

# **Chemical composition of cork, phloem and xylem of *Quercus suber* L. from different provenances**

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*“Tomorrow owes you the sum of your yesterdays.*

*No more that than. And no less.”*

Robin Hobb

## Resumo

A composição química de cortiça, floema e xilema foi determinada em árvores jovens de *Quercus suber* L. de três proveniências (Alcácer do Sal, Azeitão e Santiago do Cacém) de uma importante região de produção suberícola em Portugal, tendo em vista a análise de eventuais diferenças entre proveniências. Foram estudadas três árvores por proveniência, determinando-se o conteúdo em cinzas, extractivos (solubilizados em diclorometano, etanol e água), suberina (no caso da cortiça), lenhina e polissacáridos. Os três tecidos mostraram grandes diferenças na sua composição química. A composição química média da cortiça foi a seguinte: 0,66 % em cinzas, 11,7 % extractivos, 42,3 % suberina, 24,1 % lenhina e 16,2 % polissacáridos; do floema 2,9 % cinzas, 4,5 % extractivos, 38,0% lenhina e 49,1 % polissacáridos; e do xilema 1,1 % cinzas, 5,6 % extractivos, 23,4 % lenhina e 64,6 % polissacáridos. A análise estatística mostrou que a proveniência apenas foi um factor de variação significativo para os extractivos em etanol no caso da cortiça e os polissacáridos no floema.

A composição monomérica da lenhina de todas as amostras foi analisada por pirólise analítica a 650 °C. A lenhina dos três tecidos difere substancialmente: o rácio S/G foi 0,12 na cortiça, 1,1 no floema e 2,3 no xilema. Os compostos obtidos por pirólise foram também identificados e, sempre que foi possível determinar a sua origem, agrupados em açúcares, lenhina e suberina (apenas nas amostras de cortiça). Os açúcares representaram 58,6 %, 63,1 % e 25,4 %, a lenhina 14,4 %, 10,4 % e 12,6 % respectivamente no floema, xilema e cortiça, e a suberina na cortiça representou 33,0 % do total dos picos dos pirogramas.

Palavras-chave: *Quercus suber*, cortiça, floema, xilema, pirólise

# Abstract

The chemical composition of cork, phloem and xylem of young *Quercus suber* trees from three different provenances (Alcácer do Sal, Azeitão e Santiago do Cacém) was studied in order to evaluate possible differences between provenances. Three trees per provenance were studied and the content of ashes, extractives (soluble in dichloromethane, ethanol and water), suberin (in case of cork), lignin and polysaccharides of each tissues was quantified. There were great differences in the chemical composition between tissues. The average chemical composition in cork was 0.66 % of ashes, 11.7 % of extractives, 42.3 % of suberin, 24.1 % of lignin and 16.2 % of polysaccharides; in phloem was 2.9 % of ashes, 4.5 % of extractives, 38.0 % of lignin and 49.1 % of polysaccharides; and in xylem was 1.1 % of ashes, 5.6 % of extractives, 23.4 % of lignin and 64.6 % of polysaccharides. Statistical analysis showed that only the ethanol extractives in cork and the total polysaccharides in phloem had a significant factor of variation between provenances.

The lignin monomeric composition of all samples was studied by analytical pyrolysis at 650 °C. Lignin from the three tissues is substantially different: S/G ratio was 0.12, 1.1 and 2.3 in cork phloem and xylem respectively. The compounds obtained by pyrolysis were identified and were grouped in carbohydrates, lignin and suberin (in cork samples only). Carbohydrates accounted to 58.6 %, 63.1 % and 25.4 %, lignin 14.4 %, 10.4 % and 12.6 %, respectively in phloem, xylem and cork, and the suberin in cork represented 33.0 %.

Keywords: *Quercus suber*, cork, phloem, xylem, pyrolysis

## Resumo Alargado

Três proveniências de *Quercus suber* foram seleccionadas para a determinação da composição química sumativa da cortiça, floema e xilema com o objectivo de averiguar se existem diferença na composição química dos três tecidos entre proveniências diferentes. As mesmas são provenientes de um ensaio de proveniências estabelecido em 1998 e localizado na Herdade do Monte da Fava, em Santiago do Cacém, onde foram estabelecidas trinta e cinco proveniências de *Quercus suber* de países da Europa (Espanha, França, Itália e Portugal) e do Norte de África (Argélia, Marrocos e Tunísia) representativos da distribuição natural do sobreiro no mundo. As proveniências seleccionadas para este estudo foram as seguintes: 14 (Alcacer do Sal), 15 (Azeitão) e 19 Santiago do Cacém).

Foram escolhidas três árvores por proveniência, perfazendo um total de nove árvores estudadas. As árvores, de seis anos, foram cortadas em discos, e os três tecidos (cortiça, floema e xilema) foram separados manualmente. Os tecidos foram moídos num moinho de facas, inicialmente com uma malha de saída de 6 x 6 mm e depois com uma de 1 x 1 mm e crivados num crivo vibratório, e a fracção 20 – 80 mesh (180 µm – 850 µm) recolhida para a determinação da análise química sumativa. As cinzas, extractivos, suberina (apenas na cortiça), lenhina e polissacáridos (celulose e hemiceluloses) totais de todos os tecidos foram determinados de acordo com normas standardizadas (TAPPI). Cada análise foi feita em duplicado e os resultados expressos em percentagem do material original.

Para quantificação das cinzas, 2 gramas de amostra foram incinerados a 525 °C durante 6 horas, e o resíduo correspondeu às cinzas. Para a determinação dos extractivos totais, as amostras foram extraídas sucessivamente pelo método de Soxhlet com diclorometano (6 horas), etanol (16 horas) e água (16 horas). Após a extração das amostras procedeu-se à remoção da suberina da cortiça através de uma despolimerização por metanólise alcalina. As amostras foram refluxadas com uma solução de metóxido de sódio seguido de filtração do resíduo. A fracção líquida foi acidificada até pH 6, concentrada num rotavapor até à secura e decantada três vezes com diclorometano. A solução foi então concentrada novamente e o resíduo seco correspondido a suberina. Para a determinação da lenhina (das amostras de cortiça livres de extractivos e de suberina, e as amostras de floema e xilema livres de extractivos) foi usado o método de Klason, que consiste numa hidrólise ácida dos polissacáridos com ácido sulfúrico a 72 %. O resíduo correspondeu à lenhina Klason, e a lenhina solúvel foi estimada através da leitura do hidrolisado a 205 nm num espectrofotómetro de ultravioleta. A lenhina total correspondeu à soma da lenhina Klason e da lenhina solúvel. Para a determinação dos polissacáridos totais, os monossacáridos neutros (arabinose, galactose, glucose e xilose, os ácidos urónicos (galacturónico e glucurónico) e acético foram

determinados no hidrolisado da lenhina Klason, através de separação por cromatografia líquida.

Os três tecidos mostraram grandes diferenças na sua composição química. A composição química média (nas três proveniências) da cortiça foi 0,66 % de cinzas, 11,7 % de extractivos, 42,3 % de suberina, 24,1 % de lenhina total e 16,2 % de polissacáridos; do floema foi 2,7% de cinzas, 4,5 % de extractivos, 38,0% de lenhina de lenhina total e 49,1 % de polissacáridos; e do xilema foi 1,1 % de cinzas, 5,6 % de extractivos, 24,1 % de lenhina e 64,6 % de polissacáridos. A cortiça destaca-se pela elevada quantidade de suberina, inexistente nos restantes tecidos, e com uma grande variabilidade entre as nove árvores estudadas, variando de 35,2 % a 48,0 %; o floema pela elevada quantidade de lenhina; e o xilema pela elevada quantidade de polissacáridos.

Relativamente à variabilidade da composição química dos tecidos entre diferentes proveniências foi realizada análise estatística através de análise de variâncias (ANOVA) com comparações de médias (Teste de Tukey,  $p < 0.05$ ). Apenas os extractivos totais em etanol na cortiça, e os polissacáridos totais no floema tiveram um factor de variação significativo entre as três proveniências. Em relação aos outros componentes da análise química não houve qualquer diferença entre proveniências quer na cortiça, floema e xilema. Tal deveu-se ao pequeno número de proveniências estudadas (apenas três).

As amostras de cortiça, floema e xilema, e de cortiça sem suberina foram analisadas através de pirólise analítica. Aproximadamente 100 µg de cada amostra foram pirolisadas a 650 °C durante 10 s num pirolisador CDS Pyroprobe 5150 e a fase gasosa separada numa coluna capilar, ZB-1701, por cromatografia gasosa numa Agilent GC 7890B e os compostos identificados num detector de massa 8977B. Os compostos identificados foram agrupados em açúcares totais, lenhina total e suberina total (apenas nas amostras de cortiça).

As amostras de cortiça livres de extractivos foram caracterizadas com 25,4 % de açúcares totais, 12,6 % de lenhina total e 33,0 % de suberina (médias das três proveniências). Nas amostras de cortiça livres de extractivos e suberina, não foram encontrados compostos de suberina, o que resultou num aumento percentual na quantidade de açúcares totais e lenhina total, 37,1 % e 34,4 %, respectivamente. As amostras de floema foram caracterizadas com 58,6 % de açúcares e 14,4 % de lenhina; e nas amostras de xilema, 63,1 % de açúcares e 10,7 % de lenhina.

As amostras de cortiça extractadas e as de cortiça desuberinizadas tiveram um valor de rácio S/G bastante semelhante, 0,12 e 0,28 respectivamente, o floema 1,1 e o xilema 2,3, o que mostra um aumento no rácio S/G da periferia para o centro da árvore, ou seja, um aumento do rácio S/G da cortiça, para o floema e para o xilema.

A lenhina da cortiça é caracterizada maioritariamente por unidades G, com um valor de H:G:S de 1:2.5:0.3 e 1:2.2:0.6 respectivamente para a cortiça extractada e a cortiça desuberinizada. O floema é caracterizado por lenhina do tipo G e S (1:2.6:2.7) e o xilema por lenhina do tipo S, tendo mais do dobro de unidades S relativamente às unidades G (1:2.0:4.5).

Foi também estudada a variação da cor dos três tecidos em função das sucessivas extracções. Para tal mediu-se a cor do material inicial e após cada extracção com diclorometano, etanol e água num espectrofotómetro Minolta CM-3630. Os parâmetros de cor medidos foram o CIE  $L^*a^*b^*$ . Em relação ao parâmetro  $L^*$ , os valores foram diminuindo com cada extracção ou seja, foram ficando mais escuros, 58,8, 50,9, 48,8 e 46,0 na cortiça, e 44,5, 43,5, 42,7 e 39,8 no floema, do material originário, seguido de extracção com diclorometano, etanol e água. No xilema, o valor de  $L^*$  diminuíram com a extracção de diclorometano e etanol, mas aumentou na extracção com água, 55,8, 54,8, 53,7 e 55,8, respectivamente. Relativamente aos parâmetros  $a^*b^*$  a cortiça e o floema diminuem de valor em ambos ficando relativamente mais azulados e esverdeados do material originário para cada extracção sucessiva. No xilema o valor de  $b^*$  diminui com cada extracção ficando relativamente mais azulado, mas no valor de  $a^*$  aumenta do material originário para a última extracção, ficando relativamente mais avermelhado.



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## 1. Objectives

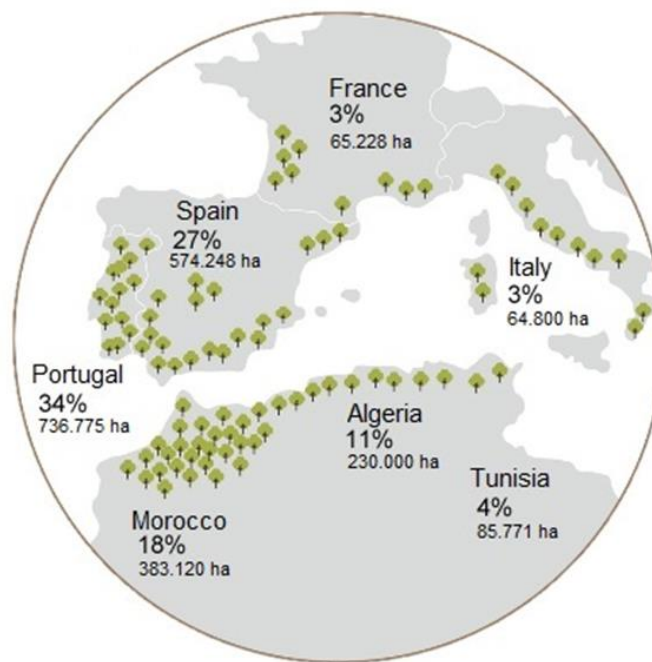
The cork oak (*Quercus suber* L.) is an important tree for the western Mediterranean and North African countries. The economic value of the tree is strongly associated with the cork production, used mainly to manufacture cork stoppers for the wine industry (Pereira 2007). Therefore, to cope with bottled wine market growth and assure the sustainability of the cork oak sector, a large number of cork oak stands was planted during the last ten years of the 20<sup>th</sup> century. Since the large distribution area of the cork oak tree in Portugal, Spain, Italy, France, Algeria, Morocco and Tunisia, encompasses very different environmental conditions, it is expected that cork oaks originated from these countries are adapted to specific sets of environmental conditions, e.g. capacity to tolerate conditions of drought or temperature variance throughout the year and resistance against pests and diseases. This means, , that a cork oak originated from one of these countries may be best suited for specific types of environmental conditions and this may be used for the installation of new cork oak stands. Due to lack of knowledge on the differences in adaptation of cork oak trees originated from different regions, *i.e.* the lack of genetic data on the cork oak, led to creation of the European Cork Oak Network, under the EU concerted action FAIR 1 CT 95-0202, involving the seven countries in the distribution area of the cork oak. The objective of this network was to enrich the cork oak genetic research by establishing provenance trials and progeny trials in the seven countries (Varela, 2000).

In the present study, three Portuguese provenances from the provenance trial located at Herdade do Monte da Fava, in Santiago do Cacém, Southern Portugal (one of the trials established by this concerted action) were selected, and three trees per provenance were sampled. The main research objective was to evaluate if there are differences in the chemical composition of cork, phloem and xylem of the cork oak trees between the selected provenances, by using summative chemical analytical methodology and analytical pyrolysis.

## 2. Introduction

### 2.1 *Quercus suber* L.

The cork oak (*Quercus suber* L.) belongs to the Fagaceae family and is distributed in the southwest Europe (Portugal, Spain, France and Italy) and northwest Africa (Morocco, Algeria and Tunisia) (Figure 1), accounting to approximately a total of 2.2 million ha, with 737 thousand ha in Portugal, representing 23 % of the total area forest area in Portugal, and 34 % of the total world cork oak area (FAO 2010; Inventário Florestal Nacional 2013).



**Figure 1.** Distribution of the cork oak in the world.  
[\(https://www.apcor.pt/montado/floresta/\)](https://www.apcor.pt/montado/floresta/)

The cork oak has a large economic importance in Portugal because of the industrial value of its outer bark *i.e.* the cork (Pereira 2007). The cork is removed from the tree in periodical intervals of time and although mainly used as a raw material for wine stoppers and floors, it has a set of properties that makes it very appealing for other applications. These properties, due to the cell structure and the chemical composition of cork, are as follows: i) low density, since more than 50% of the volume is air, and it can float, which can be used in floating devices (e.g. surfboards and fishing floats); ii) high compression and elastic behavior; iii) low permeability to liquids and gases, and combined with its elastic behavior makes cork excellent to be used as wine bottle stoppers; iv) high thermal and acoustic insulation and fire retardant, which makes it an insulator material (e.g. house walls and ceilings); v) high energy absorption, which makes it possible to be used in floors and in the shoes industry (Pereira, 2015).

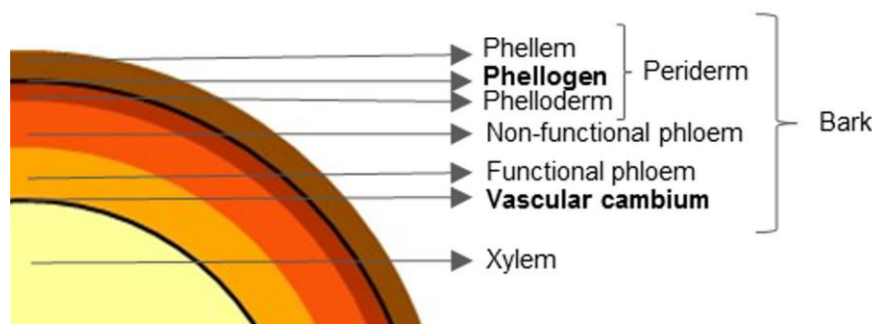
*Quercus suber* L. has been intensely studied because of the properties and applications of cork, but few studies exist about its phloem and wood (*i.e.* xylem). Some studies on cork oak wood anatomy were made by Carvalho (1996, 1997), Quilhó et al. (2003) and Sousa et al. (2009b) showing semi-ring porosity, fibres with 1.15 mm length and vessels with 133 µm diameter. Leal et al. (2005, 2006, 2007) studied the chemical composition of wood, its properties (*e.g.* density and durability), and the radial vessel size variation and reported an average wood density of 0.65 g/cm<sup>3</sup> at 12 % moisture content ; a low durability of both heartwood and sapwood. Lourenço et al. (2016) studied the chemical composition of cork, phloem and xylem of a cork oak tree and the composition and structure of the lignin in the three tissues by analytical pyrolysis and 2D-NMR spectroscopy.

## 2.2 Cork, phloem and xylem

### 2.2.1 Formation

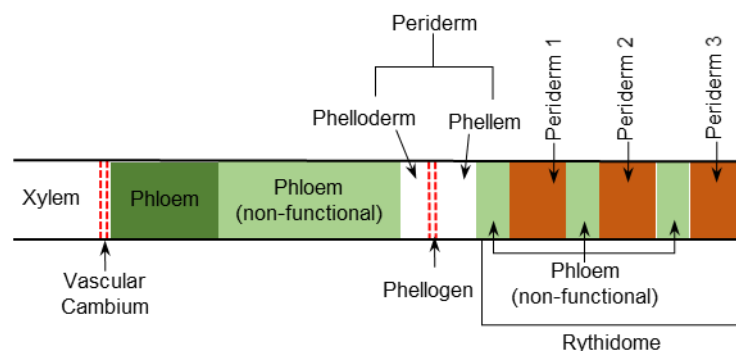
In every tree there are two radial meristems that are responsible for diameter growth: the vascular cambium and the phellogen. The vascular cambium is the innermost meristem and produces xylem cells to the inside and phloem cells to the outside. The xylem is responsible for conducting water and gives stability to the trees and the phloem is responsible for conducting nutrients. As the tree ages and new phloem cells are produced, the phloem further away from the cambium becomes inactive. The two types of phloem are known as functional phloem (near the vascular cambium) and non-functional phloem (away from the cambium) (Figure 2) (Esau, 1960).

The phellogen is the outermost meristem and produces phelloderm cells to the inside, near the non-functional phloem, and phellem to the outside (*i.e.* cork cells). The phellem, phellogen and the phelloderm together are known as the periderm, and all the tissues outside the vascular cambium (*i.e.* the phloem, phelloderm, phellogen and the phellem) are known as the bark of trees (Figure 2) (Pereira, 2007).



