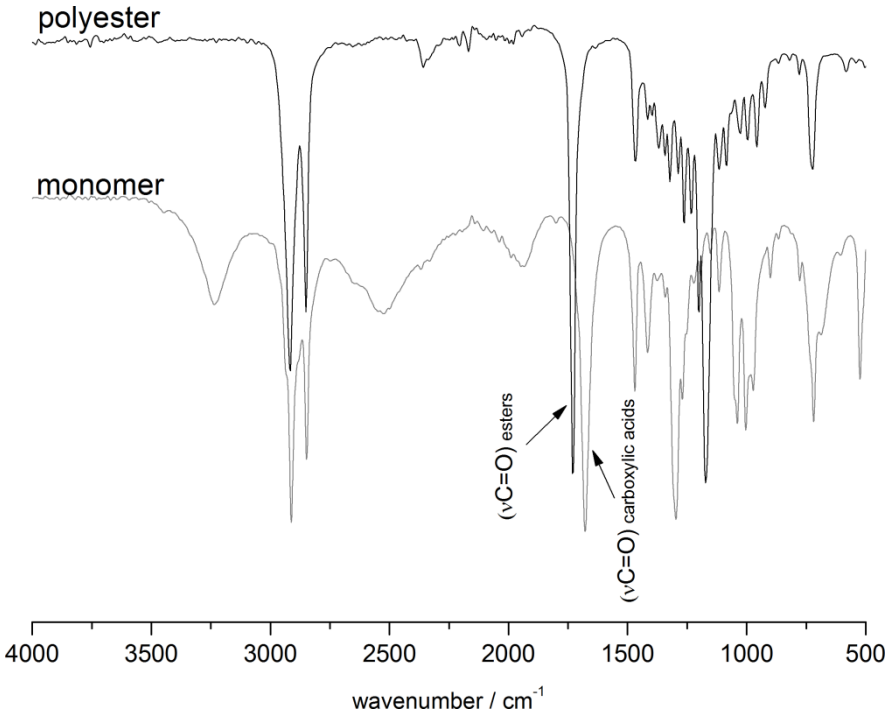

Figure 6.30 ATR FTIR spectra of 12-hydroxydodecanoic acid and poly(12-hydroxydodecanoate) formed by using $Bi(OTf)_3$ as catalyst (run 12, Table 6.16).

Figure 6.31 illustrates a typical ^1H NMR spectra of one these polyesters and of its monomer.

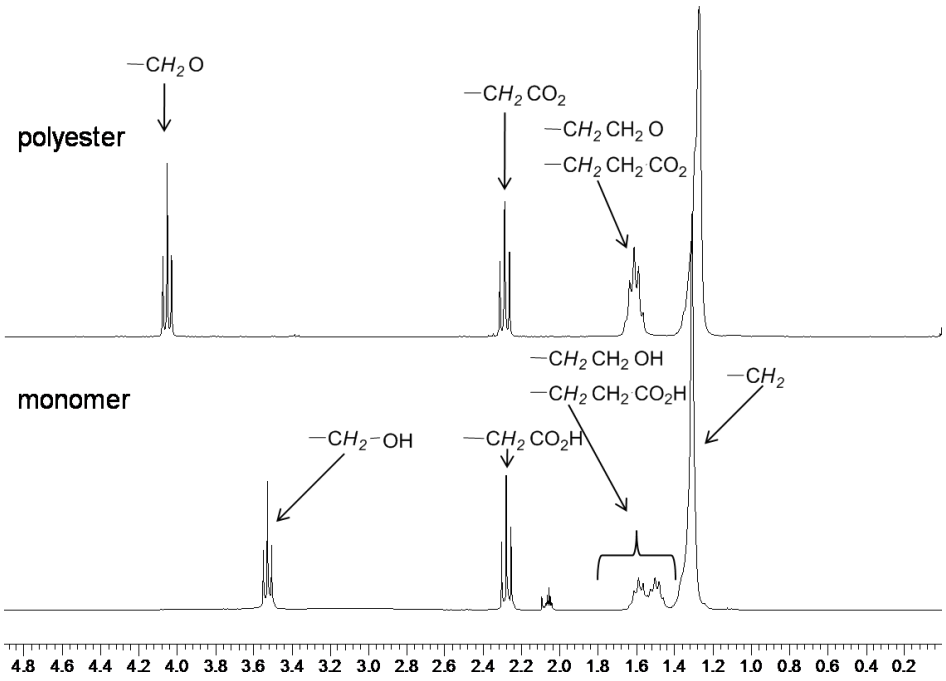

Figure 6.31 ^1H NMR spectra of 12-hydroxydodecanoic acid and the corresponding poly(12-hydroxydodecanoate) prepared using $Bi(OTf)_3$ (run 12, Table 6.16).

The comparison of the ^{1}H NMR spectra of the polyesters and of its precursors (Table 6.17 and Figure 6.31) revealed that the OC*H*$_2$ resonance was shifted downfield from δ ~3.60 ppm for the hydroxyalkanoic acid to δ ~4.05 ppm for the polymer, owing to the presence of the neighbouring carbonyl group of an ester. Additionally, the polymers prepared using the 12-hydroxyoctadecanoic acid (runs 6-7, 9-10 and 13) also showed a shift of the resonance attributed to the OC*H* proton, from δ ~ 3.60 ppm for the monomer to δ ~ 4.86 ppm for the polymers (spectra not shown). These observations confirmed the formation of the polyesters.

Table 6.17 Relevant ^{1}H NMR resonances of 12-hydroxydodecanoic acid and of the corresponding polyester (run 12, Table 6.16).

δ / ppm	**assignment**	**[a]multiplicity**	**integration**	
			12-hydroxydodecanoic acid	**poly(12-hydroxydodecanoate)**
1.27-1.31	C*H*$_2$	s	14.0	14.5
1.46-1.61	C*H*$_2$CH$_2$O C*H*$_2$CH$_2$CO$_2$	m	4.0	4.5
2.28	C*H*$_2$CO$_2$	t	-	1.9
2.35	C*H*$_2$CO$_2$H	t	2.0	tr
3.60	C*H*$_2$OH	t	2.0	-
4.05	C*H*$_2$O	t	-	2.0

[a] s = singulet; t = triplet; m = multiplet

In some instances, ^{1}H NMR spectroscopy gave additional information related to the reactivity of the different monomers, through the extent of their relative incorporation into the copolymer backbone. This issue is quite relevant to the polymerisation of suberin monomers, characterised by the presence of a mixture of different types of monomers. Thus, for example, the ^{1}H NMR spectrum of the copolymer prepared from the 12-hydroxydodecanoic acid and 12-hydroxyoctadecanoic acid (run 7, Table 6.15) displayed both the typical resonances of the corresponding homopolymers at δ 4.86 ppm (OC*H* of 12-hydroxyoctadecanoic acid units) and at δ 4.05 ppm (OC*H*$_2$ of 12-hydroxydodecanoic acid units). The ratio between 12-hydroxyoctadecanoic acid and 12-hydroxydodecanoic acid monomer units in the copolymer was then estimated by the ratio $\left[(2A_{\mathrm{OC}H})/A_{\mathrm{OC}H_2}\right]$ of the integration of the corresponding protons resonances, and it appeared that 12-hydroxydodecanoic acid was slightly more reactive than the 12-hydroxyoctadecanoic counterpart, since this ratio was 0.9, despite their initial 1:1 feed ratio.

In another experiment also involving monomers with a primary and a secondary OH group (run 9, Table 6.15), the latter showed a slightly lower extent of incorporation,

with $\left[(2\,A_{\mathrm{OC}H})/A_{\mathrm{OC}H_2}\right]$ = 0.9, suggesting, however and more importantly, that this type of monomer in the depolymerised suberins should participate without any *major* hindrance in the corresponding polycondensation reactions.

The ^{13}C NMR spectra of the model polyesters were consistent with the ^{1}H NMR results (see example of Table 6.18 and Figure 6.32).

Table 6.18 Relevant ^{13}C NMR resonances of 12-hydroxydodecanoic acid and of the corresponding polyester (run 12, Table 6.16).

assignment	***δ* / ppm**	
	12-hydroxydodecanoic acid	**poly(12-hydroxydodecanoate)**
***C*H$_2$**	25-34	25-34
***C*H$_2$OH**	63	-
***C*H$_2$O**	-	64
***C*O$_2$C**	-	174
***C*O$_2$H**	179	-

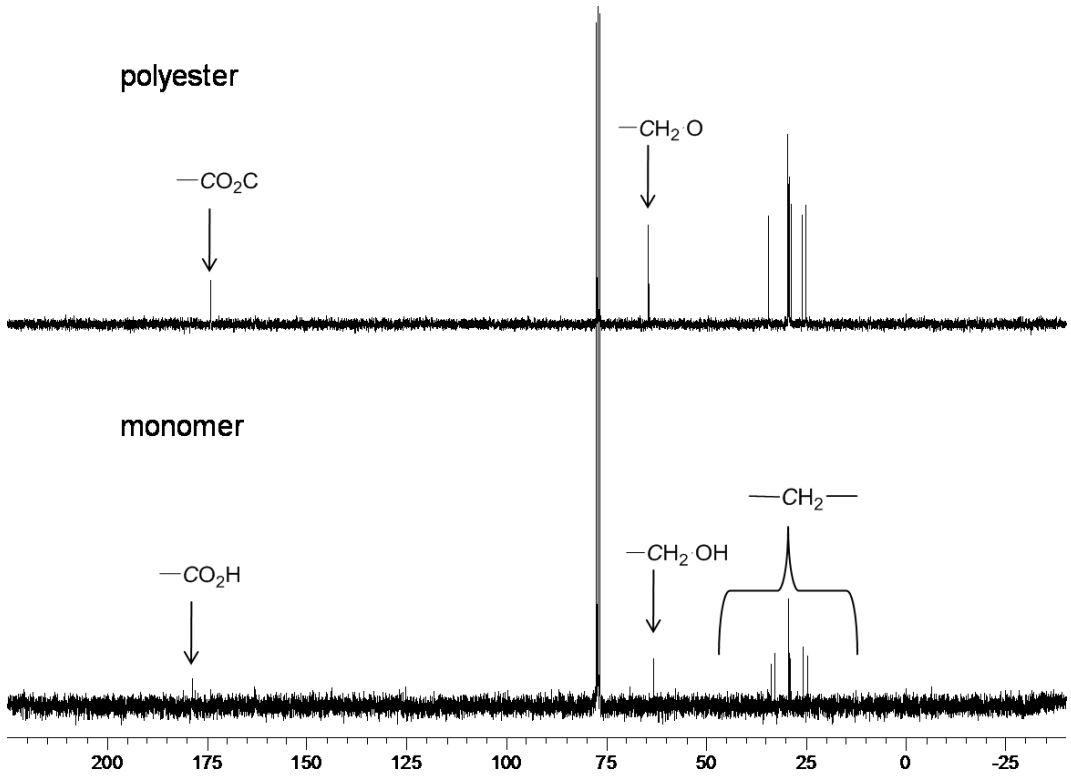

Figure 6.32 ^{13}C NMR spectra of 12-hydroxydodecanoic acid and of the corresponding poly(12-hydroxydodecanoate) (run 12, Table 6.16).

