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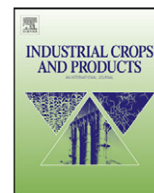

The cellular structure of cork from *Quercus cerris* var. cerris bark in a materials' perspective

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ABSTRACT

Cork in the outer bark of trees is among the valuable raw materials of biological origin due to properties that result mainly from its cellular structure. Large scale commercial utilization of cork has been only achieved with cork from *Quercus suber*. Another oak species, *Quercus cerris*, also contains substantial, albeit not continuous, regions of cork that are clearly visible to the naked eye but are so far considered as a waste material.

Bark samples of *Q. cerris* var. *cerris* trees were collected from the Andırın province, Turkey. Cork portions were separated and their cellular structure was investigated with optical and electron scanning microscopy observations. The results were compared with *Q. suber* cork.

Q. cerris cork has the typical features of cork tissues with a regular and radially aligned structure of suberized cells without intercellular voids, showing a ring structure and a distinction of earlycork and latecork cells. Solid volume fraction was estimated at 25% (22% in earlycork, 36% in latecork).

In *Q. cerris* cork cells are smaller, cell wall thickness and solid volume fraction are higher, and the tissue is less homogeneous with a higher content of lignified inclusions than in *Q. suber* cork. These factors will negatively influence quality in regard to density and mechanical properties associated to elasticity. However, this does not impair its use for production of granulates and agglomerates, e.g. for insulation and energy absorption. Separation of the cork fraction from the bark is a step required before further processing and use.

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1. Introduction

Cork is a cellular material with closed cells. The understanding of the behaviour of a cellular material requires the quantitative description of its structural features (Gibson and Ashby, 1997) since their properties depend on the way the solid is distributed in the cell faces and edges, on the geometry and dimensions of the cells, as well as on their three-dimensional arrangement.

Cork is of biological origin, formed by the secondary meristem phellogen in the outer bark of trees as part of the periderm (Esau, 1969). Each phellogen mother-cell gives by cellular division cork cells (phellem cells in plant anatomy nomenclature) that grow unidirectionally outwards in the tree's radial direction.

Cork is known world wide as the material produced by the cork oak (*Quercus suber* L.) that is used as sealant in wine bottles, as well as in insulation and surfacing products. Its structure, chemical composition and properties have been thoroughly studied and a recent review book gathered the available knowledge on formation, production, properties and uses of cork (Pereira, 2007).

Structurally cork has small thin walled cells in the form of hexagonal prisms (prism height 30–40 μm, base edge 13–15 μm and wall thickness 1–1.5 μm) that are stacked base to base in regular rows, arranged parallel without intercellular voids (Pereira et al., 1987). There are periodic variations in cell size and density during the annual growth season with formation of growth rings (Cumbre et al., 2000). Discontinuities may occur in the cork tissue namely of biological origin, i.e. the lenticular channels and inclusions of phloem tissue (Graça and Pereira, 2004; Pereira et al., 1996). Many of the properties of cork may be related to the characteristics of its cellular structure and are determinant for the use of cork products, i.e. as sealants, floating and insulation materials (Anjos et al., 2008; Pereira et al., 1992; Rosa et al., 1990; Pereira and Ferreira, 1989).

Other tree species contain cork tissues in their outer barks, i.e. *Betula pendula* (Ekman, 1983; Pinto et al., 2009), *Pinus pinaster* (Nunes et al., 1996) and *Pseudotsuga menziesii* (Hergert and Kurth, 1952; Krahmer and Wellons, 1973; Litvay and Krahmer, 1977). The bark of *Q. cerris* var. *cerris* (Turkey oak) also contains large areas of

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Fig. 1. Photograph of the bark of *Q. cerris* var. *cerris* showing the successive layers of cork (lighter coloured regions) and of phloem (dark coloured regions) in the thick rhytidome that lies to the outside of the bark phloem (shown at the bottom of the photo).

cork tissue as part of the successive periderms that constitute its thick rhytidome (Şen et al., 2011).

Q. cerris var. *cerris* grows naturally in central and south-eastern Europe and Asia Minor. In Turkey, the *Q. cerris* var. *cerris* covers about 235 000 ha in the Andirin province in Anatolia (Mıhçıoğlu, 1942). The bark of *Q. cerris* var. *cerris* contains substantial, albeit not continuous, regions of cork tissue that are clearly visible to the naked eye. It was already used in Turkey at a very small scale as an alternative to cork from *Q. suber* for production of insulation agglomerates (Mıhçıoğlu, 1942) and later for production of stoppers for the alcoholic raki drink. However, the comparisons made with *Q. suber* led to reports that the bark of *Q. cerris* was of less quality (Mıhçıoğlu, 1942; Telgeren, 1976). At present there is no specific forest management towards the exploitation of *Q. cerris* and its bark is not used.

