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STRUCTURAL AND MECHANICAL PROPERTIES OF CORK CELL WALLS FROM QUERCUS VARIABILIS BLUME (*FAGACEAE*)

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ABSTRACT

The properties of cork are strongly dependent on its cell wall properties. Thus, it is very important to characterize the cork cell walls in order to understand structure-property relationships. The reproduction cork tissue from *Quercus variabilis* Blume was examined with field-emission scanning electron microscopy to detect the structural characteristics of the cell walls. Several noteworthy anatomical features were present in the cells of *Quercus variabilis* cork. In most instances, the inner wall of cork cells was not smooth and showed an irregular surface. Solid deposits of various shapes were observed in the inner surfaces of the cell walls. Cell walls of cork tissue had severe corrugations in transverse and radial sections. Trabeculae were found for the first time in the cork tissue of *Quercus variabilis* Blume. They extended across a few cells, with a rod-like form. Nanoindentation techniques provide a new view of the mechanical properties of the cork cell walls. The hardness of cell walls of untreated and boiled reproduction cork from *Quercus variabilis* was 0.54 GPa and 0.51 GPa. The elastic modulus was 11.47 GPa and 11.81 GPa, respectively. Boiling treatment of cork could improve mechanical properties of cell walls.

KEYWORDS: Cork, *Quercus variabilis*, cell wall, structural property, mechanical property.

INTRODUCTION

Cork is the outer bark of the cork oak tree. It is a protective tissue with closed cells and can be removed from the tree stem every nine years or so, which cannot endanger the vitality of the tree (Silva et al. 2005). In China, cork is mainly from *Quercus variabilis* Blume (*Fagaceae*) and the annual output amounts to ca. 50, 000 tons (Zhao et al. 2013). As a renewable and natural material, cork has many interesting and useful properties, i.e. light weight, chemical stability, hydrophobicity, high elastic compression and dimensional recovery, and resistant to wear and fire (Pereira 2015). It has been widely used as wine stoppers, construction materials, agglomerates,

cork polymer composites and design materials, etc. (Gil 2015). Apart from *Quercus variabilis*, *Quercus suber* L., mainly distributed in the western Mediterranean countries, is another important source of cork. Especially Portugal, there was about 100, 000 tons output of cork in 2015 (Knapic et al. 2016).

Cork tissue is composed of dead cells that are, therefore, without cytoplasm and filled with gas inside, with only the thin cell walls remaining. The property of cork is always associated with chemical composition and characteristics of its cell walls. The chemical composition of cork from *Quercus variabilis* and *Quercus suber* L. has been reported in many documents (Song et al. 2016). Suberin is the major structural component and represents an average of 33-50% of the structural components. Lignin is the second structural component of cork cell walls and represents 20-25%. Cork also contains an appreciable amount of polysaccharides (12-20%) and extractives (14-18%) (Lei et al. 2012, Pereira 2013, Silva et al. 2016).

The structural features of the cork cells can be observed by scanning electron microscopy in the three principal sections. Hooke first revealed the cellular structure of cork under the microscope as early as the 17th century (Leite and Pereira 2017). The structural characteristics of cork cells from *Quercus suber* were described in detail by Pereira et al. (1987). Lei et al. (2009), Miranda et al. (2013) and Ferreira et al. (2016) analyzed the microstructure of cork from *Quercus variabilis*, respectively. Nonetheless, more refined characteristics of cell walls of *Quercus variabilis* cork have been little explored.

Cork is one of the typical cellular materials. The cell wall of cork consists of a primary wall, a relatively thick secondary wall and a thin tertiary wall on the cell lumen side under transmission electron microscopy (Song and Zhao 2017, Teixeira and Pereira 2010). The cell wall is the actual bearing structure of cork material and has the important influence on its macro mechanical properties. Although there are some reports on macro mechanical properties of cork, the data of mechanical properties at the cell wall level are scarce.

Nanoindentation testing is a powerful means to investigate mechanical properties of a material at the cell wall level (Wagner et al. 2014). It was introduced for the study of the mechanical properties of wood cell walls by Wimmer et al. and Wimmer and Lucas in 1997 (Yu et al. 2007). Over the last two decades, many researchers have investigated the modulus of elasticity and hardness of native or modified wood cell walls through nanoindentation (Brandt et al. 2010, Gindl et al. 2004, Tze et al. 2007, Yin et al. 2011, Zhang et al. 2012). Wu et al. (2010a, 2010b) and Li et al. (2013) evaluated the elastic modulus and hardness of cell walls of crop stalks and hemp stalks by nanoindentation, respectively. However, there are no literatures on using nanoindentation to evaluate the mechanical properties of the cork cell walls.

