Variability of the Chemical Composition of Cork

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The chemical composition of cork was determined, following a sampling that covered the whole production area in Portugal (29 provenances from six regions) with samples taken at cork stripping. To analyse between population variations, a more intensive sampling was made in two locations. The overall mean chemical composition of cork was: extractives 16.2% (dichloromethane 5.8%, ethanol 5.9%, water 4.5%), suberin 42.8% (long-chain lipids 41.0%, glycerol 3.8%), and lignin 22.0% (Klason 21.1%, acid soluble 0.9%). The suberin compositional ratio of long chain lipids to glycerol, LCLip:Gly, was 11.3. The proportion of neutral sugars in the polysaccharides was: glucose 46.1%, xylose 25.1%, arabinose 18.0%, mannose 3.0%, galactose 7.3%, and rhamnose 0.5%. The range of values was large and the variation between individual trees seemed to be the major factor of the differences. Geographical location of cork production was statistically significant only in a few cases when considering site and not when considering regions. The population variation in two sites was important and the absolute difference between the site mean values was small. This research covers the natural variability of cork's chemical composition and discusses the contribution of the structural compounds to the variation of cork properties.

Keywords: Cork; Quercus suber; Suberin; Lignin; Extractives; Chemical composition

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INTRODUCTION

The chemical composition of cork has been highlighted in research mainly for two reasons: first, the chemical features of cork cells are largely responsible for the properties that make this material so valuable; second, the chemical peculiarities of cork compounds make it a potentially interesting source of chemicals.

Cork is a natural cellular material characterized by a rather unique set of properties (*i.e.* low density, reduced permeability to liquids and gases, chemical and biological inertia, mechanical elasticity, and insulation properties) that have led to multiple uses, namely as closure of bottled wines, that made cork known worldwide (Pereira 2007).

Cork properties are the result of the cellular structural features and cell wall chemical composition. The distinctive chemical features of cork are the presence of suberin as the main cell wall structural component, amounting to about 40%, and a large content of lipid and phenolic extractives, while lignin is also an important structural component that together with suberin is responsible for many of the material's properties.

The chemical composition of cork has attracted researchers since the early works of Brugnatelli in 1787 and Chevreul in 1807 and 1815, and the first cork composition was given by Klauber in 1920, later complemented by other published results (*e.g.* Guillemonat 1960; Carvalho 1968; Holloway 1972; Parameswaran *et al*. 1981; Arno *et*

al. 1981; Pereira 1982), as reviewed in Pereira (2007). However, caution should be taken when comparing compositional data, since the specific chemical protocol may have a direct influence on the results obtained. The most used and trustworthy approach is an adaptation of the standard methodologies for wood with the introduction of suberin removal and determination (Marques and Pereira 1987) as a step before determination of lignin and polysaccharides (Pereira 1988). The sample preparation is also a crucial step to obtain a representative cork material: it is necessary to remove the phloemic layer at the external part of the cork plank before grinding, and a stepwise grinding and fractioning is advised to obtain cleaner cork tissue fractions (Pereira 2007).

The natural variability of the chemical composition of cork has only been very partially addressed. Cork is produced by the cork oak (*Quercus suber* L.), a species that has its distribution area concentrated around the western Mediterranean basin. Most cork-producing forests are located in Portugal, followed by Spain.

The first summative chemical analysis of cork carried out in a large number of samples of known origin (40 samples of virgin cork from four locations and 10 samples of reproduction cork from one location in Portugal) was reported by Pereira (1988). Conde *et al.* (1998), who studied the composition of corks from seven provenances in Spain with 3 to 5 trees per provenance, could not distinguish populations by chemical composition although between-population variation was found. Bento *et al.* (2001) studied the variation of cork suberin composition from five provenances (2 to 3 trees per provenance) and noted that variability mainly occurred between trees.