In this paper we describe the structure of the cork tissue from the bark of *Q. cerris* var. *cerris* from Turkey based on scanning electron microscopy observations and optical microscopy, and characterize it in relation to geometry and size of the individual cells, as well as their two- and three-dimensional arrangement. A comparison is made with the cellular characteristics of cork from *Q. suber* which is the benchmark for this type of material. It is the objective to establish basic knowledge on the cellular characteristics of *Q. cerris* var. *cerris* cork that will framework its potential utilization.

2. Materials and methods

Bark samples were collected at breast height level from nine *Quercus cerris* var. *cerris* mature trees with 70–80 years of age, in the south-eastern part of Turkey, in the Andirin Province of Kahramanmaraş (altitude of 1000 m, 662.2 mm annual rainfall and 16.5 °C mean temperature). The cork and phloem portions of the rhytidome were separated manually, and the cork fractions were kept for observation.

Small cubes with approximately 5 mm of edge were cut with a sharp razor blade. These dimensions were selected in order to have pure cork samples without phloem regions. The cubes were mounted on stubs (ProSciTech, Australia) and sputter coated (Polaron E 5100 E, USA) with gold palladium for 3 min at 20 mA with their faces oriented so that the observation surface corresponded to transverse, radial and tangential sections.

The cubes were vacuum dried and gold was vapour sprayed making up an approximately 450 Å thick coating. The surfaces were

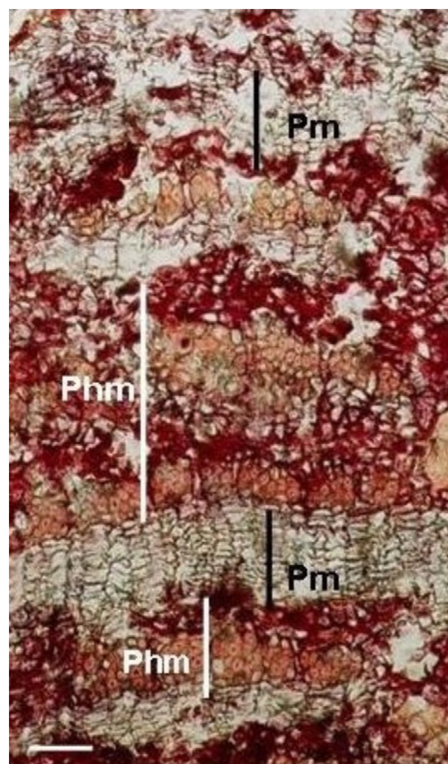

Fig. 2. Optical microscopy photograph of the bark rhytidome of *Q. cerris* var. *cerris* showing the successive layers of cork (Pm) and of phloem (Phm) in the thick rhytidome. (—) Scale bar: 125 μm.

observed in an electron scanning microscope Hitachi S-2400 at magnifications ranging from 50 to 1000×, and the images were recorded in digital format.

The cell measurements were made on the images using image analysis software (Leica Qwin Plus) on approximately 900 cell-samples. On the tangential sections, the number of edges of each cell was counted and the average cell area was calculated. On the transverse sections ring width was measured and the following measurements were made separately on earlycork and latecork cells: number of edges of each cell, radial and tangential dimensions of the lumen of each cell, radial width of the growth ring. The radial and tangential cell wall thickness was calculated as (cell dimension − lumen dimension)/2.

The distribution function of the number of edges of each cell was calculated for the tangential and the non-tangential (radial and transverse) sections as

$$f_i = \frac{N_i}{\sum N_i}$$

where N_i represents the number of cells with i edges. The dispersion of the function in relation to the mean was calculated as $\mu^2 = \sum (i-6)^2 f_i$.

Considering the average dimensions of the cellular units, it can be calculated how much of the cork volume is occupied by the solid. The individual cell is taken as a hexagonal prism, and the solid volume V_s as the difference between total volume V and the empty (lumen) volume V_0, as given by

$$V = \left(\frac{3\sqrt{3}}{2}\right) \times l^2 \times h$$

$$V_0 = \left(\frac{3\sqrt{3}}{2}\right) \times \left(l - \left(\frac{e}{\sqrt{3}}\right)\right) \times (h - e)$$