The most relevant feature was the appearance of a new resonance around δ 174 ppm, arising from the carbon of the carboxylic ester group of the polymer, instead of the carbon resonance of the free carboxylic group at 179 ppm, the former peak being absent and the latter present in the spectra of the monomers.

6.2.2 Differential scanning calorimetry analysis of the model polyesters

The thermal transitions of the model polyesters are summarised in Table 6.19.

Table 6.19 Glass transition and melting temperatures of the polyesters prepared from the model compounds.

system	[a]run	[b]T_g / °C	[b]T_m / °C
decanedioic acid + 1,12-dodecanediol	**1**	-8	80
	11		82
dodecanedioic acid + 1,12-dodecanediol	**3**		77
12-hydroxydodecanoic acid	**5**	-1	83
	12		86
12-hydroxyoctadecanoic acid	**6**		-17
	13		-26
12-hydroxydodecanoic acid + 12-hydroxyoctadecanoic acid	**7**		19, 31, 44
12-hydroxydodecanoic acid + dodecanedioic acid + 1,12-dodecanediol	**8**		78
12-hydroxyoctadecanoic acid + dodecanedioic acid + 1,12-dodecanediol	**9**	-19	26, 37

[a] See Table 6.15 and Table 6.16. [b] T_g and T_m were determined by DSC at 10 °C min^{-1} using the 1st heating trace.

These polymers, especially those prepared from non-branched linear model compounds, displayed very regular structures, as confirmed by their ^{1}H NMR spectra and as reflected in their DSC measurements (see *e.g.* Figure 6.33).

The thermograms displayed well-defined melting peaks typically at temperatures around 80 °C, in agreement with those reported for other long-chain aliphatic polyesters.[2] Their amorphous phases gave T_g values below room temperature, as expected for such flexible macromolecular backbones.

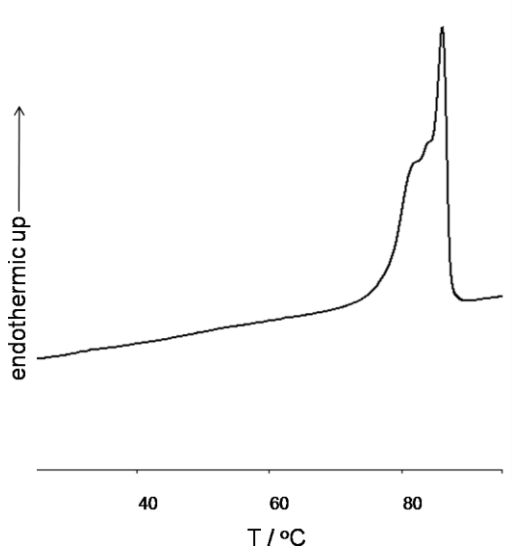

Figure 6.33 DSC thermogram of the polyester prepared by the polycondensation of 12-hydroxydodecanoic acid (run 12, Table 6.16).

On the one hand, the DSC traces of the homopolymers poly(12-hydroxyoctadecanoate) (runs 6 and 13) exhibited a very sharp intense melting peak, typical of a regular structure, but at very low temperatures (below -16 ^{o}C), which is associated with the steric hindrance induced by the bulky side chain.

On the other hand, the copolyesters incorporating 12-hydroxyoctadecanoic acid units displayed multiple melting peaks over the temperature region 19-44 ^{o}C, consistent with a much less regular structure induced by the presence of random units with and without pendant alkyl branches and hence with the formation of different crystalline assemblies and morphologies.

6.3 Polytransesterification of suberin model comonomers

The polytransesterification of 1,12-dodecanediol and dimethyl decanedioate using antimony(III) oxide as catalyst was tested. The reaction was conducted in bulk at temperatures from 100 to 160 ^{o}C, using vacuum (10^{-6} mbar) to remove the generated methanol, and vigorous stirring of the melted mixture. However, under these conditions, the monomers were volatile and the reaction did not occur, hence the reduced pressure was raised to 10^{-3} mbar, but still volatilisation of the monomers took place. This polytransesterification was not developed further with the model compounds.

Nevertheless, this approach was tested with the suberin system, since the nature of these natural mixtures was obviously different (see Section 7.3 of Chapter 7).

6.4 Conclusions

This systematic investigation showed that polyesters prepared with suberin model polyesters had a panoply of different properties depending on the suberin-model comonomers mixtures used to synthesise them. These properties should in principle mimic those of suberin polyesters counterparts, allowing thus to anticipate the features of the latter novel polymers from renewable resources. The model polyesters showed very regular structures, with melting temperatures around 80 ^{o}C (non-branched linear polymers), or significantly lower, when the linear polymers presented some degree of branching. The glass transitions were always below room temperature. Number average molecular weights varied between 700 and 5000 Da and the polydispersity index was always close to 2.

Another aspect worth mentioning was the efficient synthesis of polyesters under emulsion polymerisation conditions or by using *Candida antarctica* lipase B or even in bulk using the Bi-triflate, which were, obviously, doubly beneficial approaches from a green perspective, as they avoid the use both of methanol during the depolymerisation of suberin (hydrolysis instead of methanolysis) as well as of organic solvents as reaction media. The excellent yields and average molecular weights of the model polyesters encouraged the application of these green approaches to the synthesis of suberin polyesters as described in the following Chapter.

6.5 References

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7 Novel suberin-based biopolyesters: from synthesis to properties

Did depolymerised suberin mixtures fulfil the expectations- did they polycondense? Was it possible to control the properties of the ensuing products? How? Where they linear, branched or did they contain a cross-linked fraction? “To suberin or not to suberin”?

7.1 Introduction

The possibility of using depolymerisation mixtures of suberin fragments as monomers for the preparation of novel renewable polyesters had been strongly suggested as a result of both their characterisation study (Chapter 4) and the synthesis of suberin model polyesters (Chapter 6). Following this promising evidence and the outstanding interest both in the scientific community and in society for polymers from renewable resources,[1-9] the present key phase of this investigation focused then on the preparation and characterisation of several novel biopolyesters[10-11] from depolymerised suberin mixtures from cork oak and silver birch outer barks.

Besides the justified interest in the use of renewable raw materials, another major concern nowadays is the search for the implementation of ecofriendly polymer synthetic methods, namely the search for greener catalysts and milder reaction conditions. This issue was contemplated in this study and polycondensations under emulsion polymerisation conditions or in bulk using bismuth(III) trifluoromethane or *Candida antarctica* lipase B as catalysts were privileged. These are doubly beneficial approaches from a green perspective, as they avoid the use of both methanol during the depolymerisation of suberin as well as of organic solvents as reaction media.

Most importantly however, the suberin chemical composition was controlled and used to tailor linear or cross-linked polymers under the desired reaction conditions. The chemical composition of suberin depolymerisation products was manipulated, as explained below, by varying the suberin source (birch outer bark or cork), using different depolymerisation procedures (hydrolysis or methanolysis) and/or by a fractionation procedure where solvents of different polarity were used. The attainment of fully satisfactory results in these systems was, however, hampered by the lack of stoichiometry between OH and CO_2H groups, as the ^1H NMR analysis of depolymerised suberin samples had indicated.[12] Hence, in order to ensure optimum yields and molecular weights in polyesterification reactions, the stoichiometry between OH and CO_2H groups was mandatory (r = 1), and accordingly in this work the various depolymerised suberin mixtures were balanced through the addition of an appropriate OH-bearing comonomer for cork suberin samples, and with a CO_2H-bearing comonomer for the birch outer bark suberin counterparts. The extra functional group sources were selected by their renewable origin, namely glycerol and 2,5-furandicarboxylic acid, respectively for OH and

CO_2H moieties. Glycerol was also selected for this work because of its recent availability at very low price on the market. Additionally, the synthetic comonomers 1,12-dodecanediol and decanedioic acid, were also used, both because of their lipophilic nature and their resemblance to suberin fragments.

This is the first systematic study of the use of well-characterised samples of cork suberin[12-13] to prepare new polyesters by polycondensation (Section 7.2) and polytransesterification (Section 7.3) reactions.[10-11] The structure of the ensuing polyesters was characterised and confirmed using FTIR, ^{1}H, ^{13}C NMR, and ^{13}C high-power proton decoupling cross polarisation magic angle spinning NMR (^{13}C HPPD/CP/MAS NMR) spectroscopies. Their thermal properties were studied by DSC, DMA and TGA. XRD analysis was also employed to assess the crystalline character of these materials. All the experimental details related to the present Chapter are given in Chapter 8 (PART III Experimental).

7.2 Polycondensation reactions of suberin from cork and birch outer bark

The synthetic protocols selected for the polycondensation of the hydrolysis-depolymerised suberins (free carboxylic acids) were similar to those adopted for the model systems (Chapter 6). Reactions were conducted at low temperatures either in bulk using $Bi(OTf)_3$ as catalyst, or in emulsion using the DBSA/H_2O (Table 7.20), and involved several essays to optimise reaction conditions in terms of yields. Polycondensations catalysed by lipase B from *Candida antarctica* were also tested. The ensuing cork and birch outer bark suberin derived polyesters are referred to as $pHDS_{cork}$ and $pHDS_{birch}$, respectively.

In accordance with the results obtained for the model polyesters (Chapter 6), the highest yields of isolation of pHDSs were also obtained when using $Bi(OTf)_3$ (~34-82%). This was especially true, as expected, when the polymerisation reactions were balanced with a compensating polyol (OH functional groups greater than or equal to 2) comonomer. For example, the yields of cork polyesters increased from 39 to 57% when 1,12-dodecanediol (DD) was added (runs 1 and 2, respectively). A similar result was observed for “birch” polymers, when decanedioic acid (DDA) or 2,5-furandicarboxylic acid (FDCA) were added, *i.e.* the yield had increased from 67% to 82% or 69% (runs 6, 7 and 8, respectively). The amount of DDA and FDCA added reflects the fact that ring opening of

the epoxy moiety occurred as described below in the discussion of the ^{13}C HPPD/CP/MAS NMR results.

If DBSA was used instead, once more in accordance with the results of the model polyesters (Chapter 6), the suberin polymers were isolated in lower yields (below 13%) which persisted even when a stoichiometric quantity of 1,12-dodecanediol was added ($\eta \approx$ 12%, run 5, Table 7.20), or the time of reaction raised ($\eta \approx$ 13%, run 9, Table 7.20).

Table 7.20 Experimental data related to the polycondensation of hydrolysis-depolymerised suberin from cork or birch outer bark.

run	catalyst	[a]comonomer (%)	[b]r	η (%)	[c]Q_{ns}
pHDS$_{cork}$					
1	Bi(OTf)$_3$	-	1.27	39	85
2	Bi(OTf)$_3$	0.4% DD	1.03	57	44
3	Bi(OTf)$_3$	0.1% Gly	1.07	34	-
4	DBSA	-	1.27	8	
5	DBSA	0.5% DD	0.98	12	
pHDS$_{birch}$					
6	Bi(OTf)$_3$	-	0.33	67	66
7	Bi(OTf)$_3$	4.5% DDA	1.00	82	44
8	Bi(OTf)$_3$	3.8% FDCA	1.06	69	-
9	[d]DBSA	-	0.33	13	6

[a] Percentage of comonomer added: DD stands for 1,12-dodecanediol, Gly for glycerol, DDA for decanedioic acid and FDCA for 2,5-furandicarboxylic acid. [b] Ratio between the number of carboxylic and hydroxy groups of HDS samples and comonomer (see Subsection 4.3.3.3 of Chapter 4). [c] Percentage of fraction insoluble in TCE (see Subsection 8.12.9 of Chapter 8). [d] Reaction time 96 h.