In this study, the chemical composition of cork was determined following a field sampling covering the whole area of production in Portugal according to the relative cork oak area distribution (29 provenances). The samples were taken from mature trees under production at the time of cork stripping and subsequently analysed, carefully following the same analytical protocols to have comparable results. A more intensive sampling was made in two locations to analyse between-population variation.

The results in this study cover the natural variability of cork's chemical composition and correspond to the most extensive work so far undertaken to chemically characterize cork. The results will allow a better insight of the differences that may explain the variation in cork's properties and a focused appraisal of cork as a chemical raw material.

EXPERIMENTAL PROCEDURE

Sampling

The sampling was made in the cork oak forests in 29 locations that geographically cover the regions of cork production in Portugal. The selection was made based on the cork oak area distribution in the regions of cork production, roughly representing one sampling location per 20,000 ha of cork oak area. The location of the sampling sites is shown in Fig. 1, superposed on a schematic representation of the cork oak area.

On each location, mature cork oak trees under cork production at the time of cork stripping were randomly selected (20 trees per site), excluding trees with visible phytosanitary damage. The location of the sampling sites and the size of the selected trees are shown in Table 1. The sites were grouped in six broad national regions (North, Centre, North Alentejo, Coastal Alentejo, Interior Alentejo, and Algarve).

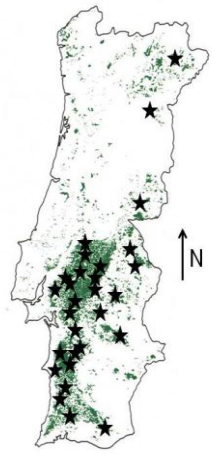

Fig. 1. Schematic representation of continental Portugal including the cork oak area distribution and the location of the cork sampling points

Table 1. Location of Sampling Sites, Mean Annual Rainfall and Temperature, and Tree Perimeter Over Cork at 1.3 m (mean of 20 trees, standard deviation)

Region	Site Code	Location Municipality, Town	Rainfall mm	Temperature ºC	Perimeter cm
North	1A	Macedo de Cavaleiros, Morais	741	11.9	98.5 (11.39)
North	2A	Idanha-a-Nova, Alcafozes	821	15.6	170.2 (57.8)
North	4A	Mêda, Longrovia	591	12.3	202.7 (38.2)
Centre	5A	Abrantes, Alvega	688	15.5	150.0 (39.9)
Centre	5B	Chamusca, Pinheiro	813	15.6	144.2 (23.7)
Centre	5C	Benavente, Samora Correia	610	16.4	122.4 (27.6)
Coastal Alentejo	6A	Alcácer Sal, Foros Albergaria	575	16.3	126.9 (23.6)
Coastal Alentejo	6B	Alcácer Sal, Palma	575	16.3	153.5 (34.8)
Coastal Alentejo	6C	Palmela, Rio Frio	610	16.4	172.5 (23.3)
Centre	7A	Ponte de Sôr, Montargil	618	16.0	136.2 (34.8)
Centre	7B	Avis, Cabeção	618	16.0	120.3 (19.2)
Centre	7C	Coruche, Santana do Mar	618	16.0	123.2 (19.1)
Centre	7D	Mora, Brotas	618	16.0	132.5 (30.4)
Centre	7E	Coruche, Chamusca	618	16.0	151.9 (20.9)
North Alentejo	8A	Montemor-o-Novo, Lavre	618	16.0	163.8 (44.7)
North Alentejo	8B	Évora, Giesteira	665	15.4	125.3 (24.3)
North Alentejo	8C	Montemor-o-Novo, Represa	643	15.6	241.0 (59.7)
Coastal Alentejo	9A	Grândola, Canal Caveira	557	15.8	150.2 (37.8)
Coastal Alentejo	9B	Grândola, Aldeia do Pico	575	16.3	119.2 (16.9)
Interior Alentejo	10A	Évora, Azaruja	643	15.6	141.3 (24.3)
Interior Alentejo	10B	Portel, Portel	706	15.9	111.3 (19.4)
Interior Alentejo	10C	Portalegre, Besteiros	908	15.1	220.9 (30.9)
Interior Alentejo	10D	Portalegre, Urra	908	15.1	269.9 (54.8)
Coastal Alentejo	11A	Sines, Sines	736	15.6	129.2 (29.6)
Coastal Alentejo	11B	Santiago Cacém, S. Bart. Serra	736	15.6	155.1 (49.8)
Coastal Alentejo	11C	Odemira, Luzianes	614	15.0	135.6 (52.6)
Coastal Alentejo	11D	Odemira, São Teotónio	614	15.0	155.3 (35.4)
Algarve	13A	S.Brás de Alportel, Bicas	511	17.3	121.7 (28.2)
Algarve	14A	Silves, São Marcos da Serra	1138	16.2	148.9(38.1)

