The Rationale behind Cork Properties: A Review of Structure and Chemistry

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Cork is a natural cellular material of biological origin with a combination of properties that make it suited for worldwide use as a wine sealant and insulation material. Cork has low density, is buoyant, is not very permeable to fluids, has a low thermal coefficient, exhibits elasticity and deformation without fracturing under compression, and has considerable durability. Such characteristics result from the features of its cellular structure, primarily its cell dimensions and topology, and from the chemical composition of the cell wall. The characteristics of the two main chemical components (suberin and lignin, which represent 53% and 26%, respectively, of the cell wall) have been analyzed. The limits of natural variation and their impacts on cork properties are discussed and used to define the material as "cork".

Keywords: Cork; Quercus suber; Suberin; Lignin; Cellular structure; Compression; Properties

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INTRODUCTION

Cork is a natural material used worldwide as the sealant for wine bottles. It has been used to "cork" glass bottles since their emergence in the beginning of the seventeenth century, and it sealed ceramic amphora many centuries earlier (Taber 2007; Pereira 2007).

Cork is of biological origin and occurs in the periderm of tree barks. It forms a protective barrier (designated phellem in plant anatomy) at the interface of the innermost living tissues and the exterior (Evert and Eichhorn 2006). Protection against temperature variation, water loss, fire, and biological attack are provided by cork as a result of its specialized cellular structure and chemical composition.

The properties of cork attracted attention long ago. It is a light material with very low permeability to liquids and gases that demonstrates buoyancy, can withstand compressive deformation without fracture, and has low heat transfer properties (Fortes *et al.* 2004; Pereira 2007). Cork has been used in various applications, including floating devices, sealing products, and insulation, energy absorption, and surfacing materials. The aesthetic character of cork in combination with its properties also led to recent applications in design products, *e.g.* for outdoor and indoor furniture, household, and personal use items. The use of cork as a biosorbent was also researched in relation to heavy metals (Chubar *et al.* 2004; Sen *et al.* 2012b), polycyclic aromatic hydrocarbons (Olivella *et al.* 2011), and oil (Pintor *et al*. 2013). Other applications of cork, such as composites, are reviewed in Silva *et al*. (2005) and Pereira (2007).

Cork is the raw material for a dedicated industrial chain of great economic importance. Commercial cork is produced in the western Mediterranean regions from the cork oak (*Quercus suber* L.) through the periodic removal of the tree bark periderm under a sustainable exploitation management system throughout the tree's lifetime (Pereira and

Tomé 2004). Cork oak forests are usually multifunctional systems that provide a rich array of environmental services and biodiversity that sustain the favorable ecological footprint of cork.

Wine stoppers are the iconic product derived from cork, but other well-known applications in insulation and surfacing consume most of the industrial cork side-streams and wastes, making the overall use of cork a highly efficient raw material utilization process. Some novel applications have received considerable attention recently, particularly those associated with its use in buildings or events that have received large media coverage, such as in the Sagrada Familia cathedral in Barcelona, the Serpentine Gallery Pavilion in London (2012), or the Portuguese pavilion in the World Exhibition of Shanghai (2010).

The cellular structure of cork was studied in the early days of experimental research (Hooke 1665) and, later on, as a bridge to understand the material's properties (Gibson *et al*. 1981; Pereira *et al.* 1987). Its chemical composition was first studied long ago (Brugnatelli 1787), but is a subject still under extensive research (as reviewed in Pereira 2007). Its structural features, chemical composition, and the molecular structures of the components of cork are the keys to better understanding the material's properties. They are the rationale behind such important performance features as the oxygen ingress into corked wine bottles and the compressive behavior underlying the bottling and maintenance of cork stoppers in the bottleneck.

This review paper presents cork's anatomy and chemistry, primarily regarding the characteristics of its two main components (suberin and lignin), that underlie the different properties that make cork special. The limits of natural variation and their impact on cork behavior are also discussed.

CELLULAR STRUCTURE OF CORK

Cork is a foam with closed cells. Its structural characteristics were briefly described by Gibson *et al.* (1981) and discussed in detail by Pereira *et al.* (1987). Its formation and development were characterized by Graça and Pereira (2004). Cork cells are formed by the phellogen, a meristematic layer (*i.e.*, with cell division capability) that produces the bark periderm.

The cork tissue is compact, without intercellular voids, and with a regular honeycomb arrangement. This biological tissue is homogeneous with regard to cell type: the cells are dead parenchymateous cells with hollow, air-filled interiors. The cells are prismatic, hexagonal on average, and are stacked base-to-base in an alignment oriented in the tree's radial direction. All cells in one radial row derive from one phellogen mother-cell: after cellular division, the cork cell differentiates and subsequently expands in the radial direction. The cell rows are arranged parallel to each other with the prism bases in staggered positions in adjacent rows.

The cellular structure appears differently in the three main sections: in a radial plane, as well as in a transverse plane, the 2-D arrangement is of a brick-layered type; in the tangential plane, the cells appear hexagonal on average in a honeycomb arrangement (Fig. 1). In spite of the different sectional layouts, the cells are topologically similar with an average of six sides (Pereira *et al.* 1987). Geometrically, the tissue is axisymmetric, with a symmetry axis along the prism's height.

It must be noted that the description of the cork structure should use the terminology of sections adopted by plant anatomy: the transverse section is the plane perpendicular to the axial direction, the tangential section is perpendicular to the radial direction, and the radial section is perpendicular to the tangential direction (see *e.g.* Pereira 2007).

The cells are small and have dimensions under those of synthetic foams. The area of the prism base is 4 to 6 x 10^{-6} cm^2 with a mean prism base edge of 13 to 15 μm; prism height is usually in the range of 30 to 40 μm. The mean cell volume is approximately 2 x 10^{-8} cm^3 and the number of cells per unit volume is 4 to 7 x 10^7 cm^{-3}. The cell walls are thin with thicknesses of 1 to 1.5 μm. The solid mass volume fraction of the cork is therefore very small, approximately 10%.

The solid mass of cork is concentrated in its cell walls. The thickness of the cell walls is constant in the different directions, with similar values in the cell edges and faces and only with a small enlargement because of rounding at face junctions (Fig. 2). There are no microscopic openings (*i.e.*, at the μm level) in the walls for cell-to-cell connection like the pits in wood cells. There are, however, minute, stuffed channels at the sub-microscopic level that occasionally cross the cell walls (Fig. 2). These are termed the plasmodesmata and are observable by transmission electron microscopy with a cross-sectional diameter of approximately 100 nm. They are remnants of the connections between the cells during division as used for cytoplasmatic exchanges (Teixeira and Pereira 2009).