The cork from *Quercus variabilis* has attracted more attention in the past few years. In this study, we explored the properties of cork cell walls from *Quercus variabilis*. The objectives were (1) to detect its key anatomical characteristics of cell walls with field-emission scanning electron microscopy (FESEM) and (2) to probe the mechanical properties of cell walls by mean of nanoindentation testing, which may enlarge the knowledge of the Chinese cork and contribute to its application.

MATERIALS AND METHODS

Cork material selection

Reproduction cork from *Quercus variabilis* was obtained from Qinling mountainous areas, located in the southwest of Shaanxi province of China (108° 10' 08' E, 33° 19' 56' N, 873.9 mm

mean annual rainfall, and 14.5 mean temperature). Cork samples with ca. 15 to 20 complete annual rings were collected in August 2015.

FESEM observations

The cork materials were processed into cubes with sides of 5 to 8 mm. The cubic samples were cut with a sharp knife to obtain a planar surface. Thin sections (ca. 120 μm) were dried and coated with 200 Å of gold palladium in the ion sputtering apparatus (Hitachi MC1000, Japan). Then, the transverse, radial, and tangential sections were observed using a FESEM (Hitachi S-4800, Japan). The images were saved in digital format.

Nanoindentation testing

Cork samples were cut into 10 × 10 × 15 mm (radial × tangential × axial) cubes. One group was cooked in boiling water for 3 h in order to investigate the influence of cooking treatment on mechanical properties of cork cell walls. The specimens were cut on transverse section, and then mounted onto an ultramicrotome (EM UC7, Leica Inc., Germany) and cross-sectioned with a glass knife. In order to obtain a smooth surface, the specimen surfaces were cut using a diamond in the end.

Nanoindentation testing was performed by the TI 950 Triboindenter (Hysitron Inc., USA). Indentations were performed in the central zone of the secondary wall of cork cells. In a force-controlled mode, the indenter tip (Berkovich type) was loaded to a peak force of 100μN at a loading rate of 40 $nm \cdot s^{-1}$. The duration of loading was 5 s and unloading was 3 s.

The elastic modulus (E) and the hardness (H) of cork cell walls were calculated from the load - displacement data. In term of Oliver-Pharr theory (1992), Nanoindentation hardness is defined as follows:

$$H = \frac{F_{max}}{A} = \frac{F_{\max}}{24.5 h_c^2}$$

where: F_{max} - the peak load in an indentation cycle,
A - the projected contact area,
h_c - the contact depth of the indentation.

The specimen's elastic modulus (E_s) was then calculated as follows:

$$\frac{1}{E_r} = \frac{1 - v_s^2}{E_s} + \frac{1 - v_i^2}{E_i}$$

where: E_i - the modulus of the diamond indenter (1141 GPa),
E_r - the reduced elastic modulus
V_i - (0.07) and V_s - the Poisson's ration of the indenter and the cork specimen, respectively. In all calculations Vs was assumed to be 0.064 for all cork.

For each samples, 40 indentations were made on the cross-section. According to the indentation morphology and position, standard indentations were selected for data analysis.

RESULTS AND DISCUSSION

Irregular surface and solid deposits of cork cell walls

The structural features of the cork cells from *Quercus variabilis* were clearly observed by FESEM in the three principal sections, as shown in Fig. 1. The cork cells appeared to have a honeycomb-type structure in the tangential section and a brick-layered structure in the non-tangential sections (transverse and radial). The three-dimensional structure of cork cells could be described as rectangular prisms (mostly pentagonal and hexagonal) that were stacked base-to-base in columns parallel to the radial direction of the tree.

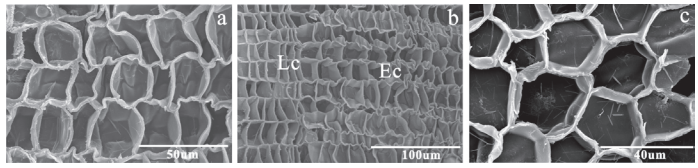

Fig. 1: Cell structure of Quercus variabilis cork under FESEM: (a) transverse section; (b) radial section; (c) tangential section; Lc: late cork, Ec: early cork

In many situations, the inner surfaces of cell walls from *Quercus variabilis* cork were not smooth and showed irregular faces and prominent ridges (Fig. 2, 3, and 4). There were some wrinkles and folds in the inner surface of cell walls. The solid deposits of various sizes presenting in the inner surfaces of the cell walls appeared in various shapes (such as particles, strips, or amorphous structures) and protruded from the inner wall into the lumen. In some regions of the cell walls, there were circular holes connecting two adjacent cells (Figs. 3d and 4b).