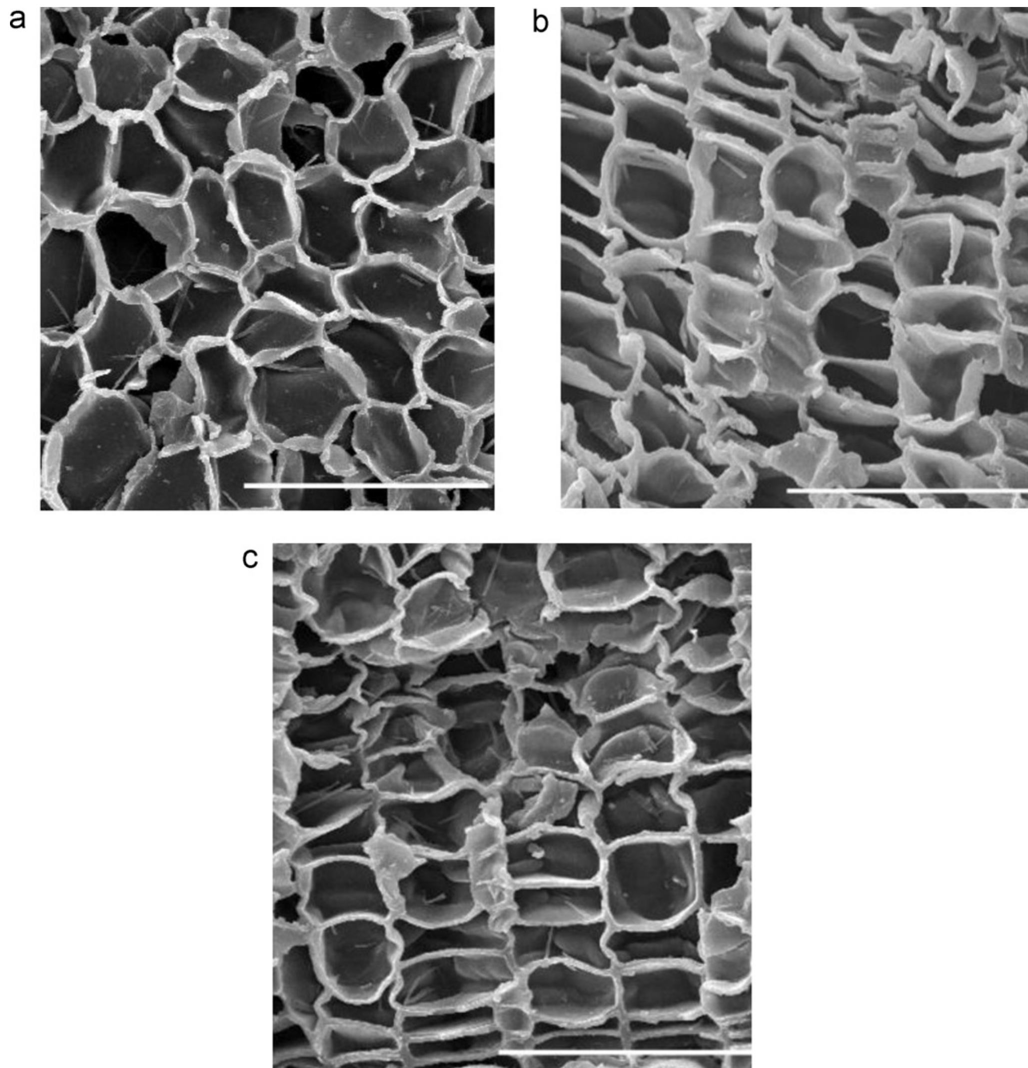

Fig. 3. Scanning electron microscopy observations of cork from the bark of *Q. cerris* var. *cerris*: (a) tangential section; (b) transverse section; (c) radial section. (—) Scale bar (a, b and c): 100 μm.

$$V_s = V - V_0$$

with l as the base edge, h the prism height and e the total wall thickness (between two cells) (Fortes et al., 2004).

Additional optical microscopic observations were also made. The rhytidome samples were impregnated with DP 1500 polyethylene glycol and transverse and longitudinal microscopic sections of approximately 17 μm thickness were prepared with a Leica SM 2400 microtome using Tesafilm 106/4106 adhesive for sample retrieval. The sections were stained with a triple staining of chrysodine/acridine red and astra blue. The sections were mounted on Kaiser glycerin and after 24 h drying were submerged in xylol for 30 min to remove the adhesive, dehydrated in 96% and 100% ethanol, and mounted on Eukitt (Quilhó et al., 1999). Sudan 4 was used for selective staining of suberin. Specimens were also taken from the bark samples and macerated in a solution of 30% H_2O_2 and CH_3COOH 1:1 at 60 °C for 48 h for cell dissociation and stained with astra blue.

The light microscopic observations were made using a Leica DM LA microscope and photomicrographs were taken with a Nikon Microphot-FXA.

3. Results

The layers of cork are part of the successive periderms that constitute the thick rhytidome of *Q. cerris* var. *cerris* and their location in the bark is shown in Fig. 1. The cork layers (phellem layers) are interspersed with phloem layers of variable thickness and do not show spatial continuity in the radial direction (Fig. 2) nor in the tangential, e.g. along the circumference, and axial directions.

3.1. Structure

The structure of the cork contained within the rhytidome of *Q. cerris* var. *cerris* as observed by scanning electron microscopy in the

Table 1
Frequency distribution of the number of edges of cells in the tangential and non-tangential sections of cork from *Q. cerris* var. *cerris*.