In general, it was observed that “birch” polyesters were isolated in higher yields than their cork counterparts, which is consistent with the GC-MS findings indicating a lower amount of monofunctional groups in birch outer bark suberin fragments (see Subsection 4.3.1 in Chapter 4), and thus a lower contribution to chain stoppage. The lipase-catalysed synthesis led to very low yields or even null yields (results not shown), both for birch outer bark and cork suberin samples.

7.2.1 Characterisation

The detailed characterisation of these novel polyesters involved first the inspection of the insoluble fractions, *i.e.* the cross-linked material, since the GC-MS results pointed out the presence of monomers with functionality higher than 2 in the HDS mixtures (see Subsection 4.3.1 in Chapter 4). The formation of cross-linked structures began by the formation of branches and then afterwards cross-links. The percentage of this fraction

(Q_{ns}) varied between 44 and 85% (Table 7.20) when $Bi(OTf)_3$ was used as catalyst. The cross-link density (as determined the swelling experiments and the application of the Flory-Rehner theory to the their results, (see Chapters 3 and 8) was equal to about 1900 g mol^{-1} for $pHDS_{cork}$-run 1, associated with an equilibrium swelling of 4% in TCE. A negligible insoluble fraction (~6%, run 9, Table 7.20) was obtained with the DBSA/water systems, probably due to the low extent of the reaction (yields ≤ 13%).

As just mentioned $Bi(OTf)_3$ catalysed polyesters were mainly composed of insoluble material, hence they were not characterised by SEC or other technique for molecular weight determination and also their contact angle with water was also not assessed. The DBSA catalysed polyesters were also not characterised in what concerns their molecular weight or contact angles with water, due to the associated low polycondensation yields.

FTIR and ^{13}C High Power Proton Decoupling Cross Polarization Magic Angle Spinning NMR analyses of the isolated product of the reaction confirmed the polymer formation. Figure 7.34 shows ATR FTIR spectra of $pHDS_{birch}$ and the HDS_{birch} counterpart.

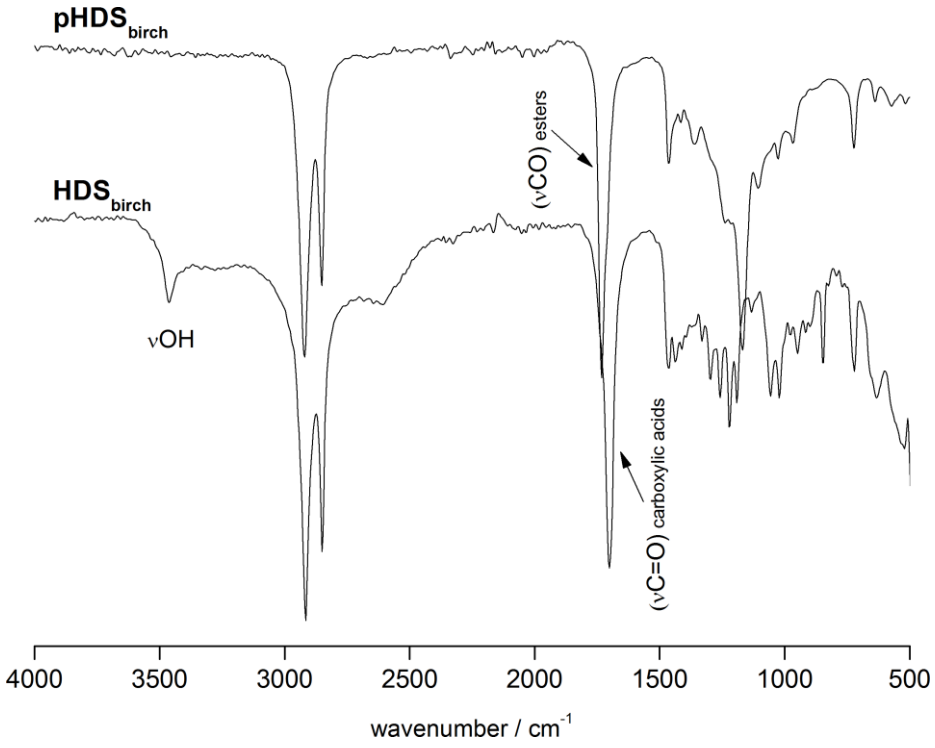

Figure 7.34 FTIR ATR spectra of $pHDS_{birch}$ polyester (run 7, Table 7.20) and of the starting depolymerised suberin mixture (HDS_{birch}).

The typical FTIR spectra of $pHDS_{birch}$ and its monomers (Figure 7.34) illustrated the differences between the polyesters and their precursors with the shift of the C=O band from the CO_2H mode in the monomers near 1703 cm^{-1} to 1731 cm^{-1}, the stretching mode of an ester group ($(\nu CO)_{esters}$), and the disappearance of the OH band around 3460 cm^{-1} (νOH).

Figure 7.35 shows typical ^{13}C HPPD/CP/MAS NMR spectra of the birch outer bark hydrolysis-depolymerised suberin mixture and of the corresponding suberin-based polyester.

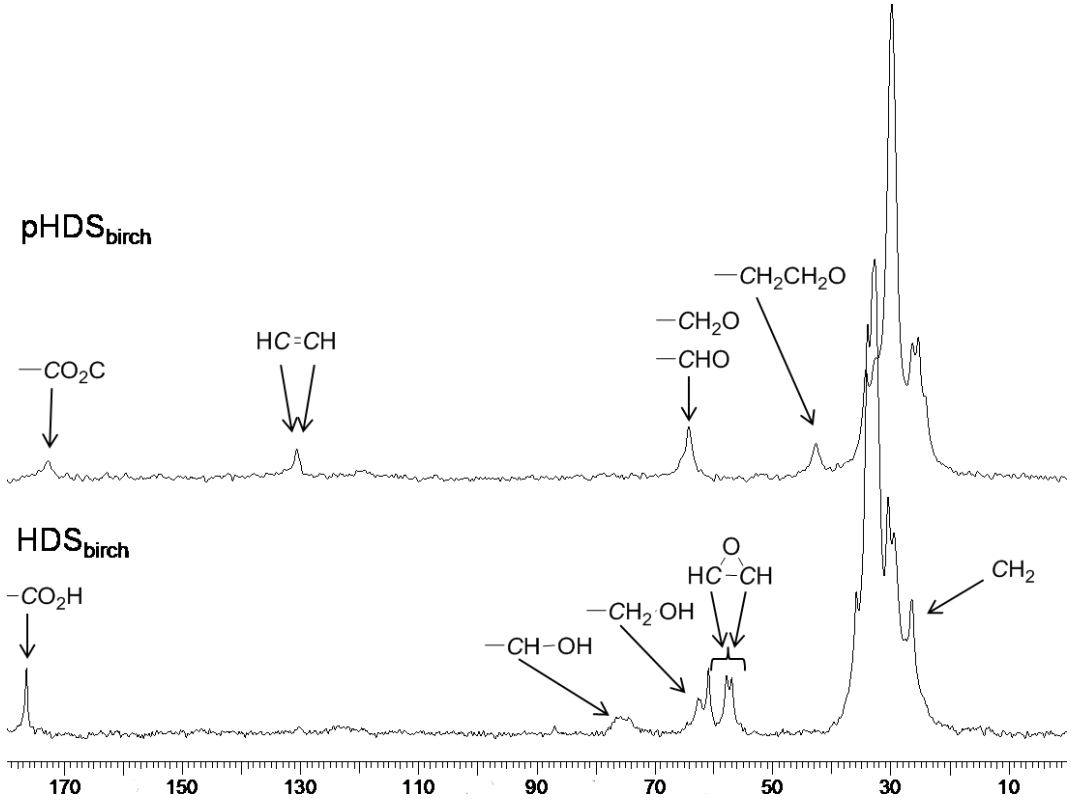

Figure 7.35 ^{13}C HPPD/CP/MAS NMR spectra of $pHDS_{birch}$ (run 7, Table 7.20) and of the starting depolymerised suberin mixture of birch outer bark.

Table 7.21 summarises the characteristic features of the ^{13}C HPPD/CP/MAS NMR spectra of the birch outer bark hydrolysis-depolymerised suberin mixture (HDS_{birch}) and of two suberin-based polyesters pHDS (runs 2 and 7, Table 7.20). pHDS's spectra exhibited the characteristic resonances of esterified structures, *i.e.* resonances at δ 64 and 173 ppm attributed to CH_2OCO and CHOCO, and CO_2C carbons, respectively. The "birch" polyester spectra did not display the resonances at δ 57-58 ppm (C*H*, epoxy ring) or at δ 76 ppm (*C*HOH, secondary alcohol) present in their starting monomer mixture, suggesting

that under the reaction conditions both functionalities had reacted forming ester linkages (*C*HOCO, δ 64 ppm). This transformation had certainly contributed to the high content of cross-linked structures found. The absence of the typical *C*H_3 carbons resonance at δ 14 ppm in the spectra of both HDS_{birch} and $pHDS_{birch}$ was consistent with GC-MS findings indicating a lower percentage of monofunctional compounds with methyl end-groups.

Table 7.21 Main peaks from the ^{13}C HPPD/CP/MAS NMR spectra of hydrolysis-depolymerised suberin and of two polyesters prepared using $Bi(OTf)_3$.[a]

assignment	δ / ppm		
	HDS_{birch}	$pHDS_{cork}$-run 2	$pHDS_{birch}$-run 7
***C*H$_3$**	-	14	-
CH_2	26-36	26-32	24-34
***C*H$_2$*C*H$_2$O**	-	43	43
***C*H (epoxy)**	57-58	-	-
***C*H$_2$OH**	62	-	-
***C*H$_2$O, *C*HO**	-	64	64
***C*HOH**	76	-	-
C*=*C	130	[b]	130
***C*O$_2$C**	-	173	173
***C*O$_2$H**	176	-	-

[a] It was not possible to analyse HDS_{cork} by ^{13}C HPPD/CP/MAS NMR due to technical problems related with the spinning of the rotor. [b] The resonance corresponding to the olefin *C*=*C* was masked by a broad signal.

The highly insoluble pHDS's exhibited accordingly a low degree of crystallinity, as indicated by their DSC and DMA thermograms (Table 7.22), corroborated by the X-ray diffraction patterns (Figure 7.36). The DSC thermograms displayed extremely broad melting peaks at relatively low temperatures compared with those observed for their suberin monomers counterpart. This was particularly evident with the $pHDS_{birch}$ polymers, whose melting peaks were always below 50 ^{o}C. The thermal features of all the pHDS polymers were quite reproducible after several heating and cooling cycles. Indeed, it was observed that after a resting period of 13 days the heating and cooling traces were equivalent to those observed in the first series of scans for a given sample, as already observed with the suberin mixtures.