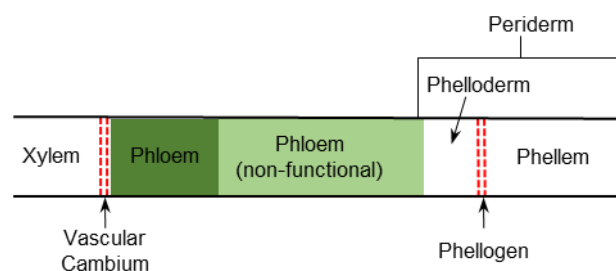
**Figure 2.** Cross section of a tree representing the wood (xylem), the functional and non-functional xylem and the periderm and its constituents. Adapted from (Leite & Pereira, 2017)

In the majority of tree species, the phellogen has a limited time of life, and a new phellogen is formed inside the non-functional phloem, when the previous phellogen dies. This process can happen many times during the life of a tree, meaning that it can have several periderms alternating with non-functional phloem. The region that includes the various periderms and non-functional phloem between them is known as the rhytidome (Figure 3) (Leite & Pereira, 2017).



**Figure 3.** Schematic representation of the radial tissue organization of a tree stem. Adapted from (Pereira, 2012a; Şen, et al., 2015).

The cork i.e. the phellem in the periderm is therefore the outer bark of the cork oak; as in other tree barks, it plays a major role in tree health (e.g. protection against animals, pathogens or environmental factors, water storage and wound healing). What is special about the outer bark of *Quercus suber* is the fact that the phellogen is continuous around the tree circumference and lives as long as the tree (Fig. 4). However when the cork is removed from the cork oak a traumatic phellogen is formed with the purpose of producing a new protective cork layer and this process is repeated through the life cycle of the cork oak (Pereira, 2007).



**Figure 4.** Representation of a tree stem of the cork oak. Adapted from (Pereira, 2012a; Şen, et al., 2015).



## 2.2.2 Chemical composition

Cork, as well as wood and phloem are chemically constituted by structural components and non-structural components. The structural components are macromolecules of polymeric nature that make up the cell wall and bestow most part of the physical and chemical properties of the tissues. In cork they are, by order of importance, suberin, lignin and polysaccharides (cellulose and hemicelluloses). Phloem and xylem do not contain suberin and are constituted by polysaccharides and lignin. The non-structural components are inorganic minerals (determined as ash) and low mass molecules, named extractives, that can be removed with solvents without compromising the cell structure. The mean chemical composition of virgin cork, phloem and xylem of *Quercus suber* is presented in table 1.

**Table 1.** Chemical composition of virgin cork, phloem and xylem from the cork oak.

% of o.d. Material	Chemical Composition		
	Cork <sup>1</sup>	Phloem <sup>2</sup>	Xylem <sup>3</sup>
<b>Ash</b>	1.2	3.1	1.5
<b>Total extractives</b>	14.2	6.2	12.7
Dichloromethane	5.4	0.1	0.3
Ethanol	4.8	1.9	7.4
Water	4.0	4.2	4.9
<b>Suberin</b>	39.4	—	—
<b>Total lignin</b>	23.0	38.4	25.3
Klason lignin	21.8	36.0	22.1
Soluble lignin	1.2	2.4	3.3
<b>Monosaccharide composition (% of total sugars)</b>			
Arabinose	13.2	2.7	1.7
Galactose	5.1	2.4	3.6
Glucose	45.4	48.8	67.5
Manose	3.2	0.3	2.7
Rhamnose	0.8	—	—
Xylose	32.3	45.9	23.9

(Adapted from: 1 - (Pereira, 1988) ; 2 - (Lourenço, et al., 2016); 3 – (Leal, et al., 2005)

### Inorganic components

The inorganic components contain a wide range of elements e.g. in wood, 80 % of these elements corresponds to calcium, magnesium and potassium (Rowel, 2005).

The inorganic fraction is quantified after total incineration at 525 °C (determined as ash). Usually barks have more ash content than wood, but in the case of the cork oak the opposite happens. The ash content of cork, phloem and xylem from *Q. suber* is, respectively, 1.2 %, 3.1 % and 1.5 % (Pereira, 1988; Leal, et al., 2005; Lourenço, et al., 2016).

## **Extractives**

Extractives are low weight molecules and are formed as a result of secondary metabolism of the cells (Pereira et al., 2003). They include a wide range and variety of molecules and are usually classified by the type of solvent e.g. polarity by which they are removed, or by chemical families. In the first they are classified in two groups: i) lipophilic compounds that are extracted by solvents with low-polarity, such as dichloromethane or chloroform; ii) polar compounds including phenolic compounds and sugars extracted by polar solvents such as ethanol and water (Pereira, 2007). Barks have a higher content of extractives than wood: for example, in softwood trees wood extractives range 2.0 – 9.0 % and barks extractives 2.0 – 25.0 %, and in hardwood trees wood has 2 - 5 % of extractives and barks 5 – 10 % (Harkin & Rowe, 1971).

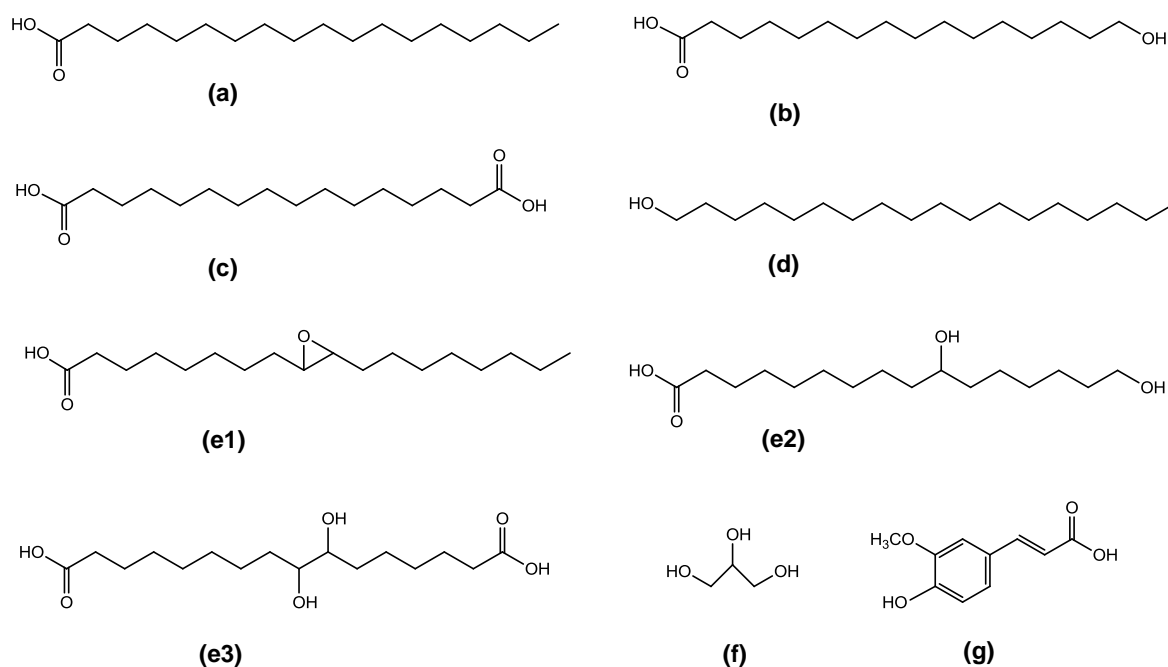
Cork from *Q. suber* has an extractives content that can go from 8 % to 24 %, with mean values ranging 14 – 18 %, and they are rich in both lipophilic and phenolic compounds (Pereira, 2007). Lipophilic compounds include triterpenes, fatty acids, n-alkanes and n-alkanols (Castola, et al., 2005; Sousa et al., 2006). Phenolic compounds are composed mainly by phenols and flavonoids.

Phloem and xylem of *Q. suber* have less extractives than cork and are mainly composed by compounds soluble in polar solvents. Leal et al. (2005) reported 12.7 % of extractives in xylem, where 12.4 % corresponded to compounds soluble in ethanol and water; Lourenço et al. (2016) reported 6.2 % total extractives in the phloem of the cork oak, where 6.1 % corresponded to polar compounds.

## **Suberin**

Suberin is the typical component of cork and therefore of barks of trees (Jansson & Nilvebrant, 2009) and depending on the species can vary from 2 – 45 % of its chemical composition (Pereira, 2012b). It is the major constituent of cork, accounting approximately to 40 % of the chemical composition, although it can vary from tree to tree (Pereira, 2013). Suberin is composed by two major type of monomers, glycerol and long chain fatty acids and alcohols; it includes also small amounts of ferulic acid (figure 5). The long chain fatty acids are composed mainly by  $\alpha,\omega$ -dicarboxylic acids,  $\omega$ -hydroxyacids and unsubstituted fatty acids, and the long chain alcohols mainly by 1-alkanols (Graça & Santos, 2006).

In virgin cork,  $\omega$ -hydroxyacids are the most abundant monomers (47.0 %) followed by  $\alpha,\omega$ -diacids (11.7 %), while in reproduction cork the last ones account to 53.0% and the  $\omega$ -hydroxyacids to 30.6 % (Leite & Pereira, 2017).

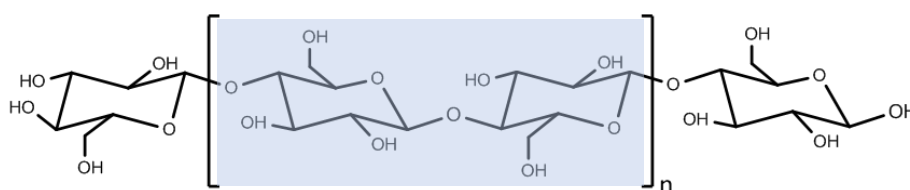


**Figure 5.** Common structures of suberin monomers obtained after cork depolymerisation followed by GC-MS separation and identification. **(a)** Unsubstituted fatty acids (C18 to C24 saturated). **(b)**  $\omega$ -hydroxy fatty acids (C16 to C26 saturated or C18 with one insaturation). **(c)**  $\alpha,\omega$ -dicarboxylic acids (C16 to C26 saturated or C18 with one or two insaturations). **(d)** 1-alkanols (C18 to C22 saturated). **(e)** Mid-chain functionalized monomers; **(e1)** Epoxy-fatty acids (C18 saturated or with one insaturation); **(e2)** Polyhydroxy-fatty acids (C18 saturated); **(e3)** Polyhydroxy  $\alpha,\omega$ -dicarboxylic acids (C18 saturated). **(f)** Glycerol. **(g)** Ferulic acid. Adapted from (Pollard, et al., 2008).

## Polysaccharides

Polysaccharides are the most abundant structural component in wood, and are formed of cellulose and hemicelluloses. Together they are known as holocellulose, accounting to 65 – 70 % in wood, with 40 – 45 % attributed to cellulose and 15 – 25 % to hemicelluloses (Rowel, 2005). In cork, polysaccharides are the least important structural component of the cell wall, representing in average 20% of its chemical composition (Pereira, 1988).

Cellulose is composed by  $\beta$ -D-glucopyranose units linked by  $\beta$ -(1 $\rightarrow$ 4) glycosidic bonds and the degree of polymerization in wood (number of glucose units in a molecule of cellulose), is usually around 9 000 – 10 000 units of  $\beta$ -D-glucopyranose but in cotton can go up to 15 000 units. The two repeating D-glucose units in cellulose is known as cellobiose (Figure 6) (Rowel, 2005; Ek et al., 2009).



**Figure 6.** Partial structure of cellulose, and cellobiose highlighted. Adapted from (Sjöström & Alén, 2013)

In consequence of the linearity and high tendency to form intramolecular and intermolecular hydrogen bonding, cellulose molecules aggregate into microfibrils forming crystalline regions (highly ordered conformation) and amorphous regions (less ordered); microfibrils aggregate into fibrils and then into cellulose fibers. This structural arrangement of cellulose confers wood important mechanical properties (e.g. high tensile strength) (Sjöström & Alén, 2013). In cork, cellulose plays a minor role in its properties, and the role conferred by cellulose to wood properties, is given in cork by suberin (Pereira, 2007).

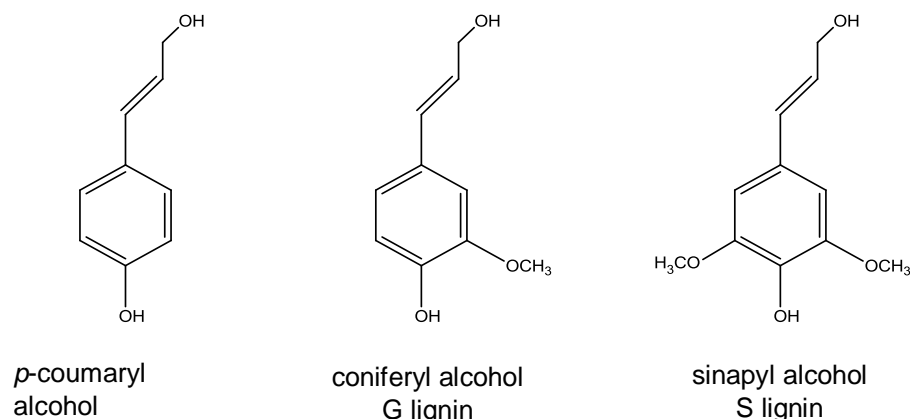
Hemicelluloses, contrary to cellulose which is a homopolysaccharide (formed only by glucose), are heteropolysaccharides (e.g. glucuronoxylans, glucomannans, galactoglucomannans, arabinoglucuronoxylans) formed by monomeric components of glucose, mannose, galactose, xylose, arabinose, rhamnose with the presence of glucuronic and galacturonic acids and with a degree of polymerization between 50 and 200 (Sjöström, 1993). The role of hemicelluloses is to strengthen the cell walls by bonding with the cellulose microfibrils (Wertz, et al., 2018).

The polysaccharide content in virgin cork ranges from 15.7 % to 21.3 %, and in reproduction cork is 19.9 %, with the monosaccharide composition being dominated by glucose, xylose and arabinose (Pereira, 1988). Lourenço et al., (2016), reported values of total polysaccharides in phloem and xylem of 33.8 % and 44.7 %, respectively, with glucose and xylose as the main monosaccharides in both tissues. The monosaccharide composition of cork is dominated by glucose and xylose, and with a considerable amount of arabinose (45.4 %, 32.3 % and 13.2 % of total polysaccharides, respectively (Pereira, 1988)); and phloem and xylem by glucose and xylose (48.8 % and 45.9 % in phloem, and 67.5 % and 23.9 % in xylem, respectively (Leal, et al., 2005; Lourenço, et al., 2016)).

## **Lignin**

Lignin is the second most important structural component in wood and in cork of *Q. suber*. The mean lignin content reported in cork, phloem and xylem of the cork oak is 23.0 %, 38.4 % and 25.3 %, respectively (Pereira, 1988; Leal, et al., 2005; Lourenço, et al., 2016).

Lignin is a polyphenolic polymer mainly constituted by three monomers, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol that are distinguished by the lack or presence of one or two methoxyl groups (Figure 7). In wood, lignin is distributed in the secondary wall, mainly in the middle lamella and its function is to serve as the “glue” that binds the cells and microfibrils. It confers rigidity, strength, hydrophobicity and defense against pathogens in both wood and cork cells (Pereira, 2007; Wertz, et al., 2018). In addition to the three precursors, recent studies have shown that the polymerization of lignin may include other precursors e.g. hydroxycinnamates (Lourenço & Pereira, 2018).



**Figure 7.** Basic monomers that make up lignin structure.

The lignin can be distinguished in wood and barks by the proportion of the three monomers. Lignin composition also varies with species; for example, lignin in softwoods is mainly composed by G units with a low amount of H units, and in hardwoods it is mainly composed by G and S units (some species of trees with an equal amount of this monomers and others species that can go up to percentages of S units, three times higher that G units) and with minor percentage of H units (Ek, et al., 2009).

The mean percentage of lignin reported in cork, phloem and xylem of the cork oak is 23.0 %, 38.4 % and 25.3 %, respectively (Pereira, 1988; Leal, et al., 2005; Lourenço, et al., 2016). Lignin from cork is characterized by a G type lignin, with a H:G:S molar composition of 2:85:13, while lignin from phloem as less G units, and from xylem is enriched in S units (H:G:S of 1:58:41 and 1:45:55, respectively) (Lourenço, et al., 2016).

### 2.3 *Quercus suber* chemical variability

The chemical composition variation between provenances of cork from *Q. suber* has been only researched by i) Conde et al. (1998a) that studied the variability of chemical composition of cork from seven provenances and found no significant differences that could allow to distinguish provenances of *Q. suber* by its chemical composition, only between trees from the same provenance; ii) by Conde et al. (1998b), that studied the polyphenolic extractives of cork from seven provenances with no significantly differences in relation to site in the content of total tannins and ellagitannins, and report differences between the content of some individual compound that could discriminate provenances (*i.e.* gallic acid, caffeic acid and protocatechuic aldehyde); and iii) by Pereira (2013), that studied the chemical variability of cork between 29 provenances from six cork production regions where no significant differences in relation to provenances and regions, and only in relation between individual trees.

Other studies relating the chemical composition of cork from *Q. suber* from different sites and locations have been made. Bento et al. (2001) studied the suberin composition in cork of trees from five different sites and found no differences between them, only between trees. Pereira (1998) studied the chemical composition of virgin cork from four different sites and concluded that there were only significant differences in the content of extractives and polysaccharides in relation to location, but there was a large variability between trees from the same location and even within-tree. Jové et al. (2011) studied the variability in chemical composition of bark layers of cork from six locations and concluded that there were significant differences in suberin and holocellulose contents with respect to the bark layers but no significant differences were found between the different production areas.

There are no studies in relation to the chemical composition differences between provenances of phloem or xylem from *Quercus suber*.

### 3. Material and methods

#### 3.1 Sampling

This study was performed with *Quercus suber* L. samples from a provenance trial located on Herdade do Monte da Fava (in Santiago do Cacém), Southern Portugal. Monte da Fava is characterized by a Mediterranean climate, located at an altitude of 79 m and with a sandy soil texture. The mean annual temperature and precipitation are 16.2 °C and 577 mm, respectively (Sampaio, et al., 2016).

The trial was established in March 1998 as part of an European Cork Oak Network, and the trees in this trial originated from seeds collected in 35 cork oak provenances from countries of Europe and North Africa, representing the cork oak natural distribution. Table 2 presents the details of the location of the selected provenances and the trees used in this study: the code of provenances and the corresponding code used in the trial, the identification of the trees (ID), the region of seed collection, geographical variables and climate data for the seed source (Sampaio, et al., 2016).

**Table 2.** Details of the *Quercus suber* L. provenances used in this study.

Provenance Code*	Provenance code (Trial)	Tree ID	Region	Latitude	Longitude	Tm (°C)	PPT (mm)
P14	PT18	89	Alcacer do Sal	38°29'N	8°35'W	16.3	577
		399					
		630					
P15	PT19	416	Azeitão	38°30'N	9°02'W	14.3	681
		1194					
		3293					
P19	PT23	188	Santiago do Cacém	38°01'N	8°42'W	15.6	736
		1608					
		3235					

\*Code of the provenances attributed in this study; Tm – mean temperature; PPT – precipitation.