Number of edges	Tangential	Non-tangential
4	0.029	0.280
5	0.296	0.385
6	0.486	0.312
7	0.168	0.036
8	0.019	0.002
μ_2	0.656	0.754

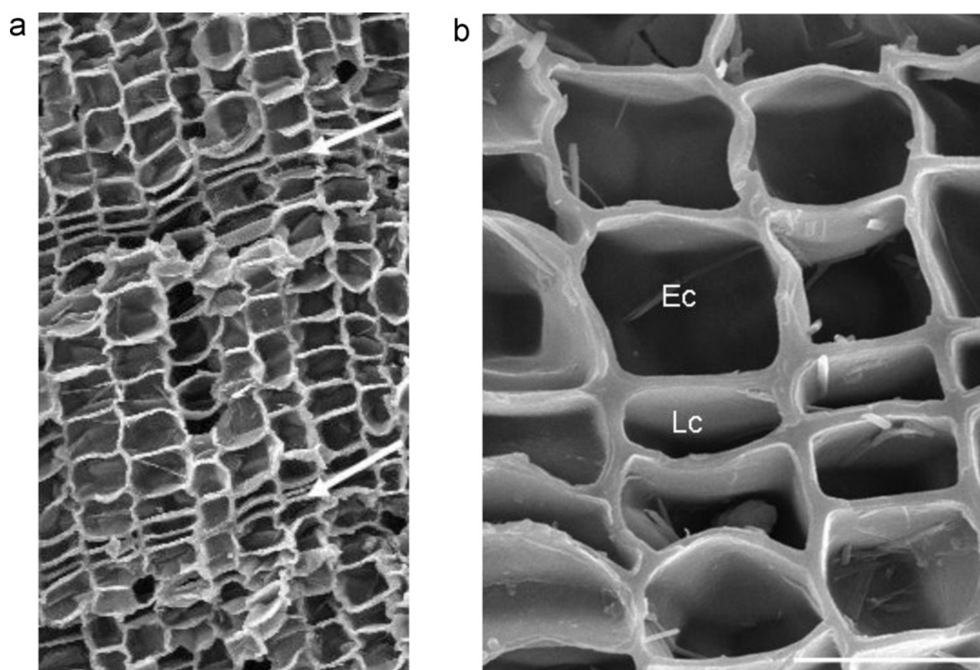

Fig. 4. SEM images of cork from the bark of *Q. cerris* var. *cerris* showing a ring organization with alternating layers of earlycork (Ec) and latecork (Lc) cells (a); a ring boundary (b). Scale bar: (a) 300 μm; (b) 30 μm.

three principal sections is shown in Fig. 3. In each section the cells formed a bidimensional network of edges and vertices, arranged without gaps or intercellular voids.

In the tangential section, the cork cells appear as polygons arranged in a honeycomb-type structure (Fig. 3a). About half of the cells have six sides, with five- and seven-sided polygons making up most of the other cases (Table 1). The dispersion around the average of the number of polygonal edges is low, reflecting a large homogeneity of cell shape in this section. This means that in the majority of cases three cells meet at each vertex of the network.

The radial and transverse sections of cork look very similar and may be referred to as non-tangential sections (Fig. 3b and c). They differ from the tangential section, since the individual cells are approximately rectangular and aligned in parallel rows with a brick wall appearance. Their topological description shows some differences in relation to the tangential section (Table 1): the number of sides of each cell is on average five with a distribution that shows a substantial fraction of cells with four edges. Therefore in these sections and in most of the cases four cells meet at each vertex.

3.2. Cork rings

The cork shows a layered structure of rings with a cell size variation within each ring that distinguishes the cells formed in the first period of growth from the cells formed at the end of the previous growing season, named, respectively, earlycork and latecork cells (Fig. 4a). Earlycork cells are larger and have thinner walls while latecork cells have thicker walls and a much smaller prism height (Fig. 4b). Optical microscopy observations showed that 1–2 layers of the phellem cells in the limit of each growth ring may be heavily lignified (Fig. 5).

The cork rings comprised approximately 7–9 earlycork cells and 2–4 latecork cells in each radial row. Typical measurements of the ring width averaged 201 μm, with a range from 160 μm to 236 μm.

3.3. 3D-structure

The three sections observed in cork allow visualizing its three-dimensional structure (Fig. 3, Table 1). In general terms, cork may be described as being formed by hexagonal prisms that are stacked base-to-base forming rows. The cell rows are assembled in a parallel position in a compact space-filling arrangement. The rows are aligned in the radial direction and therefore the individual cells have the prism height oriented in the radial direction and the prism base in a tangential plane. In adjacent rows the prism bases are frequently coincident and in other cases lay in staggered positions.