The formation process and cellular arrangement of *Quercus variabilis* cork are clearly similar to those of *Quercus suber* cork (Bai et al. 2014, Graça and Pereira 2004). It was noteworthy that the inside walls of *Quercus variabilis* cork were much rougher, with more deposits on the inner surface, while the inner surfaces of cell lumens in *Quercus suber* cork are smooth with occasional deposited materials (Ferreira et al. 2016).

Another significant feature of the cell walls was corrugation. In some cell walls, the corrugation followed a regular folding pattern (Fig. 1a), while it was a random distortion in others (Fig. 1b). The undulate lateral walls of cells were roughly oriented along the radial direction.

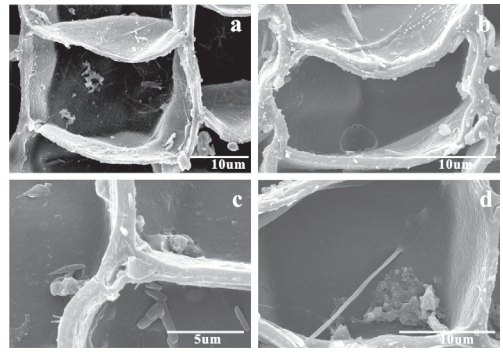

Fig. 2: Cell walls of Quercus variabilis cork observed by FESEM in transverse sections: (a, b) Non-smooth surface of cell walls; (c, d) granular and amorphous deposits.

The corrugation of cell walls was not usually observed in the tangential section (Fig. 1c). The cork cells were approximately prismatic. The base surfaces of cells in the tangential section were occasionally uneven and showed faces bending. It was widely believed that the corrugations stem from compression during cork cell growth (Rosa et al. 1990).

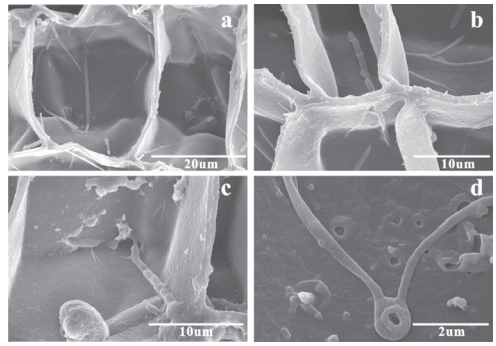

Fig. 3: Cell walls of Quercus variabilis cork observed by FESEM in radial sections: (a) uneven cell wall; (b) strip deposits without a certain direction; (c) globular and strip deposits; (d) substances around the holes protruding the surface of cell wall.

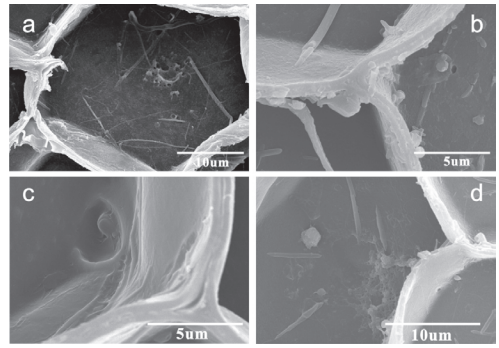

Fig. 4: Cell walls of Quercus variabilis cork, observed by FESEM in tangential sections: (a) uneven base face of cork cell; (b) a hole and scattered deposits; (c) irregular surface; (d) amorphous deposits.

Trabeculae

Trabeculae have been reported in fibers, vessel elements, and axial parenchyma of a wide variety of angiosperm and gymnosperm woods (Butterfield and Meylan 1972). In the course of this research, a number of trabeculae were observed in the transverse and radial sections from *Quercus variabilis* cork (Fig. 5). They could extend to several cells and were slightly expanded at the point of insertion into the cell wall (Fig. 5b). The trabeculae appeared to be rod-shaped extensions of the cell wall substance.

Although most examples of trabeculae have been described in wood cells, it has not been the major focus of wood anatomy. Rare reports have been made on trabeculae in the plant periderm. As for cork cells, to our knowledge, only one report had been made on trabeculae in phellem cells of *Quercus suber* L. (Pereira 1989). In this study, we observed trabeculae in cork cells of *Quercus variabilis*. However, the mechanism of its formation was still unclear. In wood cells, trabeculae were thought to originate from the secondary cambium (Butterfield 1979). The fact that trabeculae observed in cork cells might also stem from cork cambium was intriguing.