*Quercus suber* L. trees with 6 years of age were used in this study. The samples were taken from discs collected between 1.0 and 1.3 m of the stem height. The cork, phloem and xylem tissues were manually separated using a chisel and a hammer. The cork and xylem tissues were milled in two cutting mills, passing through a 6 x 6 mm sieve in a Retsch SM 2000, and then through a 1 x 1 mm sieve in a Thomas Willey lab mill. The phloem was directly milled in the Thomas Willey Lab Mill, passing through the 1 x 1 mm sieve. All the tissues were sieved

in a Retsch AS 200, and samples from the >20-80 mesh fractions (180  $\mu\text{m}$  – 850  $\mu\text{m}$ ) were taken for chemical analysis. All the analyses were made in duplicate and average results reported as percentage of initial mass.

## 3.2 Chemical analysis

### 3.2.1 Ash determination

Ash content was determined by TAPPI standard method T15 os-58. An amount of 2 g of each tissue was incinerated for no less than 6 h at 525  $^{\circ}\text{C}$ , and the residue after incineration weighed as ash. The formula applied for the content of ashes is as follows, where o.d. material stands for oven-dried material.

$$\text{Ashes (\%)} = \frac{\text{Residue}}{\text{o.d.material}} * 100$$

### 3.2.2 Extractives determination

The extractives are low weight molecules that are not part of the cell-wall structure and can be removed with solvents. The most used solvents are dichloromethane, ethanol and water. The first solvent is used to remove mainly substances such as resins, fats and waxes; the second to remove phenolic substances; and the last to remove carbohydrates. The samples were submitted to a successive soxhlet extraction using dichloromethane (6 h), ethanol (16 h) and water (16 h), (TAPPI standard method T211 om-02), in extraction thimbles. After the extractions samples were oven-dried at 100  $^{\circ}\text{C}$ , weighed and the extractive content determined gravimetrically:

$$\text{Extractives (\%)} = \frac{\text{Extracted Residue}}{\text{o.d.material}} * 100$$

### 3.2.3 Cork methanolysis

The cork was depolymerized by alkaline methanolysis. Extractive-free cork samples of 1.5 g were refluxed in a 100 ml solution of sodium methoxide (50mM)

during 3 h and filtrated in a G3 crucible. The residue was refluxed again during 15 m in 100 ml of methanol ( $\text{CH}_3\text{OH}$ ), and, after filtration, the combined filtrates were acidified to pH 6 with 2M sulfuric acid ( $\text{H}_2\text{SO}_4$ ) in methanol and evaporated to dryness in a rotary evaporator. This residue was suspended in 50 ml of water and extracted 3 times with 50 ml of dichloromethane ( $\text{CH}_2\text{Cl}_2$ ). The extract was dried with anhydrous sodium sulfite ( $\text{Na}_2\text{SO}_3$ ), filtrated and



evaporated to dryness. The residue, which corresponds to the long chain fatty components of suberin was oven-dried at 100 °C, weighed, and the suberin content calculated as:

$$\text{Suberin (\%)} = \frac{\text{Residue}}{\text{o.d.extracted material}} * (100 - \text{Extractives})$$

The solid residue after the methanolysis was called cork<sub>des</sub> (the cork without suberin).

### 3.2.4 Klason and soluble lignin determination

The Klason lignin from the extractive-free phloem and xylem, and from the cork residue after methanolysis was determined by TAPPI standard method T222 om-88. This method consists in hydrolyzing and solubilizing the polysaccharides with 72 % sulfuric acid, and the residue after the hydrolysis corresponding to the Klason lignin, was oven dried and weighed (formula A was applied for cork, and B for phloem and xylem). The soluble lignin was determined by TAPPI method UM 250, by measuring the absorbance of ultraviolet radiation at a wavelength of 205 nm, in a Shimadzu A160 spectrophotometer, of the filtrate obtained after the hydrolysis for the Klason lignin determination. The formula used for soluble lignin was formula C (in the cork tissue) and D (in phloem and xylem tissues), where A<sub>205</sub> is the absorbance at 205 nm, V<sub>i</sub> the initial volume, f is the dilution factor (10), ε is the absorptivity (110 cm/g), and m is the mass of oven dried material of cork, phloem and xylem used in the Klason method.

$$\text{A. Klason lignin (\%)} = \frac{\text{Residue}}{\text{o.d.extracted material}} * (100 - \text{Extractives} - \text{Suberin})$$

$$\text{B. Klason lignin (\%)} = \frac{\text{Residue}}{\text{o.d.extracted material}} * (100 - \text{Extractives})$$

$$\text{C. Soluble lignin (\%)} = \frac{A_{205} * V_i * f}{\epsilon * m} * (100 - \text{Extractives} - \text{Suberin})$$

$$\text{D. Soluble lignin (\%)} = \frac{A_{205} * V_i * f}{\epsilon * m} * (100 - \text{Extractives})$$

### 3.2.5 Neutral monosaccharides, acetate and uronic acids determination

The polysaccharides composition was determined as neutral monosaccharides, acetate and uronic acids in the hydrolysate from the Klason lignin. The monosaccharides and the uronic acids were separated by High Pressure Ion-exchange Chromatography using a Dionex ICS3000 equipped with a PAD detector; the column used was Thermo Carbopac PA10 (250 x 4 mm) + Aminotrap and the mobile phase was NaOH + CH<sub>3</sub>COONa with a flow of 1 ml/min at 30 °C. The acetic acid content was separated by High Pressure Ion-exclusion Chromatography using a Thermo Finnigan Surveyor and measured at a wavelength of 210 nm by a UV/Vis

detector; a Biorad Aminex 87H (300 x 7.8 mm) column was used and the eluent was H<sub>2</sub>SO<sub>4</sub> (10 mN) with a flow of 0.6 ml/min at 30 °C.

### 3.3 Statistical analysis

The statistical analysis was made in StatSoft Statistica 10 Enterprise and differences between provenances 14, 15 and 19 were tested with one-way ANOVA, pairwise analysis (Tukey test,  $p < 0.05$ ).

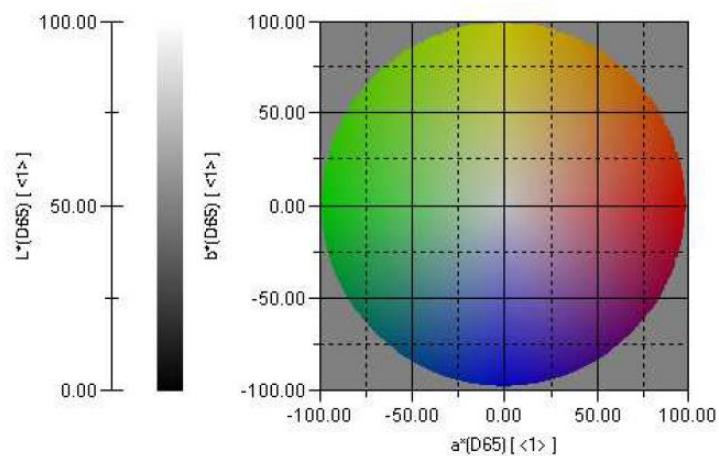
### 3.4 Analytical pyrolysis (PY-GC/MS)

The extractive-free samples of cork, phloem and xylem, and the cork<sub>des</sub> sample were milled to a fine powder on a Retsch MM200 mixer mill, and dried under vacuum over phosphorus pentoxide. The samples were then weighed (approximately 100 µg) and pyrolysed at 650 °C for 10 s in a CDS Pyroprobe 5150 Pyrolyzer connected to an Agilent GC 7890B coupled to a mass detector system 5977B, and using a fused-silica capillary column ZB-1701 (60 m x 0.25 mm i.d. x 0.25 µm film thickness), and helium as carrier gas (total flow of 1 mL/min). The oven heating program started at 40 °C (held for 4 min), increased to 70 °C at a rate of 10 °C/min, then to 100 °C at 5 °C/min, next to 265 °C at 3 °C/min (held for 3 min), and last to 270 °C at 5 °C/min (held for 9 min). The temperature of the injector and the GC/MS interface were kept at 270 °C and 280 °C, respectively.

The compounds were identified using the Wiley/NIST libraries, and literature (Faix, et al., 1990; Ralph & Hatfield, 1991).

### 3.5 Color measurements

The samples of cork, phloem and xylem were subjected to optical measurements before the extractions and between each extraction with dichloromethane, ethanol and water, with a, in order to evaluate a possible relation between color and the removal of extractives. The tissues were characterized by color parameters of the CIE L\*a\*b\* scale (CIELAB), measured in a spectrophotometer Minolta CM-3630. The L\* represents lightness, varying between 0 (black) and 100 (white); a\* and b\* parameters varies between -100 and 100, varying from green (negative values) and red (positive values) in parameter a\*, and from blue (negative values) and yellow (positive values) in parameter b\* (Figure 8).



**Figure 8.** CIE L\*a\*b\* color scale.

## 4. Results

### 4.1 Chemical composition

The summative chemical composition of cork from the three provenances is presented in Table 3 as provenance means and mean values of the nine trees studied (% of the original material). The standard deviation, in brackets, and the results of the pairwise analysis (Tukey test,  $p < 0.05$ ) are also included. The chemical composition of cork from the nine individual trees is presented in the annex section (Annex 1).

The mean values for the ash content in cork was quite similar between provenances (0.69 %, 0.63 % and 0.66 % for provenances 14, 15 and 19, respectively), with a small variation between the nine trees as can be seen by the low standard deviation values (Table 3).

**Table 3.** Chemical composition of cork samples from the three cork oak provenances (% of oven-dried material). Mean values, standard deviation and pairwise Tukey test.

% of original material	Cork			
	P14	P15	P19	Mean (STDEV)
<b>Ash</b>	<b>0.69 (0.05) a</b>	<b>0.63 (0.03) a</b>	<b>0.65 (0.07) a</b>	<b>0.66 (0.06)</b>
<b>Total extractives</b>	<b>10.4 (0.3) a</b>	<b>12.7 (0.7) a</b>	<b>12.0 (1.8) a</b>	<b>11.7 (1.4)</b>
Dichloromethane	4.7 (0.3) a	5.3 (0.6) a	5.1 (0.7) a	5.1 (0.5)
Ethanol	2.4 (0.6) a	4.1 (0.6) b	3.1 (0.6) ab	3.1 (1.0)
Water	3.3 (0.4) a	3.3 (0.6) a	3.8 (0.5) a	3.5 (0.6)
<b>Suberin</b>	<b>42.7 (2.7) a</b>	<b>43.3 (7.0) a</b>	<b>41.0 (5.1) a</b>	<b>42.3 (4.7)</b>
<b>Total lignin</b>	<b>24.9 (1.3) a</b>	<b>23.4 (3.2) a</b>	<b>23.9 (2.3) a</b>	<b>24.1 (2.2)</b>
Klason lignin	24.2 (1.3) a	22.7 (3.0) a	23.0 (2.3) a	23.3 (2.1)
Soluble lignin	0.71 (0.09) a	0.68 (0.16) a	0.85 (0.08) a	0.75 (0.13)
<b>Polysaccharides</b>	<b>16.8 (1.6) a</b>	<b>15.2 (4.4) a</b>	<b>16.6 (4.1) a</b>	<b>16.2 (3.2)</b>
Arabinose	2.8 (0.2) a	2.7 (0.5) a	2.7 (0.2) a	2.8 (0.3)
Galactose	1.1 (0.1) a	1.0 (0.1) a	1.1 (0.03) a	1.05 (0.06)
Glucose	7.3 (0.9) a	6.6 (2.3) a	7.2 (1.9) a	7.0 (1.6)
Xylose	4.9 (0.7) a	4.3 (2.4) a	5.2 (1.9) a	4.8 (1.6)
Galacturonic acid	0.51 (0.01) a	0.45 (0.01) a	0.44 (0.09) a	0.47 (0.06)
Glucuronic acid	0.05 (0.01) a	0.05 (0.01) a	0.06 (0.02) a	0.05 (0.01)

The same letter in a row means that no significant differences were found between provenances.

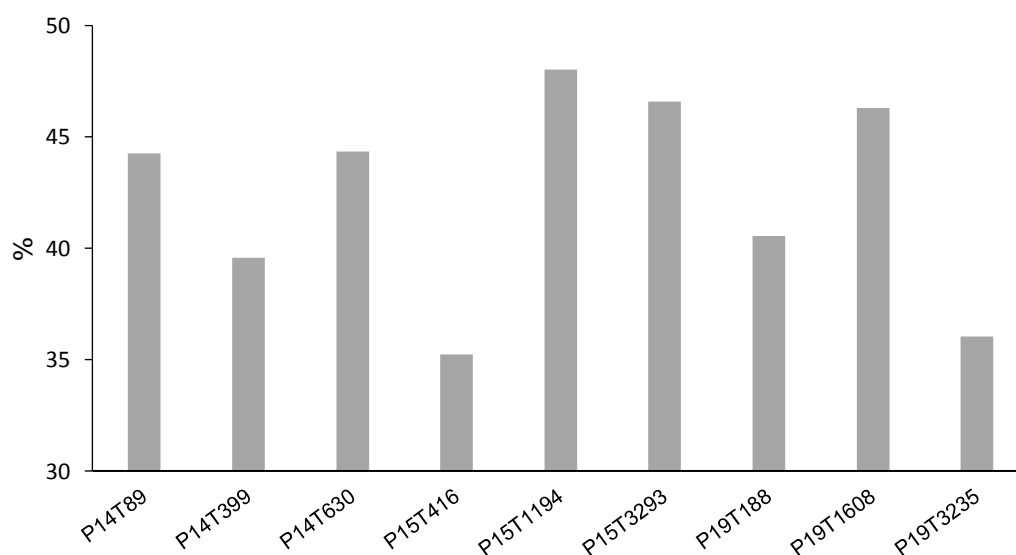
Cork has a high content of total extractives (Table 3) with values of 10.4 %, 12.7 % and 12.0 % for provenances 14, 15 and 19 respectively. Total extractives ranged from 10.1 % in Tree 630 (provenance 14) to 13.3 % in Tree 1608 (provenance 19, Annex 1). In Table 4 is presented the percentage of total extractives by solvent, and it can be seen that compounds soluble in dichloromethane correspond to almost 50 % of total extractives (average of 43.6 % in the nine

trees), with the polar compounds representing 26.2 % and 30.2 % in ethanol and water extracts respectively. Note that, ethanol extractives are higher than water extractives for two trees (Trees 1194 and 3293), in opposite to what happens in the other trees (Annex 1).

**Table 4.** Proportion of the extractives solubilized by dichloromethane, ethanol and water, as % of total extractives in the cork tissue.

% of total extractives	Cork			
	P14	P15	P19	Mean
<b>Dichloromethane</b>	46.3 (3.0)	42.0 (2.6)	42.5 (5.2)	<b>43.6 (3.1)</b>
<b>Ethanol</b>	20.7 (5.8)	32.2 (10.3)	25.7 (9.9)	<b>26.2 (6.1)</b>
<b>Water</b>	33.0 (3.4)	25.8 (8.5)	31.8 (7.9)	<b>30.2 (4.9)</b>

Suberin is exclusive to the cork tissue, and its mean content was 42.7 %, 43.3 % and 41.0 %, respectively in provenances 14, 15 and 19 (Table 3), but with a large variation among the nine trees, ranging from 35.2 % (P15, tree T416) to 48.0 % (P15, tree T1194), as it can be seen in Figure 9. After suberin, lignin was the second highest component in cork, accounting to 24.1 %, 23.4 % and 23.9 % in provenances 14, 15 and 19 respectively. Klason lignin ranged from 22.7 % (P15) to 24.2 % (P14), and the soluble lignin represented less than 1 % (Table 3).



**Figure 9.** Suberin content of cork from the nine trees studied (% o.d. material).

Total polysaccharides in cork accounted to 16.7 %, 15.2% and 16.6 % P14, P15 and P19 respectively (Table 3) with a mean value of 16.2 %. Table 5 shows the carbohydrate

composition as the monosaccharides and the uronic acids proportion in relation to total polysaccharides distributed by provenances. Glucose, xylose and arabinose were the principal monosaccharides of cork polysaccharides, representing 43.3 %, 29.0 % and 17.7 % in the nine trees studied. Galactose, galacturonic and glucuronic acids make up the remaining polysaccharides, accounting to 6.7 %, 3.0 % and 0.34 % of the total. Although the values of total polysaccharides in cork varied in the nine trees studied, the proportion of each monosaccharide was very similar in all the trees (Table 5). For example, the main monosaccharides glucose and xylose varied from 42.9 % to 43.7 %, and 27.1 % to 30.5 %, respectively. The same behavior was found for arabinose (17.0 % to 19.0 %), galactose (6.4 % to 7.0 %), and for galacturonic (2.7 % to 3.1 %) and glucuronic acid (0.33 % to 0.36 %).

A statistical analysis using all the values for the cork chemical characterization showed that the only significant differences were for the ethanol extractives between provenances 14 and 15.

**Table 5.** Composition of monosaccharides, uronic and acetic acids in the cork tissue of the three cork oak provenances (% of total monosaccharides).

% of total monosaccharides	Cork			
	P14	P15	P19	Mean
<b>Arabinose</b>	17.1 (2.1)	19.0 (6.9)	17.0 (3.0)	<b>17.7 (4.0)</b>
<b>Galactose</b>	6.4 (1.0)	7.0 (1.8)	6.5 (1.3)	<b>6.7 (1.3)</b>
<b>Glucose</b>	43.7 (1.6)	43.4 (2.6)	42.9 (0.8)	<b>43.3 (1.6)</b>
<b>Xylose</b>	29.3 (1.8)	27.1 (7.0)	30.5 (3.5)	<b>29.0 (4.3)</b>
<b>Galacturonic acid</b>	3.1 (0.3)	3.1 (0.7)	2.7 (0.2)	<b>3.0 (0.5)</b>
<b>Glucuronic acid</b>	0.33 (0.03)	0.33 (0.05)	0.36 (0.05)	<b>0.34 (0.04)</b>

The mean summative chemical composition and the standard deviation of phloem from the three provenances, and the mean value of the nine trees is presented in Table 6. The results of the pairwise analysis (Tukey test,  $p < 0.05$ ) are also included. The chemical composition of phloem for the nine trees studied is presented in the annex section (Annex 2).

**Table 6.** Chemical composition of phloem samples from the three cork oak provenances (% of oven-dried material). Mean, standard deviation, and pairwise Tukey test.