The glass transition temperatures of the powdered polyesters, as well as of the suberin mixtures (Chapter 4), were determined by the highly T_g sensitive technique- DMA, at two frequencies (1 and 10 Hz), in order to confirm the nature of the relaxation processes. As shifts were in the range of 8 ^{o}C per decade of frequency they were most certainly glass transitions. The pHDS materials exhibited T_g values between -18 and 39 ^{o}C (Table 7.22), whereas those of their monomers were less than or equal to -31 ^{o}C (see Table 4.10, Chapter 4). It is well known that the cross-link density influences the T_g,

through the frequency of the segmental motion, which makes this property a frequently investigated parameter to characterise cross-linked materials.[5,7] The highest T_g temperature was observed for $pHDS_{cork}$ run 2 (39.0 ^oC), indicating a correspondingly higher cross-link density, although the percentage of fraction of insoluble in TCE was not the highest value obtained.

Table 7.22 The glass transition and melting temperatures of the pHDS polyesters and of their monomer mixtures.

run	[a] T_g / ^oC	[b] T_m / ^oC
$pHDS_{cork}$		
1		43, 52, 73
2	39	42, 51
3		33-78
5		81
$pHDS_{birch}$		
6	-18	17, 39
7	-11	16, 39
8	-9	15, 38

[a] T_g was determined by DMA at 1 Hz using the tan δ maximum. [b] T_m was determined by DSC at 10 ^oC min^{-1} using the 1st heating trace.

The XRD patterns of the highly insoluble pHDS polyesters ($Bi(OTf)_3$-catalysed systems) showed a pronounced amorphous halo centred at 2θ ~20^o, and a poorly resolved peak at 2θ ~22^o (Figure 7.36).

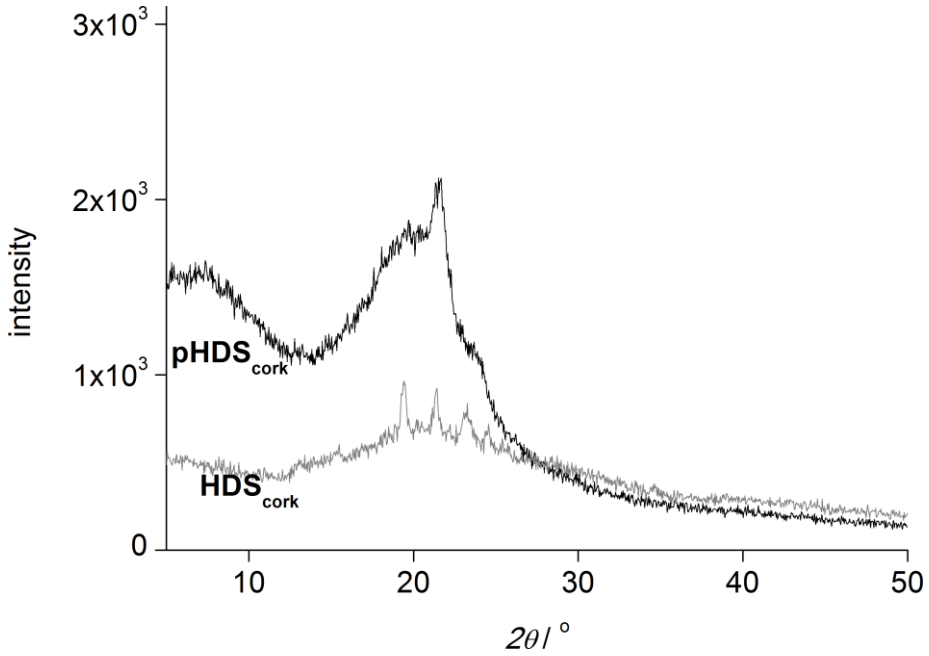

Figure 7.36 XRD pattern of a $pHDS_{cork}$ polyester (run 2, Table 7.20) and of the starting depolymerised suberin mixture (HDS_{cork}).

The other pHDS polymers with lower fractions of insoluble products (DBSA catalysis, runs 4, 5 and 9, Table 7.20) showed important crystalline domains through their XRD patterns with well-resolved peaks and their DSC thermograms displayed well-defined melting features around 80 ^{o}C.

The evidences just described corroborate the picture where amorphous cross-linked structures were formed when the extent of the reaction between HDS monomers was high (both primary and secondary OH groups reacted in the presence of $Bi(OTf)_3$). However, if the extent of the reaction was lower (DBSA catalyst) the ensuing polymers presented a higher extent of crystalline domains.

TGA was used to investigate the thermal decomposition behaviour of these polyesters under a nitrogen atmosphere (Table 7.23 and Figure 7.37).

Table 7.23 Degradation temperatures of pHDS polyesters.

run	T_d / ^{o}C
pHDS$_{cork}$	
1	414, 458, 647
2	406, 457, 628
5	410, 466, 637
pHDS$_{birch}$	
6	329, 434, 485, 612
7	345, 446, 474, 615
8	447, 598

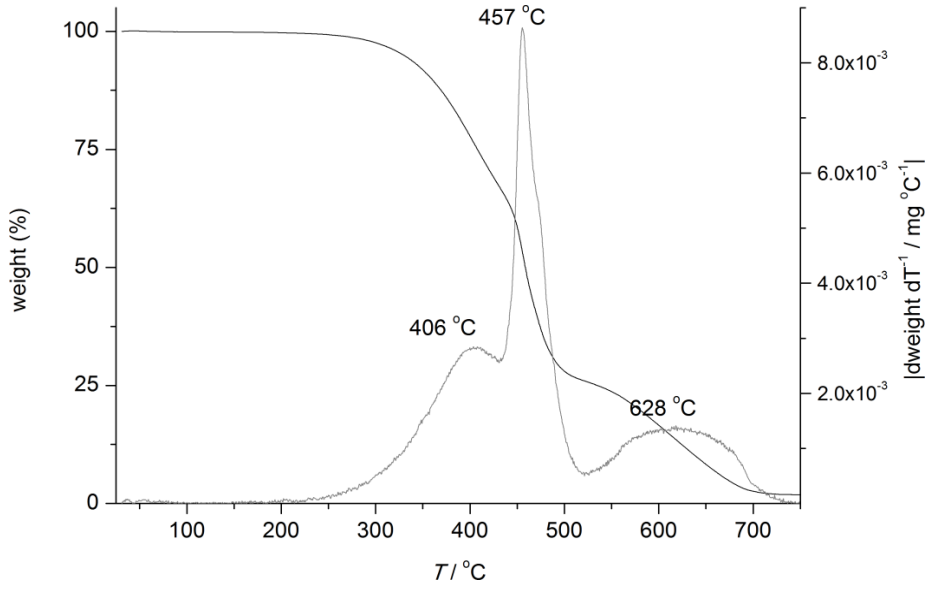

Figure 7.37 TGA and derivative TGA (dweight dT^{-1}) thermograms of pHDS$_{cork}$ (run 2, Table 7.20).

All polymers appeared to be thermally stable up to 290 ^{o}C (less than 2% weight loss), and to degrade in three distinct steps (Table 7.23). Figure 7.37 shows a typical TGA thermogram of a pHDS$_{cork}$ (run 2, Table 7.20), with three characteristic features at about 406 ^{o}C, followed by another weight loss around 457 ^{o}C, and a third at about 628 ^{o}C. These TGA measurements revealed a total 98% weight loss at temperatures between 290 and 800 ^{o}C. The thermogram profiles of suberin polyesters were quite similar to those observed for their suberin monomers counterpart, since both presented a degradation in three steps, although the degradation temperatures were consistently higher for the polyesters (see Subsection 4.4.3 of Chapter 4).

7.3 Polytransesterification reactions of suberin from cork

The bulk polytransesterifications of methanolysis-depolymerised suberins (methyl esters of carboxylic acids extracted with *n*-hexane or with DCM) was also studied, using Sb_2O_3 as catalyst (Table 7.24) and were found to be relatively rapid (typically around 9 hours). The ensuing cork polyesters are referred as pHEX-MDS$_{cork}$ and pDCM-MDS$_{cork}$ for HEX-MDS$_{cork}$ or DCM-MDS$_{cork}$, respectively.

Table 7.24 Experimental data related to the polytransesterification of methanolysis-depolymerised suberin from cork using antimony(III) oxide as catalyst.

run	[a]DD (%)	[b]*r*	*η* (%)	[c]M_w	[d]M_w/M_n
pHEX-MDS$_{cork}$					
10	-	1.67	14	3 800	1.4
11	1.0	1.06	40	4 800	1.1
12	1.7	0.85	23	4 600	1.1
pDCM-MDS$_{cork}$					
13	-	1.43	50	-	-
[e]**14**	-	1.43	54	-	-

[a] Percentage of DD added. [b] Ratio between the number of carboxylic and hydroxy groups of MDS samples and of DD added (see Chapter 4). [c] M_w were determined by SEC in $CHCl_3$. [d] Polydispersity index determined by SEC in $CHCl_3$. [e] Reaction carried out using K_2CO_3 as catalyst as described in Section 8.11 of Chapter 8.

These polyesters were isolated with yields comprised between 14 and 54%, weight-average molecular weights from 3800 to 4800 Da, and a polydispersity index unexpectedly close to 1 (Table 7.24). This latter value must be an artefact both caused by the procedure followed to recover the polymer, where only the methanol insoluble polyesters were recovered and the oligomeric species remained dissolved in methanol (ATR FTIR spectra of the viscous residue obtained after evaporating methanol from the

filtered medium corroborated this explanation) and by some extent of insolubility of these polyesters in chloroform for the concentrations used in the SEC analyses.

Once more, these polytransesterification results were strongly related to the addition (or not) of a stoichiometric quantity of DD to balance the suberin known intrinsic lack of stoichiometry (Subsection 4.3.3.3 of Chapter 4). By this correction, both the η and M_w improved from *e.g.* 14% and 3800 (run 10, Table 7.24) to 40% and 4800 (run 11, Table 7.24). However, if DD was not added in the precise amount to make r approach unity, as in the case of run 12, the yield decreased drastically to 23%, yet the M_w decreased only to 4600. Additionally, the polytransesterification catalysts tested, antimony(III) oxide and potassium carbonate, led to the formation of pDCM-MDS_{cork} polyesters in comparable yields (both around 50%, see *e.g.* runs 13 and 14, Table 7.24).

They were ointment-yellow to brown, and in the case of pHEX-MDS_{cork}, they were completely soluble in non-polar organic solvents, suggesting the formation of linear chains although not excluding some degree of branching. pDCM-MDS_{cork} polyesters, like the pHDSs catalysed by $Bi(OTf)_3$, contained an insoluble fraction, suggesting the formation of cross-linked structures. These results were in tune with the GC-MS chemical composition of depolymerised suberin mixtures indicating that the HEX-MDS_{cork}, compared with the other samples, had a lower amount of monomers with functionality higher than 2 (Chapter 4).

7.3.1 Characterisation

All the soluble polyesters synthesised by potransesterification, independent of the specific structure examined, presented obviously hydrophobic character, with water contact angles ranging between 95 and 100^o, similarly to their monomer counterpart.[14] This was not surprising given the fact that all the materials bear long aliphatic chains, which orient themselves at the surface of the polymer film; that is, the water drop always comes into contact with the same nonpolar moieties. It follows that these new suberin-based polyesters were indeed highly hydrophobic materials.