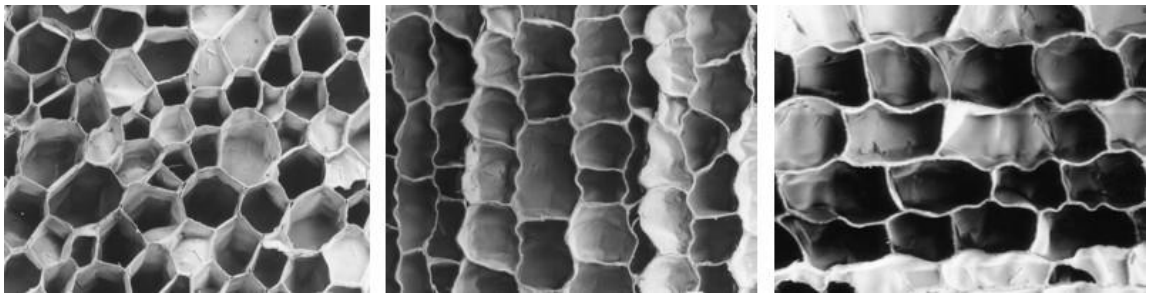

Fig. 1. Structure of cork as observed by scanning electron microscopy in the three main sections: (left) tangential section, perpendicular to the tree's radial direction; (middle) transverse section, perpendicular to the tree's axial direction; and (right) radial section, the tree's radial section

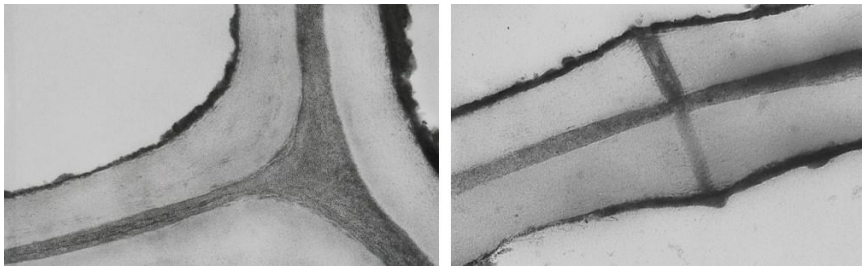

Fig. 2. Cross-section of the cell wall of cork as observed by transmission electron microscopy, showing one plasmodesma (right)

Despite the overall regularity of cork's structure, it contains natural heterogeneity given by the formation of the annual rings that represent the yearly growth rhythm of cork, similar to what happens in wood. Cork formation stops in October or November and starts a new growth season in April or May (Costa *et al.* 2002). The last few cells that are

produced in a year are called latecork cells and have a smaller prism height (10 to 15 μm) and thicker cell walls (2 to 3 μm). In a cork annual ring, the number of latecork cells is small (4 to 8 cells in one radial growth ring), while the so-called earlycork cells represent about 40 to 200 cells in a row (Pereira *et al.* 1992). Although the cellular characteristics of cork are largely dominated by earlycork (which represents 90 to 95% of the total volume), the presence of the latecork layers, with their approximately 20% volume fraction, influences the overall properties of cork.

Another factor of the natural variation in cork cells is the undulation of their cell walls. The lateral faces of the cell prisms are not straight and usually exhibit undulations, often 2 per face, that run rather uniformly and parallel. This pattern varies, and stronger undulations or corrugations can appear such that, in special cases, near cell collapse can occur. This is often the case in the first cells formed in the early spring of a growth year as these cells grow radially against the previous season's latecork cells. Figure 3 shows an example of the transition between two cork rings and of this type of undulation. The capacity of the corrugation of cork cell walls without fracture is a consequence of the cell wall's chemical composition, as will be discussed.

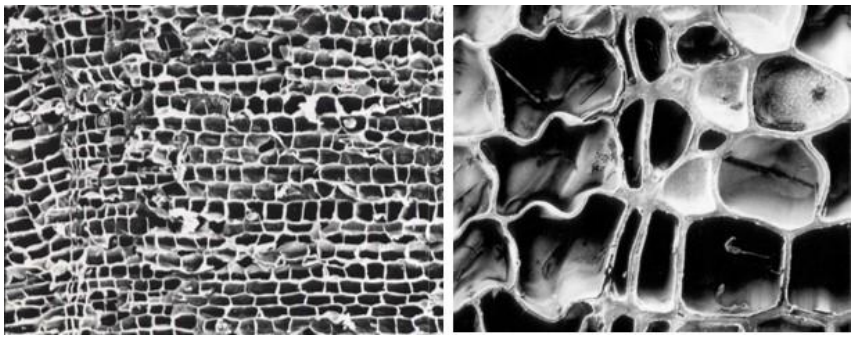

Fig. 3. Transition between two annual growth rings (left) and a magnified view of the ring boundary region between earlycork cells of one year and latecork cells of the previous year

Another natural heterogeneity in the cork tissue is the presence of conspicuous lenticular channels that radially cross the cork layer. These are of natural origin and are thought to ensure the gas exchange between the below-cork tissues and the exterior. They visually appear as small rounded spots in the tangential sections and as radially aligned strips in the other sections, the so-called cork porosity. The lenticular channels are filled with a loose cellular material and are often bordered by thick-walled sclereid cells (Fig. 4). The lenticular channels vary largely in number and dimensions, depending on tree genetics, from minute pores less than 0.1 mm^2 in cross-sectional area to over 100 mm^2.

The lenticular channels are usually quantified by a porosity coefficient calculated as the proportion of pores in the total area. The porosity coefficients of cork range from below 2% to over 15%, and have been determined on cork planks (Pereira *et al.* 1996), wine stoppers (Costa and Pereira 2007; Oliveira *et al.* 2012) and discs for champagne stoppers (Lopes and Pereira 2000). Surface image analysis of the cork stoppers and porosity quantifications are the basis for the visual classification of cork into quality grades (Costa and Pereira 2006; Oliveira *et al.* 2015a).

Recently, a 3-D rendering of the interior of a cork stopper made with X-ray microtomography allowed observation of the internal lenticular architecture (Oliveira *et al.* 2015c). The observation of cork stoppers with a medical tomography equipment also

made possible visualizing and identifying some defects of wine stoppers (Oliveira *et al.* 2015b). Other non-destructive methods have been also applied to cork, *e.g.* neutron imaging (Lagorce-Tachon *et al.* 2015), Synchotron (Donepudi *et al.* 2010), Compton (Brunetti *et al.* 2002), and Terahertz (Hor *et al.* 2008; Mukherjee and Federici 2011) tomography.

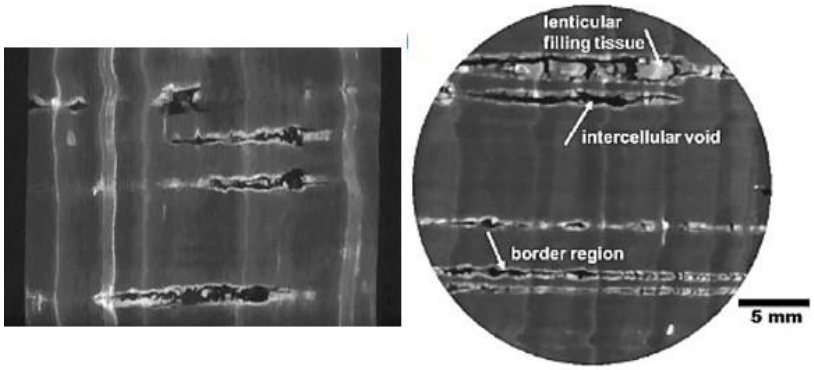

Fig. 4. Lenticular channels as observed by microtomography within a cork stopper in the radial (left) and transverse (right) sections, showing the loose filling tissue and their high-density border; the denser regions (lighter shaded) of the latecork layers at the growth ring boundary are also shown

CHEMICAL COMPOSITION OF CORK

The nature of cork is also a function of its chemical composition, especially the presence of suberin as a structural component of its cell walls. Suberin exists only in cork tissues in the periderm of barks, apart from minor occurrence in specialized bodies (*e.g.*, in Casparian bands). The chemical reaction of suberin with aliphatic-sensitive stains (such as Sudan dyes) is used in plant anatomy to detect cork tissues (Machado *et al.* 2013).