% of original material	Phloem			
	P14	P15	P19	Mean (STDEV)
<b>Ash</b>	<b>3.1 (0.7) a</b>	<b>2.7 (1.1) a</b>	<b>2.8 (0.3) a</b>	<b>2.9 (0.7)</b>
<b>Total extractives</b>	<b>3.9 (0.5) a</b>	<b>4.8 (1.4) a</b>	<b>5.0 (0.8) a</b>	<b>4.5 (1.0)</b>
Dichloromethane	0.14 (0.03) a	0.17 (0.06) a	0.18 (0.04) a	0.17 (0.04)
Ethanol	1.5 (0.4) a	1.6 (0.3) a	1.4 (0.3) a	1.5 (0.3)
Water	2.3 (0.2) a	2.8 (1.7) a	3.4 (1.1) a	2.8 (1.1)
<b>Total lignin</b>	<b>38.4 (1.2) a</b>	<b>37.8 (3.1) a</b>	<b>37.9 (1.1) a</b>	<b>38.0 (1.8)</b>
Klason lignin	35.9 (1.2) a	35.4 (3.3) a	35.5 (0.8) a	35.6 (1.8)
Soluble lignin	2.5 (0.04) a	2.4 (0.2) a	2.4 (0.3) a	2.5 (0.2)
<b>Polysaccharides</b>	<b>48.5 (1.2) ab</b>	<b>51.6 (2.1) a</b>	<b>47.3 (1.1) b</b>	<b>49.1 (2.3)</b>
Arabinose	1.1 (0.1) a	1.4 (0.3) a	1.1 (0.2) a	1.2 (0.2)
Galactose	0.9 (0.03) a	1.2 (0.3) a	0.8 (0.1) a	0.97 (0.23)
Glucose	21.2 (2.1) a	22.6 (2.9) a	20.3 (2.0) a	21.4 (2.3)
Xylose	18.7 (0.3) a	19.2 (1.0) a	19.4 (0.9) a	19.1 (0.8)
Galacturonic acid	1.0 (0.3) a	1.3 (0.4) a	0.84 (0.2) a	1.1 (0.3)
Glucuronic acid	0.16 (0.2) a	0.25 (0.2) a	0.07 (0.01) a	0.16 (0.14)
Acetic acid	5.4 (0.5) a	5.6 (0.8) a	4.8 (0.7) a	5.3 (0.7)

The same letter in a row means that no significantly differences were found between provenances.

Ash content in phloem accounted to 3.1 %, 2.7 % and 2.8 % in provenance 14, 15 and 19 respectively, ranging from 1.8 % (Tree 416) to 4.0 % (Tree 1194, Annex 2). The content of total extractives in phloem was 3.9 %, 4.8 % and 5.0 % in provenances 14, 15 and 19 respectively, with a mean value of 4.5 %. As it can be seen from Table 7, in phloem the dichloromethane extracts represented a minor fraction of total extractives (mean of 3.7 % in the three provenances), while the polar compounds were the major part representing 96.3% of total extractives, with ethanol extracts accounting for 33.4% (28.3 % - 37.7 %) and the water extracts for 62.9 % (58.6 % - 68.1 %).

Total lignin in phloem accounted to 38.4 %, 37.8 % and 37.9 % in provenances 14, 15 and 19 respectively. For all the trees, total lignin ranged from 34.4 % (Tree 1194) to 40.1 % (Tree 3293, Annex 2), Klason lignin ranged from 31.8 % (Tree 1194) to 37.9 % (Tree 3293) and soluble lignin from 2.1 % (Tree 188) to 2.7 % (Tree 1608).

**Table 7.** Proportion of the extractives solubilized by dichloromethane, ethanol and water (% of total extractives) in the phloem samples of the three cork oak provenances.

% of total extractives	Phloem			
	P14	P15	P19	Mean
<b>Dichloromethane</b>	3.7 (1.1)	3.8 (1.2)	3.6 (1.6)	<b>3.7 (1.0)</b>
<b>Ethanol</b>	37.7 (5.9)	34.3 (1.3)	28.3 (6.8)	<b>33.4 (12.8)</b>
<b>Water</b>	58.6 (5.5)	61.9 (0.4)	68.1 (8.5)	<b>62.9 (13.3)</b>

In Table 8 is presented the proportion of monosaccharides, uronic and acetic acids, in relation to total polysaccharides from the phloem tissue. Polysaccharides represented 48.5 % (P14), 51.6 % (P15) and 47.3 % (P19), with a low standard deviation (1.2, 2.1 and 1.1 respectively), and ranging from 46.4 % (Tree 3235) to 53.9 % (Tree 1194, Annex 2). Glucose and xylose were the major monosaccharides, accounting respectively to 21.2 % and 18.7 % (P14), 22.6 % and 19.2 % (P15) and 20.3 % and 19.4 % (P19) of the oven dried material, and both monosaccharides represented more than 80 % of the total sugars in phloem (Table 8). Acetic acid accounted to 4.8 % (P19) to 5.4 % (P14) of oven dried material, while arabinose, galacturonic and glucuronic acids made up the remaining monosaccharides, accounting to less than 6 % (Table 6). In phloem there were statistically significant differences only in the total polysaccharides content between provenances 15 and 19.

**Table 8.** Composition of monosaccharides, uronic and acetic acids in the phloem tissue (% of total monosaccharides) of the three cork oak provenances

% of total monosaccharides	Phloem			
	P14	P15	P19	Mean
<b>Arabinose</b>	2.3 (0.2)	2.6 (0.6)	2.3 (0.5)	<b>2.4 (0.4)</b>
<b>Galactose</b>	1.8 (0.01)	2.3 (0.5)	1.7 (0.2)	<b>2.0 (0.4)</b>
<b>Glucose</b>	43.7 (3.3)	43.8 (4.4)	42.8 (3.3)	<b>43.4 (3.2)</b>
<b>Xylose</b>	38.5 (1.5)	37.5 (3.1)	41.0 (2.8)	<b>39.0 (2.7)</b>
<b>Galacturonic acid</b>	2.1 (0.6)	2.5 (0.7)	1.8 (0.2)	<b>2.1 (0.6)</b>
<b>Glucuronic acid</b>	0.33 (0.33)	0.49 (0.31)	0.14 (0.03)	<b>0.32 (0.27)</b>
<b>Acetic acid</b>	11.2 (1.3)	10.8 (1.8)	10.2 (1.4)	<b>10.7 (1.4)</b>



The summative analysis of the xylem samples from the three provenances is presented in Table 9, also including the pairwise analysis (Tukey test,  $p < 0.05$ ). The detailed results of the chemical composition of xylem for the nine trees studied is presented in the annex section (Annex 3).

The xylem samples presented an ash content of 1.1 % (P14), 1.2 % (P15) and 1.1 % (P19), therefore with a small variability between provenances as seen by the low values of standard deviation (0.2, 0.1 and 0.1, respectively).

**Table 9.** Chemical composition of xylem samples from the three cork oak provenances (% of oven-dried material). Mean, standard deviation values and pairwise Tukey test.

% of original material	Xylem			
	P14	P15	P19	Mean (STDEV)
<b>Ash</b>	<b>1.1 (0.2) a</b>	<b>1.2 (0.1) a</b>	<b>1.1 (0.1) a</b>	<b>1.1 (0.1)</b>
<b>Total extractives</b>	<b>4.9 (0.8) a</b>	<b>5.7 (0.2) a</b>	<b>5.9 (0.7) a</b>	<b>5.6 (0.7)</b>
Dichloromethane	0.29 (0.06) a	0.31 (0.04) a	0.37 (0.05) a	0.32 (0.06)
Ethanol	1.2 (0.5) a	1.9 (0.1) a	1.5 (0.6) a	1.6 (0.5)
Water	3.4 (1.2) a	3.5 (0.3) a	4.0 (0.7) a	3.7 (0.8)
<b>Total lignin</b>	<b>22.6 (0.5) a</b>	<b>24.4 (1.4) a</b>	<b>23.0 (1.0) a</b>	<b>23.4 (1.2)</b>
Klason lignin	19.7 (0.7) a	21.6 (1.5) a	20.4 (0.7) a	20.6 (1.3)
Soluble lignin	2.9 (0.3) a	2.8 (0.2) a	2.6 (0.4) a	2.8 (0.3)
<b>Polysaccharides</b>	<b>66.9 (1.7) a</b>	<b>64.7 (5.1) a</b>	<b>62.0 (2.3) a</b>	<b>64.6 (3.6)</b>
Arabinose	1.0 (0.1) a	0.9 (0.2) a	1.0 (0.08) a	1.0 (0.1)
Galactose	1.6 (0.4) a	1.6 (0.5) a	1.4 (0.2) a	1.5 (0.4)
Glucose	40.4 (1.7) a	37.5 (3.0) a	36.3 (0.9) a	38.1 (2.6)
Xylose	17.1 (0.6) a	18.0 (0.6) a	18.4 (1.4) a	17.8 (1.0)
Galacturonic acid	1.2 (0.1) a	1.2 (0.3) a	0.88 (0.07) a	1.1 (0.2)
Glucuronic acid	0.24 (0.1) a	0.23 (0.1) a	0.06 (0.01) a	0.18 (0.13)
Acetic acid	5.4 (0.5) a	5.3 (1.3) a	4.0 (0.8) a	4.9 (1.1)

The same letter in a row means that no significantly differences were found between provenances.

Total extractives amounted to 4.9 % (P14), 5.7 % (P15) and 5.9 % (P19), with a total mean value of 5.6 %. The extracts were mainly from water (60.9 % to 69.3 %) followed by ethanol (24.9 % to 33.7 %); the compounds soluble in dichloromethane ranged from 5.4 % to 6.2 % (Table 10). Total lignin in xylem was 22.6 % (P14), 24.4 % (P15) and 23.0 % (P19), and ranged from 22.0 % (Tree 3235) to 25.8 % (Tree 3293, Annex 3). The content of Klason lignin varied from 19.0 % (Tree 630) and 23.2 % (Tree 3293), while soluble lignin between 2.4 % (Tree 188) and 3.3 % (Tree 630).

**Table 10.** Proportion of the extractives solubilized by dichloromethane, ethanol and water (% of total extractives) in the xylem of the three cork oak provenances.

% of total extractives	Xylem			
	P14	P15	P19	Mean
Dichloromethane	5.9 (1.9)	5.4 (1.3)	6.2 (0.9)	<b>5.8 (1.2)</b>
Ethanol	24.9 (13.4)	33.7 (11.6)	25.8 (8.1)	<b>28.1 (9.2)</b>
Water	69.3 (15.1)	60.9 (12.6)	67.9 (8.1)	<b>66.0 (10.0)</b>

The content of total polysaccharides in xylem was 66.9 %, 64.7 % and 62.0 % in provenances 14, 15 and 19 respectively, and ranged from 59.5 % (Tree 3235) and 70.3 % (Tree 1194, Annex 3). The monosaccharides composition was dominated by glucose and xylose, reaching almost 86.6 % of total monosaccharides, with arabinose, galactose and galacturonic, glucuronic and acetic acids making up 13.3 % of total monosaccharides (Table 11).

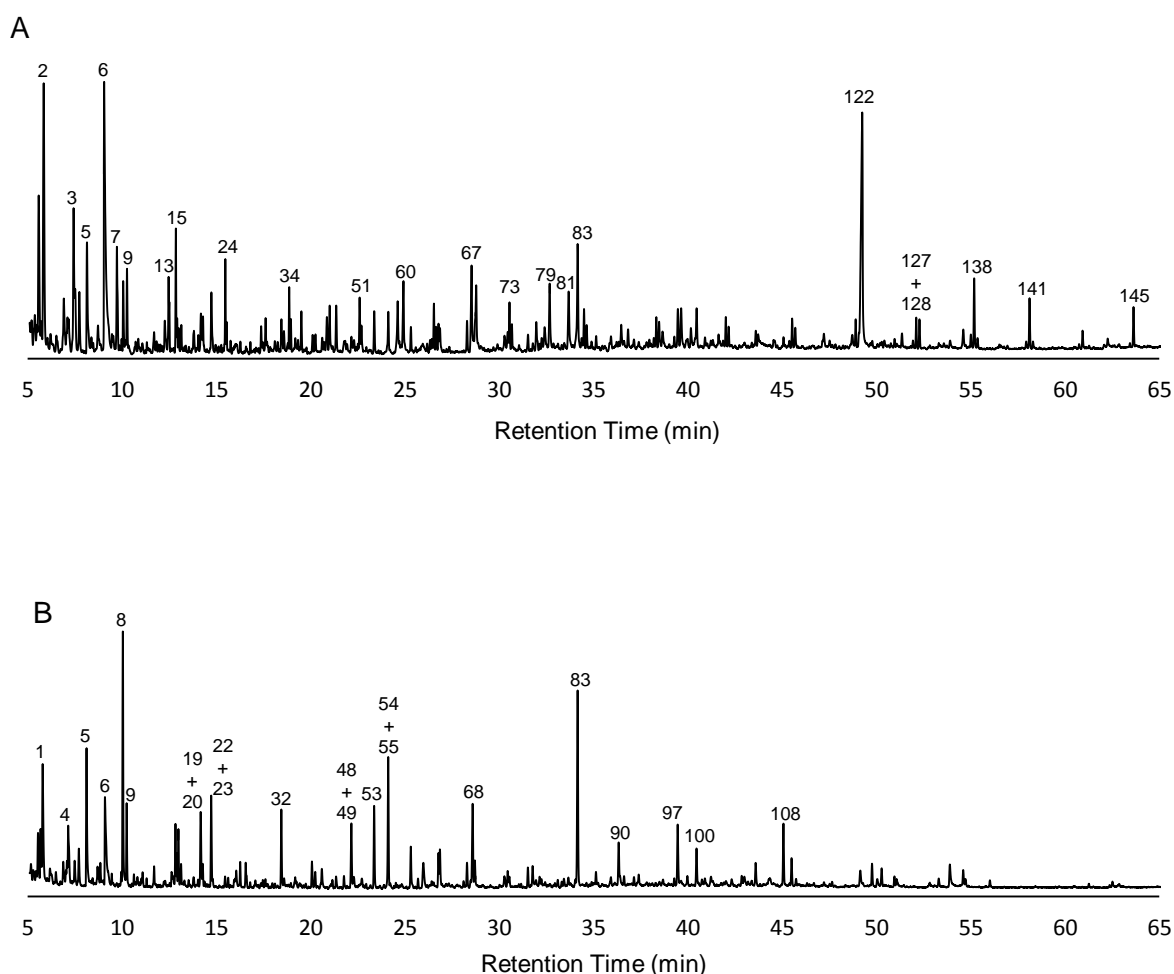
**Table 11.** Composition of monosaccharides, uronic and acetic acids in the xylem tissue (% of total monosaccharides) of the three cork oak provenances.

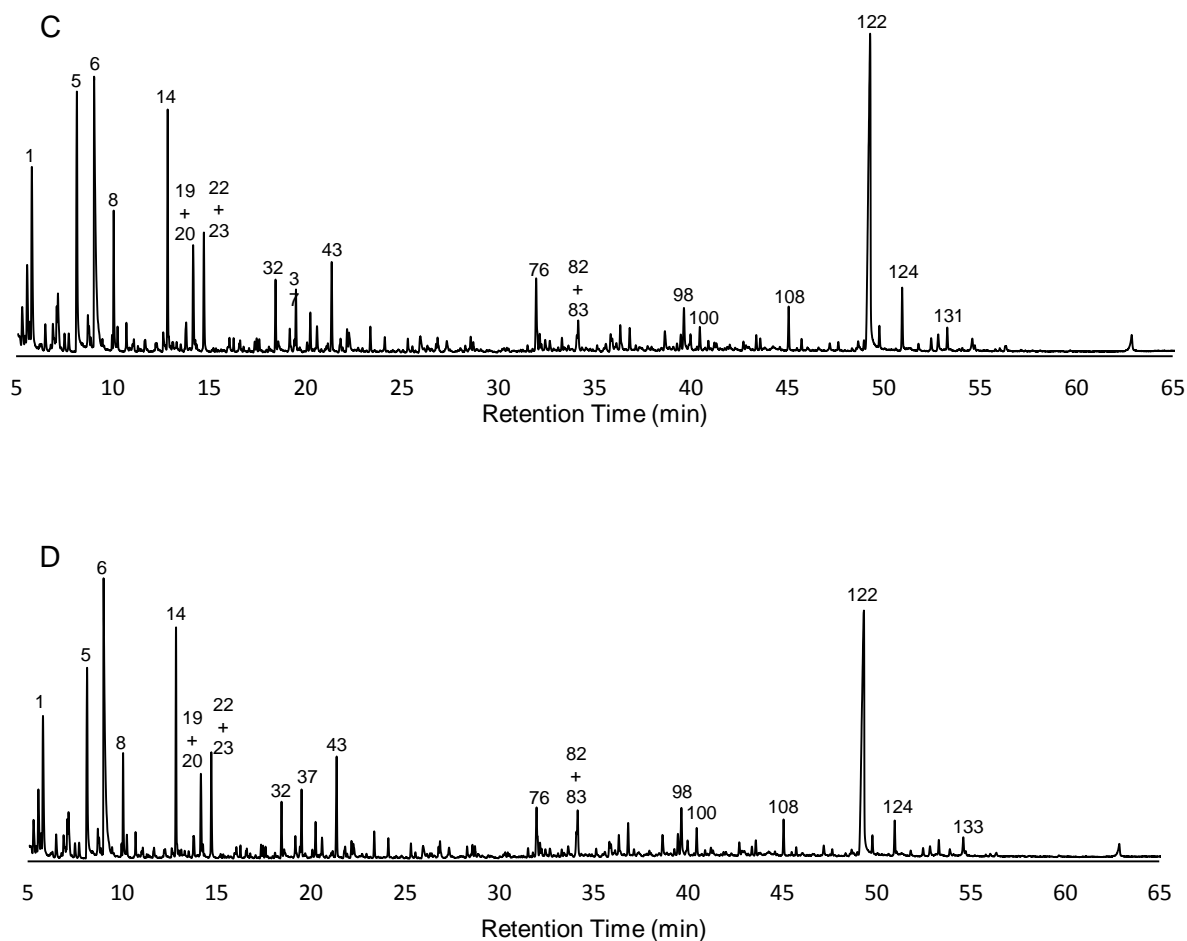
% of total monosaccharides	Xylem			
	P14	P15	P19	Mean
Arabinose	1.5 (0.2)	1.4 (0.3)	1.7 (0.2)	<b>1.5 (0.2)</b>
Galactose	2.4 (0.6)	2.4 (0.7)	2.2 (0.3)	<b>2.3 (0.5)</b>
Glucose	60.4 (1.1)	57.9 (0.5)	58.5 (2.1)	<b>58.9 (1.6)</b>
Xylose	25.6 (1.5)	27.8 (1.5)	29.7 (1.3)	<b>27.7 (2.0)</b>
Galacturonic acid	1.7 (0.2)	1.8 (0.3)	1.4 (0.1)	<b>1.7 (0.3)</b>
Glucuronic acid	0.36 (0.21)	0.34 (0.20)	0.10 (0.02)	<b>0.27 (0.19)</b>
Acetic acid	8.0 (0.7)	8.2 (1.7)	6.4 (1.1)	<b>7.5 (1.4)</b>

From the statistical point of view, there were no significant differences in the summative chemical composition of the xylem samples between the three studied provenances.

## 4.2 Pyrolysis

The pyrograms of cork, cork<sub>des</sub>, phloem and xylem are presented in Figure 10. The beginning of the pyrograms is composed mainly by carbohydrates derived compounds (carbohydrates and suberin in the case of cork), and after 23 min the lignin derived compounds start to appear. The chromatograms of cork and cork<sub>des</sub> are relatively different, especially for the fact that in cork<sub>des</sub> there were no suberin related peaks. As for phloem and xylem, both chromatograms are nearly identical, with just some compounds that were found in phloem and not in xylem (*peak 15*, 1,3-dimethyl-benzene; *peak 110*, guaiacylacetone; *peak 125*, *cis*-coniferyl alcohol; *peak 132*, *trans*-coniferyl alcohol; and *peak 135*, *trans*-sinapyl alcohol) and one compound that was identified in xylem but not in phloem (*peak 65*, a compound similar to 5-(hydroxymethyl)dihydro-2(3*H*)-furanone). The list of the 147 identified compounds in each tissue (cork, cork<sub>des</sub>, phloem and xylem) of the three provenances and their % (of total chromatographic area) is presented in the annex section (Annex 4).