Cork samples were taken at breast height (1.3 m above ground) from the cork plank (reproduction cork) at the time of cork stripping and taken to the laboratory where they were allowed to air dry under well-ventilated conditions.

The study of between-provenance variations of chemical composition was made on two trees per provenance (total 58 samples). Between tree variation was studied within two provenances (codes 5A and 8C, Table 1) from two important cork production sites on 20 trees per site.

Sample Preparation

A sample with approximately 15x15 cm^2 was cut, and the cork back (the lignocellulosic layer of phloemic tissue that remains to the outside during cork growth) was removed with an additional 3 mm of the underlying cork to ensure complete separation of the lignocellulosic layer.

The cork sample was milled using a knife mill (Retsch SM 2000) with an output sieve of 10x10 mm^2, and the granulated material was screened using a vibratory sieving apparatus (Retsch AS 200 basic) using standard Tyles sieves with the following mesh sizes: 80 (0.180 mm), 60 (0.250 mm), 40 (0.425 mm), 20 (0.850 mm), 15 (1.0 mm), and 10 (2.0 mm). The fractions above 1 mm (15 mesh) were milled again and fractionated. The fractions below 60 mesh (0.250 mm) were discarded to avoid contamination from lenticular material and eventual woody inclusions since the finer fractions are enriched in these materials that have a chemical composition different from cork (Pereira 2007).

For the chemical analysis, the 40-60-mesh granulometric fraction was used.

Chemical Analysis

Chemical summative analyses included determination of extractives, suberin, lignin, and polysaccharides as referred in the literature (Pereira 2007). All determinations were made in duplicate aliquots.

The extraction with organic solvents and water was performed in a Soxhlet apparatus successively with dichloromethane, ethanol, and water for 4 h, 6 h, and 8 h, respectively. The solvents were recovered under vacuum, and the extractives content was determined from the mass of the solid residue after drying at 60 ºC overnight. The results are reported as a percentage of the original oven dry cork mass. The sum of dichloromethane-, ethanol-, and water-extracted material represents the total extractives of cork.

Suberin content was determined in the extractive-free material by use of methanolysis for depolymerisation (Pereira 1988). A 1.5 g sample of extractive-free material was refluxed with 100 mL of a 3% methanolic solution of $NaOCH_3$ in CH_3OH for 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 were recovered with dichloromethane in three successive extractions, each with 50 mL of dichloromethane. The combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness. The suberin extracts, including the long chain fatty acid and fatty alcohol monomers of suberin (named LCLip), were quantified gravimetrically, and the results are expressed in a percent of the initial dry mass.

The water extract was used for determination of the glycerol content. This was made by high-pressure liquid chromatography (HPLC) on a cation exchange column (Sugar Pack, Waters), with water as eluent coupled to a refractometric detector. The concentration was determined after calibration with a glycerol standard.

Total suberin was calculated as the sum of the long-chain lipids (LCLip) and the glycerol (Gly) contents. The mass ratio of LCLip-to-Gly was calculated as an indicator of suberin macromolecular structure. This ratio was also calculated in terms of molar ratio taking into account the molecular weights of glycerol and of the most frequent fatty acid monomer as the reference for LCLip (9-epoxioctadecanedioic acid, MW = 328).