^{1}H NMR spectroscopy was used to confirm the formation of the expected pHEX-MDS_{cork} polyesters (Table 7.25 and Figure 7.38). ^{13}C HPPD/CP/MAS NMR spectroscopy was used instead in the case of the insoluble polyesters pDCM-MDS_{cork}.

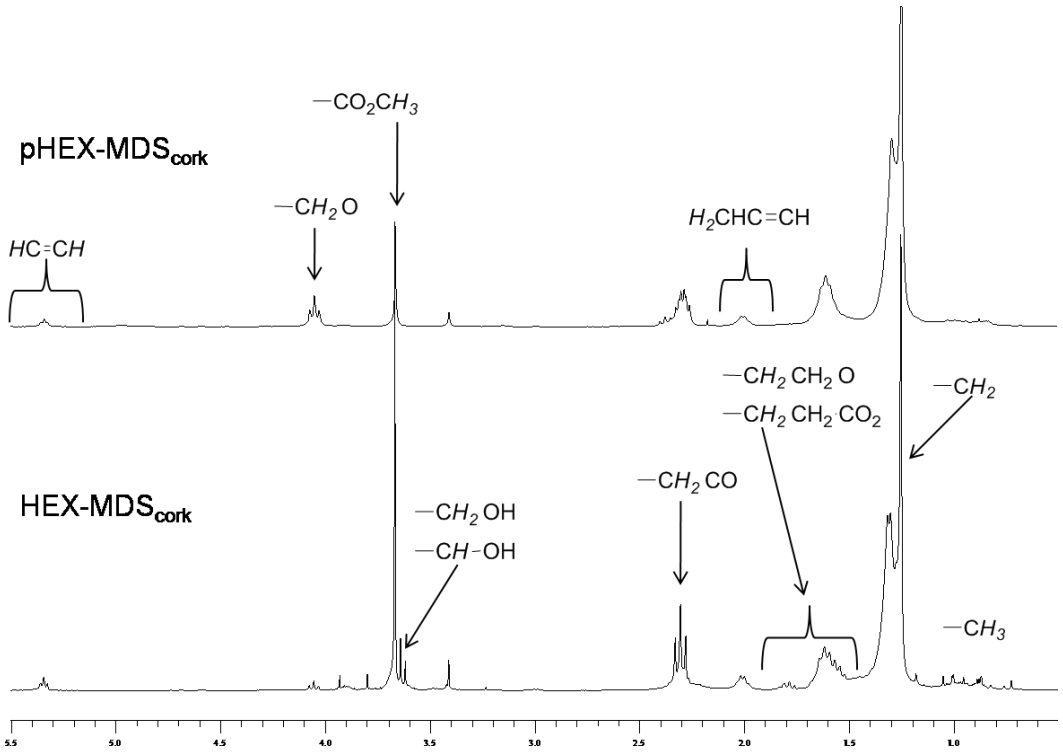

Figure 7.38 ^{1}H NMR spectra of pHEX-MDS$_{cork}$ (run 11, Table 7.24) and of the starting depolymerised suberin mixture (HEX-MDS$_{cork}$).

The ^{1}H NMR spectra of pHEX-MDS$_{cork}$ displayed some important differences compared to those of their precursors (Table 7.25 and Figure 7.38), namely a substantial decrease in the relative intensity of the singlet assigned to the protons of CO_2CH_3 (δ 3.67 ppm), the disappearance of the resonance assigned to the protons of CH_2OH and $CHOH$ (δ 3.64 ppm), and an increase in the relative intensity of the triplet attributed to the protons of the OCH_2 groups of an ester (δ 4.05 ppm).

Table 7.25 Main peaks in the ^{1}H NMR spectra of methanolysis-depolymerised suberin and of one of its typical polyesters (run 11, Table 7.24).

δ/ ppm	**[a]multiplicity**	**assignment**	**integration**	
			HEX-MDS$_{cork}$	**pHEX-MDS$_{cork}$**
0.72-1.05	m	CH_3	6	6
1.25-1.30	d	CH_2	56	78
1.61-1.78	m	CH_2CH_2O, CH_2CH_2CO	14	21
2.00	m	CH_2CH=CH	3	3
2.29	t	CH_2CO	8	10
2.38	t	CH_2CO_2H	tr	tr
3.64	t	CH_2OH,$CHOH$	2	-
3.67	s	OCH_3	9	4
4.05	t	OCH_2	tr	4
5.34	m	CH=CH	1	1

[a] multiplicity: s= singlet; d=doublet; t=triplet; m=multiplet.

The ^{13}C NMR spectra were consistent with these ^{1}H NMR results and the ^{13}C HPPD/CP/MAS/NMR spectra of the pDCM-MDS_{cork} further confirmed the same structural features relative to the corresponding polyester networks.

The FTIR spectra of all the polyesters were essentially the same as those of their precursors, except, as expected, for the disappearance of the band attributed to the stretching mode of the OH group at 3460 cm^{-1} and the disappearance of a small shoulder attributed to the vibration associated with the methyl group stretching mode around 3001 cm^{-1}.

The pHEX-MDS_{cork} materials displayed a high degree of crystallinity, as indicated by their DSC thermograms (Table 7.26 and Figure 7.39) and XRD patterns (Figure 7.40).

Table 7.26 Glass transition and melting temperatures of pHEX-MDS_{cork} polyesters.

run	[a] T_g / °C	[a] T_m / °C
pHEX-MDS_{cork}		
10	-	39, 54, 73
11	-	46, 64, 72
12	-13	39, 52, 72

[a] T_g and T_m were determined by DSC at 10 °C min^{-1}.

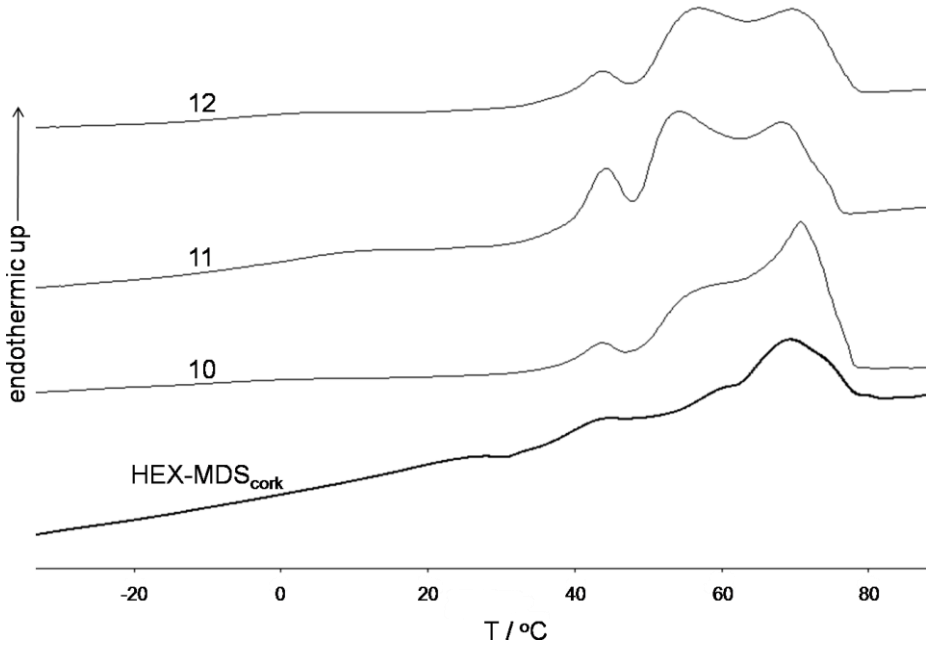

Figure 7.39 DSC thermogram of pHEX-MDS_{cork} polyesters (runs 10-12, Table 7.24) and of the corresponding depolymerised suberin mixture HEX-MDS_{cork}.

Their first common feature was the presence of multiple melting points ranging from 35 to 80 °C in their DSC heating traces, consisting of a reasonable well-defined

melting peak, typically around 40 ^{o}C, and a broader peak between 50 and 80 ^{o}C, with an indication of two small peaks around 55 and 70 ^{o}C. Comparing these polyester traces with those of their monomer mixtures they showed slightly higher melting temperatures for the former, as could be observed in Figure 7.39. The second common feature was a glass transition below room temperature, as expected for macromolecules bearing long aliphatic segments in their backbone.

On the one hand, the XRD patterns (Figure 7.40) of pHEX-MDS$_{cork}$ polyesters clearly suggested their crystalline character with intense peaks at *2θ* ~21 [110], 24 [200], 28, 30 [210], 36 [020], and 41º [310], assigned to the orthorhombic system. This pattern compares favourably with literature results for polyethylene[15-16] or long chain hydrocarbon mixtures.[17]

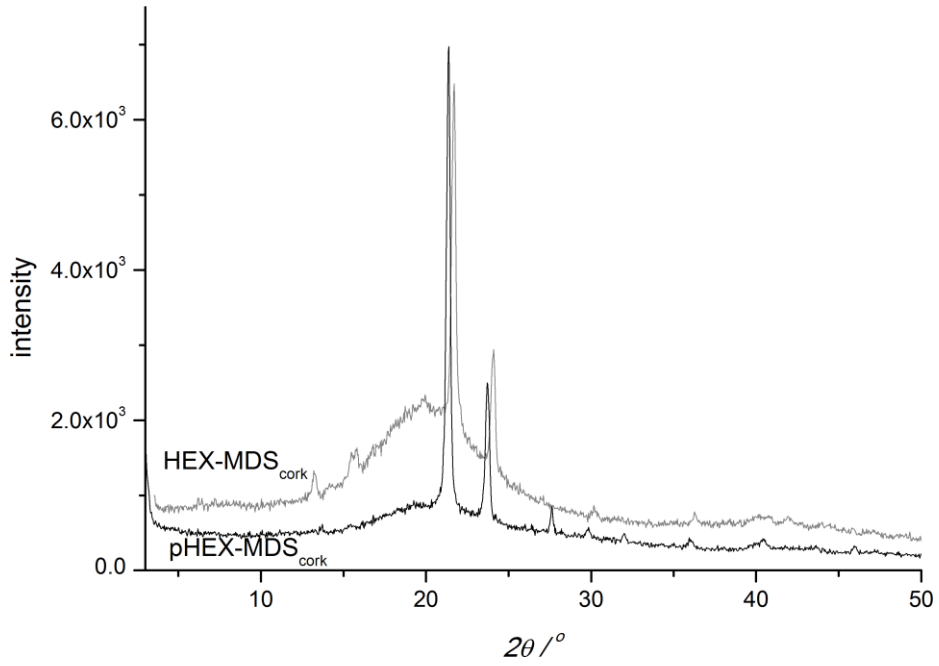

Figure 7.40 XRD pattern of pHEX-MDS$_{cork}$ polyester (run 12, Table 7.24) and of the corresponding depolymerised suberin mixture HEX-MDS$_{cork}$.

On the other hand, pDCM-MDS$_{cork}$ patterns (not presented) displayed a prominent amorphous halo centred on *2θ* ~20^{o}. Also, the peaks representing a crystalline phase, appearing at the same *2θ* angles, were not so distinct, indicating a lower degree of crystallinity for pDCM-MDS$_{cork}$.