**Figure 10.** Py-GC/MC pyrograms of cork (A), desuberinized cork (B), phloem (C) and xylem (D) from *Q. suber*.

Tables 12 and 13 show the pyrolysis results presented as the sum of the main chemical families of cork, cork<sub>des</sub> (Table 12), phloem and xylem (Table 13). The identified carbohydrates include pyran, furan, low molecular compounds and other type of carbohydrates compounds. The lignin compounds include syringyl (S), guaiacyl (G), *p*-hydroxyphenyl (H) lignin type of units and other compounds that can be from any of those three monomers. Suberin compounds belong only to cork, and were divided in fatty acids, alkanes, alkenes, alkadienes and in non-identified suberin compounds.

In cork, the mean values of total carbohydrates accounted to 25.6 %, total lignin to 12.6 % and total suberin to 33.0 %. In cork<sub>des</sub> (after methanolysis) there were no suberin compounds identified, leading to an increase in total carbohydrates and total lignin, 37.1 % and 34.4 %, respectively (Table 12).

In carbohydrates, the low molecular compounds represented 10.8 % in cork and 21.5 % in cork<sub>des</sub>. Annex 4 presents the list of identified compounds, where the main compounds with low molecular weight were acetic acid (*peak 6*; 6.6 % and 4.2 %, respectively) and hydroxyacetaldehyde (*peak 5*; 1.6 % and 3.4 %). In cork<sub>des</sub> there's also 2-hydroxypropanone (*peak 8*) accounting to 6.1 % but in cork just reach 0.81 %, and 2-oxo-propanal (*peak 1*) with 4.8% in cork<sub>des</sub> but was not identified in cork. Pyran compounds represented 7.2 % in cork, and only 2.0 % in cork<sub>des</sub> with levoglucosane (*peak 122*) representing 5.9 % and 0.53 % respectively. Furan compounds represented 4.4 % and 5.8 %, in cork and cork<sub>des</sub> respectively. The main compound in cork was *peak 81* (1,5-anhydro-arabinofuranose) representing 1.7 % (it represented only 0.40 % in cork<sub>des</sub>). *Peak 22* (furfural) and *peak 54* (2-(propan-2-one)-tetrahydrofuran) were the major furan compounds in cork<sub>des</sub> representing 1.3 % and 2.1 %, respectively (in cork the compounds represented only 0.43 % and 0.25 %, respectively). Other carbohydrates (including not identified compounds) make up the remained of total carbohydrates, accounting to 3.2 % and 7.8 % in cork and cork<sub>des</sub>, respectively.

**Table 12.** Composition of pyrolysis products grouped by derivative families of extracted cork and desuberized cork (% of total chromatogram area) as determined by Py-GC/MS.

	Cork				Cork <sub>des</sub>			
	P14	P15	P19	Mean	P14	P15	P19	Mean
Total lignin	13.2	12.5	12.1	<b>12.6</b>	32.3	33.5	37.5	<b>34.4</b>
S	1.0	0.78	0.47	<b>0.76</b>	5.4	4.2	4.11	<b>4.6</b>
G	7.0	6.5	5.4	<b>6.3</b>	15.9	16.8	17.4	<b>16.7</b>
H	2.5	2.5	2.6	<b>2.5</b>	6.4	7.6	9.0	<b>7.6</b>
Others	2.7	2.7	3.6	<b>3.0</b>	4.6	4.9	7.0	<b>5.5</b>
S/G	0.14	0.12	0.09	<b>0.12</b>	0.34	0.25	0.24	<b>0.28</b>
H:G:S	1:2.9:0.4	1:2.6:0.3	1:2.0:0.3	<b>1:2.5:0.3</b>	1:2.5:0.9	1:2.2:0.6	1:1.9:0.5	<b>1:2.2:0.6</b>
Total carbohydrates	26.4	24.4	25.7	<b>25.6</b>	40.6	33.6	37.1	<b>37.1</b>
Pyran	7.7	7.0	6.8	<b>7.2</b>	2.4	1.8	1.8	<b>2.0</b>
Furan	4.5	4.2	4.5	<b>4.4</b>	5.8	5.8	5.9	<b>5.8</b>
Low molecular	11.0	10.3	11.0	<b>10.8</b>	24.0	18.5	21.9	<b>21.5</b>
others	3.2	2.9	3.4	<b>3.2</b>	8.4	7.5	7.5	<b>7.8</b>
Total suberin	33.2	33.0	32.9	<b>33.0</b>	-	-	-	-
Fatty acids	7.4	7.4	7.9	<b>7.6</b>	-	-	-	-
Alkane	1.9	2.1	1.6	<b>1.9</b>	-	-	-	-
Alkene	18.1	17.5	17.5	<b>17.7</b>	-	-	-	-
Alkadiene	4.2	4.4	4.4	<b>4.3</b>	-	-	-	-
Not identified	1.7	1.6	1.5	<b>1.6</b>	-	-	-	-

Lignin derived compounds, represented in cork 12.6 % of total pyrogram area, and in cork<sub>des</sub> 34.4 %. The G-units dominated in both cork and cork<sub>des</sub>, respectively 6.3 % and 16.7 %, majorly represented by guaiacol (*peak 55*), 4-vinylguaiacol (*peak 83*), *trans*-isoeugenol (*peak 97*) and vanillin (*peak 100*) and accounting respectively to 0.36 %, 2.0 %, 0.74 % and 0.90 % in cork samples and 2.1 %, 6.0 %, 2.0 % and 1.2 % in cork<sub>des</sub> samples (Annex 4). H-units accounted to 2.5 % in cork and 7.6 % in cork<sub>des</sub> with phenol (*peak 53*) accounting to 0.51 % and 2.7 %, and *o*-cresol (*peak 57*) 0.28 % and 1.3 %, respectively. The third major compound of H lignin in cork was *o*-xilenol (*peak 69*) with 0.61 % and accounting 0.89 % in cork<sub>des</sub>. S-lignin units only represented 0.76 % in cork (S-units derived compounds identified ranged between 0.05 % and 0.16%) and 4.6 % in cork<sub>des</sub>. In the last one, the main compounds were syringol (*peak 90*) and 4-vinylsyringol (*peak 108*) with values of 1.5 % and 1.3 % respectively, but only 0.08 % and 0.16 % in cork. The other lignin derived compounds group represented 3.0 % and 5.5 % in cork and cork<sub>des</sub>, respectively. In this group the main compounds were benzene (*peak 4*; 0.94 % and 1.3 %) and toluene (*peak 9*; 0.97 % and 2.4%). Overall, the lignin from cork was mainly constituted by G units, with a H:G:S distribution of 1:2.5:0.3 in cork and 1:2.2:0.6 in cork<sub>des</sub>. Consequently, the S/G ratio attained values of 0.12 and 0.28 in cork and cork<sub>des</sub> respectively.

Regarding the suberin derived compounds, only identified in cork samples, alkenes accounted to 17.7 %, followed by fatty acids (7.6 %), alkadienes (4.3 %) and alkanes (1.9 %). Alkenes were dominated by 1-hexene (*peak 2*, 4.9 %), 1-heptene (*peak 3*, 2.5 %) and 1-octene (*peak 7*, 1.7 %); the remained alkene compounds represented less than 1 % each).

**Table 13.** Composition of pyrolysis products grouped by derivative families of extracted phloem and xylem (% of total chromatogram area) as determined by Py-GC/MS.

	Phloem				Xylem			
	P14	P15	P19	Mean	P14	P15	P19	Mean
Total lignin	15.0	14.5	13.5	<b>14.4</b>	10.4	11.9	9.9	<b>10.7</b>
S	6.3	5.6	4.6	<b>5.5</b>	6.3	7.2	5.4	<b>6.3</b>
G	5.5	5.2	4.9	<b>5.2</b>	2.5	3.0	2.6	<b>2.7</b>
H	1.8	2.1	2.3	<b>2.0</b>	1.2	1.3	1.6	<b>1.4</b>
Others	1.4	1.6	1.7	1.6	0.35	0.40	0.33	0.36
S/G	1.2	1.1	0.92	<b>1.1</b>	2.5	2.4	2.1	<b>2.3</b>
H:G:S	1:3.1:3.5	1:2.5:2.7	1:2.2:2.0	<b>1:2.6:2.7</b>	1:2.1:5.1	1:2.3:5.4	1:1.6:3.3	<b>1:2.0:4.5</b>
Total carbohydrates	57.8	57.4	60.8	<b>58.6</b>	64.1	62.6	62.5	<b>63.0</b>
Pyran	17.3	15.7	17.7	<b>16.9</b>	24.1	20.9	22.2	<b>22.4</b>
Furan	5.6	5.7	5.9	<b>5.7</b>	6.0	6.2	6.0	<b>6.1</b>
Low molecular	27.1	28.5	28.8	<b>28.1</b>	25.9	27.2	26.0	<b>26.3</b>
others	7.8	7.5	8.4	<b>7.9</b>	8.1	8.3	8.3	<b>8.2</b>

Phloem presented a total lignin of 14.4 % and xylem of 10.7 % (Table 13). S-units accounted to 5.5 % and 6.3 % respectively; where the major compounds were 4-vinylsyringol (*peak 108*; 0.92 % and 0.96 %), syringaldehyde (*peak 124*; 0.78 % and 1.1 %) and *trans*-sinapaldehyde (*peak 136*; 0.83 % and 0.72 %, Annex 4). G-units in phloem and xylem represented respectively 5.2 % and 2.7 %, and the main compounds were 4-vinylguaiacol (*peak 83*; 0.89 % and 0.36 %), *trans*-isoeugenol (*peak 97*; 0.72 % and 0.45 %) and vanillin (*peak 100*; 0.80 % and 0.48%). The H-units represented only 2.0 % and 1.4 % in phloem and xylem respectively, followed by the group of other lignin compounds in amounts of 1.6 % in phloem and 0.36 % in xylem.

The lignin monomeric composition was different in the two tissues: phloem was constituted with a similar amount of G and S units (1:2.6:2.7) and xylem was dominated by S units (1:2.0:4.5). Therefore, the S/G ratio values were 1.1 and 2.3 in phloem and xylem, respectively.

The phloem and xylem pyrograms were largely dominated by carbohydrates, accounting to 58.6 % in phloem and 63.0 % in xylem (Table 13). Low molecular compounds reached 28.1 % and 26.3 % respectively, dominated by acetic acid (10.3 % and 8.5 %), hydroxyacetaldehyde (5.5 % and 6.2 %) and 2-oxo-propanal (4.0 % and 4.1 %, Annex 4). Pyran compounds accounted to 16.9 % and 22.4 % and were composed mainly by levoglucosane (12.9 % and 18.3 %). Furan compounds represented 5.7 % and 6.1 %, with furfural accounting to 1.2 % and 1.1 %. Other carbohydrates corresponded to 7.9 % and 8.2 % in phloem and xylem respectively.

Table 14 presents a comparison between the chemical contents obtained by wet chemical analysis and by pyrolysis in respect to carbohydrates, lignin and suberin. In cork, the values of total carbohydrates determined by wet chemical analysis were lower compared with pyrolysis (16.4 % *versus* 25.4 %), while total lignin (24.1 % *versus* 12.6 %) and suberin (42.3 % *versus* 33.0 %) were overestimated. In phloem and xylem, total lignin was overestimated by wet chemical analysis (38.0 % *versus* 14.4 %, and 23.4 % *versus* 10.7 %, respectively), and in total carbohydrates the results obtained in phloem by wet chemical analysis were lower compared with pyrolysis results (49.1 % *versus* 58.6 %). On the contrary, in xylem the values of carbohydrates obtained by the two technics were very similar (64.6 % *versus* 63.0 %).

**Table 14.** Comparison of content in carbohydrates, lignin and suberin determined by wet chemical analysis and pyrolysis of cork, phloem and xylem. Mean of the three cork oak provenances.

	Cork		Phloem		Xylem	
	Chemical	Pyrolysis	Chemical	Pyrolysis	Chemical	Pyrolysis
Carbohydrates	16.4	25.4	49.1	58.6	64.6	63.0
Lignin	24.1	12.6	38.0	14.4	23.4	10.7
Suberin	42.3	33.0	-	-	-	-

### 4.3 Color measurements

The color characterization of each tissue based on the CIE L\*a\*b\* parameters is presented in Table 15, for the raw material and after each successive extraction, distributed by provenances.

Cork was characterized by a mean lightness (L\*) of 58.8, varying from 57.6 (P14) and 60.1 (P15, Table 15). Each successive extraction reduced the L\* value varying from 50.0 (P14) to 51.5 (P15) after dichloromethane extraction, from 48.2 (P14) to 49.4 (P19) after ethanol extraction and from 45.3 (P14) to 47.2 (P15) after water extraction. For the a\* and b\* parameters, cork presented quite similar values: 11.6 and 25.4 (P14), 11.6 and 25.8 (P15), 11.4 and 26.0 (P19). The effect of each extraction upon the parameter a\* from the raw material to the last extraction was the same for all provenances, increasing slightly *i.e.* the extracted sample became reddish than the original material. For example, in provenance 14 a\* slightly increased with dichloromethane extraction from 11.6 to 12.1 and was maintained after ethanol and water extraction. Regarding the b\* parameter, each extraction reduced its value with cork becoming bluer, with values ranging from the starting material to the last extractions as follows: 25.4 to 21.9 (P14), from 25.8 to 22.7 (P15), from 26.0 to 22.0 (P19).

Phloem was characterized by a mean L\* of 44.5, varying from 43.0 in P15 and 45.5 in P14 (Table 15). As in cork, extraction reduced the L\* value of all provenances to values of 40.7 (14), 39.3 (15 and 19). Regarding a\* and b\* parameters in the starting phloem, P14 presented values of 9.4 and 21.2, P15 values of 10.0 and 21.3 and P19 values of 11.9 and 23.6. The extraction procedure induced a slight decrease in a\* and b\* parameters to values of 8.8 and 18.7 (14), 8.8 and 19.0 (15), 10.4 and 19.8 (19) respectively.

Xylem was characterized by a mean L\* of 55.9, varying from 54.5 (P15) and 56.9 (P15 and P19, Table 15). The L\* values slightly decreased with dichloromethane and ethanol extractions in all provenances, varying from 53.6 (P15) to 56.2 (P19) in the first extraction, and then from 52.0 (P15) to 55.1 (P19) in the second extraction; however after the third extraction (water) the



L\* slightly increased to values similar to the starting xylem. The a\* and b\* parameters were for P14 8.2 and 21.6, for P15 8.3 and 21.5 and for P19 9.8 and 23.5. The parameter a\* slightly decreased from the raw material to the last extraction *i.e.* the xylem become greener, with a smooth decrease from 8.2 to 8.0 (P14), from 8.3 to 7.7 (P15) and from 9.8 to 9.0 (P19). In parameter b\*, and as in cork and phloem, each extraction reduced its value with xylem becoming bluer, varying from the starting xylem to the last extractions as follows: 21.6 to 19.8 (P14), 21.5 to 18.9 (P15) and 23.5 to 20.2 (P19).

Overall, there were no large changes in the color of the starting material and the color of the extracted material.

**Table 15.** Mean values of the L\*, a\* and b\* color parameters determined in cork, phloem and xylem for the three cork oak provenances before and after extraction with dichloromethane, ethanol and water.

		Cork			Phloem			Xylem		
		L*	a*	b*	L*	a*	b*	L*	a*	b*
P14	Raw	57.6	11.6	25.4	45.5	9.4	21.2	56.2	8.2	21.6
	Dichloromethane	50.0	12.1	24.2	44.4	9.0	20.5	54.6	8.2	21.2
	Ethanol	48.2	12.1	23.4	43.5	8.8	19.8	53.9	8.2	20.7
	Water	45.3	12.1	21.9	40.7	8.8	18.7	56.7	8.0	19.8
P15	Raw	60.1	11.6	25.8	43.0	10.0	21.3	54.5	8.3	21.5
	Dichloromethane	51.5	12.3	24.5	42.2	9.0	20.5	53.6	7.7	20.5
	Ethanol	48.8	12.1	23.5	41.4	8.9	19.8	52.0	7.8	19.6
	Water	47.2	12.5	22.7	39.3	8.8	19.0	54.6	7.7	18.9
P19	Raw	58.7	11.4	26.0	45.0	11.9	23.6	56.9	9.8	23.5
	Dichloromethane	51.3	12.0	25.0	44.0	11.4	22.5	56.2	9.5	22.9
	Ethanol	49.4	11.6	24.1	43.1	10.9	21.8	55.1	9.0	21.8
	Water	45.6	11.8	22.0	39.3	10.4	19.8	56.2	9.0	20.2

## 5. Discussion

The chemical differences between cork, phloem and xylem in *Quercus suber* have only been very scarcely researched. Knowledge on the specific composition of each tissue is available in more extent, namely about cork (as compiled in Pereira 2007). Lourenço et al. (2016) studied the anatomy and the chemical composition of the three tissues, and analyzed their isolated lignin by analytical pyrolysis and by 2D-NMR spectroscopy.

### 5.1 Chemical composition of cork, phloem and xylem

The chemical composition of the cork samples is in general agreement with the literature regarding virgin and reproduction cork (Pereira 1988, Pereira 2015, Lourenco, et al., 2016). The total extractives content reported in this study (on average 11.7 %, Table 3) is close to the 10.4 % reported by Lourenço et al. (2016) but lower than the 14.1 % to 16.9 % range mentioned by Pereira (1988). Suberin is the most important structural component of cork, corresponding in this study to an average of 42.3 %, in the variation range, 30.1 % to 44.8 %, reported by Pereira (2015). There was a large variability in the suberin content between the nine trees studied (ranging from 35.2 % to 48.0 %, Figure 9), as also found by Pereira (1988), who reported a larger variability ranging from 27.9 % to 49.4 %.

Lignin is the second most important component in cork and the values reported here are in agreement with Pereira (1988), with 21.7 % in virgin cork and 23.0 % in reproduction cork, and under the 27.1 % reported by Lourenço, et al. (2016).

Polysaccharides correspond to the third fraction of the structural components in cork, in agreement with the 15.7 % to 21.3 % (Pereira, 1988) and 19.8 % (Lourenço, et al., 2016) in virgin cork.

The chemical composition of cork is specific to the species (Leite and Pereira 2017) e.g. regarding the suberin content: *Quercus cerris* (28.5 %), *Betula pendula* (36.2 %), *Pseudotsuga menziesii* (36.2%) and *Quercus variabilis* (39.2 %) (Şen, et al., 2010; Miranda, et al., 2013; Ferreira, et al., 2016b, 2017).

The chemical composition of the phloem from *Q. suber* was studied for the first time by Lourenço et al. (2016) who reported values similar to those found in the present study (Table 6). In the mentioned study, 3.1 % of ashes and 38.4 % of lignin were reported, in agreement with the values in the present study (2.9 % and 38.0 %, respectively), but a slightly content of extractives was reported by Lourenço et al. (2016) (6.2 % versus 4.5 %).