Klason and acid-soluble lignin contents were determined on the extracted and desuberinised materials. Sulphuric acid (72%, 3.0 mL) was added to 0.35 g of the sample, and the mixture was placed in a water bath at 30 ºC for 1 h after which it 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. Klason lignin was determined as the mass of the solid residue after drying at 105 ºC. The acid-soluble lignin was determined on the combined filtrate by measuring the absorbance at 206 nm using a UV/VIS spectrophotometer. Total lignin was calculated as the sum of the Klason lignin and acid soluble lignin contents.

The polysaccharides in cork were estimated by determining the neutral mono-saccharide monomers released by the total acid hydrolysis used for lignin determination in the extractive-free and suberin-free cork sample. The neutral sugars were determined after derivatisation as alditol acetates and separation by gas chromatography with a method adapted from TAPPI method 249 cm-00. The separation was done by GC (HP 5890A gas chromatograph) equipped with a FID detector, using helium as the carrier gas (1 mL/min) and a fused silica capillary column S2330 (30 m x 0.32 mm i.d. x 0.20 µm film thickness). The column program temperature was 225 to 250 ºC, with a 5 ºC/min heating gradient, and the temperature of injector and detector was 250 ºC. For quantitative analysis, the GC was calibrated with pure reference compounds, and inositol was used as an internal standard in each run.

Statistical Analysis

In order to evaluate if the measured variables presented differences between cork provenances, several one-way analyses of variance were carried out followed by a Tukey multiple comparison test. The normality assumption for all variables was tested by the Shapiro-Wilk test recommended for use with small samples (*e.g.* <50). In a few cases, small deviations from normality occurred, but it was considered that ANOVA is robust enough. The equality of variances assumption was validated with the Levene test, and only the Klason lignin content presented a p-value inferior to 0.05 (p=0.02). It was considered that effects were statistically significant for a p-value less than or equal to 0.05. All the statistical analysis were performed using SPSS® statistical software (version 20.0; SPSS Inc., Chicago IL).

RESULTS

Cork Chemical Composition

The overall mean chemical composition of cork was as follows, in % of oven dried initial material: extractives 16.2% (dichloromethane 5.8%, ethanol 5.9%, and water

4.5%), suberin 42.8% (long-chain lipids 41.0% and glycerol 3.8%), and lignin 22.0% (Klason lignin 21.1% and acid-soluble lignin 0.9%). The suberin compositional ratio of LCLip:Gly was 11.3 and the ratio of suberin to lignin contents was 2.0. The composition of polysaccharides in relation to neutral sugars was the following: glucose 46.1%, xylose 25.1%, arabinose 18.0 %, mannose 3.0 %, galactose 7.3 %, and rhamnose 0.5 %.

Table 2 shows the overall mean, maximal and minimal values, standard deviation, and coefficient of variation of the mean for the chemical composition data of all the cork samples. The range of values is large but the coefficients of variation of the mean are rather small with the exception of those for the ethanol and water extractives and of the monosaccharides present in minor amounts (mannose and rhamnose).

Table 2. Mean, Maximal and Minimal Values, Standard Deviation (Std), and Coefficient of Variation (CV) of the Mean for the Chemical Composition Data of all the Cork Samples (n = 58)