The suberin mixture XRD patterns (see *e.g.* Figure 7.40) were similar to those of their polyesters counterparts, although with peaks slightly shifted to higher angles,

indicating closer crystallographic packing. Additionally, the suberin patterns indicated a higher proportion of amorphous regions.

All the TGA thermograms of these polyesters displayed a steep degradation feature beginning at about 400 ^{o}C (~50% weight loss) and in the case of the pHEX-MDSs polyesters, an additional step at approximately 615 ^{o}C (Table 7.27). These polymers were found to be thermally stable up to 300 ^{o}C (less than 2% weight loss), which is approximately 100 ^{o}C more that the value obtained for the corresponding suberin monomers (see Subsection 4.4.3 of Chapter 4).

Table 7.27 Degradation temperatures of $pMDS_{cork}$ polyesters and of their monomer mixtures.

run	T_d / ^{o}C
pHEX-MDS$_{cork}$	
11	439, 616
pDCM-MDS$_{cork}$	
14	422

The present results showed that it is possible to synthesise novel polyesters entirely based on renewable resources with predominantly linear chains (with some degree of branching) or, instead, with variable amounts of cross-linked structures (pHEX-MDS_{cork} *vs.* pDCM-MDS_{cork}).

7.4 Relative interest and drawbacks of the mechanistic approaches

The polycondensation and polytransesterification reactions performed in this study behaved according to a classical stepwise mechanism, depending obviously on factors such as the functionality of the suberin monomers and their stoichiometric balance, the presence of monofunctional impurities, and, of course, on the effective shifting of the reaction equilibria towards polyester formation (Chapter 3). Additionally, the structure and properties of the products of these reactions are obviously quite dependent on the suberin mixture input used to prepare them, and hence quite dependent on the natural variability of suberin.

The knowledge of the chemical composition of the two different species, *viz.*, *Quercus suber* L. or *Betula pendula* Roth, confirmed a higher presence of polyfunctional

epoxy derivatives in the latter. However, together with the adequate suberin isolation protocol and the experimental conditions adopted here, it was possible to apply an appropriate control of the ensuing macromolecular architectures and thus obtain either linear or cross-linked polyesters.

This suberin polycondensation and polytransesterification reactions were also quite affected by the lack of stoichiometric balance of these monomer mixtures, as could be easily estimated by the corrected form of the Carothers equation. However, this was overcome by adding an appropriate amount of a balancing comonomer.

The presence of monofunctional groups in depolymerised suberin was another factor, which affected some of the polymerisation reactions by stopping them abruptly, except for HDS_{birch} suberin, where the monofunctional compounds were thoroughly extracted before the suberin depolymerisation.

7.5 Conclusions

This first systematic investigation showed that suberin, a ubiquitous but still sub-exploited natural polymer, is a valuable renewable resource for the preparation of novel hydrophobic materials, whose properties resemble those of petroleum-based aliphatic polyesters. Moreover, it was possible to synthesise two types of polyesters in high yields (up to 82%), under mild reaction conditions. Improved results in terms of yields and molecular weights were obtained when reactions were carried out under stoichiometrically balanced conditions by the addition of an appropriate amount of a compensating OH-bearing comonomer for cork suberin samples, and with a CO_2H-bearing comonomer for the birch suberin counterparts.

These polyesters bore either linear or branched/network structures depending on their synthetic conditions and the specific nature of the monomer mixture employed. Both the polycondensation and polytransesterification approaches were doubly beneficial from a green perspective, as they avoided the use of organic solvents as reaction media and employed adequate catalysts.

These polymers may have potential applications, as for example in terms of latex arising from the emulsion polymerisation and hence in coatings or in paints.[18] Other possible applications are related to the cross-linked polymers in thermosetting coatings;

although this would require that the cross-linking reactions take place *in situ,* during the actual processing. Although, applications were not the major focus of this study, the fundamental investigation carried out here, covering polymer characterisations and the optimisation of their synthesis, was definitely the first step towards the development of future possible practical issues.

7.6 References

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[PART III EXPERIMENTAL]

8 Substrates, chemicals, general procedures and techniques

What were the main materials used in this study? ? How was the suberin depolymerisation carried out? How was the ash content determined? How was lignin isolated from cork or birch outer bark? And what about carbohydrates? How were suberin mixtures of monomers and their model systems polycondensed? How were suberin mixtures and their polyesters characterised? How were suberin model polyesters characterised? And the other products?

8.1 Introduction

In this Chapter, a synthetic but hopefully clear description of all procedures and techniques adopted in this work is provided.

The suberin depolymerisation products, as earlier mentioned in Chapter 4, were isolated by either alkaline hydrolysis or methanolysis (Section 8.4), and their products characterised using different techniques (Section 8.12). The overall chemical compositions of cork, birch outer bark and industrial cork powder were also determined, using different experimental procedures to isolate and characterise them (Sections 8.3-8.7 and 8.12).

The synthesis of polyesters from suberin monomers or model monomers was carried out using polycondensation (Section 8.10) or polytransesterification (Section 8.11) reactions. The ensuing products were characterised by means of various techniques (Sections 8.12).

8.2 Substrates, reagents and solvents

Quercus suber L. cork planks, “amadia grade”, were supplied by Corticeira Amorim mill (Herdade da Moinhola, Portugal, March, 2005). These included cork planks after a resting period in the field (cork), and boiled cork planks after a cork cooking stage (Bcork). Outer and inner fractions of cork and boiled cork planks were hand-cut corresponding to surface fractions 3-5 mm and 5-10 mm thick, respectively.

The industrial cork powder (ICP) was also supplied by Corticeira Amorim mill (Santa Maria de Lamas, Portugal, February, 2005). Black condensates (Bcond) were sampled in the Amorim Revestimentos mill (Lourosa, Portugal, November, 2004). Cork samples were ground in a Retsch SK hammer mill, sieved and the granulometric fraction of 40-60 mesh used for analysis.

Betula pendula Roth outer bark was a generous gift of Professor Christer Eckerman (Vaasa, Finland, June, 2005). Bark was ground in a laboratory mill to pass a 6 mm screen, separated in water into inner (sedimented) and outer bark (floating). The ensuing outer bark was air dried, sieved and the granulometric fraction of to 40-60 mesh used for analysis.

Sodium methoxyde ($NaOCH_3$, 97%), sodium hydroxide (NaOH, ≥98%), sodium borohydride (99%), *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide (≥99%), trimethylchlorosilane (≥98%), trichloroacetyl isocyanate (TAI, ≥97%), hexadecanoic acid (99%), docosanoic acid (99%), *n*-tetracosane (99%), *n*-hexadecane (≥99%), stigmastanol (97%), betulinic acid (90%) oleanolic acid (97%), ellagic acid (97%), 1,12-dodecanediol (DD, 99 %), decanedioic acid (DDA, 99%), dodecanedioic acid (98%), 12-hydroxydodecanoic acid (97%), 12-hydroxyoctadecanoic acid (99%), glycerol (Gly, 99%), *p*-dodecylbenzene-sulfonic acid (DBSA, 90%), bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$, ≥99%), antimony(III) oxide (Sb_2O_3, ≥99%), and lipase from *Candida antarctica* immobilized in an acrylic resin were purchased from Sigma-Aldrich Chemicals. Potassium hydroxide (KOH, 85%) and potassium carbonate (K_2CO_3) were purchased from Merck. 1-methylimidazole was purchased from Fluka (≥99%). 2-Deoxy-*D*-glucose (99%) was purchased from Acros. Indium (99.999%) and lead (99.999%) were purchased from Perkin-Elmer. 2,5-Furandicarboxylic acid (FDCA) was a gift from Prof. Antoine Gaset of Toulouse National Polytechnic Institute. All solvents used were analytically pure or higher grade.

All the above chemicals were used without further purification.

8.3 Isolation of extractives

Soxhlet extraction of cork and cork by-products. Extractives from cork, industrial cork powder and black condensate samples were removed from solid samples (~ 20 g) by sequentially Soxhlet extraction with dichloromethane (70 mL), methanol (70 mL), and water (70 mL) during 10 h for each solvent. The resulting extracts were then freed from the solvent in a rotary evaporator, vacuum-dried, and weighed. Each of these solvent extractions was carried out in triplicate. The ensuing dichloromethane, methanol and water extractives are referred in the text as DCM-EXT, MeOH-EXT, water-EXT, respectively.

Soxhlet extraction of birch outer bark samples. Birch outer bark extractives were removed from solid samples (~20 g) by Soxhlet extraction with ethanol (750 mL) during 20 h. This extraction was carried out in triplicate. The resulting extracts were then dried to constant weight and quantified gravimetrically.

Alkaline hydrolysis of DCM extracts from cork and cork by-products. Alkaline hydrolysis of the DCM extracts from cork, boiled cork, industrial cork powder and from

black condensate was carried out in order to evaluate the presence of esterified structures in these samples. Each extract (20 mg) was heated at 100 °C with a KOH solution (0.5 M, 10 mL) in methanol/water (1:9 v/v), under a nitrogen atmosphere, for 1 h. The ensuing mixture was cooled to room temperature, acidified with aqueous hydrochloric acid (1 M) to pH 2, and extracted three times with DCM (5 mL). The resulting DCM extract was then passed over anhydrous sodium sulphate, freed from this solvent in a rotary evaporator, vacuum-dried, and weighed.

8.4 Depolymerisation of suberin

Alkaline hydrolysis of suberin from cork and industrial cork powder. The alkaline hydrolyses[1] of both cork and industrial cork powder were carried out by heating the powdered sample (16 g) with a KOH solution (0.5 M, 2 L) in ethanol/water (9:1 v/v) at 70 ^{o}C for 1.5 h. The ensuing mixture of hydrolysed suberin fragments was cooled to room temperature, acidified with aqueous hydrochloric acid (2 M) to pH 3-3.5, and extracted three times with 500 mL of diethyl ether, this latter solution being then extracted once with 500 mL of water in order to remove any water-soluble compound. The resulting diethyl ether extract was then passed over anhydrous sodium sulphate, freed from this solvent in a rotary evaporator, vacuum-dried, and weighed. The ensuing mixtures of hydrolysed suberin fragments are referred in the text as HDS_{cork} or HDS_{ICP} (hydrolysis-depolymerised suberin of cork or of ICP, respectively).

Alkaline hydrolysis of birch outer bark suberin. The alkaline hydrolysis[2] of the powdered birch outer bark was conducted by refluxing the sample (~100 g) with a NaOH solution (1L, 0.6 M) in 2-propanol/water (9:1 v/v) for 1 h, followed by filtration of the solid residue. The residue was then further refluxed for 15 min with 2-propanol (0.5 L) and again filtered. The combined liquid fractions were allowed to stand overnight at -18 ^{o}C and the precipitate formed was filtered and dried under vacuum. The ensuing precipitate of sodium salts of suberin monomers was mixed with water, acidified to pH 3-3.5, and promptly extracted three times with methyl tert-butyl ether. The extract was passed over anhydrous sodium sulphate, the solvent removed in a rotary evaporator and the residue weighed. This residue is referred in the text as HDS_{birch} (birch outer bark hydrolysis-depolymerised suberin).