The comparison with the composition of phloem in other species shows that the phloem from *Betula pendula* has a similar content of ashes (3.6 %) and lignin (35.4 %), but a higher content of extractives (8.1%, where 75 % correspond to compounds soluble in dichloromethane) and a lower content of polysaccharides (43.3 %) (Ferreira, et al., 2017); for *Q. cerris* phloem, the content of ash and extractives is higher, respectively 13.0 % and 6.5 %, but presents a similar content in lignin (35.4 %) and a lower content of sugars (30.6 %) (Şen, et al., 2010). *P. menziesii* presented a lower content of ashes and polysaccharides (0.9 % and 31.8 %, respectively), a similar value of lignin (35.1 %) and a higher content in extractives (28.4 %). In pine species, phloem presents a variable composition of lignin ranging from 32.4 % (*P. radiata*), 23.7 % (*P. pinaster*) and 12.9 % (*P. taeda*) (Pimentel, et al., 2017).

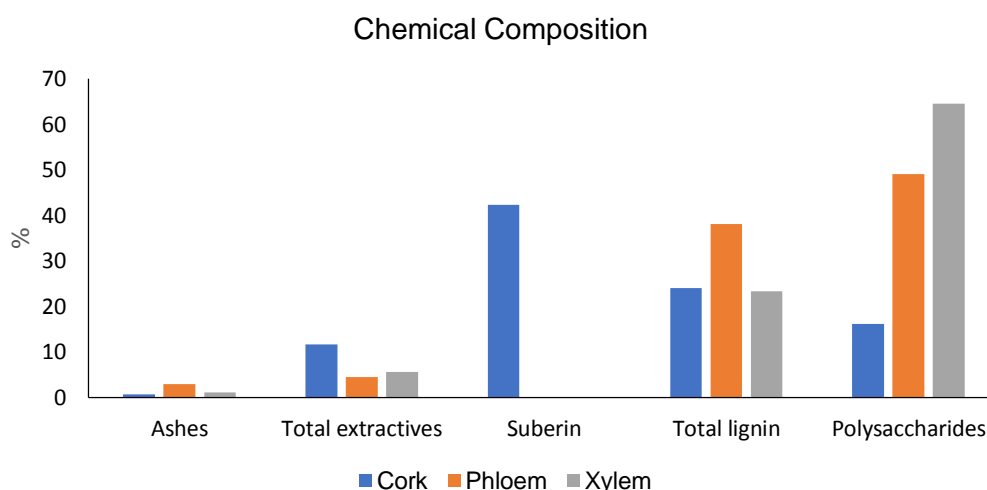
Regarding the chemical composition of xylem from *Q. suber* literature is scarce, and only a few studies were found (Leal, et al., 2005; Lourenço, et al., 2016). The extractives content is lower when compared with the 12.7 % reported by Leal et al. (2005) and the 8.4 % of Lourenço et al. (2016) and the mean lignin content of 23.4 % is in agreement with the 23.6 % and 25.4 % of lignin reported by Lourenço et al. (2016) and Leal et al. (2005), respectively.

The xylem from other oak species has a distinct chemical composition compared with cork oak wood. *Q. faginea* xylem has higher content of extractives (14.5 %) and a similar content of lignin (24.5 %) (Sousa, et al., 2009a); *Q. cerris* xylem presented 6.7 % of extractives and 26.4 % of lignin (Bajraktari, et al., 2018) and *Q. rubra* xylem has 4.4 % of extractives and, 20.2 % of lignin (Pettersen, 1984).

## 5.2 Chemical differences between cork, phloem and xylem

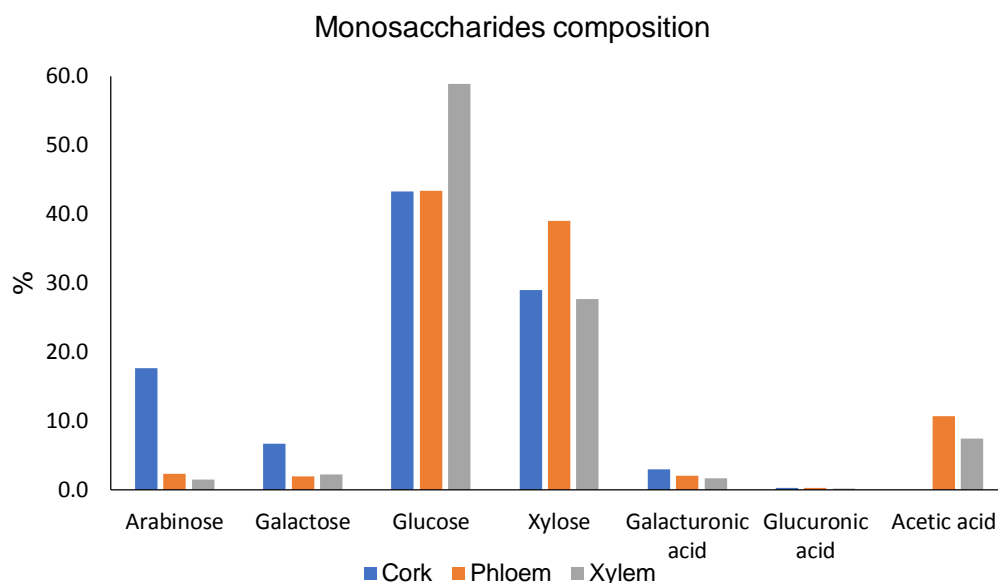
Figure 11 resumes the mean summative chemical composition of cork, phloem and xylem from the nine trees. The three tissues presented great differences between them, being the most obvious the presence of suberin in cork (with a mean value of 42.3 %) and the lack of it in phloem and xylem. Phloem was the tissue with the highest content of ashes (2.9 %), followed by xylem (1.1 %) and cork (0.6 %). Total extractives were higher in cork (11.7 % vs. 5.6 % in xylem and 4.5 % in phloem), and characterized by a different distribution of extracts e.g. in cork dominated the apolar extracts, while in phloem and xylem the polar extracts (Tables 4, 7 and 10). In cork and xylem, the mean content of total lignin was similar, 24.1 % and 23.4 % respectively (but with cork having a higher content of Klason lignin (23.3 % *versus* 20.6 %, Tables 3 and 9), and lower content of soluble lignin (0.7 % *versus* 2.5 %). The phloem has a higher content of total lignin (38.0 %) in accordance with the high content of lignified fibers and sclereids, as discussed by Lourenço et al. (2016). Phloem has less polysaccharides than xylem in consequence of the higher content of total lignin and inorganic material. Cork presented a

minor content of total polysaccharides when compared with phloem and xylem (respectively 16.2 %, 49.1 % and 64.6 %) as a consequence of the presence of suberin and lignin as the main components of the cell walls, protecting the tree against biotic agents and limiting the transport of water and nutrients (Franke & Schreiber, 2007; Pereira, 2015).



**Figure 11.** Summative chemical composition of cork, phloem and xylem (mean values of nine trees, as % o.d. material).

Figure 12 presents the mean composition of the neutral monosaccharides, uronic and acetic acids of cork, phloem and xylem of the nine trees studied. The composition of the polysaccharides was different between the three tissues. Cork was dominated by glucose, xylose and arabinose (43.3 %, 29.0 % and 17.7 % in percentage of total polysaccharides respectively), while phloem and xylem were dominated by glucose and xylose, with phloem attaining a similar content of glucose and xylose (43.4 % and 39.0 %, respectively, in percentage of total polysaccharides) but xylem attaining a higher percentage of glucose than xylose (58.9 % and 27.7 %, respectively). The presence of a large content of xylose in the three tissues indicates that hemicelluloses are mainly constituted by xylans, especially in the phloem tissue. The phloem of *Quercus cerris* has a similar monosaccharide composition as discussed by Şen et al. (2010). The tree tissues presented a low percentage of uronic acids, 3.3 %, 2.4 % and 2.0 % in cork, phloem and xylem respectively. Rocha et al. (2004) mentioned a higher content of uronic acids in the cork of *Q. suber* (12 %). Phloem and xylem presented a moderate amount of acetyl groups (10.7 % and 7.5 %, respectively), while in cork they were not detected.



**Figure 12.** Monosaccharides and uronic and acetic acids composition of cork, phloem and xylem (mean values of nine trees, as percentage of total polysaccharides).

### 5.3 Provenances variation

Tables 3, 6 and 9 show that there were almost no statistically significant differences between provenances 14, 15 and 19.

In cork, the differences were observed only in ethanol extractives between provenances 14 and 15. In phloem, there were significant differences between provenances in respect to total polysaccharides, with the results of provenance 15 significantly different from provenance 19. In xylem, there were no differences between the provenances.

Although there was no statistical significant differences in the present study, these results must be taken with a cautious regard, considering that only three provenances were studied and only three trees per provenance, and considering that the provenances chosen are not so far from each other (the distance between P15, Azeitão, to P19, Santiago do Cacem is 124 Km).

Nevertheless, a similar trend was reported by Conde et al. (1998a) that studied corks from seven provenances in Spain; and by Pereira (2013) that chemical characterized cork samples from 52 different provenances, concluding that there are variations in the chemical composition of cork, but could not be attributed to the geographical origin of provenances. In fact, even in the same tree, the cork obtained from different parts show a variability in the chemical composition as discussed by Pereira (1988).

However, the study of more provenances of *Q. suber* from the same provenance trial (Herdade do Monte da Fava) would enrich this study, originating a stronger statistical analysis and, could

or not, support the lack of significant differences between provenances of the cork oak verified in the present study.

## 5.4 Py-GC/MS

Analytical pyrolysis is a very useful tool to evaluate the chemical composition of lignocellulosic materials, especially the lignin monomeric composition (Lourenço, et al., 2018)

There are various studies mentioned in the literature of *Q. suber* analyzed by analytical pyrolysis. Marques & Pereira (2013) and Marques et al. (2016) studied the cork and wood from *B. pendula*, *Q. cerris* and *Q. suber* by analytical pyrolysis at a temperature of 550 °C, and studied the extractive-free cork and desuberized cork and the isolated lignin from the same samples of *Q. suber* by pyrolysis and 2D-HSQC-NMR spectroscopy, respectively. Lourenço et al. (2016) analysed the isolated lignin of cork, phloem and xylem from the cork oak by pyrolysis and 2D-NMR spectroscopy.

The lignin monomeric composition of cork, phloem and xylem is very different (Tables 12 and 13). The S/G ratio increases from cork to phloem and then to xylem (0.12, 1.1 and 2.3 respectively).

The results obtained in the present work for the lignin composition using analytical pyrolysis on the extracted materials are similar to those obtained when using milled lignin of cork, phloem and xylem e.g. a S/G ratio of 0.10, 0.62 and 1.66 respectively (Lourenço, et al., 2016). This composition was also confirmed by 2D-NMR spectroscopy with S/G of 0.1, 0.7 and 1.2 respectively (Lourenço, et al., 2016). According to the same author, the lignin monomeric composition is specific to each tissue, types of cell and function e.g. cork is formed by a different meristem than phloem and xylem, which have a different type of lignification. In the lignification process of the cell wall, H units are deposited first, then G units and lastly S units, meaning that a rapid lignification of the cell wall will lead to a lignin richer in G units than S units. The lignification process of the cork cell wall occurs at a faster way than of the phloem and xylem, which explains the less content of G units in phloem and xylem. Another reason is the fact that phloem and xylem are respectively constituted by a large amount of sclereids and fibers, both composed by a higher content of S units than G units (Lourenço, et al., 2016).

There were great differences in the results of samples from cork and cork<sub>des</sub>. In the last sample, there was a higher content of total carbohydrates (37.1 % versus 25.6 %) and total lignin (34.4 % versus 12.6 %), in consequence of the absence of suberin compounds, that are the majority of compounds identified in cork samples. In spite of this, the ratio S/G and the H:G:S relation are similar (0.12 and 1:2.5:0.3 in cork, and 0.28 and 1:2.2:0.6 in cork<sub>des</sub>, respectively). The

pyrolysis of phloem and xylem, showed similarities, with phloem attaining more lignin (14.4 *versus* 10.7) and less carbohydrates (58.6 % *versus* 63.0 %) compared to xylem. These results are in agreement with the summative chemical composition, where phloem presented the more lignin and low polysaccharides than xylem.

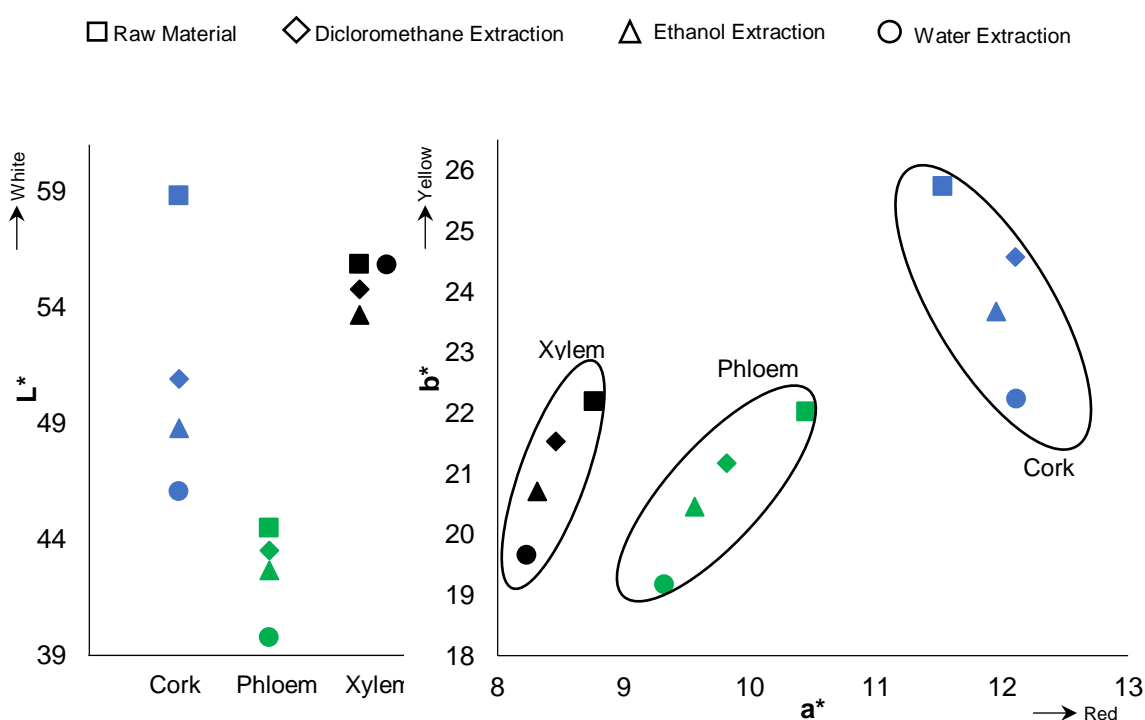
There were major differences in the results obtained from the two technics on the three tissues (Table 14), especially in cork and phloem samples, meaning that pyrolysis is not a useful tool if the goal is to quantify with precision the chemical composition of biomass, since the results of pyrolysis can be very variable depending on the temperature, time of the analysis and GC column used (Lourenço, et al., 2018). The differences found in the present study between the wet chemical analysis and pyrolysis may be attributed to the temperature used in the pyrolysis analysis (650 °C). Literature mention that pyrolysis of cork samples at lower temperatures (550 °C for example) induce an increase of carbohydrates and lignin compounds, and a decrease in suberin derivatives, while the opposite happens as the temperature of pyrolysis is increased (Marques & Pereira, 2014). In phloem and xylem the pyrolysis temperature applied (650°C) induce a decrease of lignin and an increase in carbohydrates compounds comparatively to wet chemical analysis, except on the content of carbohydrates in xylem, which attained a similar value (64.6 % *versus* 63.0 %, respectively in wet chemical analysis and pyrolysis). As discussed by Lourenço et al. (2016), rising pyrolysis temperature (>400°C) leads to lignin secondary reactions, with the formation of compounds of cresol type and due to the liberation of metoxy groups can consequently form compounds attributed to carbohydrates which may explain the results attained in the present study.

## 5.5 Color

Figure 13 presents the mean values of the CIE L\*a\*b\* of cork, phloem and xylem. Phloem is the darkest of the three tissues with a mean L\* value of 44.5, followed by xylem with 55.9 and then cork with 58.8. Regarding a\* b\* parameters there is not a great difference between the tissues. Xylem is greener with a mean a\* value of 8.8, phloem with 10.4 and cork is slightly reddish with 11.5. Regarding the b\* parameter, phloem and xylem are very similar with values of 22.0 and 22.2 respectively, and cork is slightly yellower with a value of 25.7. Leal et al. (2005) reported a lightness of 54.9 for the cork oak wood, ranging from 33.9 to 69.4, and an a\* of 8.7 (6.6 to 10.6) and b\* of 19.1 (12.5 to 24.5). The values CIE L\*a\*b\* from the xylem reported in the present study are in range of values reported by Leal et al. (2005).

The solvent extraction showed some changes in the L\*a\*b\* values of the samples. Regarding the L\* parameter, and as mentioned above, cork starts by being the lighter tissue (with high lightness values) and phloem the darker tissue (with low value of lightness). After the final

extraction, the xylem becomes the lighter tissue (55.8), occurring a great reduction in the cork tissue (from 58.8 to 46.0), while phloem continued the darkest of the three (39.8). All the tissues decreased in  $b^*$ , becoming slightly bluer with each extraction: cork with mean values of 24.6, 23.7 and 22.2, phloem with 21.2, 20.5 and 19.2, and xylem 22.2, 21.5 and 19.5 after dichloromethane, ethanol and water extraction respectively (Figure 13). In the case of the  $a^*$  parameter, both phloem and xylem presented the same behavior, i.e. becoming slightly greener with each extraction (values of 9.8, 9.6 and 9.3 in phloem and 8.5, 8.3 and 8.2 in xylem, respectively after each successive extraction). Cork tends to become reddish after the last extraction, going from 11.5 (raw material) to 12.1 (dichloromethane extraction), 12.0 (ethanol extraction) and finally to 12.1 (water extraction).



**Figure 13.** Mean values of the three provenances of  $L^*$  parameter (left figure) and  $a^*$   $b^*$  parameters (right figure) from cork, phloem and xylem (values of the raw material and each successive extraction).



## 6. Conclusion

In this study the summative chemical composition of the three tissues - cork, phloem and xylem - from nine cork oak trees from three different provenances was determined, in order to assess if the geographical origin of the trees plays a role in the chemical variability of *Q. suber* trees between provenances. All the tissues were also studied by analytical pyrolysis to assess lignin monomeric composition and carbohydrates derivatives. The cork from *Q. suber* has been intensively studied for a long time due to its importance, but little is known about the other tissues in the cork oak stem, especially the phloem.

The three tissues have great chemical differences between them. Cork is predominantly constituted by suberin and lignin, with lower amounts of polysaccharides and extractives. Phloem and xylem have a similar content of total extractives, and both are dominated by polysaccharides and lignin, with phloem having more lignin and less polysaccharides than xylem.

The results from pyrolysis showed also differences between cork, phloem and xylem, especially in relation to lignin composition from the three tissues. Cork lignin is dominated by G units, with some amount of H units; phloem lignin is mainly composed by a similar amount of G and S units, while xylem lignin is mainly constituted by S units.

There were almost no differences in the chemical composition of the tissues between provenances. Lignin composition also showed no differences between the provenances. The chemical composition of trees depends on various factors, not only by geographical origin, and some variability was found in tissues from trees of the same provenances. However the results have a weak statistical significance due to the small number of provenances studied (only three), and more provenances from the cork oak should be studied to confirm or not these findings.

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## Annex

**Annex 1.** Chemical composition of cork samples from three trees of each provenance (mean values of two replicates for each tree).