Chemical parameter	Mean	Max – Min	Std.	% CV
% o.d. cork				
Extractives, total	16.2	32.9 – 8.6	3.9	24.1
Dichloromethane	5.8	7.4 – 3.5	0.8	13.8
Ethanol	5.9	22.0 – 1.7	3.0	50.8
Water	4.5	11.2 – 1.0	1.6	35.6
Suberin, total	42.8	54.2 – 23.1	6.2	14.5
Long chain lipids	41.0	50.5 – 23.0	5.2	12.7
Glycerol	3.8	5.1 – 2.7	0.6	15.8
Lignin, total	22.0	36.4 – 17.1	3.3	15.0
Klason lignin	21.1	35.5 – 16.1	3.3	15.6
Acid soluble lignin	0.9	1.5 – 0.5	0.2	22.2
Ratio LCLip:Gly	11.3	14.5 – 8.2	1.6	14.2
Monosaccharide composition, % of total neutral sugars				
Glucose	46.1	53.6 - 41.8	3.6	7.8
Xylose	25.1	31.7 – 21.4	3.7	7.3
Arabinose	18.0	24.4 – 12.7	3.0	16.7
Mannose	3.0	12.4 – 2.1	2.8	93.3
Galactose	7.3	10.4 – 5.2	1.2	16.4
Rhamnose	0.5	1.1 – 0.0	0.5	100.0

Variation of Chemical Composition

The mean chemical composition of cork from the 29 provenances is summarized in Table 3. An analysis of variance showed that the provenance is a significant factor of variation only for the content in water extractives ($p = 0.001$), glycerol ($p = 0.002$), klason lignin ($p = 0.001$), and total lignin ($p = 0.001$). However, the locations that showed significant chemical differences in relation to the others were only the following: site 5A for water extractives, sites 5B and 10C for Klason and total lignin, and site 7B for glycerol.

The chemical data was also analysed after considering the location clustering by regions. The analysis of variance showed that there were no differences of cork chemical composition between regions of provenance.

The more intense analysis in two sites (5A and 8C) with a population sampling of 20 trees per site showed the results summarised in Table 4. The between-tree variation was large in both sites, but significant site differences were found for ethanol and water

extractives and for suberin content, although the absolute differences were of small magnitude.

Table 3. Mean Chemical Composition (% of oven dry mass) of the Cork Samples Collected in 29 Sites (2 trees per site) Covering the Natural Distribution of the Cork Oak Area in Portugal

Site	Extractives				Suberin			Lignin			Polysac.
	Dichlor.	Ethanol	Water	Total	LCLip	Glyc	Total	Klason	Soluble	Total	*
1A	6.0	4.9	3.3	14.2	45.3	4.8	50.1	19.8	0.9	20.7	13.1
2A	4.2	5.7	3.4	13.3	42.1	4.2	46.2	21.1	0.9	22.0	13.3
4A	6.0	4.8	3.2	14.0	46.4	4.6	51.0	19.1	0.9	20.0	6.0
5A	4.9	9.6	8.9	23.4	38.3	3.6	41.9	16.4	1.0	17.4	9.1
5B	6.0	3.9	5.7	15.6	33.1	4.0	37.1	26.9	0.9	27.8	12.7
5C	6.1	6.8	4.7	17.6	43.3	3.7	47.0	19.8	0.9	20.7	10.5
6A	5.4	6.8	3.5	15.7	39.2	3.5	42.7	23.2	0.8	24.0	13.5
6B	6.7	5.0	5.3	17.0	41.1	3.3	44.4	21.2	0.9	22.1	10.0
6C	5.3	9.6	6.9	21.8	39.9	3.0	42.9	17.6	1.2	18.8	9.4
7A	5.8	4.4	3.9	14.1	40.3	3.8	44.1	22.0	1.0	23.0	14.8
7B	6.4	5.6	4.9	16.9	37.2	2.7	39.9	21.4	1.0	22.4	15.8
7C	6.1	5.5	4.3	15.9	41.1	3.8	44.9	22.0	1.0	23.0	12.0
7D	6.5	5.9	4.7	17.0	41.6	4.0	45.6	21.0	0.9	21.9	10.6
7E	5.9	5.7	4.4	16.0	43.7	3.9	47.6	20.0	0.7	20.7	12.1
8A	5.3	4.3	3.6	13.2	45.5	4.8	50.3	21.1	0.7	21.8	7.5
8B	5.6	5.3	3.3	14.2	36.5	2.7	39.2	20.9	0.8	21.7	11.8
8C	6.3	12.1	4.7	23.1	40.1	3.8	43.9	16.1	1.1	17.2	10.2
9A	6.1	4.3	3.8	14.2	42.7	4.3	47.0	20.2	0.9	21.1	14.3
9B	5.4	7.1	4.1	16.6	46.0	3.6	49.6	19.3	0.7	20.0	11.4
10A	6.1	4.9	5.2	16.2	36.9	3.7	40.6	21.4	1.3	22.7	18.8
10B	5.4	3.8	3.7	11.9	46.9	4.7	51.6	19.2	0.9	20.1	10.7
10C	5.4	3.7	3.4	12.5	43.3	4.1	47.4	31.8	0.8	32.7	10.8
10D	5.3	8.5	4.6	18.4	38.7	3.8	42.5	18.3	1.2	19.4	9.3
11A	5.6	4.3	2.6	12.5	48.7	4.6	53.3	20.9	0.6	21.5	6.3
11B	6.0	4.2	4.2	14.4	37.9	4.1	42.0	22.1	1.1	23.2	10.8
11C	6.4	4.6	5.1	16.1	41.9	4.0	45.9	19.7	0.9	20.6	13.0
11D	6.3	4.4	4.1	14.8	48.4	3.8	52.2	19.0	0.9	19.9	11.4
13A	5.8	5.0	3.8	14.6	38.9	3.7	42.6	21.6	1.0	22.6	14.9
14A	6.9	4.4	3.9	15.2	42.8	3.8	46.6	19.8	0.9	20.7	11.8