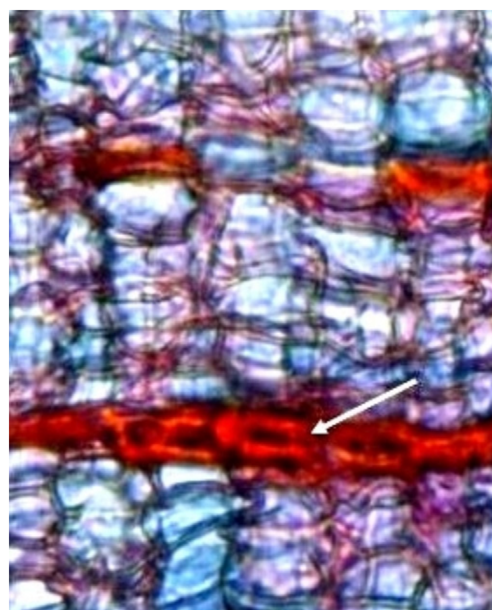

Fig. 5. Optical microscopy observation showing thick lignified phellem cells (arrow) in the limit of the growth ring. (—) Scale bar: 25 μm.

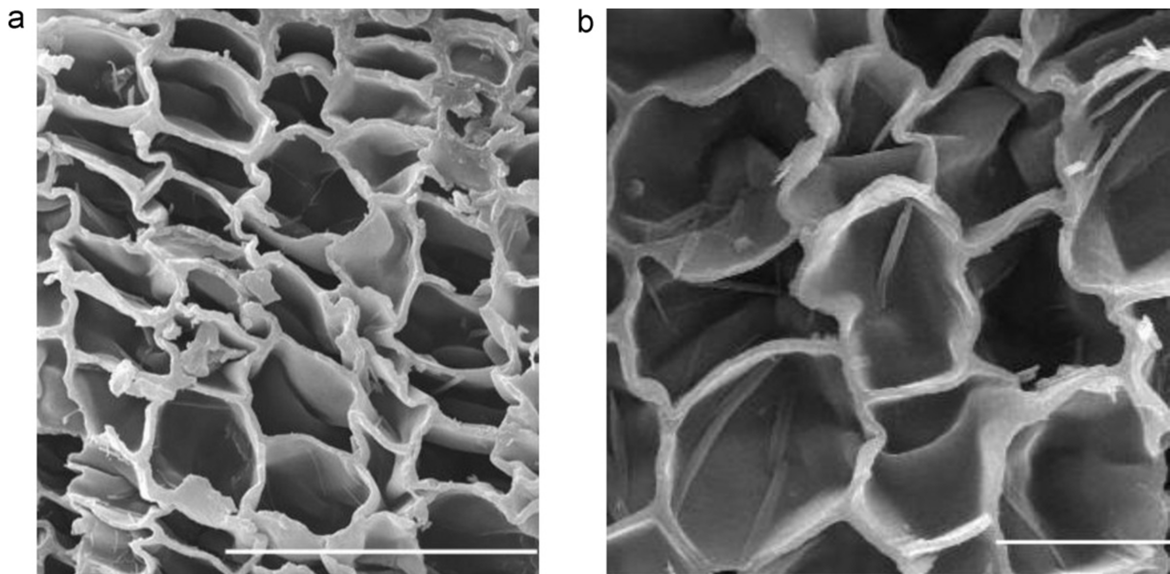

Fig. 6. Scanning electron microscopy observations of cork from the bark of *Q. cerris* var. *cerris* showing the undulations of cell walls. Scale bar: (a) 100 μm; (b) 30 μm.

3.4. Undulation

In the radial and transverse sections of cork it can be observed that the cell sides that are roughly oriented along the radial direction, i.e. the prism lateral faces, in most cases are not straight but show some cell wall undulation (Fig. 6a and b). Usually one to two corrugations per face can be seen especially in the earlycork cells but the amplitude of the corrugations is variable within and between samples. In some regions the cells were heavily compressed in the radial direction or may show distortion of cells in other directions (Fig. 7).

In the tangential section the sides of the cells did not usually show corrugations, although some buckling may occur.

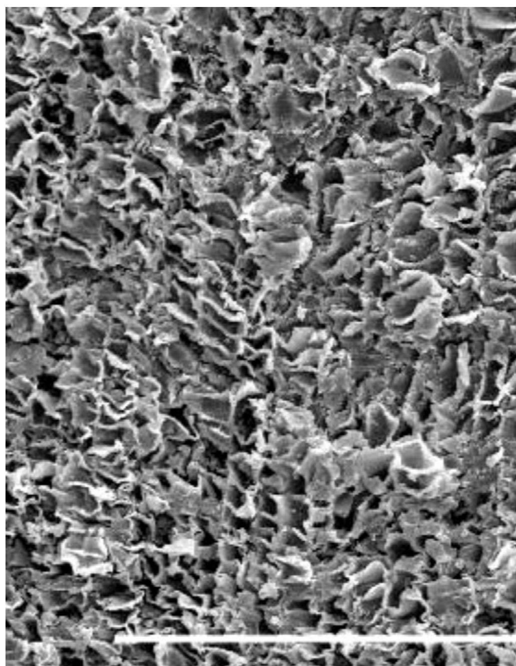

Fig. 7. Scanning electron microscopy observations in the transverse section of cork from the bark of *Q. cerris* var. *cerris* showing heavy cell corrugation and distortion. (—) Scale bar: 300 μm.