Cork									
% of original	Provenance 14			Provenance 15			Provenance 19		
material	Tree 89	Tree 399	Tree 630	Tree 416	Tree 1194	Tree 3293	Tree 188	Tree 1608	Tree 3235
<b>Ash</b>	<b>0.72</b>	<b>0.70</b>	<b>0.63</b>	<b>0.62</b>	<b>0.66</b>	<b>0.60</b>	<b>0.69</b>	<b>0.57</b>	<b>0.69</b>
<b>Total extractives</b>	<b>10.3</b>	<b>10.7</b>	<b>10.1</b>	<b>11.8</b>	<b>12.9</b>	<b>13.0</b>	<b>13.0</b>	<b>13.3</b>	<b>10.0</b>
Dichloromethane	5.1	4.9	4.4	4.6	5.8	5.5	5.2	5.8	4.4
Ethanol	1.6	2.1	2.7	3.3	4.5	4.3	3.5	3.5	2.4
Water	3.6	3.8	2.9	3.9	2.6	3.3	4.2	4.1	3.2
<b>Suberin</b>	<b>44.3</b>	<b>39.6</b>	<b>44.4</b>	<b>35.2</b>	<b>48.0</b>	<b>46.6</b>	<b>40.5</b>	<b>46.3</b>	<b>36.0</b>
<b>Total lignin</b>	<b>24.1</b>	<b>26.5</b>	<b>24.2</b>	<b>27.1</b>	<b>21.9</b>	<b>21.3</b>	<b>24.6</b>	<b>21.2</b>	<b>25.7</b>
Klason lignin	23.4	25.7	23.6	26.2	21.3	20.7	23.9	20.4	24.7
Soluble lignin	0.78	0.74	0.60	0.87	0.59	0.59	0.77	0.86	0.92
<b>Polysaccharides</b>	<b>14.9</b>	<b>17.7</b>	<b>17.7</b>	<b>20.2</b>	<b>11.8</b>	<b>13.7</b>	<b>15.7</b>	<b>13.1</b>	<b>21.1</b>
Arabinose	2.9	2.7	3.0	2.2	2.7	3.1	2.9	2.5	2.9
Galactose	1.1	0.97	1.1	0.99	0.97	1.1	1.0	1.0	1.1
Glucose	6.3	8.0	7.7	9.2	5.1	5.6	6.7	5.5	9.2
Xylose	4.1	5.4	5.3	7.0	2.6	3.3	4.6	3.7	7.3
Galacturonic acid	0.52	0.50	0.52	0.46	0.44	0.46	0.45	0.35	0.52
Glucuronic acid	0.05	0.06	0.05	0.06	0.04	0.05	0.06	0.04	0.08

**Annex 2.** Chemical composition of phloem samples from three trees of each provenance (mean values of two replicates for each tree).

<b>Phloem</b>									
% of original	<b>Provenance 14</b>			<b>Provenance 15</b>			<b>Provenance 19</b>		
material	Tree 89	Tree 399	Tree 630	Tree 416	Tree 1194	Tree 3293	Tree 188	Tree 1608	Tree 3235
<b>Ash</b>	<b>3.8</b>	<b>2.4</b>	<b>3.3</b>	<b>1.8</b>	<b>4.0</b>	<b>2.4</b>	<b>3.1</b>	<b>2.9</b>	<b>2.6</b>
<b>Total extractives</b>	<b>3.4</b>	<b>3.9</b>	<b>4.4</b>	<b>5.9</b>	<b>4.8</b>	<b>3.1</b>	<b>5.7</b>	<b>5.1</b>	<b>4.1</b>
Dichloromethane	0.17	0.11	0.15	0.12	0.24	0.16	0.23	0.16	0.15
Ethanol	1.1	1.4	1.9	1.2	1.6	1.9	1.0	1.5	1.7
Water	2.1	2.4	2.3	4.5	3.0	1.0	4.4	3.5	2.2
<b>Total lignin</b>	<b>37.3</b>	<b>39.7</b>	<b>38.2</b>	<b>39.1</b>	<b>34.4</b>	<b>40.1</b>	<b>36.8</b>	<b>39.0</b>	<b>37.8</b>
Klason lignin	34.9	37.1	35.7	36.6	31.8	37.9	34.6	36.3	35.5
Soluble lignin	2.5	2.5	2.5	2.6	2.6	2.2	2.1	2.7	2.3
<b>Polysaccharides</b>	<b>49.9</b>	<b>47.4</b>	<b>48.5</b>	<b>50.2</b>	<b>53.9</b>	<b>50.2</b>	<b>48.5</b>	<b>46.9</b>	<b>46.4</b>
Arabinose	1.2	0.98	1.2	1.0	1.7	1.3	1.0	0.93	1.3
Galactose	0.92	0.87	0.89	1.2	1.4	0.86	0.91	0.70	0.85
Glucose	23.4	19.1	21.2	19.4	25.1	23.2	22.6	18.9	19.3
Xylose	18.4	18.8	19.0	20.4	18.5	18.9	18.3	20.1	19.7
Galacturonic acid	0.89	1.3	0.86	1.3	1.7	0.88	0.91	0.62	1.0
Glucuronic acid	0.07	0.34	0.06	0.34	0.35	0.07	0.07	0.05	0.08
Acetic acid	5.1	6.0	5.3	6.5	5.2	5.0	4.7	5.5	4.2



**Annex 3.** Chemical composition of xylem samples from three trees of each provenance (mean values of two replicates for each tree).

Xylem									
% of original	Provenance 14			Provenance 15			Provenance 19		
material	Tree 89	Tree 399	Tree 630	Tree 416	Tree 1194	Tree 3293	Tree 188	Tree 1608	Tree 3235
<b>Ash</b>	<b>1.3</b>	<b>1.0</b>	<b>1.1</b>	<b>1.3</b>	<b>1.1</b>	<b>1.3</b>	<b>1.04</b>	<b>1.2</b>	<b>0.97</b>
<b>Total extractives</b>	<b>4.4</b>	<b>5.8</b>	<b>4.6</b>	<b>6.0</b>	<b>5.6</b>	<b>5.8</b>	<b>5.3</b>	<b>6.7</b>	<b>5.8</b>
Dichloromethane	0.28	0.24	0.36	0.29	0.35	0.30	0.33	0.35	0.43
Ethanol	0.95	0.88	1.9	1.9	2.1	1.9	0.92	1.6	2.1
Water	3.2	4.7	2.4	3.8	3.1	3.6	4.1	4.7	3.3
<b>Total lignin</b>	<b>23.2</b>	<b>22.3</b>	<b>22.3</b>	<b>24.6</b>	<b>23.1</b>	<b>25.8</b>	<b>23.5</b>	<b>23.8</b>	<b>22.0</b>
Klason lignin	20.5	19.5	19.0	21.5	20.1	23.2	21.0	20.6	19.6
Soluble lignin	2.7	2.8	3.3	3.1	2.6	2.7	2.4	3.1	2.4
<b>Polysaccharides</b>	<b>65.2</b>	<b>68.5</b>	<b>67.0</b>	<b>63.7</b>	<b>70.3</b>	<b>60.4</b>	<b>62.7</b>	<b>63.9</b>	<b>59.5</b>
Arabinose	1.2	0.96	0.90	0.71	1.1	1.0	0.98	1.0	1.1
Galactose	1.7	1.2	2.0	1.1	2.2	1.5	1.5	1.2	1.3
Glucose	38.7	42.2	40.3	36.5	41.0	35.2	37.3	35.9	35.7
Xylose	17.8	16.6	16.9	17.5	18.7	17.8	18.1	19.9	17.2
Galacturonic acid	1.0	1.3	1.2	1.2	1.5	0.89	0.96	0.87	0.81
Glucuronic acid	0.08	0.34	0.31	0.33	0.28	0.07	0.07	0.05	0.07
Acetic acid	4.7	5.9	5.4	6.4	5.7	3.9	3.7	4.9	3.3

**Annex 4.** Identified compounds and % of total area of carbohydrate, lignin and suberin derived products from de pyrolysis of cork, cork<sub>des</sub>, phloem and xylem of *Quercus suber* L. C = carbohydrate derivative, CL = Low molecular carbohydrates, CP = Pyran compounds, CF = Furan compounds. Lignin derived compounds: H = *p*-hydroxyphenyl lignin, G = guaiacyl lignin, S = syringyl lignin units. Sub = suberin derived compounds. NI = not identified compound.

Peak no.	Compound	Origin	Cork				Cork <sub>des</sub>				Phloem				Xylem			
			P14	P15	P19	Mean	P14	P15	P19	Mean	P14	P15	P19	Mean	P14	P15	P19	Mean
1	2-oxo-propanal	CL	-	-	-	-	5.4	3.8	5.3	<b>4.8</b>	3.9	4.0	4.0	<b>4.0</b>	4.1	4.4	3.7	<b>4.1</b>
2	1-hexene	Sub (alkene)	5.1	4.6	5.0	<b>4.9</b>	-	-	-	-	-	-	-	-	-	-	-	-
3	1-heptene	Sub (alkene)	2.5	2.4	2.5	<b>2.5</b>	-	-	-	-	-	-	-	-	-	-	-	-
4	benzene	L	0.84	0.83	1.2	<b>0.94</b>	1.0	1.1	1.8	<b>1.3</b>	-	-	-	-	-	-	-	-
5	hydroxyacetaldehyde	CL	1.7	1.5	1.6	<b>1.6</b>	3.8	3.0	3.4	<b>3.4</b>	5.1	5.7	5.6	<b>5.5</b>	6.4	6.2	5.9	<b>6.2</b>
6	acetic acid	CL	6.6	6.4	6.8	<b>6.6</b>	4.7	4.0	3.9	<b>4.2</b>	10.0	10.1	10.7	<b>10.3</b>	7.9	8.8	8.8	<b>8.5</b>
7	1-octene	Sub (alkene)	1.8	1.8	1.4	<b>1.7</b>	-	-	-	-	-	-	-	-	-	-	-	-
8	2-hydroxypropanone (Acetol)	CL	0.75	0.78	0.90	<b>0.81</b>	6.67	5.46	6.15	<b>6.1</b>	1.9	2.2	2.2	<b>2.1</b>	2.1	2.1	2.2	<b>2.2</b>
9	toluene	L	0.84	0.88	1.2	<b>0.97</b>	2.0	2.2	3.0	<b>2.4</b>	0.48	0.56	0.75	<b>0.60</b>	-	-	-	-
10	HOCH=CHOH	CL	0.11	0.10	0.05	<b>0.09</b>	0.13	0.05	0.07	<b>0.08</b>	0.43	0.43	0.45	<b>0.44</b>	0.52	0.45	0.47	<b>0.48</b>
11	2-propenoic acid	Sub (fatty acid)	0.46	0.53	0.70	<b>0.56</b>	-	-	-	-	-	-	-	-	-	-	-	-
12	1-nonene	Sub (alkene)	0.82	0.81	0.85	<b>0.83</b>	-	-	-	-	-	-	-	-	-	-	-	-
13	1,8-nonadiene	Sub (alkadiene)	0.46	0.45	0.49	<b>0.47</b>	-	-	-	-	-	-	-	-	-	-	-	-
14	3-hydroxypropanal	CL	1.1	0.98	1.2	<b>1.1</b>	0.76	0.55	0.68	<b>0.66</b>	3.7	3.8	4.1	<b>3.9</b>	3.0	3.3	3.2	<b>3.2</b>
15	1,3-dimethyl-benzene	L	0.30	0.30	0.42	<b>0.34</b>	0.63	0.69	1.00	<b>0.77</b>	0.41	0.42	0.46	<b>0.43</b>	-	-	-	-
16	pyrrole	Protein	0.28	0.27	0.30	<b>0.28</b>	1.32	1.58	1.81	<b>1.57</b>	-	-	-	-	-	-	-	-
17	furan-2-one isomer	CF	0.23	0.22	0.24	<b>0.23</b>	0.20	0.17	0.21	<b>0.19</b>	0.38	0.38	0.41	<b>0.39</b>	0.32	0.40	0.41	<b>0.38</b>
18	3-furaldehyde	CF	0.23	0.22	0.24	<b>0.23</b>	0.20	0.17	0.21	<b>0.19</b>	0.38	0.38	0.41	<b>0.39</b>	0.32	0.40	0.41	<b>0.38</b>
19	CH <sub>3</sub> -CO-CHOH-CHO	CL	0.34	0.22	0.20	<b>0.25</b>	1.3	0.86	1.2	<b>1.1</b>	1.0	1.1	0.86	<b>1.0</b>	0.92	0.96	0.82	<b>0.90</b>
20	CHO-CH <sub>2</sub> -CH <sub>2</sub> -CHO	CL	0.34	0.22	0.20	<b>0.25</b>	1.3	0.86	1.2	<b>1.1</b>	1.0	1.1	0.86	<b>1.0</b>	0.92	0.96	0.82	<b>0.90</b>
21	styrene	L	0.45	0.44	0.60	<b>0.50</b>	0.54	0.55	0.76	<b>0.62</b>	-	-	-	-	-	-	-	-
22	furfural	CF	0.46	0.38	0.44	<b>0.43</b>	1.4	1.1	1.3	<b>1.3</b>	1.1	1.2	1.2	<b>1.2</b>	1.1	1.1	1.0	<b>1.1</b>
23	2-cyclopenten-1-one	C	0.46	0.38	0.44	<b>0.43</b>	1.4	1.1	1.3	<b>1.3</b>	1.1	1.2	1.2	<b>1.2</b>	1.1	1.1	1.0	<b>1.1</b>
24	1-decene	Sub (alkene)	0.95	0.91	0.98	<b>0.95</b>	-	-	-	-	-	-	-	-	-	-	-	-

25	similar to 1,9-decadiene	Sub (alkadiene)	0.30	0.30	0.34	<b>0.31</b>	-	-	-	-	-	-	-	-	-	-	-	-
26	2-methyl-2-cyclopenten-1-one	C	0.13	0.07	0.15	<b>0.12</b>	0.74	0.77	0.98	<b>0.83</b>	-	-	-	-	-	-	-	-
27	hexanoic acid	Sub (fatty acid)	0.36	0.37	0.38	<b>0.37</b>	-	-	-	-	-	-	-	-	-	-	-	-
28	4-cyclopentene-1,3-dione	C	0.14	0.15	0.14	<b>0.14</b>	0.24	0.17	0.17	<b>0.19</b>	-	-	-	-	-	-	-	-
29	similar to 4-cyclopentene-1,3-dione	C	0.21	0.25	0.26	<b>0.24</b>	0.23	0.18	0.13	<b>0.18</b>	-	-	-	-	-	-	-	-
30	4-pentenoic acid	Sub (fatty acid)	0.21	0.25	0.26	<b>0.24</b>	-	-	-	-	-	-	-	-	-	-	-	-
31	1-ethenyl-2-methyl-benzene	L	0.11	0.14	0.13	<b>0.13</b>	0.05	0.06	0.05	<b>0.05</b>	-	-	-	-	-	-	-	-
32	2-hydroxy-2-cyclopenten-1-one	C	0.36	0.29	0.30	<b>0.32</b>	2.0	1.7	1.9	<b>1.9</b>	1.2	1.3	0.85	<b>1.1</b>	1.1	1.1	0.87	<b>1.0</b>
33	undecane	Sub (alkane)	0.16	0.18	0.13	<b>0.16</b>	-	-	-	-	-	-	-	-	-	-	-	-
34	1-undecene	Sub (alkene)	0.66	0.66	0.70	<b>0.67</b>	-	-	-	-	-	-	-	-	-	-	-	-
35	1,10-undecadiene	Sub (alkadiene)	0.23	0.19	0.21	<b>0.21</b>	-	-	-	-	-	-	-	-	-	-	-	-
36	NI suberin derivative	Sub	0.23	0.19	0.21	<b>0.21</b>	-	-	-	-	-	-	-	-	-	-	-	-
37	NI carbohydrate derivative	C	0.64	0.60	0.88	<b>0.70</b>	0.07	0.06	0.06	<b>0.06</b>	1.4	1.4	2.4	<b>1.7</b>	1.4	1.7	1.9	<b>1.7</b>
38	indene	Protein	0.23	0.21	0.29	<b>0.24</b>	0.81	0.79	1.03	<b>0.88</b>	-	-	-	-	-	-	-	-
39	dihydro-2(3 <i>H</i> )-furanone	CF	0.23	0.22	0.38	<b>0.28</b>	0.61	0.51	0.62	<b>0.58</b>	0.59	0.62	0.99	<b>0.73</b>	0.60	0.67	0.78	<b>0.68</b>
40	2(5 <i>H</i> )-furanone	CF	0.19	0.19	0.22	<b>0.20</b>	0.77	0.67	0.64	<b>0.69</b>	0.48	0.51	0.41	<b>0.46</b>	0.44	0.52	0.39	<b>0.45</b>
41	heptanoic acid	Sub (fatty acid)	0.60	0.59	0.64	<b>0.61</b>	-	-	-	-	-	-	-	-	-	-	-	-
42	5-hexenoic acid	Sub (fatty acid)	0.74	0.77	0.92	<b>0.81</b>	-	-	-	-	-	-	-	-	-	-	-	-
43	4-hydroxy-5,6-dihydro-(2 <i>H</i> )-pyran-2-one	CP	0.75	0.59	0.65	<b>0.66</b>	0.46	0.28	0.24	<b>0.33</b>	2.5	2.6	2.2	<b>2.4</b>	2.1	2.4	2.0	<b>2.2</b>
44	2-hydroxybenzaldehyde	H	0.10	0.12	0.09	<b>0.10</b>	0.18	0.21	0.26	<b>0.22</b>	0.20	0.21	0.24	<b>0.21</b>	0.16	0.19	0.22	<b>0.19</b>
45	2 <i>H</i> -pyran-2-one	CP	0.08	0.11	0.09	<b>0.10</b>	0.18	0.21	0.26	<b>0.22</b>	0.20	0.21	0.24	<b>0.21</b>	0.16	0.19	0.22	<b>0.19</b>
46	2-hydroxy-3-methyl-2-cyclopenten-1-one	C	0.12	0.11	0.10	<b>0.11</b>	0.94	0.83	0.94	<b>0.90</b>	0.51	0.52	0.39	<b>0.48</b>	0.43	0.45	0.40	<b>0.43</b>
47	methyl-dihydro-(2 <i>H</i> )-pyran-2-one	CP	0.12	0.11	0.10	<b>0.11</b>	0.94	0.83	0.94	<b>0.90</b>	0.21	0.19	0.17	<b>0.19</b>	0.21	0.22	0.23	<b>0.22</b>
48	2-hydroxy-1-methyl-1-cyclopentene-3-one isomer	C	0.09	0.08	0.07	<b>0.08</b>	0.20	0.19	0.08	<b>0.16</b>	0.21	0.19	0.17	<b>0.19</b>	0.21	0.22	0.23	<b>0.22</b>
49	3-methyl-(5 <i>H</i> )-furan-2-one	CF	0.09	0.08	0.07	<b>0.08</b>	0.20	0.19	0.08	<b>0.16</b>	0.21	0.19	0.17	<b>0.19</b>	0.21	0.22	0.23	<b>0.22</b>
50	dodecane	Sub (alkane)	0.12	0.11	0.08	<b>0.10</b>	-	-	-	-	-	-	-	-	-	-	-	-
51	1-dodecene	Sub (alkene)	0.79	0.76	0.79	<b>0.78</b>	-	-	-	-	-	-	-	-	-	-	-	-
52	1,11-dodecadiene	Sub (alkadiene)	0.38	0.39	0.43	<b>0.40</b>	-	-	-	-	-	-	-	-	-	-	-	-
53	phenol	H	0.51	0.45	0.55	<b>0.51</b>	2.2	2.6	3.2	<b>2.7</b>	0.56	0.65	0.70	<b>0.63</b>	0.36	0.40	0.46	<b>0.41</b>
54	2-(propan-2-one)-tetrahydrofuran	CF	0.31	0.26	0.18	<b>0.25</b>	1.9	2.2	2.2	<b>2.1</b>	-	-	-	-	-	-	-	-