The chemical composition of cork has been reported from various authors, starting with the composition given by Klauber (1920) with suberin representing 58% of the cork mass. The first attempt to characterize the chemical composition of cork using a large number of samples was made by Pereira (1988) with a total of 50 samples, and later by Conde *et al.* (1998) with about 30 samples, and recently by Dehane *et al.* (2014) with 60 samples.

The widest coverage of cork chemical composition was made by Pereira (2013) who analyzed a total of 96 cork samples from 29 locations, therefore allowing calculation of a robust average and range of variation. Table 1 shows the chemical composition of cork relative to the oven-dry mass (Pereira 2013) and as proportion of the structural components. Suberin represents an average of 53% of the structural components and lignin represents 26%. Cellulose and hemicelluloses represent approximately 10 and 11% of the structural cell wall components, respectively. Cork also contains an appreciable amount of extractives that include both non-polar and polar compounds (6 and 10% of the oven-dry cork mass, respectively) (Pereira 2013). The inorganic materials content, determined as ash, is approximately 1% (Pereira 1988) and has been the subject of a recent review (Ponte-e-Sousa and Neto-Vaz 2011).

Table 1. Summative Chemical Composition (% o.d. cork mass), Monosaccharide Composition (% of total neutral sugars), and Proportion of Cell Wall Structural Components of Cork (% of the structural components mass) (calculated from Pereira 2013)

	% on OD Cork Mean (std)	% of Structural Components
Extractives, Total	16.2 (3.9)	
Dicholoromethane	5.8 (0.8)	
Ethanol	5.9 (3.0)	
Water	4.5 (1.6)	
Suberin, Total	44.8 (6.2)	52.8 (7.3)
Long Chain Lipids	41.0 (5.2)	48.3 (6.1)
Glycerol	3.8 (0.6)	4.5 (0.7)
Lignin, Total	22.0 (3.3)	25.9 (3.9)
Klason Lignin	21.1 (3.3)	24.9 (3.9)
Acid Soluble Lignin	0.9 (0.2)	1.0 (0.2)
Monosaccharide Composition (% of Total Neutral Sugars)		
Glucose	46.1 (3.6)	
Xylose	25.1 (3.7)	
Arabinose	18.0 (3.0)	
Mannose	3.0 (2.8)	
Galactose	7.3 (1.2)	
Rhamnose	0.5 (0.5)	

Suberin

Suberin is a macromolecule of aliphatic nature. It is a structural component of the cell wall, and its removal destroys cell integrity (Pereira and Marques 1988). Suberin is polymeric and contains two types of monomers, glycerol and long chain fatty acids and alcohols, which are linked by ester bonds between hydroxyl and carboxylic groups.

The monomeric composition of cork suberin is well-established. Numerous studies have used chemical depolymerisation followed by GC-MS separation and identification of the solubilized monomers (Graça and Pereira 2000) to make such determinations. Pyrolysis was also used in some studies (Bento *et al.* 1998). Table 2 shows the main suberinic monomers and their average proportions, by mass of the total solubilized products (Graça and Pereira 2000) and in molar percentages of the identified compounds (Pereira 2007) found in pure cork tissue (*i.e.*, without any lenticular filling material and phloemic inclusions). Several studies describe the monomeric composition of suberin (Arno *et al*. 1981; Holloway 1983; Garcia-Vallejo *et al*. 1997; Bento *et al*. 1998; Cordeiro *et al.* 1998; Lopes *et al*. 2000a; Ferreira *et al.* 2012), but Graça and Pereira (2000) more closely analyzed only the suberised cork tissue and quantified the monomers present using standards and their response factors under the chromatographic conditions used.

Glycerol is the most important single monomer in cork, representing 40.8% of the molecules released by methanolysis (14.2% of the mass of the solubilised products). The long chain monomers are mainly α,ω-diacids and represent 36.4% of the monomers (45.5% of the total mass); ω-hydroxyacids make up 21.0% of the monomers (26.3% of the total mass). The most abundant single monomers are 9-epoxyoctadecanedioic acid (22.9% of the total mass), 22-hydroxydocosanoic acid (7.9%), 9,10-dihydroxyoctadecanodioic acid (7.7%), and 9-epoxy-18-hydroxyoctadecanoic acid (7.3%). Other important monomers are 9-octadecenoic acid (6.2%) and 18-hydroxy-9-octadecenoic acid (5.4%). In terms of chain length, most of the fatty acids have 18 carbons, corresponding to 56.8% of

all monomers. The second-most important chain length is 22 carbons, corresponding to 12.4% of the monomers. Only the C18-diacids and the C18-hydroxyacids exhibited mid-chain functionalization.

Table 2. Monomeric Composition of Suberin in the Cork of *Quercus suber* as Determined after Depolymerisation by Methanolysis, as the Mass Proportion of the Total Solubilized Products and as the Molar Proportion of the Identified Monomers (Graça and Pereira 2000; Pereira 2007)

Chemical classes and compounds	Formula	Mass %	Mol %
Glycerol	$CH_2OHCHOHCH_2OH$	14.2	40.8
1-Alkanols	$CH_3\ (CH_2)_n\ CH_2OH$	1.1	0.8
Alkanoic acids	$CH_3\ (CH_2)_n\ COOH$	1.1	0.7
Saturated diacids	$COOH\ (CH_2)_n\ COOH$	8.7	6.7
Hexadecanedioic acid	$COOH\ (CH_2)_{14}\ COOH$	2.0	1.8
Octadecanedioic acid	$COOH\ (CH_2)_{16}\ COOH$	0.5	0.4
Eicosanedioic acid	$COOH\ (CH_2)_{18}\ COOH$	1.0	0.8
Docosanedioic acid	$COOH\ (CH_2)_{20}\ COOH$	4.5	3.2
Tetracosanedioic acid	$COOH\ (CH_2)_{22}\ COOH$	0.7	0.5
Substituted diacids		36.8	29.7
9-octadecenedioic acid	$COOH\ (CH_2)_7\ CH{=}CH(CH_2)_7\ COOH$	6.2	5.3
9-epoxioctadecanedioic acid	$COOH\ (CH_2)_7\ CHOCH(CH_2)_7\ COOH$	22.9	18.5
9,10-dihydroxyoctadecanedioic acid	$COOH\ (CH_2)_7CHOHCHOH(CH_2)_7COOH$	7.7	5.9
Saturated ω-hydroxyacids	$COOH\ (CH_2)_n\ COOH$	11.4	8.6
16-hydroxyhexadecanoic acid	$CH_2OH\ (CH_2)_{14}\ COOH$	0.4	0.4
18-hydroxyoctadecanoic acid	$CH_2OH\ (CH_2)_{16}\ COOH$	0.1	0.1
20-hydroxyeicodecanoic acid	$CH_2OH\ (CH_2)_{18}\ COOH$	0.5	0.4
22-hydroxydocosanoic acid	$CH_2OH\ (CH_2)_{20}\ COOH$	7.9	5.9
24-hydroxytetracosanoic acid	$CH_2OH\ (CH_2)_{22}\ COOH$	2.4	1.7
26-hydroxyhexacosanoic acid	$CH_2OH\ (CH_2)_{24}\ COOH$	0.1	0.1
Substituted ω-hydroxyacids	$COOH\ (CH_2)_n\ COOH$	14.9	12.4
18-hydroxy-9-octadecenoic acid	$CH_2OH\ (CH_2)_7\ CH{=}CH(CH_2)_7\ COOH$	5.4	4.7
9-epoxi-18-hydroxyoctadecanoic acid	$CH_2OH\ (CH_2)_7\ CHOCH(CH_2)_7\ COOH$	7.3	6.0
9,10,18-trihydroxyoctadecanoic acid	$CH_2OH\ (CH_2)_7CHOHCHOH(CH_2)_7COOH$	2.2	1.7
Ferulic acid		0.5	0.6
Others and unidentified*		12.0	
Total		100	100