3.5. Cell dimensions

The cell dimensions in earlycork and latecork are summarized in Table 2. On average in earlycork the prism height is 25 μm, the base edge 16 μm and the cell wall thickness 2–3 μm; in latecork the prism height is reduced to 14 μm, and the cell wall thickness increased to 3–5 μm. However, the cork dimensions were far from uniform and there were large variations within the sample and between samples.

The aspect ratio of the cells (h/l) is about 1.5–2 in earlycork cells and close to 1 in latecork cells.

3.6. Solid volume

The solid distribution in faces and edges is uniform and the cell edges have substantially the same cell wall thickness as faces, apart from a slight rounding that is observed at the inner side of cells at face junction (Fig. 8a and b). The wall thickness differs for the cell faces lying in the tangential plane (the prism base) which are thinner than those in the radial direction (prism sides), corresponding, respectively, to 2.1 and 2.9 μm in earlycork and 2.8 and 5.2 μm in latecork.

Considering the average dimensions of the cellular units, it can be calculated how much of the cork volume is occupied by the solid. The solid fraction in the cork calculated in percent volume is approximately 22.3% in the earlycork and 35.8% in the latecork region.

Table 2
Mean and standard deviation (in parenthesis) of the cellular dimensional characteristics of the cork cells from *Q. cerris* var. *cerris* measured on transverse sections in the radial and tangential directions.

	Earlycork	Latecork
Radial width, μm	24.6 (5.6)	14.0 (6.6)
Radial lumen width, μm	20.5 (5.9)	8.5 (4.2)
Radial cell wall thickness, μm	2.1 (1.3)	2.8 (2.0)
Tangential width, μm	30.2 (6.3)	30.0 (5.8)
Tangential lumen width, μm	24.4 (6.8)	20.0 (7.8)
Tangential cell wall thickness, μm	2.9 (1.6)	5.2 (3.8)

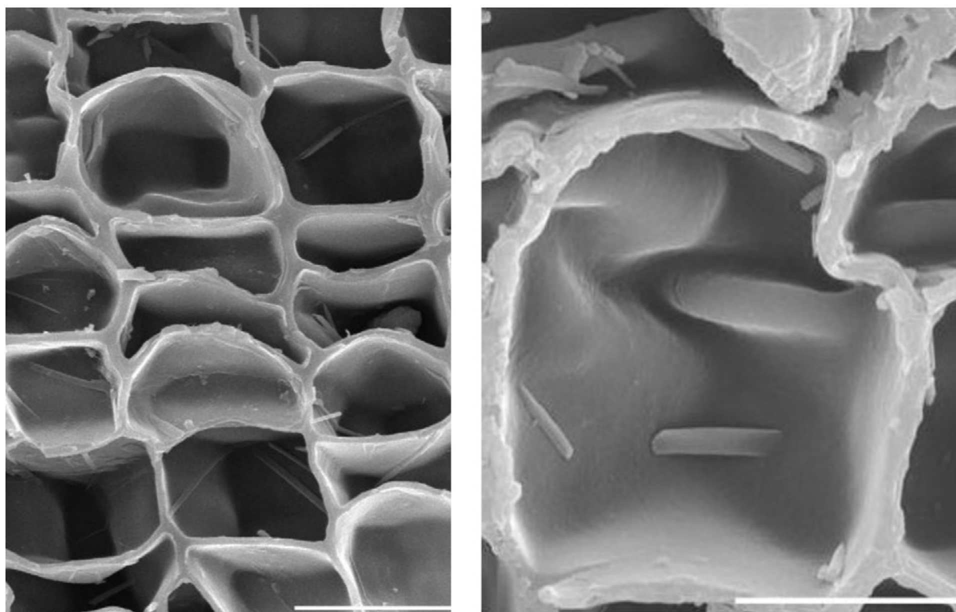

Fig. 8. Scanning electron microscopy observations in the transverse section of cork from the bark of *Q. cerris* var. *cerris* showing the solid distribution in the cell wall of contiguous cells. Scale bar: (a) 30 μm; (b) 10 μm.

3.7. Structural discontinuities

Within the cork tissue there are lenticular discontinuities with an approximate circular section in the tangential section as shown in Fig. 9, and extending as channels in the radial direction. These channels are filled with thin walled cells with a loose arrangement.