* Polysaccharides correspond to neutral sugars after total hydrolysis

Table 4. Mean and Standard Deviation (in parenthesis) for the Chemical Composition Data of Cork Samples from Two Sites (5A and 8C, 20 trees per site)

	Site 5A	Site 8C
% od cork		
Extractives, total	16.2 (4.0)	17.7 (5.8)
Dichloromethane	5.7 (0.7)	5.8 (0.8)
Ethanol	5.6 (2.6)	7.7 (4.9)
Water	5.5 (2.2)	4.3 (1.8)
Suberin, total	44.8 (4.0)	41.7 (6.4)
Long chain lipids	42.1 (3.8)	37.9 (6.3)
Glycerol	3.9 (0.6)	3.8 (0.7)
Lignin, total	21.6 (2.9)	22.7 (4.0)
Klason lignin	20.6 (2.8)	21.7 (3.9)
Acid soluble lignin	0.9 (0.2)	1.0 (0.3)

DISCUSSION

The sampling and chemical determinations that were made in this work are the largest ones made for cork and therefore should allow a solid insight regarding the mean value and range of variation for the chemical components of cork, as summarized in Table 2. Overall, the values obtained encompass the published values for cork chemical composition (Pereira 1988; Conde *et al.* 1998). It is noteworthy that the range of variation is wide, especially regarding extractives (from 8.6 to 32.9%), but also of suberin (from 23.1 to 54.2%) and lignin (from 17.1 to 36.4%). However, the distribution of values is rather concentrated, and the coefficients of variation of the mean values are moderate (Table 2), with the largest values found for extractives and especially for the polar extractives (that may be extracted either by ethanol or water).

The known features of cork chemical composition are therefore firmly established. Suberin is the main structural component of the cell wall: in percent of the structural components (calculated as % of extractive-free cork), suberin represents, on average, 49% (ranging from 35% to 57%). Lignin is the second most important structural component of the cells with 25% of extractive-free cork (from 19% to 35%). Therefore the properties of cork should be related mostly to the combined presence of these two polymers, as has been discussed by Pereira (2007).