* Unidentified compounds represented 10.0%

Ferulic acid is also found in the solution of depolymerised aliphatic products. The amounts of solubilized compounds reported varied from 0.5% (Table 2, Graça and Pereira 2000) to 1.3% to 1.5% (Graça and Pereira 1997; Lopes *et al*. 2000a) and 5% to 8% (Bento *et al*. 1998, 2001a,b; Conde *et al*. 1998). Experimental conditions certainly play an important role in such quantifications. The most recent determination of the amount of ferulic acid released by suberin depolymerization showed that it represented 2.7% of the suberin (Marques *et al*. 2015).

With respect to the macromolecular assembly, it is clear that suberin is a glyderidic polyester with glycerol as the bridge between its long-chain monomeric units as the basis for the three-dimensional development of the polymer (Graça and Pereira 1997). The macromolecule includes glyceryl-acyl-glyceryl, glyceryl-acyl-acyl-glyceryl, and glyceryl-acyl-feruloyl moieties, among other possibilities. Most of the aliphatic monomers in cork suberin are functionalised at the mid-chain (Table 2), which adds stereochemical constraints to the spatial development of the macromolecule.

The molar ratio of the long-chain lipids-to-the glycerol content (LCLip:Gly) has been proposed as a chemical parameter to characterize the macromolecular structure of

suberin because it may be associated with the proportion of LCLip-intermonomeric linkages in the macromolecule (Pereira 2013). The average ratio was found to be 3.2.

The degree of polymerization is not known, although mild depolymerization yielded solubilized fragments containing up to approximately 40 long-chain components (Bento *et al.* 2001b). Similarly, suberin solubilization using ionic liquids allowed researchers to obtain polymeric, film-forming suberin fragments (Ferreira *et al.* 2012, 2013; Garcia *et al.* 2014).

A 3-D representation of a model structure proposed by Pereira (2007) for a suberinic oligomer called attention to the fact that the structure is not linear and does not undergo compact, space-filling development. However, an overall strip configuration seems probable. This is still a subject of active research.

Figure 5 (left) represents the chemical structural of a hypothetical polymer of glycerol and 9-epoxyoctadecanedioic acid (the main suberin monomer) showing a spatially turning strand of repeating moieties. Figure 5 (right) also shows a possible arrangement for an oligomer with various types of fatty acid monomers (using the main monomers of suberin, although not in the proportions given by Table 2) as well as ferulic acid. It is clear that the spatial arrangement strongly depends on the specific monomers assembled and on the locations of their linkages. For instance, mid-chain functionalization (*e.g.*, epoxy or double-bond) leads to diverse stereochemical organizations. Further, the overall dimension of the macromolecule causes spatial constraints.

Notwithstanding the hypothetical nature of the models presented, it is evident that the suberin macromolecule occupies considerable space because of the long chain moieties, and that glycerol acts as an anchoring and structuring point for the different monomeric units.

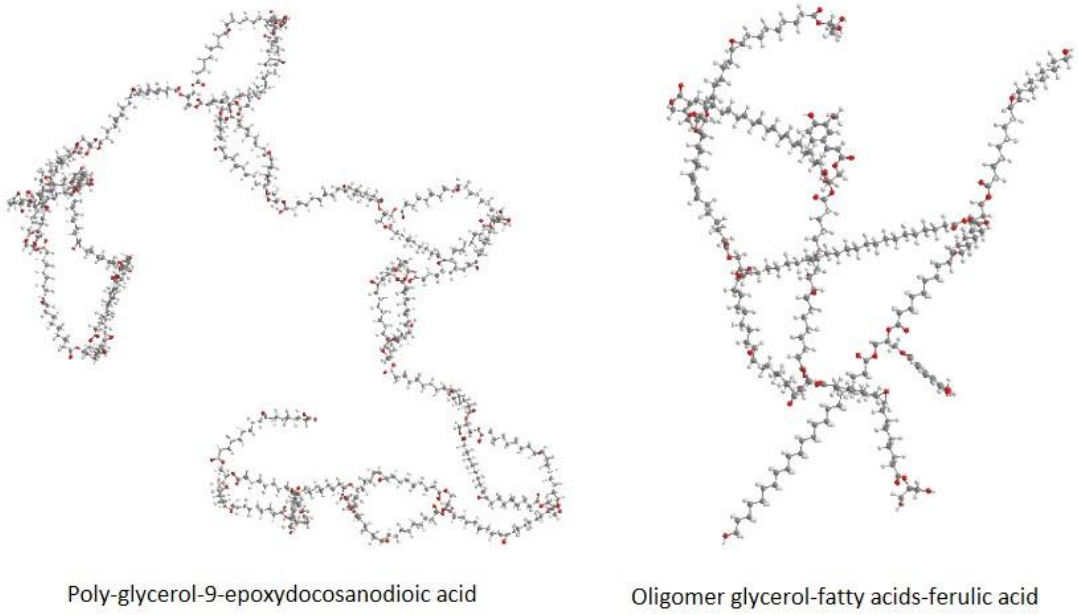

Fig. 5. Schematic 3-D representation of (left) a hypothetical polymer of glycerol and 9-epoxyoctadenadioic acid, including 20 glycerol and 30 fatty acid monomers (molecular mass of 10632) and (right) suberin oligomer containing 8 glycerol, 10 different long-chain acids, and 2 ferulic acid monomers (molecular mass 4150)

Lignin

Lignin is the second most important structural cell wall component in cork (Table 1). Different from suberin, lignin is not specific to cork and is present in most of the secondary cellular tissues of plants. It has been studied for many decades due to its importance in wood pulping, and more recently, for biomass deconstruction (Achyuthan *et al*. 2010).

Lignin is of aromatic nature. It is a polymer made up of three types of phenylpropane monomers (*p*-coumaryl, coniferyl, and sinapyl alcohols) linked by a free-radical reaction initiated *via* enzymatic phenoxy radical formation. The inter-unit linkages in the polymer can be of various types due to the different reactive sites present on the monomers: β-O-4', α-O-4', β–β', β–5', 5-5', 4-O-5', or β-1'. The specific proportions of the monomers and intermonomeric linkages depend on the material.

The presence of lignin in cork was first shown by Marques *et al.* (1994), who isolated and characterized a milled cork lignin (MCL), showing that it fulfills the chemical requirements of what is considered lignin (Marques *et al.* 1996, 1999; Pascoal Neto *et al.* 1996). Cork lignin has a monomer composition of 95% guaiacyl units (G), 3% syringyl units (S), and 2% 4-hydroxyphenyl units (H), with a methoxyl content of 14% (Marques *et al.* 1996). The nature of cork lignin as G-type lignin was recently confirmed by Py-GC-MS/FID (Marques and Pereira 2013). The inter-unit linkages in cork lignin are primarily β-O-4' alkyl-aryl ether bonds (around 80%) and β-5' phenylcoumarans, with small amounts of β–β' resinols and 5-5' dibenzodioxocins (Fig. 6) (Marques *et al.* 2015). Ferulic acid linked by ether linkages with lignin was found to represent about 3% of the lignin (Marques *et al.* 2015).