The cork tissue frequently also includes lignified phloem cells (fibers and sclereids). Sclereids may occur in the phellem mass in aggregates of a few cells, sometimes forming prominent nodules with large tangential or radial diameters (Fig. 10). In general sclereids are approximately isodiametric although with various shapes and sizes, and have almost no lumen and very thickened and polilamellated cell walls transversed by minute pit channels (Fig. 10). They frequently include large prismatic crystals and phenolic compounds (Fig. 11).

4. Discussion

The anatomy of the bark of *Q. cerris* var. *cerris* was recently described (Şen et al., 2011) and shown to contain a thick rhytidome formed by successive periderms with conspicuous cork layers as depicted in Fig. 1. It is this important content of cork in the bark of *Q. cerris* that has triggered interest in its possible utilization and on the characterization of its cellular structure as a determining factor for defining properties and performance, as shown in detail for *Q. suber* cork (Pereira, 2007) and in general for cellular materials (Gibson and Ashby, 1997).

The results of this paper bring a description of *Q. cerris* var. *cerris* cork cells and structure that was not yet available, therefore allowing a comparison with the known features of the commercial valuable *Q. suber* cork. However, taking into account the bark and rhytidome structure (Figs. 1 and 2), the use of the *Q. cerris* cork fraction will require a fractionation process and be limited to cork granulates. The need for a careful bark fractionation was already advised when considering the chemical

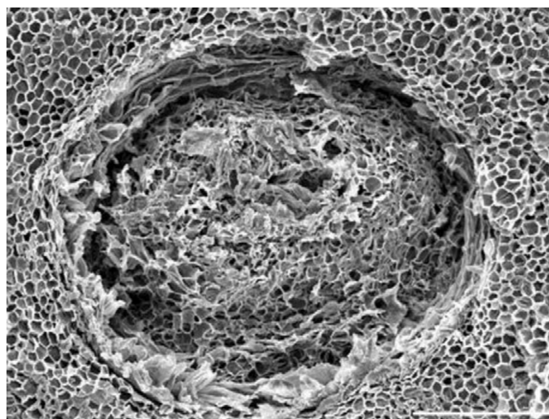

Fig. 9. Scanning electron microscopy observations in the tangential section of cork from the bark of *Q. cerris* var. *cerris* showing one lenticular pore. Scale bar: 300 μm.

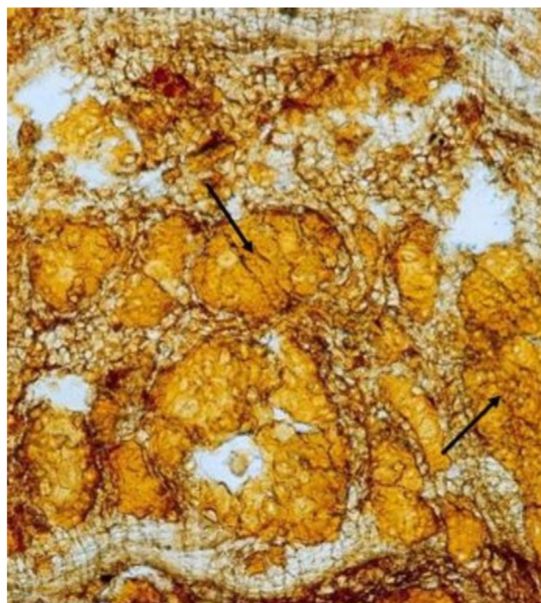

Fig. 10. Inclusions of lignified cells within the cork tissue as observed by optical microscopy in thin sections. Scale bar: 50 μm.

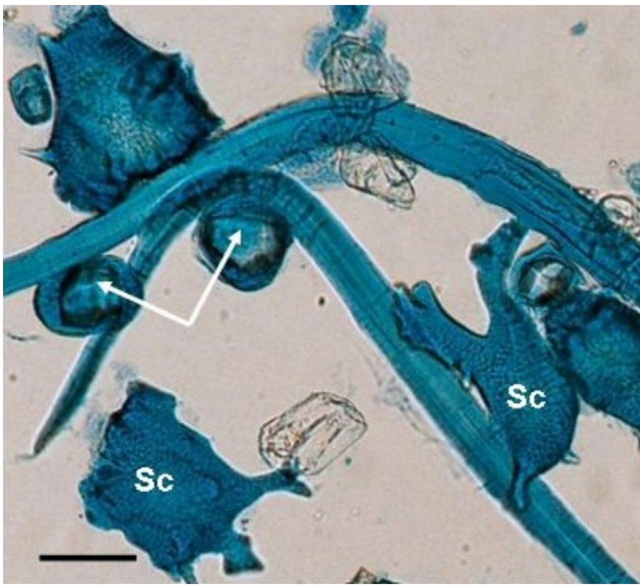

Fig. 11. Sclereids (Sc) observed by optical microscopy in dissociated cells and prismatic crystals (arrows). Scale bar: 50 μm.

composition of the *Q. cerris* rhytidome components (Sen et al., 2010).

