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The chemical composition of cork and phloem in the rhytidome of *Quercus cerris* bark

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ABSTRACT

Quercus cerris is an important oak species in Eastern Europe and Minor Asia that has a thick bark with a substantial content of cork tissues in its rhytidome. The chemical composition of the cork and of the interspersed phloemic tissues in the rhytidome of *Q. cerris* var. *cerris* from mature trees from Turkey was investigated in relation to summative composition, monomeric composition of suberin, non-polar extractives composition, elemental analysis and ash composition. *Q. cerris* cork has 2.6% ash, 16.7% extractives, 28.5% suberin (fatty monomers) and 28.1% lignin. The non-cellulosic monosaccharide composition shows the predominance of xylose (27.8% of total neutral sugars) with arabinose and galactose (11.5% and 7.9%). Suberin is composed mainly by long-chain ω-hydroxyacids representing 90% of all long-chain monomers and include α,ω-diacids (less than 8%), and small amounts of alkanoic acids in C16 and C18 and alkanols in C20, C22 and C24.

The phloemic tissues have a different composition with a high content of ash (13.0%) and a lignin–cellulose–hemicellulose cell wall composition. Separation of cork from phloem will therefore be a pre-requisite for use of *Q. cerris* cork.

1. Introduction

Tree barks are complex biomass components that have a large potential for utilization mostly based on their chemical composition.

In the outer bark, the periderm results from the activity of the phellogen, a secondary meristem that produces cork to the outside and phelloderm to the inside (Esau, 1977; Fahn, 1990). In most species, the phellogen has a short lifespan of a few years and is periodically renewed in internal regions of the phloem. This originates layers of successive periderms interspersed by phloemic tissues that constitute the so-called rhytidome of tree barks.

This structural organisation has been shown in many tree species, and in some the cork layers contained in the successive periderms are substantial and conspicuous to the naked eye, i.e. in *Pinus pinaster* (Nunes et al., 1996), *Betula pendula* (Ekman, 1983; Pinto et al., 2009), *Pseudotsuga menziesii* (Hergert and Kurth, 1952; Krahmer and Wellons, 1973; Litvay and Krahmer, 1977). This is also the case of *Quercus cerris* where successive periderms are seen in the outer bark with a clearly differentiated cork tissue, as described for the first time by Şen et al. (submitted for publication). *Quercus suber* makes up a special case with production of a very thick cork layer in a persistent periderm (Pereira, 2007).

Cork is a closed-cell material with a set of specific properties that result to a large extent from its chemical composition, i.e. very low permeability, hydrophobic behaviour, biological inertia, large elastic compression and dimensional recovery (Silva et al., 2005). Cork has been extensively studied in the cork oak (*Q. suber*), due to its economic importance for production of wine stoppers and insulation materials, and its structure, chemistry and properties have been recently reviewed in a reference book (Pereira, 2007).

Chemically cork is characterized by the presence of suberin as a major cell wall structural component. Its content varies with species, i.e. 40% in *Q. suber* (Pereira, 1988a), 33% in *P. menziesii* (Graça and Pereira, 2000b), 5% in *Calotropis procera* (Pereira, 1988b), 45% in *B. pendula* (Pinto et al., 2009).

Suberin is a macromolecule formed by the ester coupling of fatty alcohols, fatty acids and diacids, hydroxy fatty acids and glycerol, including also ferulic acid and eventually other phenolic components (Graça and Pereira, 1997, 1998, 2000a; Pereira, 2007). The monomeric composition of suberin is also species dependent as shown, i.e. for *Q. suber* (Graça and Pereira, 2000b), *P. menziesii* (Graça and Pereira, 1999), *B. pendula* (Holloway and Deas, 1973; Ekman, 1983; Gandini et al., 2006) or *Solanum tuberosum* (Graça and Pereira, 2000c). The cork cell wall further includes as structural

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components lignin and the polysaccharides cellulose and hemicelluloses, and a considerable amount of extractives (Pereira, 1988a; Conde et al., 1998). The soluble compounds that may be extracted from cork include mainly polar compounds, i.e. low molecular weight phenolics and tannins (Conde et al., 1997; Cadahía et al., 1998) as well as a lipophilic fraction contain waxes and triterpenes (Conde et al., 1999; Castola et al., 2002, 2005; Sousa et al., 2006).

There are relatively few chemical studies of tree bark periderms, namely of their cork tissues, which could constitute a basis for their use. This study aims to enlarge this knowledge by chemically analysing the cork fractions contained in the periderms of *Q. cerris* bark rhytidome. *Q. cerris* is an important oak species in Eastern Europe and Minor Asia that has a thick bark with a substantial content of cork tissues in its rhytidome, namely the var. *cerris* that includes large, albeit not continuous, regions of cork tissue, clearly visible to the naked eye. Since significant amounts of phloemic tissues are present between the cork layers, these were also chemically analysed. This study constitutes a basis for a possible utilization of *Q. cerris* bark, namely of its cork fraction, as a suggestion made a few decades ago (Mıhçıoğlu, 1942) but not followed by any research. Nothing was published so far on the cork from *Q. cerris* and this will be the first report on its chemical composition.