The average molecular formula of MCL was calculated as $C_9H_{8.74}O_{2.82}$ $(OCH_3)_{0.85}$ with a mean degree of polymerization of approximately 40 (Marques *et al*. 1996).

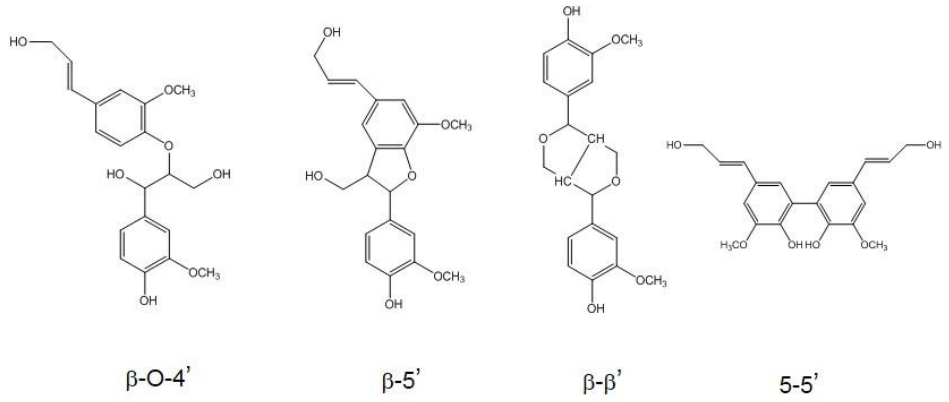

Fig. 6. Main inter-unit linkages in cork lignin (using coniferyl alcohol as the monomer)

With respect to the macromolecule, cork lignin's structure is largely a result of the fact that the main inter-monomeric links are of the β-O-4' type. This results in a rather linear structure that curves helicoidally but has anchor points at its aromatic rings. Figure 7 is a schematic representation of a lignin oligomer with 11 guaiacyl rings, eight β-O-4' bonds, and two β-5' inter-unit linkages that approximates the main known features of cork lignin.

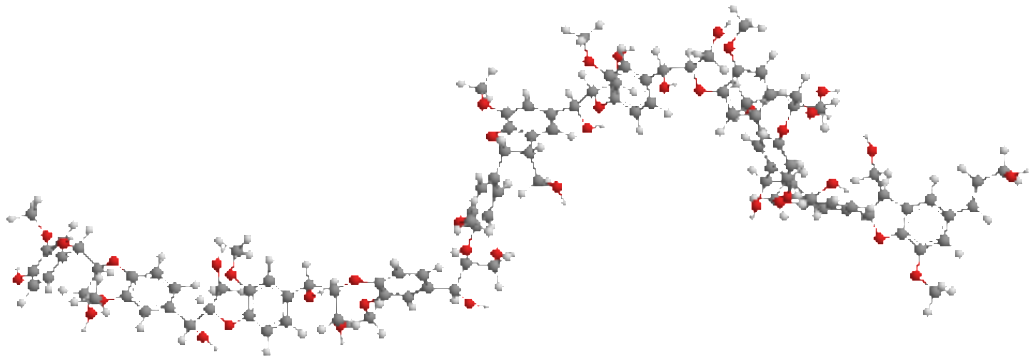

Fig. 7. Schematic representation of a possible lignin oligomer (corresponding to a molecular mass of 2106) containing 11 aromatic guaiacyl rings and eight β-O-4' and two β-5 inter-monomeric bonds

Cellulose and Hemicelluloses

Cork also includes cellulose and hemicelluloses as structural components, but in a proportion much lower than their occurrence in wood (about 20% in cork *vs.* 70 to 80% in wood).

The cellulose content in cork has been estimated at approximately 10% of the mass of structural components and the hemicelluloses content has been estimated at about 12% (Pereira 1988, 2013). The ratio of cellulose-to-hemicelluloses in cork, about 1:1.2, is very different from the 1:0.4 ratio in wood, stressing the much less important role of cellulose in cork.

Upon total hydrolysis, the extractives and suberin-free cork yields neutral sugars and uronic acids. Glucose corresponds to 46% of the total neutral sugars, xylose to 25%, and arabinose to 18%, accompanied by smaller amounts of galactose, mannose, and rhamnose (Table 1). The uronic acid content of cork polysaccharides is approximately 12% (Rocha *et al*. 2004). The hemicelluloses of cork include three xylans: 4-O-methylglucuronoxylan, arabino-4-O-methylglucuronoxylan, and 4-O-methylglucurono-arabinogalactoglucoxylan (Asensio 1987a,b, 1988a,b).

Many aspects of cork polysaccharides are unknown, such as the degree of polymerization, the crystallinity of the cellulose, and the fibrillar orientation.

Topochemistry of Cork Cell Walls

The structural components of cork cell walls have a different chemical nature and polymer features, as described previously. Table 3 summarizes their main characteristics. It is clear by their proportions that most of the properties of cork are imparted by suberin and lignin (together they represent an average of 79% of the total structural components) and that their macromolecular features play a key role in the arrangement and assembly in the cork cell wall.

Suberin is the primary component in the secondary wall of cork cells. Its deposition begins very quickly after cell formation and continues along a few cells during their radial expansion (Teixeira and Pereira 2009). Although many aspects of the macromolecular structure of suberin are unknown, evidence given by transmission electron microscopy and by chemical composition studies, as reviewed and discussed by Pereira (2007), suggest that suberin has ribbon-like development with spaces between the monomers because of the stereochemical arrangement of its structure (Fig. 5). The dimension in the direction

perpendicular to the cell wall is about 4 to 6 nm, which is consistent to the arrangement shown in Fig. 5. The suberin molecule therefore includes carbons with two mobilities: most have a higher mobility and a smaller proportion are more rigid (Lopes *et al*. 2000b), corresponding the long chain CH_2 carbons and the glyceridic carbons, respectively.

Lignin is incorporated into the cell wall and the middle lamella by occupying available spaces between the suberin "ribbons" or becoming entangled with them during their spatial development. The structure of lignin is characterized by the presence of aromatic rings that give the molecule a rather concentrated spatial development and impart bulk and rigidity. However the overall macromolecule should have a helically curving structure favored by the main β-O-4' inter-monomeric links (Fig. 7). Therefore, the lignin molecule is somewhat flexible, and it can be speculated that the lignin and the suberin macromolecules can be somewhat paired within the secondary wall assembly. The removal of suberin from cork cells therefore substantially reduces the secondary wall thickness to about half (Teixeira and Pereira 2010) and disrupts the wall structure (Pereira and Marques 1988).

Chemical links between aromatic and aliphatic regions occur, which explains the analytical difficulty of isolating cork lignin (Marques *et al.* 1994, 1999). It has been recently shown that ferulic acid plays a role in the cross-linking between the cork structural polymers: it is esterified and bound to the suberinic monomers, and by ether links to lignin, thereby acting as a bridge between them (Marques *et al.* 2015).