Alkaline methanolysis of suberin from cork and industrial cork powder. The alkaline methanolysis[3] of both cork and industrial cork powder were conducted by refluxing the

powder sample (16 g) in a dry methanol $NaOCH_3$ solution (0.6 M, 2.0 L) for 4 h, followed by filtration of the solid residue. The residue was then refluxed for 1 h with dry methanol (0.5 L) and filtered again. The combined liquid fractions were acidified to pH 3-3.5 with diluted hydrochloric acid (2.0 M), and then extracted three times with 500 mL of dichloromethane. The resulting extract was then passed over anhydrous sodium sulphate and freed from this solvent in a rotary evaporator, vacuum-dried, and weighed. The ensuing mixtures are referred in the text as DCM-MDS_{cork} or DCM-MDS_{ICP} (dichloromethane methanolysis-depolymerised suberin of cork or ICP, respectively).

A sample of the DCM-MDS_{cork} extract (~8 g) was further fractionated by refluxing it with *n*-hexane (250 mL) for 4 h, cooling to room temperature, and isolating the *n*-hexane soluble fraction. The *n*-hexane was removed in a rotary evaporator and thereafter the residue was vacuum-dried and weighed. This sample is referred in the text as HEX-MDS_{cork}.

8.5 Determination of the ash content

The ash content of cork, birch outer bark and industrial cork powder samples was determined according to TAPPI standard T 221 om-93. The solid samples were burned in a furnace at approximately 525 ^{o}C, during 1 h. The ensuing ash residue was cooled to room temperature and weighted.

8.6 Determination of the Klason lignin

The acid-insoluble (Klason) lignin of all solid residues, obtained after suberin isolation (by alkaline hydrolysis), was determined by the Klason method[4] according to TAPPI standard T222 om-88. The free-suberin residue (1 g) was suspended with concentrated H_2SO_4 (72%, 15 mL) at 20 ^{o}C, followed by dilution with water (300 mL), and refluxing for 2.5 h. The ensuing solid Klason lignin was filtered and dried.

8.7 Determination of carbohydrates

The total carbohydrates content of all solid residues, obtained after suberin isolation (by alkaline hydrolysis), were determined by quantification of the neutral sugars released by

Saeman-based hydrolysis.[5] The acid hydrolysis was conducted by treating the free-suberin residue (10 mg) with concentrated H_2SO_4 (72% w/w, 400 µL) under nitrogen, at room temperature, during 3 h, followed by dilution with water (4.4 mL) and heating at 100 ^oC during 2.5 h. The ensuing solution was cooled to room temperature, neutralised with ammonia solution (25% v/v), and then 2-deoxy-*D*-glucose (20 mg mL^{-1}, 0.05 mL) was added as an internal standard for GC-FID analysis. Aliquots (1 mL) were reduced and acetylated to yield alditol acetates. Monosaccharides were reduced by adding a solution of sodium borohydride in ammonia (0.1 mL of 0.15 g mL^{-1}) with heating at 30 ^oC, for 1 h. Then, the excess of sodium borohydride was decomposed by adding concentrated CH_3CO_2H (0.1 mL, 18 M). The acetylation of the reduced monosaccharides was conducted by adding 1-methylimidazole (0.45 mL), and acetic anhydride (3 mL), and heating at 30 ^oC for 30 min. The excess of acetic anhydride was decomposed by adding water to the mixture (5 mL) in an ice bath. Then, the mixture was extracted with DCM (1 mL) and the ensuing alditol acetates mixtures analysed by GC-FID.

8.8 Derivatisation of depolymerised suberin and extracts

Prior to all GC-MS analysis, each suberin or extractives sample (~20 mg) was trimethylsilylated:[6] the residue dissolved in pyridine (250 µL), and components containing hydroxy and carboxylic acid groups converted to their trimethylsilyl (TMS) ethers and esters, respectively, by adding *N,O-bis*(trimethylsilyl)trifluoroacetamide (250 µL) and trimethylchlorosilane (50 µL), and the adequate amount of internal standard. After the mixture had stood at 70 °C for 30 min, the TMS derivatives were analysed by GC-MS.

8.9 Derivatisation of depolymerised suberin with trichloroacetyl isocyanate

The suberin sample (~15 mg) was dissolved in $CDCl_3$ (500 µL) in a NMR tube, and an excess of TAI (varying between 45-200 µL) was added at room temperature, in a controlled argon atmosphere to avoid side reactions with moisture.[7] The mixtures were stirred until complete dissolution and the ^1H NMR spectrum promptly recorded.

8.10 Polycondensation of suberin and model suberin monomers

Bismuth(III) trifluoromethanesulfonate-catalysed polycondensations. Typically, reactions were carried out using approximately 1 g of suberin-like monomers or using approximately 0.5 g of HDS_{cork} or HDS_{birch} and an adequate quantity of a comonomer to adjust the stoichiometry between the OH and CO_2H groups (1,12-dodecanediol or glycerol for HDS_{cork}, and decanedioic acid or 2,5-furandicarboxylic acid for HDS_{birch}), and $Bi(OTf)_3$ ($5x10^{-3}$ mmol mol^{-1} or $6.5x10^{-2}$ mmol g^{-1}, respectively). The mixture was stirred with 2 mL of 1,4-dioxan at 90 ^oC for 1 h before applying vacuum gradually (~10^{-3} mbar) for 48 h, so that 1,4-dioxan and water were slowly removed.[8] Then, the mixture was dissolved in DCM (~25 mL) and the polymer precipitated, by pouring the solution into an excess of cold methanol (~1 L) to remove the $Bi(OTf)_3$ and the soluble oligomers, filtered, dried under vacuum, and weighted. These polymers are referred in the text as model or suberin polyesters ($pHDS_{cork}$ and $pHDS_{birch}$).

Enzymatic polycondensation. Typically, reactions were conducted in bulk by mixing 1 g of suberin-like monomers or 1 g of hydrolysis-depolymerised suberin and the *Candida antarctica* lipase B (5-25% w/w) at 70 ^oC for 48 h, with vigorous stirring.[9] Then, the mixture was dissolved in DCM (100 mL), and the insoluble enzyme was separated by filtration. The excess of dichloromethane was then removed in a rotary evaporator, and the polymer was precipitated, by pouring the solution into an excess of cold methanol (~1 L) to remove the soluble oligomers, filtered, dried under vacuum, and weighted. These polymers are referred in the text as model or suberin polyesters ($pHDS_{cork}$ and $pHDS_{birch}$).

Emulsion polycondensation. Typically, reactions were carried out using approximately 1 mmol of suberin-like monomers, or approximately 0.5 g of HDS_{cork} or HDS_{birch} and a stoichiometric quantity of a comonomer (1,12-dodecanediol for HDS_{cork}), suspended in water in the presence of DBSA (0.49 mmol $mmol^{-1}$ and 6 mmol g^{-1}, respectively).[10] The mixture was stirred at 80 ^oC for 48 h. Then, DCM (~25 mL) was added to the mixture and the polymer precipitated, by pouring into an excess of cold methanol (~1 L) to remove the DBSA and the soluble oligomers, filtered, dried under vacuum and weighted. These polymers are referred in the text as model or suberin polyesters ($pHDS_{cork}$ and $pHDS_{birch}$).

8.11 Polytransesterification of suberin and model suberin monomers

Reactions were carried out in bulk typically using approximately 1 g of suberin-like monomers or using approximately 0.5 g of MDS_{cork}, a stoichiometric quantity of a comonomer (1,12-dodecanediol), and 2% w/w of Sb_2O_3. The mixture was heated progressively from 100 ^oC to 160-190 ^oC during 2 h, and then kept for 7 h at that maximum temperature under high vacuum (~10^{-6} mbar) with constant stirring. The ensuing $pMDS_{cork}$ polymers were dissolved in DCM (~25 mL), acidified to pH 4-5 with concentrated HCl, and then precipitated, by pouring into an excess of cold methanol (~1 L) to remove the Sb_2O_3 and the soluble oligomers, filtered, dried under vacuum and weighted. These polymers are referred in the text as $pMDS_{cork}$.

Reactions with K_2CO_3 were carried out following a similar procedure.

8.12 Analyses

The suberin depolymerisation products, as already mentioned in Chapter 4, were characterised in detail using several chemical and physical means, including chromatographic, spectroscopic and thermal techniques. The conditions adopted when using these techniques will be described briefly below, but their fundamentals will be skipped. The lipophilic extractives of cork and their by-products, and also of birch outer bark were characterised by GC-MS.

8.12.1 Gas chromatography analyses

GC-MS analyses of depolymerised suberin and lipophilic extractives. GC-MS analyses of the TMS-derivatised samples were performed using a Trace GC 2000 gas chromatograph coupled with a mass-selective Finnigan Trace MS detector, using helium as carrier gas (35 cm s^{-1}) and equipped with a DB-1 J&W capillary column (30 m x 0.32 mm i.d., 0.25 µm film thickness). The chromatographic conditions were as follows:[3] isothermal at 80 °C for 5 min, ramped from 80 to 285 °C (4 °C min^{-1}), and then isothermal at 285 °C for 15 min; injector temperature, 250 °C; transfer line temperature, 285 °C; split ratio, equal to 1:50.[1] The MS was operated in the electron impact mode with electron impact energy of 70 eV and data collected at a rate of 1 scan s^{-1} over a range of m/z 33-800. The ion source was maintained at 200 °C.

For quantitative analyses, GC-MS was calibrated with pure reference compounds representative of the major classes of components present in the suberin or extractives samples, relative to the internal standard, *n*-tetracosane (hydrolysis products) or *n*-hexadecane (methanolysis products). The reference compounds were hexadecanoic acid, docosanoic acid or stigmastanol, oleanolic acid, betulinic acid, and ellagic acid, for suberin or extractives samples, respectively. The corresponding multiplication factors needed to obtain correct quantification of the peak areas were calculated as an average of six GC-MS runs. Quantitative results were obtained as the average of four concordant injections.

GC-FID analyses of the monosaccharide alditol acetates. Gas chromatography with flame ionisation detector (GC-FID) analyses of the monosaccharide alditol acetates were carried out using a Varian 3350 gas chromatograph, using helium (35 cm s^{-1}), as carrier gas, and equipped with a DB-225 J&W column (30 m x 0.25 mm i.d., x 0.15 µm film thickness). The chromatographic conditions were as follows:[11] isothermal at 220 °C for 5 min; ramped from 220 to 230 °C (2 °C min^{-1}); and then isothermal at 230 °C for 5 min; injector temperature, 230 °C; transfer line temperature, 230 °C. The quantification was made using calibration curves with reference compounds (rhamnose, arabinose, xylose, mannose, galactose e glucose).

8.12.2 Attenuated total reflection Fourier transform infrared spectroscopy

ATR FTIR spectra were run with a Brücker IFS FTIR spectrophotometer equipped with a single horizontal Golden Gate ATR cell. The resolution was 8 cm^{-1} after 256 scans.

8.12.3 Nuclear magnetic resonance spectroscopy

^{1}H and ^{13}C NMR analysis. ^{1}H and ^{13}C NMR spectra of suberin samples dissolved in $CDCl_3$ were recorded using a Brücker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. The ^{1}H NMR spectra ($CDCl_3$) were acquired at 300.13 MHz with at least 64 scans. All chemical shifts were expressed as parts per million (ppm) downfield from tetramethylsilane used as the internal standard.