2. Materials and methods

2.1. Samples

Bark samples were collected from *Q. cerris* var. *cerris* mature trees with 70–80 years of age, in the South-eastern part of Turkey, in the Andırın Province of Kahramanmaraş. The cork and phloem portions were separated manually, ground in a Retsch SK hammer mill, sieved and the 40–60 mesh fractions were kept for analysis. The fractions were purified by suspending in water for a short time for further separation of the cork (floating layer) and phloem (sedimenting material). The resulting fractions were dried at 60 °C for 2 days before chemical analysis.

2.2. Chemical summative analyses

Chemical summative analyses included determination of ash, extractives, suberin, klason and acid-soluble lignin, and monomeric composition of polysaccharides.

Ash content was determined according to TAPPI Standard T 15 os-58 using 2.0 g of cork and phloem materials that were incinerated at 450–500 °C overnight and the residues weighted.

Extractives were determined by successive Soxtec extractions with dichloromethane, ethanol and water during 1.5 h with each solvent. The solvents were recovered and the extractives content determined from mass of residue after drying at 105 °C and reported as a percentage of original samples.

Suberin content was determined in extractive-free material by use of methanolysis for depolymerisation. A 1.5 g of extractive-free material was refluxed with 100 ml of a 3% methanolic solution of $NaOCH_3$ in CH_3OH during 3 h. The sample was filtrated and washed with methanol. The filtrate and the residue were refluxed with 100 ml CH_3OH for 15 min and filtrated again. The combined filtrates were acidified to pH 6 with 2 M H_2SO_4 and evaporated to dryness. The residues were suspended in 50 ml water and the alcoholysis products recovered with dichloromethane in three successive extractions, each with 50 ml dichloromethane. The combined extracts were dried over anhydrous sodium sulphate (Na_2SO_4), and the solvent was evaporated to dryness (Pereira, 1988a). Suberin extracts were quantified gravimetrically, and results were expressed in percent of cork dry weight. These include the fatty acid and fatty alcohol monomers of suberin.

Klason and acid-soluble lignin, and carbohydrates contents were determined on the extracted and desuberinised materials. Sulphuric acid (72%, 3.0 ml) was added to 0.35 g of extracted and desuberinised sample and the mixture was placed in a water bath at 30 °C for 1 h after which the sample was diluted to a concentration of 3% H_2SO_4 and hydrolysed for 1 h at 120 °C. The sample was vacuum filtered through a crucible and washed with boiling purified water. Acid-soluble lignin was determined on the combined filtrate by measuring the absorbance at 206 nm using a UV–vis spectrophotometer. Klason lignin was determined by the mass of residue after drying at 105 °C. Measurements were reported as a percentage of the original sample and klason lignin and acid-soluble lignin were combined to give the total lignin content.

The polysaccharides were calculated based on the amount of the neutral sugar monomers released by total hydrolysis, after derivatisation as alditol acetates and separation by gas chromatography with a method adapted from Tappi 249 cm. The hydrolysed carbohydrates were derivatized as alditol acetates and separated by GC (HP 5890A gas chromatograph) equipped with a FID detector, using helium as carrier gas (1 ml/min) and a fused silica capillary column S2330 (30 m × 0.32 mm ID; 0.20 μm film thickness). The column program temperature was 225–250 °C, with 5 °C/min heating gradient, and the temperature of injector and detector was 250 °C. For quantitative analysis the GC was calibrated with pure reference compounds and inositol was used as an internal standard in each run.

2.3. Composition of lipophilic extractives

Aliquots of the dichloromethane extracts (1–5 ml) were taken and filtered through Anatop 10 membranes (pore dimensions 0.2 μm, Merck). The filtrate was evaporated under N_2 flow and dried under vacuum at room temperature. The residues were dissolved in 250 μl of pyridine per mg of dry mass and the compounds containing hydroxyl and carboxyl groups were trimethylsilylated into trimethylsilyl (TMS) ethers and esters, respectively, by adding 250 μl of bis(trimethylsily)-trifluoroacetamide. The reaction mixture was heated at 60 °C for 30 min in an oven. The derivatized extracts were immediately analysed by GC–MS.

The derivatized solution was injected in a GC–MS (Agilent 5973 MSD) with the following GC conditions: DB5-MS column (60 m, 0.25 mm; ID, 0.25 μm film thickness), injector 320 °C, oven temperature program, 100 °C (5 min), rate of 8 °C/min up to 250 °C, rate of 2.5 °C/min up to 320 °C (20 min). The MS source was kept at 220 °C and the electron impact mass spectra (EIMS) taken at 70 eV of energy.

Compounds were identified as TMS derivatives by comparing their mass spectra with a GC–MS spectral library (Wiley, NIST), and by comparing their fragmentation profiles with published data (Eglinton and Hunneman, 1968; Kolattukudy and Agrawal, 1974). For semi-quantitative analysis, the area of peaks in the total ion chromatograms of the GC–MS analysis were integrated and their relative proportions expressed as percentage.

2.4. Suberin composition

Aliquots of methanolic cork extract and the respective organic phases (ca. 1 ml) were evaporated under N_2 flow and dried under vacuum at room temperature. The residue was dissolved in 250 μl of pyridine and components containing hydroxyl groups were trimethylsilylated into their trimethylsilyl (TMS) ethers with 250 μl bis-(trimethylsilyl)trifluoroacetamide) during 15 min in an oven at 60 °C. The methyl ester TMS ethers were immediately analysed by GC–MS to avoid degradation of the sample. The acidic monomers