There are also links between aromatic units and hemicelluloses, forming lignin-carbohydrate complexes (LCC) (Marques *et al*. 1994, 1996).

Cellulose is considered to constitute a tertiary wall lining the cells on the lumen-side; hemicelluloses are also present in the primary wall. However, evidence for the polysaccharides' cell wall topochemistry and their specific interactions with the other structural components is scarce.

Table 3. Main Characteristics of Cork Cell Wall Structural Components

	Suberin	**Lignin**	**Cellulose**	**Hemicelluloses**
Mass Proportion	53%	26%	10%	12%
Chemical Nature	lipid	aromatic	saccharide	saccharide
Main Monomers	glycerol α,ω-diacids ω-hydroxyacids	coniferyl alcohol	glucose	xylose arabinose glucuronic acid
Minor Monomers	alkanols alkanoic acids ferulic acid	sinapyl alcohol coumaryl alcohol ferulic acid		galactose mannose rhamnose
Main Intermonomeric Links	ester	β-O-4' β-5'	β(1-4) glycosidic	β(1-4) glycosidic α(1-2) glycosidic
3-D Development	ribbon-like	helical strand	linear	linear branched
Main Cell Wall Location	secondary wall	middle lamella secondary wall	primary wall tertiary wall	primary wall tertiary wall
Chemical Affinity	hydrophobic	hydrophobic	hydrophilic	hydrophilic

CELLULAR AND CHEMICAL RATIONALE FOR CORK PROPERTIES

The cellular features of cork and the chemical composition of its cell walls determine the material's properties. Some of the most iconic characteristics of cork are described below, showing how the structure and chemical features of the structural

components explain the functionality of cork. Density, buoyancy, thermal insulation, fire behavior, compression, and permeability are discussed.

Density and Buoyancy

The density of cellular materials is expressed as their solid mass fraction and the density of the solid. The density of air-dried cork is usually about 150 to 160 kg m^{-3}, but a broader range of values can be observed in nature, as influenced by several factors.

The density of the solid (*i.e.*, cell walls) is estimated as 1250 kg m^{-3} (Flores *et al.* 1992). As the cell wall density varies only slightly, the differences in cork density are derived from its structural features such as cell size and cell wall corrugation (Fig. 1), the proportion of earlycork and latecork in the annual ring (Fig. 3), the extent of porosity (Fig. 4), and inclusions and discontinuities.

The average dimensions of earlycork and latecork cells indicate densities of 110 and 420 kg m^{-3}, respectively. The higher density of the latecork layer is clearly seen in Fig. 4. Large annual rings and thin annual rings have different densities, according to their differing proportions of earlycork and latecork cells (95:5 and 75:25, respectively): 126 and 188 kg m^{-3}, respectively.

The corrugation of the lateral prism walls of the earlycork cells also impacts the material's density. The effect on density depends on the corrugation parameter (the quotient between the length of the corrugated wall and the length of the wall if it were straightened) in a way such that the density is higher when cells are more corrugated. The straightening of the cell walls, by thermal treatments or boiling in water, will decrease cork density; on the contrary, treatments that increase the cellular corrugation will yield denser corks (*e.g.*, the compression of a stopper in the neck of a bottle).

Regarding the porosity resulting from lenticular channels, the general tendency is toward higher density values in corks with more and larger lenticular channels. In fact, lenticular channels contain a filling material, and in most cases they are bordered by thicker cells (Fig. 4).

Cork has been used since antiquity as a floatation device. The buoyancy of cork is derived from its low density and the fact that the cells in cork are closed and without open connections to one another at μm level. Another reason for the floating capacity of cork is the very small diffusion of water into it: the diffusion coefficient of water in cork has been found to be between 1.4×10^{-10} m^2 s^{-1} (Fonseca *et al.* 2013), 2×10^{-11} m^2 s^{-1} at 20 °C (Rosa and Fortes 1993), and 7×10^{-13} m^2 s^{-1} at 25 °C (Marat-Mendes and Neagu 2004).

Thermal Insulation and Fire Behavior

The rate of heat transfer through cork is very low because of the material's structural characteristics. Its solid fraction is small, and the gas enclosed in the cells of cork has low thermal conductivity. The cells are small and closed, which eliminates convection. Radiation is reduced through repeated absorption and reflection at the numerous cork cell walls.

In comparison with other synthetic insulation foams, cork has smaller cells but higher density, which results in comparable heat transfer properties. The chemical composition of the cell wall of cork imparts appreciable thermal stability as compared to that of synthetic polymers (*e.g,* polystyrene or polyurethane), which degrade and melt at comparatively low temperatures. In cork, the small polysaccharides content and the thermal stability of suberin (Sen *et al.* 2012a, 2014) facilitate better performance at elevated temperatures. At 350 °C, cork maintains its cellular structure but has expanded cells and

thinner cell walls, as shown in Fig. 8. Even at very high temperatures over 2000 °C, the cork structural backbone is maintained (Reculusa *et al.* 2006). This allows cork to be used as an insulation layer in case of fire.

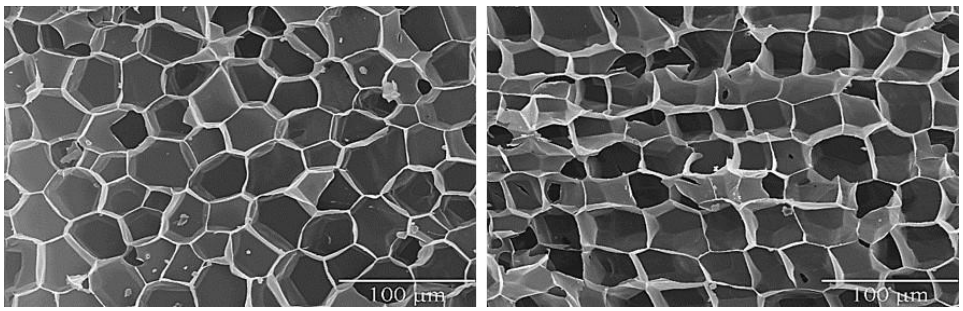

Fig. 8. Scanning electron micrographs of cork treated at 350 °C in air: tangential section (left) and radial section (right)

Compression Behavior

Under compression, cork exhibits a behavior typical of cellular materials, with some peculiarities. The stress-strain curves of cork have three phases associated with different deformation processes (Gibson *et al.* 1981; Rosa and Fortes 1988; Anjos *et al.* 2008).

The first phase represents small stress and deformation values up to a strain of approximately 5 to 7%, corresponding to the elastic bending of the cells. This process is practically fully reversible. The second region starts after the yield stress point and forms a large plateau with a small slope until strains up to about 50%. This region corresponds to the buckling of cells. The last phase, above strains of about 70%, shows a sharp increase in stress and a steep slope, corresponding to the densification of the material and the crushing of cells; the buckled cell walls touching each other; and the disappearance of the empty volumes of the lumen. The full densification of the material occurs at a deformation of about 85%.

Figure 9 exemplifies what occurs in cork, at the cellular level, during compression along the stress-strain curve. Three strain levels are important for the use of corks in wine bottling: 20, 30, and 50%, corresponding approximately to the deformation of a cork stopper inside a wine bottle, in the bottling machine, and in a champagne bottle, respectively. Although each point is located in the plateau region of the stress-strain curve, they correspond to different intensities of cellular buckling.