The ratio of suberin to lignin content was calculated with a mean of 2.0 (+/- 0.4), ranging from 3.2 to 1.0. This chemical parameter is important since it relates to the contribution of both polymers to the physical behaviour of the cells, namely under mechanical stress, *i.e.* compression resistance and dimensional recovery after stress relief. The mechanical role of both polymers should be different as a result of their different macromolecular spatial development: a) lignin is a networked 3-D polymer with numerous more or less isotropically distributed C-C and other intermonomeric bonds, which should be responsible for the resistance values under compression; b) suberin is a polymer with an anisotropic molecular development that includes glyceridic linked long-chain aliphatics forming rather flexible planar ribbon-like structures, and is the preferential contributor to the elasticity and relaxation properties (Pereira 2007). It is also probable that both components contribute at differing degrees to permeation and diffusion of liquids and gases through cork.

It is proposed here a chemical parameter relating to the macromolecular structure of suberin: the ratio of the long-chain lipids and the glycerol content (LCLip:Gly). This ratio is important since it relates to the spatial development of the suberin macromolecule. The mass ratios that were calculated (ranging from 8.2 to 14.5, Table 2) correspond to approximate mean molar ratios of 3.2, with a range from 2.3 to 4.1. For polymerization, glycerol has three functional groups, while most of the long-chain lipids are bifunctional, and a small amount have only one functional group (Pereira 2007). Therefore higher values of LCLip:Gly should correspond to a higher proportion of LCLip-intermonomeric linkages that will result into a higher molecular mobility leading to higher flexibility of the structure.

Taken together, all the structural chemical features of cork show a variability in the composition of the cell wall that may explain, or contribute to explain, the variability that is found in the performance of cork products, namely of cork stoppers.

Another chemical characteristic of cork is the significant proportion of extractives (Table 2). This was also previously reported by several authors, and has been the rationale for various proposals for the use of cork as a source of extractable chemicals

(Pereira 2007). The non-polar extractives that include long-chain lipid molecules (fatty acids and alcohols) and triterpenes (*i.e.* cerin, friedelin) represented, on average, 38% of the total extractives (range from 52% to 18%). There was a trend to have the lowest proportion of non-polar compounds when total extractives content was higher. The more polar extractives (ethanol and water solubles), which include mostly low and high molecular mass phenolic compounds, represented the majority of the potentially solubilisable compounds from cork (Cadahia *et al*. 1998; Conde *et al.* 1997; Mazzoleni *et al.* 1998). This is also a chemical factor that may be involved in the natural variability of cork performance, *e.g.* in the cork stoppers role in bottle wine aging.

As regards to the chemical variation in relation to the geographical location of cork production (Tables 1 and 3), statistically significant differences were found only in a few cases when considering site and none when considering regions. This is in accordance with the findings of Conde *et al.* (1998) for seven provenances.

Variation between trees seems to be the major factor of differences. Between-tree variation in a site was important (Table 4), and even if the two studied sites were statistically different in relation to polar extractives and suberin, the absolute difference of the mean values was of small magnitude. The major role of genetics in cork oak growth and cork formation has been repeatedly stressed leading to large between-tree variation in cork features (Pereira 2007). Similar findings were obtained in Spain (Conde *et al.* 1998).

The provenance differentiation of cork based on chemical composition therefore does not appear possible, unless some compositional differences or compound markers (for instance in the nonpolar or polar extractives) are found. This study was not addressed here. However, given the large variability found in the content of extractives between samples and their potential for resource valorisation, such study constitutes an interesting further research line.

CONCLUSIONS

1. The mean chemical composition of cork and its range of variation were firmly established in this work, and covered the natural variability of *Quercus suber* cork's chemical composition.
2. Suberin is the main structural cell wall component of cork, and lignin is the second structural component. The properties of cork largely rely on these two polymers, and their proportional variation should account for the natural variability that is found in cork properties.
3. Cork has a high content of extractives that show a wide range of values, especially regarding the polar components. Such variability is also a factor that may influence some performance differences of cork.
4. There is a natural variation in the chemical composition of cork that could not be traced back to geographical origin of production, and differences between individual trees are the most important variation factor.

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