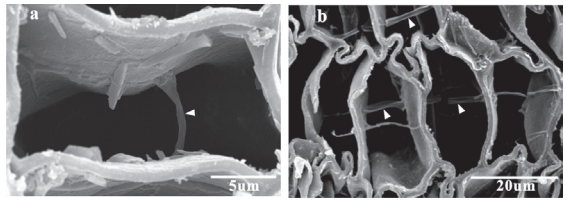

Fig. 5: Trabeculae in cork cells of Quercus variabilis observed by FESEM: (a) transverse section; (b) radial section, arrowhead: trabeculae showing insertion in the cell walls.

Mechanical characteristics of cork cell walls

Average values of the hardness and elastic modulus of cork cell walls were shown in Tab. 1. Nanoindentation tests showed an average hardness of 0.54 GPa and an elastic modulus of 11.47 GPa for untreated cell walls of reproduction cork. After the reproduction cork was boiled at 100°C for 3h, an increase in the elastic modulus of about 3% to 11.81 GPa and a reduction in the hardness of about 6% to 0.51 GPa were observed. This proved clearly that boiling treatment of cork could improve mechanical properties of cell walls.

Tab. 1 also listed the previous results on the nano-mechanical properties of hardwood, softwood and crops stalk. It was apparent from Tab. 1 that the values of elastic modulus of cork cell walls were lower than those of some woods and crops stalk. Compared with the hardwood and softwood, the average hardness values of cork cell walls were a litter higher, but less than that of crops stalk. The different cell wall structure and chemical components could be significant contributors to the nano-mechanical difference between cork and other materials.

Boiling treatment can increase the elasticity of cork cell walls. The hardness, reduced by the boiling treatment, can be explained by a small number of decomposition of hemicelluloses and the extraction of water-soluble matter (mostly low and high molecular mass phenolic compounds), which reduces the connections among the polymers of the cork and softens the structure of the cork cell wall.

Tab. 1: Comparison of nano-mechanical properties of reproduction cork, hardwood, softwood and crops stalk measured by nanoindentaion.

Species	Elastic modulus (GPa)	Hardness (GPa)
Reproduction cork	11.47 (12.19)	0.54 (14.67)
Boiled reproduction cork	11.81(11.72)	0.51(12.56)
Hardwood	16.9-24.6 (5.69-11.8)	0.44-0.56 (4.03-10.6)
Softwood	14.2-18.0 (1-15)	0.34-0.53 (2.0-7.0)
Crops stalk	18.4 (10.8)	0.60 (10.2)

Note: a) The nano-mechanical values of hardwood and softwood are from the document of Wu et al 2010b and the nano-mechanical values of crops stalk are from Wu et al 2010a.

b) The coefficient of variation (CV, in bracket; units in percentage) represents the variability of the elastic modulus and hardness.

Nanoindentation has been a well-established technique to characterize mechanical properties of materials at the cell wall level (Eder et al. 2013). However, nanoindentation on cork is a very young experimental technique. In order to evaluate the mechanical properties of cell walls, there was not the process of embedding for cork samples in this study. Compared with wood cell walls, cork cell walls were liable to yield compressional deformation. In order to obtain qualified

samples, the slicing velocity of cork sample was slightly faster than that of wood sample and minimizing slicing area of each cutting was also crucial.

Cork material is anisotropic particularly at small length scales. The values of mechanical properties are affected by the heterogeneous nature of the cork cell walls. Due to the complex deformation field under the indenter tip, the indentation modulus of cork can be known as being a sort of geometric average of the elastic tensor. Much deep research of elastic parameters of cork cell walls requires more information about molecular structure of suberin and layered-space configuration of components of cork cell walls. More experiments adopting different load, indenter shapes and orientations is needed to exploration (Vincent et al. 2014).

CONCLUSIONS

This paper provides a detailed description of the structural and mechanical characteristics of cell walls from *Quercus variabilis* cork. The specific conclusions can be drawn as follows:

1. In many cases, the inside walls of cork cells were not smooth and there were many deposits with various shapes on the inner surface. The cell side walls had prominent corrugations in the transverse and radial sections.
2. Trabeculae were discovered in the cork tissue of *Quercus variabilis*. They extended across a few cells, with a rod-like form.
3. Nanoindentation can be used to investigate the mechanical properties of cork cell walls at nanoscale. The hardness of cell walls of untreated and boiled reproduction cork was 0.54 GPa and 0.51 GPa. The elastic modulus was 11.47 GPa and 11.81 GPa, respectively. Boiling treatment could increase the elasticity of cork cell wall and decreased its hardness.

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