Compression does not cause failure of the cork cells, and even in the densification phase, the cell walls do not fracture. The recovery of the original dimensions after stress removal is rapid and is associated with the unfolding of buckled cell walls. Permanent deformation after 50% strain is small (-3 to -9%) and may be related to the lignocellulosic cells that line the pores (Fig. 4) (Anjos *et al.* 2014).

Although anisotropic, the compressive behavior of cork in different directions is similar. It does exhibit higher strength in the radial direction than in the non-radial (*i.e.*, axial and tangential) directions. The values reported in the literature for the Young's modulus of cork are in the range of 10 to 20 MPa, with the same type of anisotropy between radial and non-radial directions (Rosa *et al.* 1990; Rosa and Pereira 1994; Pereira *et al.* 1992; Anjos *et al.* 2008). In a recent comprehensive study of 200 cork samples, the Young's

moduli averaged 10.4 and 9.2 MPa in the radial and non-radial directions, respectively (Oliveira *et al.* 2014).

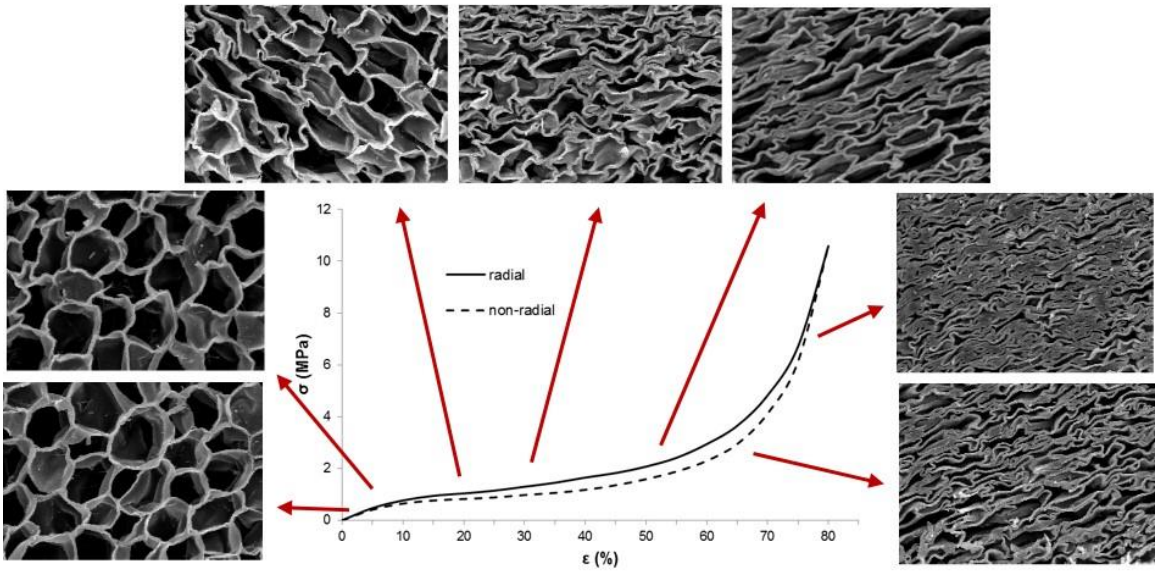

Fig. 9. Stress-strain curves for compression of cork with scanning electron micrographs of cork's cellular features, shown in the tangential section, at various axial compression strains

The variation in the dimensions in the directions perpendicular to the direction of compression (*i.e.*, the Poisson effect), is very small in cork (Fortes and Nogueira 1989). This is related to the material's ability to undulate its cell walls, allowing for large deformation without lateral expansion.

It is logical that the relative proportion of cell walls, or in other words, the solid fraction as given by cork density, influences compression. Cork samples with higher density exhibit overall larger resistances to compression: their Young's modulus and the energy consumed to densify them increases with density, and densification tended to occur earlier (at around 75%) (Anjos *et al.* 2008; Oliveira *et al.* 2014).

The chemical structure of the cork cell wall explains this behavior. The flexible suberin macromolecule, with its long-chain linear monomers as shown in Fig. 5, allows for cell wall undulation even to complete folding without fracture (Fig. 10).

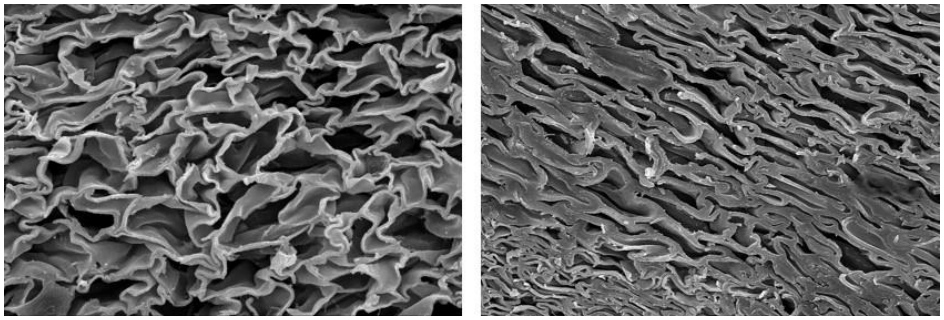

Fig. 10. Scanning electron micrographs of cork's cellular features after compression in the axial direction at strains of approximately 50% (left) and 70% (right)

At the same time, the lignin macromolecule can accompany this deformation because most inter-unit linkages are of the β-O-4 type, which allows for flexibility (Fig. 7)

while the aromatic rings give compressive strength to the cell wall. Therefore, cork compression should be related to the relative proportions of suberin and lignin in the cork, and cork samples with relatively higher suberin contents require less stress for deformation (Oliveira *et al.* 2014).

Permeability

Cork is used for sealing purposes because of its low permeability and high flexibility under compression. The permeability of cork to helium and other non-condensable gases (oxygen, nitrogen, and carbon dioxide) was studied using a considerable number of cork samples without macroscopic inhomogeneities such as lenticular channels (Faria *et al.* 2011). The permeability coefficients were low but varied widely across three orders of magnitude. Water-boiled cork (the pre-treatment that all raw cork planks undergo before stopper production) exhibited lower permeability than non-boiled cork. For oxygen permeation through boiled cork, the most probable permeability (distribution peak or mode) is around 5 µmol/cm·atm·day and the 95th percentile is 223 µmol/cm·atm·day. For non-boiled cork, the peak is around 25 µmol/cm·atm·day and the 95th percentile is around 593 µmol/cm·atm·day. Such large range of variation was also found for cork permeability to oxygen when studying disc samples cut from stoppers (Lequin *et al.* 2012).

The mechanism for the permeability of cork to gases was established as transport processes between cells through the small plasmodesmata channels (Fig. 2) under a molecular flow regime (Brazinha *et al.* 2013). The transport followed a Knudsen molecular flow mechanism with negligible contributions of viscous transport to the total flux. The driving force that regulates gas transport through cork is the gradient of the partial pressure of the gas. A model was developed, based on the morphology of the cork cell structure (the cell dimensions and the plasmodesmata features) that fitted well the determined experimental values. Others have considered that the limiting step for oxygen transport is the diffusion in cell walls (Lagorce-Tachon *et al.* 2014).

The permeation of vapors and liquids through cork was found to differ from the described permeation of non-condensable gases (Fonseca *et al.* 2013). From studies with ethanol and water vapors and liquids, it was found that these species permeate not only through the small channels of the plasmodesmata but also through the walls of the cork by sorption and diffusion, as schematically represented in Fig. 11. The overall permeation of water was higher than that of ethanol by approximately 4 times in the vapor phase and 14 times in the liquid phase due to the larger size of the ethanol molecule.