^{13}C High Power Proton Decoupling Cross Polarization Magic Angle Spinning NMR spectra of polyesters samples containing an insoluble fraction were recorded at 9,4 T on a Brüker 500 spectrometer using a 4 mm double-bearing probe, 9 kHz spinning rate and MAS with proton 90^{o} pulses of 4 µs. Chemical shifts are given in ppm from glycine.

8.12.4 Size-exclusion chromatography analysis

SEC analyses were conducted with a Polymer Laboratories PL-GPC110 system equipped with a Refractive Index detector, using a set of two Tosoh G2000HHR columns (30.0 cm x 7.8 mm i.d.) and one Tosoh HHR-L guard column (4.0 cm x 6.0 mm i.d.), kept at 40 ^{o}C and previously calibrated with polystyrene standards (Polymer Laboratories) in the range 580-7000 Da. Chloroform was used as the mobile phase with a flow of 0.7 mL min^{-1}. All polymer samples were dissolved in chloroform (*ca.*12.5 mg mL^{-1}).

8.12.5 Vapour pressure osmometry

VPO analyses of model polyesters were carried out with a Knauer K7000 instrument, operating with dichloromethane at 35 ^{o}C (calibrated with *n*-tetracosane standard), using polymer solutions with concentrations ranging from 3.9 to 10.3 mg mL^{-1}. M_n results were calculated as the average of three concordant determinations.

8.12.6 Thermal analysis

Differential scanning calorimetry analysis. DSC thermograms of suberin samples were obtained with a Pyris Diamond DSC calorimeter from Perkin-Elmer, using nitrogen as purging gas (20 mL min^{-1}), and aluminium pans (30 µL, 3 bar) to encapsulate the samples (~5 mg). The calorimeter was calibrated for heating temperature with approximately 10 mg of each of the following metals: 99.999% pure indium, T_f = 156.60 ^{o}C, and 99.999% pure lead, T_f = 327.47 ^{o}C. Typically, scans were conducted with a heating rate of 10 ^{o}C min^{-1} in the temperature range of -90 to 100 ^{o}C and then isothermal at 100 °C for 3 min. Followed by cooling at 10 ^{o}C min^{-1} in the temperature range of 100 to -90 ^{o}C The heating/cooling cycles were repeated for up to four times.

Polarised light thermal microscopy. The hot stage/DSC video microscopy analyses were performed using a Linkam system DSC 600. The optical observations were conducted with a Leica DMRB microscope and registered using a Sony CCD-IRIS/RGB video camera. The image analysis used a Linkam system software with Real Time Video Measurement System. The images were obtained combining the use of polarised light with wave compensators, at 200 x magnification.

Suberin mixtures samples were dispersed in a covered 7 mm quartz crucible. Scans were conducted under nitrogen with a heating and cooling rate of 10 ^{o}C min^{-1} in the temperature range of -90 to 100 ^{o}C. The heating/cooling cycles were repeated for up to three times.

Dynamic mechanical analysis. DMA measurements were carried out with a 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 -150 to 120 ^{o}C at 3 ^{o}C min^{-1}. A small amount of the powder samples were dispersed in a foldable stainless still sheet from Materials Pocket of Triton technology.

Thermogravimetric analysis. TGA analyses were carried out with a Shimadzu TGA50 analyser equipped with a platinum cell, using platinum pans to encapsulate the samples (~5 mg). Typically, samples were heated at a constant rate of 10 ^{o}C min^{-1} from room temperature up to 800 ^{o}C, under a nitrogen flow of 20 mL min^{-1}. The thermal decomposition temperatures was taken at the onset of significant (≥ 0.2%) weight loss from the heated sample.

8.12.7 X-ray diffraction

XRD analyses were performed using a Philips X'pert MPD instrument operating with CuK_{α} radiation (λ = 1.5405980 Å) at 40 kV and 50 mA. Samples were scanned in the 2θ range of 3 to 50^{o}, with a step size of 0.04^{o}, and time per step of 50 s.

8.12.8 Contact angles measurements

Contact angles with water were measured at room temperature with a "Surface Energy Evaluation System" commercialised by Brno University, Czech Republic. Each θ value reported here was the average of three measurements carried out on a flat film of the polyester substrate deposited onto a glass plate.

8.12.9 Cross-linked fraction and swelling experiments

Approximately 100 mg of several pHDS samples were mixed with 25 mL of 1,1,2,2-tetrachloroethane (TCE) and stirred for 24 h at room temperature. The insoluble fraction was then filtered, dried and weighted. The percentage of fraction insoluble (Q_{ns}) was determined by the following equation,

$$Q_{ns} = \frac{(m_{\mathrm{o}} - m_{24\mathrm{h}})}{m_{\mathrm{o}}}\ 100$$

where m_{o} and $m_{24\mathrm{h}}$ stand for the polymer masses before and after 24 h mixing with TCE.

A compact piece of this fraction (~18 mg) was allowed to swell into 10 mL of TCE at room temperature. The polymer was taken out of this solvent at regular intervals, its

surface wipe-cleaned with filter paper, and weighted. This procedure was repeated until a constant weight was reached, which suggested that the swelling equilibrium had been reached. The percentage swelling was calculated using the expression,

$$\frac{V_W - V_0}{V_0}$$

where V_w and V_0 stand for the polymer volumes (calculated from the corresponding measured weights) in wet and dry conditions, respectively. The cross-link density was calculated using the Flory-Rehner theory (equations 3.10 and 3.11 of Chapter 3), considering that the fraction of dangling ends defects was negligible; taking the molar volume of TCE as V_1 = 105.8 $cm^3\ mol^{-1}$, the polymer volume at the swelling equilibrium ϕ_e ≈ 0.27, the density of the dry polymer (assimilated to that of branched amorphous polyethylene) ρ ≈ 0.92 g cm^{-3},[12] and the interaction parameter of the same polyethylene in TCE as χ ≈ 0.24.[12]

8.13 References

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[PART IV FINAL REMARKS]

9 Final remarks

What are the main achievements of this investigation? What could still be done in the future about suberin? What perspectives?

And, for the last time, the basic question- “to suberin or not to suberin”?

9.1 Epilogue

This fundamental study concerning both the detailed characterisation of depolymerised suberin from the two particularly suberin-rich species, cork from *Quercus suber* L. and the outer bark of *Betula pendula* Roth, and its use in the synthesis of biopolyesters has afforded interesting insights into the chemistry, physics and applicability of suberin in the synthesis of novel polyesters. One of the main challenges of this study was to control the chemical composition of depolymerised suberin mixtures in order to tailor the ensuing properties of the polyesters, either linear, with a significant amount o crystalline material, or, otherwise, polyesters essentially cross-linked with an amorphous character. This was achieved by applying different isolation protocols and more importantly using suberin from cork of *Quercus suber* L. and the outer bark of *Betula pendula* Roth.

Moreover, this study was encouraging, because it gave clear indications about the possibility of developing new suberin-based materials, in the context of relatively simple and green processes and with the major interest of valorising suberin isolated from industrial residues from the cork and the pulp and paper industries. Additionally, the use of suberin from cork could be an important contribution to cork valorisation, since its traditional use in cork stoppers is being challenged by synthetic substitutes and therefore polymers could be a promising alternative.[1]

This investigation confirmed the valuable and rare chemical composition of depolymerised suberin from both cork and silver birch outer bark. The comparative study between these samples revealed some important similarities between them, namely the presence in both species of considerable amounts of ω-hydroxyalkanoic acids and α,ω-alkanedioic acids. For example, 22-hydroxydocosanoic acid, 18-hydroxyoctadec-9-enoic acid, octadec-9-enedioic acid and hexadecanedioic acid were some of the most abundant suberin fragments identified by GC-MS. However, the amount of epoxy derivatives was found to be significantly higher in the “birch” sample, with 9,10-epoxy-18-hydroxyoctadecanoic acid as the most abundant compound identified.

These samples also showed interesting thermal and crystallographic properties with the *n*-hexane fraction of the methanolysis-depolymerised suberin of cork and the hydrolysis-depolymerised suberin of birch outer bark displaying a high degree of crystallinity with melting temperatures typical of compounds with long aliphatic chains. On

the contrary, both the methanolysis-depolymerised suberin (DCM extract) and the hydrolysis-depolymerised suberin from cork exhibited an essentially amorphous character.

Cork, its industrial residues and birch outer bark samples were also found to contain a significant fraction of extractives. The lipophilic extractives of cork and the industrial cork powder were rich in triterpene compounds, such as betulinic acid and friedeline. The black condensate was found to be composed of both important fractions of triterpenes and oligomeric alkanols, ω-hydroxyalkanoic acids and alkanoic acids.

It was possible to prepare successfully novel polyesters entirely based on renewable resources, as confirmed by ATR FTIR and NMR spectroscopy. They were synthesised by a simple green procedure based on the direct polycondensation of hydrolysis-depolymerised suberin fragments from cork or birch outer bark, in bulk, using $Bi(OTf)_3$ as catalyst. This is a doubly beneficial approach from a green perspective, as it avoids the use of both methanol during the depolymerisation of suberin as well as of organic solvents as reaction media. Some of these materials possessed a high amount of cross-linked structures.

Polyesters with predominantly linear chains (with some degree of branching) were also synthesised when using methanolysis-depolymerised suberin from cork and Sb_2O_3 as catalyst, or, instead, when using hydrolysis-depolymerised suberin also from cork but catalysed in emulsion using a DBSA/water system. Their hydrophobic character was confirmed by water contact angles measurements.

These polyesterification reactions are obviously dependent on the presence of a balancing comonomer, since they are naturally unbalanced, presenting different molar amounts of hydroxyl and carboxyl groups. Hence, reactions were carried out in the presence of a stoichiometric amount of glycerol, 1,12-dodecanediol, 2,5-furandicarboxylic acid or decanedioic acid, selected for their renewable origin and/or resemblance to suberin. Additionally, these reactions are obviously dependent on the presence of monomeric impurities, which could be removed, in some cases, by a pre-extraction procedure before suberin depolymerisation.

9.2 Perspectives

The study carried out during this Thesis contributed to the development of novel suberin biopolyesters, following this pertinent interest in fine chemicals from renewable origin. Nevertheless, after this Thesis (as indeed at the completion of any Thesis) several questions remained unanswered and, more importantly, other research topics could be envisaged, as briefly mentioned below:

- the implementation of greener suberin depolymerisation methods;
- the biodegradability of suberin-based polyesters;
- the development of *in situ* polycondensation approaches to prepare the cross-linked polyesters;
- the study of the feasibility of using these polyesters in practical applications;
- development of methodologies to isolate and adequately purify promising compounds/fractions of the lipophilic extracts of cork and its industrial residues;
- and, finally, the assessment of the bioactivity of the lipophilic extracts of cork and its industrial residues.

Finally, a few words about the underlying question of this study, raised repeatedly all along the manuscript (maybe trivial, but inevitable) - ***"to suberin or not to suberin: that is the question"***. The actual results answered this question: from suberin depolymerisation and characterisation to suberin application in biopolyesters. Thus in retrospect, the answer seems now obviously affirmative, although, still much should be done to advance in this fascinating field.

9.3 Reference

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