Overall the cellular structure of *Q. cerris* cork is similar to that found in corks of other species namely in *Q. suber* (Pereira et al., 1987; Pereira, 2007) with a regular and compact arrangement of small closed cells. Some differences are, however, present as discussed in sequence.

The cell dimensions of *Q. cerris* cork (Table 2) are smaller than those of *Q. suber* in relation to the prism height in the earlycork cells (25 μm vs. 30–40 μm), while cell wall thickness is higher (2–3 μm vs. 1–1.5 μm). The fraction of solid material in *Q. cerris* cork (22% and 36%, respectively, in earlycork and latecork, Table 3) can be estimated at an average of about 25% by assuming that the volume ratio earlycork:latecork is about 0.82:0.18, taking into account the number of both cell types and their dimensions. This solid fraction is much higher compared with the 10% of *Q. suber* cork (Pereira, 2007). In consequence *Q. cerris* cork has a higher density and therefore looses the competitive advantage of a very low density of *Q. suber* cork and other cellular materials used for insulation.

The topology of *Q. cerris* cork as regards the distribution of the number of cells in each section (Table 1) shows a higher dispersion in the non-tangential sections. In these sections the cells are mostly 5- and 6-sided but with a substantial proportion of 4-sided cells, leading to the frequent location of the prism base of cells in contiguous rows in the same tangential plane (Fig. 3a). This differs from *Q. suber* cork where the three sections are topologically very similar (Pereira et al., 1987).

Another difference in the cellular characteristics of *Q. cerris* cork lies in a more irregular undulation of the cell walls (Figs. 6a,b and 7) in comparison to *Q. suber*. This is a consequence of the thicker cell walls but also of the less regular stress distribution of radial growth

Table 3
Dimensional characteristics of the cork cells from *Q. cerris* var. *cerris*.

	Earlycork	Latecork
Prism height, μm	24.6	14.0
Prism base edge, μm	15.5	15.5
Average base area, μm^2	620.9	620.9
Total cell volume, μm^3	15355	8738
Number of cells per cm^3	6.5×10^7	11.4×10^7
Solid volume fraction, %	22.25	35.84

in the bark tissue due to the presence of phloemic layers between the cork regions (Figs. 1 and 2).

Rings were visible in the phellem layer in each periderm (2–5 rings, Fig. 4a). Previous anatomical studies of *Q. cerris* var. *cerris* bark estimated that the phellogen lifespan in each periderm was about 25 years (Şen et al., 2011). This means that the phellogen activity will not have an annual regularity and the rings shown in the phellem are not annual rings as it is the case in *Q. suber*. The lignified layers of phellem cells in the limit of each ring (Fig. 5) are not noticed in *Q. suber* cork although they are observed in other species, i.e. *E. globulus* (Quilhó et al., 1999) and various tropical barks (Roth, 1981).

The intensity of phellem growth in each periderm was moderate, with only 9–13 cells produced by one phellogen mother cell during one ring period. This differs markedly from *Q. suber* where each phellogen mother cell produces annually about 10–20 phellem cells in young plants and many more (up to about 100 cells) in mature trees (Graça and Pereira, 2004; Pereira et al., 1992).

Q. cerris var. *cerris* also showed a great amount of lignified and sclerified phloem cells within the cork tissue as compared with *Q. suber* (Fig. 10). Sclereids originate from axial and radial parenchyma, which enlarge and thicken and compact masses of sclereids are clearly visible on cut surfaces of *Quercus* barks (Trockenbrodt, 1991; Howard, 1977; Graça and Pereira, 2004). From a material's point of view, the sclerified nodules are defects that will negatively impact on the mechanical properties of the cork tissue and are one of the causes for the reported inferior quality of *Q. cerris* var. *cerris* bark in comparison with *Q. suber* (Mıhçıoğlu, 1942).

On the contrary very few lenticular channels were observed crossing the cork layers (Fig. 9). Therefore they will not be the determining quality parameter as it is the case for *Q. suber* cork (Pereira et al., 1996).

The combined characteristics of *Q. cerris* cork cells and structure will influence the material's properties, behaviour during processing and performance in use, as detailed for *Q. suber* cork (Pereira, 2007). Overall the quality may be considered as lower than that of *Q. suber* in what relates to density and mechanical properties associated to elasticity. However, consequences should not be overwhelming since the potential use of *Q. cerris* cork has to be directed to the production of granulates and agglomerates, where such properties will have less impact.

5. Conclusions

The cork of *Q. cerris* var. *cerris* shows typical features of bark cork tissues with a regular and radially aligned structure of suberised cells without intercellular voids. These characteristics support its use as a cellular material namely for insulation and energy absorption. Separation of the cork fraction from the rhytidome is a step required before further processing and use.

Taking as reference the cork from *Q. suber*, the cells of *Q. cerris* var. *cerris* are smaller leading to a higher solid volume fraction, and the tissue is less homogeneous with a higher content of lignified inclusions.

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