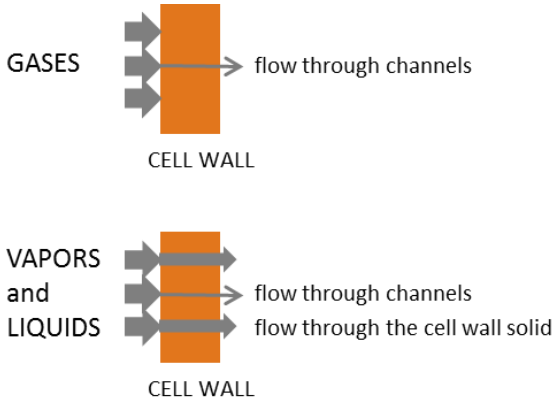

Fig. 11. Schematic representation of the flow of non-condensable gases, vapors, and liquids through the cork cell wall (Fonseca *et al.* 2013)

The permeation of liquids was higher than the permeation of vapors by a factor of 2.5 for water and of 1.2 for ethanol. It was also interesting that wetting *via* exposure to liquid water or ethanol caused an irreversible decrease of the cork's permeability to gases. This explains the lower permeability of water-boiled cork than that of the non-boiled cork (Faria *et al.* 2011).

The permeability of cork is of major practical interest for its use as a wine stopper. Under use conditions, when a stopper is inserted in the neck of a bottle, a recent study (Oliveira *et al.* 2014) examined the oxygen ingress rates into the bottle for a large number of samples. Although the kinetics were similar, a large variation was found, which is in line with the findings of Faria *et al.* (2011).

It is clear that permeability of cork to gases is related to its anatomical features (namely the cell wall plasmodesmata, their number, and their orientation) in conjunction with the cell's dimensional features. The permeation of vapors and liquids is associated with the cork cell wall's chemical composition and topochemistry. It is probable that a large part of the natural variation found in cork's performance as a wine sealant is related to such fundamental characteristics.

THE NATURE OF CORK

The properties of cork are based, as previously discussed, on the features of its cellular structure and its chemical composition. Together, these properties endow the material with its "cork" nature. As discussed, the existing natural variation in cork influences the material's properties to a certain extent but does not impair its overall performance. The limits of this natural variation are important to define the material as cork.

Regarding the structure of cork, a quantified appraisal of the existing variation in the cell dimensions and topology has not been made beyond the works of Pereira *et al.* (1987, 1992). A large part of the variability in cork performance will be related to the cell prism height and the frequency distribution of its values. Knowledge as to the factors that may impact cork growth, such as climatic conditions, will allow for better understanding of cork's structural variability and the influence of this variability on its properties.

One aspect of interest is the estimate of the macroscopic dimensional limit required for the material to exhibit cork-like performance. The minimum particle size required to maintain such performance would be interesting to determine. When the dimensions of cork particles are reduced, the number of closed cells decreases, the external surface of the particle is enlarged, and consequently, the number of open, through-cut cells increases. An extreme case is illustrated in Fig. 12 in which cork was finely ground to particles less than 0.1 mm in size, showing that the cells were destroyed and mostly cell fragments remained. The chemical components of the cork are preserved but the material's structure is not. Consequently, the overall "cork" nature is lost.

This is of interest due to the increasing production and use of composites in which cork particles are bound with adhesives or combined with other materials. A cork particle of volume 0.015 mm^3 (*e.g.*, a cube of edge length 0.25-mm of edge) contains about 500 cells (7 to 9 cells per one row), of which only a fraction (6 to 8 cells in one row) will be closed. This particle size should likely be the smallest size to maintain the typical cork behavior, even when used in cork-derived composites. Figure 12 shows an example of a

cork granulate fraction obtained by separation between 0.18- and 0.25-mm sieves in which the effect of the particle size on the number of cells can be observed.

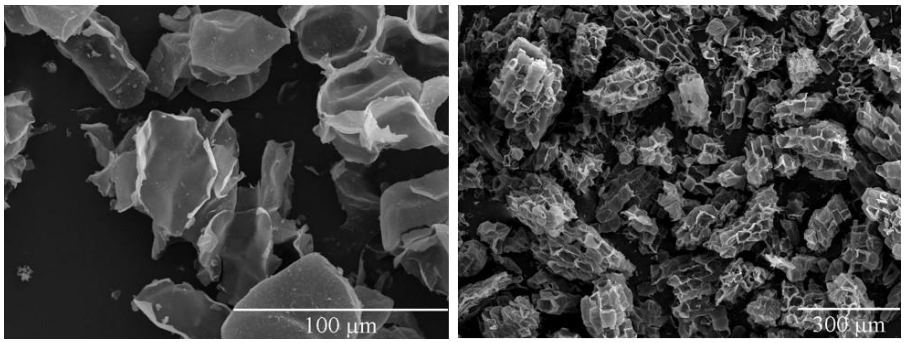

Fig. 12. Scanning electron micrographs of cork granules: (left) particles ground to below 0.1 mm in size and (right) granulometric fraction retained between 0.18- to 0.25-mm sieves.

Data regarding the natural variation of the chemical composition of the cork cell wall exists. Large sampling and chemical analyses of reproduction cork (96 samples, Pereira 2013; 10 samples, Pereira 1988) and virgin cork (40 samples, Pereira 1988) allow for insight into the natural variation found in cork and into its overall average chemical composition. The content of suberin in the cell wall is the most important chemical attribute of cork since this is its most unique feature and is directly related to most of the typical properties of cork.

The suberin content is, on average, 52.8% of the mass of the structural components of cork, with a rather narrow standard deviation of 7.3% (Table 1). It is true that some samples have suberin contents outside this interval. Although this leads to variation in its properties, such as its compression variables, abnormal suberin contents still allow the material to behave as cork.

When cork is mixed with other materials, such as in composites, it would be interesting to understand how the cork fraction influences the composite's properties and at what point the material loses its "cork" performance; in other words what is the minimum content of cork in a mixture to still have a cork-like behavior. Unfortunately, experimental data are not available. However, some estimates may be made using existing chemical data and statistics (Table 1). If one considers that the minimum suberin content required to impart the required cork properties is the mean value (52.8% of the mass of the structural components) minus two standard deviation values (two times 7.3%), than this value will correspond to a suberin content of 38.2% of the mass of the structural components, which is a rather rare value as compared to the natural occurrence range. In the case of mixtures of cork with other materials in composites, a minimum cork content of 72% of an average cork would be required to maintain such a minimum suberin content, and therefore to preserve the general cork properties of the composite. Therefore, a composite material with such a proportion of cork would still exhibit the known cork performance. This is certainly a matter for which targeted experimental research is needed.

CONCLUDING REMARKS

Cork is a natural cellular material of biological origin with an interesting and unique combination of properties. It has low density, buoyancy, very low permeability, low

thermal coefficients, elasticity, and withstands large deformation without fracture under compression. These properties are the reason for the material's various applications, namely as a sealant and insulator.

Cork's properties are the combined result of the features of its cellular structure, particularly its cell dimensions and topology, its cell wall ultrastructure, and the cell wall chemical composition. The chemicals in the cell wall include suberin, the major chemical component and cork's fingerprint. Together, these properties define cork's behavior.

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