55	guaiacol	G	0.46	0.39	0.22	<b>0.36</b>	1.9	2.2	2.2	<b>2.1</b>	0.35	0.26	0.22	<b>0.28</b>	0.15	0.18	0.12	<b>0.15</b>
56	6-heptenoic acid	Sub (fatty acid)	1.3	1.2	1.3	<b>1.3</b>	-	-	-	-	-	-	-	-	-	-	-	-
57	<i>o</i> -cresol	H	0.27	0.24	0.32	<b>0.28</b>	1.1	1.3	1.5	<b>1.3</b>	0.28	0.32	0.34	<b>0.31</b>	0.20	0.21	0.26	<b>0.22</b>
58	NI sugar	C	-	-	-	-	-	-	-	-	0.61	0.62	0.52	<b>0.58</b>	0.69	0.67	0.63	<b>0.66</b>
59	tridecane	Sub (alkane)	0.18	0.21	0.21	<b>0.20</b>	-	-	-	-	-	-	-	-	-	-	-	-
60	1-tridecene	Sub (alkene)	0.62	0.60	0.65	<b>0.62</b>	-	-	-	-	-	-	-	-	-	-	-	-
61	1,12-tridecadiene	Sub (alkadiene)	0.40	0.40	0.44	<b>0.42</b>	-	-	-	-	-	-	-	-	-	-	-	-
62	<i>p</i> -cresol	H	0.35	0.33	0.38	<b>0.35</b>	0.84	1.02	1.19	<b>1.02</b>	0.23	0.26	0.28	<b>0.26</b>	0.12	0.14	0.16	<b>0.14</b>
63	<i>m</i> -cresol	H	0.32	0.30	0.33	<b>0.32</b>	0.84	0.98	1.19	<b>1.00</b>	0.30	0.39	0.40	<b>0.36</b>	0.22	0.24	0.31	<b>0.26</b>
64	4-methyl-(5 <i>H</i> )-furan-2-one	CF	0.04	0.03	0.04	<b>0.04</b>	0.09	0.11	0.13	<b>0.11</b>	0.13	0.10	0.10	<b>0.11</b>	0.09	0.09	0.08	<b>0.09</b>
65	similar to 5-(hydroxymethyl) dihydro-2(3 <i>H</i> )-furanone	CF	-	-	-	-	-	-	-	-	-	-	-	-	0.37	0.33	0.32	<b>0.34</b>
66	4-methylguaiacol	G	0.54	0.50	0.35	<b>0.46</b>	0.64	0.90	1.02	<b>0.85</b>	0.30	0.25	0.32	<b>0.29</b>	0.16	0.17	0.15	<b>0.16</b>
67	octanoic acid	Sub (fatty acid)	1.5	1.5	1.5	<b>1.5</b>	-	-	-	-	-	-	-	-	-	-	-	-
68	NI sugar	C	-	-	-	-	2.3	2.0	1.6	<b>2.0</b>	0.40	0.42	0.31	<b>0.37</b>	0.36	0.39	0.29	<b>0.35</b>
69	2,3-dimethyl-phenol ( <i>o</i> -xlenol)	H	0.55	0.65	0.63	<b>0.61</b>	0.77	0.92	0.96	<b>0.89</b>	0.23	0.24	0.29	<b>0.25</b>	0.17	0.16	0.20	<b>0.17</b>
70	7-octenoic acid	Sub (fatty acid)	0.83	0.97	1.0	<b>0.94</b>	-	-	-	-	-	-	-	-	-	-	-	-
71	3-ethyl-phenol	H	0.15	0.15	0.15	<b>0.15</b>	0.32	0.35	0.48	<b>0.38</b>	-	-	-	-	-	-	-	-
72	tetradecane	Sub (alkane)	0.15	0.15	0.15	<b>0.15</b>	-	-	-	-	-	-	-	-	-	-	-	-
73	1-tetradecene	Sub (alkene)	0.56	0.56	0.58	<b>0.57</b>	-	-	-	-	-	-	-	-	-	-	-	-
74	1,13-tetradecadiene	Sub (alkadiene)	0.29	0.33	0.35	<b>0.32</b>	-	-	-	-	-	-	-	-	-	-	-	-
75	4-ethylguaiacol	G	0.10	0.09	0.08	<b>0.09</b>	0.82	1.01	0.81	<b>0.88</b>	0.11	0.13	0.10	<b>0.11</b>	0.07	0.06	0.06	<b>0.07</b>
76	NI sugar	C	0.63	0.54	0.66	<b>0.61</b>	0.20	0.25	0.20	<b>0.22</b>	1.5	1.3	1.9	<b>1.6</b>	2.3	2.1	2.2	<b>2.2</b>
77	similar to dihydro-6-methyl-2 <i>H</i> -Pyran-3(4 <i>H</i> )-one	CP	-	-	-	-	-	-	-	-	0.37	0.44	0.23	<b>0.35</b>	0.34	0.34	0.20	<b>0.29</b>
78	nonanoic acid	Sub (fatty acid)	0.66	0.54	0.55	<b>0.58</b>	-	-	-	-	-	-	-	-	-	-	-	-
79	8-nonenoic acid	Sub (fatty acid)	0.65	0.65	0.61	<b>0.63</b>	-	-	0.16	-	-	-	-	-	-	-	-	-
80	1,4:3,6-dianhydro- $\alpha$ -D-glucopyranose	CP	-	-	-	-	-	-	-	-	0.19	0.20	0.10	<b>0.16</b>	0.26	0.28	0.21	<b>0.25</b>
81	1,5-anhydro-arabinofuranose	CF	1.7	1.6	1.7	<b>1.7</b>	0.38	0.53	0.27	<b>0.40</b>	0.37	0.55	0.39	<b>0.44</b>	0.26	0.35	0.27	<b>0.29</b>
82	2,3-dihydrobenzofuran	O	0.13	0.11	0.10	<b>0.11</b>	0.31	0.32	0.37	<b>0.33</b>	0.52	0.64	0.54	<b>0.57</b>	0.34	0.40	0.33	<b>0.36</b>
83	4-vinylguaiacol	G	2.3	1.9	1.7	<b>2.0</b>	5.7	5.8	6.6	<b>6.0</b>	0.90	0.96	0.81	<b>0.89</b>	0.34	0.40	0.33	<b>0.36</b>
84	pentadecane	Sub (alkane)	0.27	0.23	0.18	<b>0.23</b>	-	-	-	-	-	-	-	-	-	-	-	-

85	1-pentadecene	Sub (alkene)	0.64	0.59	0.63	<b>0.62</b>	-	-	-	-	-	-	-	-	-	-	-	-
86	1,14-pentadecadiene	Sub (alkadiene)	0.45	0.42	0.43	<b>0.43</b>	-	-	-	-	-	-	-	-	-	-	-	-
87	eugenol	G	0.29	0.23	0.27	<b>0.26</b>	0.38	0.35	0.36	<b>0.36</b>	0.26	0.23	0.21	<b>0.23</b>	0.11	0.12	0.12	<b>0.12</b>
88	5-hydroxymethylfurfural	CF	0.13	0.11	0.17	<b>0.14</b>	0.12	0.18	0.17	<b>0.16</b>	0.44	0.44	0.34	<b>0.40</b>	0.59	0.46	0.54	<b>0.53</b>
89	4-allylphenol	H	0.21	0.25	0.17	<b>0.21</b>	0.12	0.18	0.17	<b>0.16</b>	-	-	-	-	-	-	-	-
90	syringol	S	0.13	0.10	0.01	<b>0.08</b>	1.6	1.3	1.4	<b>1.5</b>	0.64	0.56	0.38	<b>0.53</b>	0.60	0.67	0.41	<b>0.56</b>
91	NI carbohydrate derivative	C	0.42	0.43	0.39	<b>0.41</b>	0.12	0.15	0.13	<b>0.13</b>	0.72	0.60	0.76	<b>0.69</b>	0.52	0.58	0.67	<b>0.59</b>
92	cis-isoeugenol	G	0.14	0.13	0.09	<b>0.12</b>	0.29	0.30	0.20	<b>0.27</b>	-	-	-	-	-	-	-	-
93	hexadecane	Sub (alkane)	0.07	0.10	0.08	<b>0.08</b>	-	-	-	-	-	-	-	-	-	-	-	-
94	1-hexadecene	Sub (alkene)	0.41	0.44	0.41	<b>0.42</b>	-	-	-	-	-	-	-	-	-	-	-	-
95	1,15-hexadecadiene	Sub (alkadiene)	0.40	0.50	0.47	<b>0.46</b>	-	-	-	-	-	-	-	-	-	-	-	-
96	2-hydroxymethyl-5-hydroxy-2,3-dihydro-(4H)-pyran-4-one	CP	0.31	0.46	0.33	<b>0.37</b>	-	-	-	-	0.64	0.44	0.85	<b>0.64</b>	0.93	0.89	1.1	<b>0.98</b>
97	trans-isoeugenol	G	0.81	0.74	0.68	<b>0.74</b>	2.0	2.0	2.0	<b>2.0</b>	0.73	0.69	0.73	<b>0.72</b>	0.40	0.52	0.43	<b>0.45</b>
98	similar to 1,5-anhydro-arabinofuranose	CF	0.91	0.84	0.90	<b>0.88</b>	-	-	-	-	1.3	1.2	1.3	<b>1.2</b>	1.4	1.4	1.3	<b>1.4</b>
99	4-methylsyringol	S	0.16	0.15	0.11	<b>0.14</b>	0.30	0.30	0.26	<b>0.29</b>	0.52	0.45	0.47	<b>0.48</b>	0.47	0.49	0.47	<b>0.48</b>
100	vanillin	G	0.91	0.97	0.83	<b>0.90</b>	1.3	1.2	1.2	<b>1.2</b>	0.83	0.77	0.80	<b>0.80</b>	0.44	0.56	0.43	<b>0.48</b>
101	1-(4-hydroxy-3-methoxyphenyl)propyne	G	0.28	0.25	0.20	<b>0.25</b>	0.51	0.32	0.48	<b>0.44</b>	-	-	-	-	-	-	-	-
102	heptadecane	Sub (alkane)	0.05	0.13	0.03	<b>0.07</b>	-	-	-	-	-	-	-	-	-	-	-	-
103	1-heptadecene	Sub (alkene)	0.41	0.42	0.41	<b>0.41</b>	-	-	-	-	-	-	-	-	-	-	-	-
104	1,16-heptadecadiene	Sub (alkadiene)	0.25	0.32	0.26	<b>0.28</b>	-	-	-	-	-	-	-	-	-	-	-	-
105	homovanillin	G	-	-	-	-	-	-	-	-	0.43	0.38	0.38	<b>0.40</b>	0.25	0.31	0.24	<b>0.27</b>
106	4-ethylsyringol	S	-	-	-	-	0.27	0.27	0.09	<b>0.21</b>	0.12	0.09	0.03	<b>0.08</b>	0.08	0.14	0.08	<b>0.10</b>
107	acetoguaiacone	G	0.31	0.27	0.22	<b>0.27</b>	1.07	0.86	0.91	<b>0.95</b>	0.43	0.42	0.39	<b>0.41</b>	0.21	0.27	0.20	<b>0.22</b>
108	4-vinylsyringol	S	0.24	0.14	0.11	<b>0.16</b>	1.5	1.2	1.3	<b>1.3</b>	1.03	0.97	0.74	<b>0.92</b>	0.98	1.13	0.77	<b>0.96</b>
109	octadecane	Sub (alkane)	0.07	0.06	0.08	<b>0.07</b>	-	-	-	-	-	-	-	-	-	-	-	-
110	guaiacylacetone	G	0.21	0.20	0.19	<b>0.20</b>	0.52	0.88	0.85	<b>0.75</b>	0.06	0.06	0.08	<b>0.07</b>	-	-	-	-
111	1-octadecene	Sub (alkene)	0.21	0.20	0.19	<b>0.20</b>	-	-	-	-	-	-	-	-	-	-	-	-
112	1,17-octadecadiene	Sub (alkadiene)	0.18	0.20	0.19	<b>0.19</b>	-	-	-	-	-	-	-	-	-	-	-	-
113	4-allylsyringol	S	0.13	0.13	0.13	<b>0.13</b>	0.27	0.11	0.11	<b>0.16</b>	0.12	0.10	0.10	<b>0.11</b>	0.14	0.17	0.13	<b>0.14</b>
114	4-propylsyringol	S	-	-	-	-	-	-	-	-	0.12	0.10	0.10	<b>0.11</b>	0.14	0.17	0.13	<b>0.14</b>

115	<i>trans</i> -coniferyl alcohol	G	0.18	0.16	0.11	<b>0.15</b>	0.20	0.21	0.16	<b>0.19</b>	0.21	0.18	0.20	<b>0.20</b>	0.11	0.12	0.10	<b>0.11</b>
116	guaiacyl vinyl ketone	G	0.18	0.16	0.11	<b>0.15</b>	0.20	0.21	0.16	<b>0.19</b>	0.21	0.18	0.20	<b>0.20</b>	0.11	0.12	0.10	<b>0.11</b>
117	<i>cis</i> -4-propenylsyringol	S	-	-	-	-	-	-	-	-	0.17	0.15	0.17	<b>0.16</b>	0.27	0.24	0.21	<b>0.24</b>
118	nonadecane	Sub (alkane)	0.34	0.44	0.31	<b>0.36</b>	-	-	-	-	-	-	-	-	-	-	-	-
119	1-nonadecene	Sub (alkene)	0.39	0.44	0.38	<b>0.40</b>	-	-	-	-	-	-	-	-	-	-	-	-
120	4-propenylsyringol	S	-	-	-	-	-	-	-	-	0.12	0.04	0.02	<b>0.06</b>	0.11	0.14	0.10	<b>0.11</b>
121	1,18-nonadecadiene	Sub (alkadiene)	0.12	0.19	0.13	<b>0.14</b>	-	-	-	-	-	-	-	-	-	-	-	-
122	1,6-anhydro- $\beta$ -D-glucopyranose (levoglucosan)	CP	6.4	5.7	5.6	<b>5.9</b>	0.77	0.47	0.36	<b>0.53</b>	13.2	11.7	13.9	<b>12.9</b>	20.2	16.6	18.2	<b>18.3</b>
123	<i>trans</i> -4-propenylsyringol	S	0.13	0.10	0.03	<b>0.09</b>	0.65	0.47	0.55	<b>0.55</b>	0.61	0.56	0.46	<b>0.55</b>	0.73	0.86	0.65	<b>0.74</b>
124	syringaldehyde	S	0.16	0.10	0.06	<b>0.11</b>	0.27	0.18	0.16	<b>0.20</b>	0.88	0.79	0.68	<b>0.78</b>	1.0	1.3	0.90	<b>1.1</b>
125	<i>cis</i> -coniferyl alcohol	G	-	-	-	-	-	-	-	-	0.08	0.10	0.06	<b>0.08</b>	-	-	-	-
126	eicosane	Sub (alkane)	0.13	0.12	0.09	<b>0.11</b>	-	-	-	-	-	-	-	-	-	-	-	-
127	1-eicosene	Sub (alkene)	0.43	0.45	0.39	<b>0.42</b>	-	-	-	-	-	-	-	-	-	-	-	-
128	1,19-eicosadiene	Sub (alkadiene)	0.43	0.44	0.41	<b>0.43</b>	-	-	-	-	-	-	-	-	-	-	-	-
129	homosyringaldehyde	S	-	-	-	-	-	-	-	-	0.28	0.25	0.22	<b>0.25</b>	0.32	0.48	0.36	<b>0.39</b>
130	1,6-anhydro- <i>B</i> -D-glucofuranose	CF	-	-	-	-	-	-	-	-	0.21	0.15	0.21	<b>0.19</b>	0.34	0.27	0.22	<b>0.27</b>
131	acetosyringone	S	0.07	0.07	0.02	<b>0.05</b>	0.38	0.23	0.18	<b>0.26</b>	0.41	0.37	0.30	<b>0.36</b>	0.46	0.49	0.37	<b>0.44</b>
132	<i>trans</i> -coniferyl alcohol	G	0.12	0.17	0.11	<b>0.13</b>	0.11	0.19	0.12	<b>0.14</b>	0.19	0.30	0.13	<b>0.20</b>	-	-	-	-
133	<i>trans</i> -coniferaldehyde	G	0.18	0.30	0.17	<b>0.22</b>	0.32	0.40	0.26	<b>0.33</b>	0.37	0.33	0.31	<b>0.34</b>	0.21	0.22	0.30	<b>0.24</b>
134	syringylacetone	S	-	-	-	-	0.19	0.18	0.11	<b>0.16</b>	0.17	0.17	0.15	<b>0.16</b>	0.16	0.17	0.14	<b>0.16</b>
135	<i>trans</i> -sinapyl alcohol	S	-	-	-	-	-	-	-	-	0.17	0.12	0.11	<b>0.13</b>	-	-	-	-
136	<i>trans</i> -sinapaldehyde	S	-	-	-	-	-	-	-	-	0.98	0.89	0.63	<b>0.83</b>	0.75	0.76	0.64	<b>0.72</b>
137	heneicosane	Sub (alkane)	0.2	0.2	0.2	<b>0.2</b>	-	-	-	-	-	-	-	-	-	-	-	-
138	1-heneicosene	Sub (alkene)	1.1	1.1	0.97	<b>1.1</b>	-	-	-	-	-	-	-	-	-	-	-	-
139	1,20-heneicosadiene	Sub (alkadiene)	0.16	0.17	0.14	<b>0.15</b>	-	-	-	-	-	-	-	-	-	-	-	-
140	docosane	Sub (alkane)	0.12	0.11	0.09	<b>0.11</b>	-	-	-	-	-	-	-	-	-	-	-	-
141	1-docosene	Sub (alkene)	0.69	0.69	0.60	<b>0.66</b>	-	-	-	-	-	-	-	-	-	-	-	-
142	1,21-docosadiene	Sub (alkadiene)	0.10	0.09	0.08	<b>0.09</b>	-	-	-	-	-	-	-	-	-	-	-	-
143	NI suberin derivative	Sub	0.30	0.26	0.20	<b>0.25</b>	-	-	-	-	-	-	-	-	-	-	-	-
144	NI suberin derivative	Sub	0.18	0.23	0.22	<b>0.21</b>	-	-	-	-	-	-	-	-	-	-	-	-

145	NI suberin derivative	Sub	0.44	0.37	0.40	<b>0.40</b>	-	-	-	-	-	-	-	-	-	-	-	-
146	NI suberin derivative	Sub	0.20	0.15	0.10	<b>0.15</b>	-	-	-	-	-	-	-	-	-	-	-	-
147	NI suberin derivative	Sub	0.34	0.43	0.36	<b>0.38</b>	-	-	-	-	-	-	-	-	-	-	-	-