

STUDY OF THE VARIABILITY IN CHEMICAL COMPOSITION OF BARK LAYERS OF *QUERCUS SUBER* L. FROM DIFFERENT PRODUCTION AREAS

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Cork is the bark of the cork oak tree (*Quercus suber* L), a renewable and biodegradable raw bioresource concentrated mainly in the Mediterranean region. Development of its potential uses as a biosorbent will require the investigation of its chemical composition; such information can be of help to understand its interactions with organic pollutants. The present study investigates the summative chemical composition of three bark layers (back, cork, and belly) of five Spanish cork samples and one cork sample from Portugal. Suberin was the main component in all the samples (21.1 to 53.1%), followed by lignin (14.8 to 31%), holocellulose (2.3 to 33.6%), extractives (7.3 to 20.4%), and ash (0.4 to 3.3%). The Kruskal-Wallis test was used to determine whether the variations in chemical composition with respect to the production area and bark layers were significant. The results indicate that, with respect to the bark layer, significant differences were found only for suberin and holocellulose contents: they were higher in the belly and cork than in the back. Based on the results presented, cork is a material with a lot of potential because of its heterogeneity in chemical composition.

Keywords: Bioresource; *Quercus suber* L; Chemical composition; Bark layers; Suberin; Lignin; Holocellulose; Extractives

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INTRODUCTION

Cork is the bark of the cork oak (*Quercus suber* L.) and is a natural, renewable, and biodegradable raw material. The periodic extraction of cork (stripping) is fundamental to the environment and to the economic and social sustainability of some rural areas in the Mediterranean basin. Because trees are not cut down, cork oak harvesting is an environmentally friendly process: the bark is stripped for the first time when the tree is 20 to 25 years old; afterwards the stripping is carried out every 9 to 10 years in most regions and every 12 years in Catalonia (Costa et al. 2003). The lifespan of a cork oak tree ranges from 170 to 200 years; therefore the tree may be stripped around 15 to 18 times.

Cork forests cover an area of almost 2.2 million hectares, concentrated mainly in the Mediterranean region: Portugal, Spain, southern France, part of Italy (Sicily and Sardinia), Algeria, Morocco, and Tunisia. These areas have the weather conditions necessary for its growth – dry summers and mild winters (Riboulet et al. 1986) – and their cork oak habitats have been ranked among the most valuable in Europe and are

listed in the EC Habitats Directive. Cork forests prevent desertification and are home to many species of animals and plants (Gil 2009).

Europe has about 60% of the world's cork forests and accounts for over 80% of global cork production (Barberis et al. 2003). Cork is a suitable material for wine bottle stoppers. In fact, production of cork stoppers for wine represents almost 70% of the cork market. Besides the production of cork stoppers, the annual production of cork waste reaches around 50,000 tons, corresponding, on average, 25 to 30% of the quantity used in cork stopper manufacturing (Silva et al. 2005). In this study, the cork samples used came from remaining cork obtained after punching out cork strips to obtain cork stoppers.

Cork is mainly composed of lignin and suberin (hydrophobic biopolymers) together with the hydrophilic polysaccharides cellulose and hemicellulose. Suberin consists of a polyester structure composed of long chain fatty acids, hydroxy fatty, and phenolic acids, linked by ester groups (Pereira 2007). According to the model proposed by Kolattukudy (1980), the aromatic domain of suberin is bonded to the lignin/cellulosic matrix. This heterogeneous chemical composition provides numerous bonding possibilities for a wide range of pollutants (Barrera-Garcia et al. 2008; Chubar et al. 2003; Domingues et al. 2005, 2007; Karbowski et al. 2010; Machado et al. 2002; Psareva et al. 2005; Olivella et al., submitted for publication a; Villaescusa et al. 2000).

The distribution of these biopolymers is quite variable within the cork bark due to the different characteristics of its bark layers (back, cork, and belly). The possibility of selecting high proportions of lignin, suberin, and/or polysaccharides from the different cork bark layers makes this a very interesting natural raw material to explore biopolymer/pollutant interactions and a promising biosorbent for pollutants. However, the characterization of the chemical composition of bark layers is needed to determine potential uses.

Although the chemical composition of cork has been studied since the end of the 18th century (Conde et al. 1988; Holloway 1972; Pereira 1988a; Pinto et al. 2009), the chemical composition of the different layers of the bark has yet to be studied. The purposes of this study were: (1) to analyze the chemical composition (ash, extractives, lignin, suberin, and holocellulose) of three different bark layers (back, cork, and belly) of five Spanish cork samples and one cork sample from Portugal; and (2) to determine the chemical composition variation in relation to the different origins of cork production and the bark layers using the non-parametric Kruskal-Wallis test.

EXPERIMENTAL

Materials

Reagents and samples

For standard solutions and batch experiments deionized water was used. All solvents used were Super Purity grade from Romil (Cambridge).

Selected samples came from the cork strips supplied by a cork factory after stoppers had been punched out of them. The cork strips were cut into three sections with a hand saw: the back (the outermost bark), 6 to 10 mm thick; the cork (the middle part, used for cork stoppers), 26 to 32 mm thick; and the belly, 3-5 mm thick. The back was

previously scraped with a knife. Each section was separately cut into small pieces (< 10 mm), ground with a ZM-200 ultra centrifugal mill (Retsch, Netherlands), and filtered with a sieve shaker (Cisa, Spain) to obtain granulometric fractions of 40 to 60 mesh (0.25 to 0.42 mm grain size) that were used for the subsequent analyses. The three analyzed bark layers came from different production areas: five from Spain and one from Portugal (Table 1). A total of eighteen samples were analyzed. The cork thickness of the cork strips and of each bark layer were measured before the chemical analyses.

Table 1. Geographical Origin and Code Samples

Geographical origin	Region	Sample code
Catalonia	Girona	CAT
Castilla de Mancha	Ciudad Real	CMANCH
Extremadura	Badajoz	EXT-4
Extremadura	Cáceres	EXT-6
Portugal	Alentejo	POR
Toledo	Talavera de la Reina	TOL

Methods

Chemical composition

The summative chemical analyses included the determination of ash, extractives, suberin, Klason lignin and acid soluble lignin, and holocellulose. All experiments were performed in duplicate. The ash content was determined by incinerating 2 g of cork at 525 °C during 1 h with a muffle furnace (Faenza, Italy). Extractives were removed by successive Soxhlet extractions with dichloromethane (6 h), ethanol (8 h), and hot water (20 h). After each extraction step the solution has been evaporated and the solid residue was weighed with an analytical balance (Sartorius, Germany).

The suberin content was determined in extractive-free material by methanolysis for depolymerization (Pereira 1988a). A 1.5 g sample of extractive-free cork was refluxed with 250 mL of 3% NaOCH₃ in CH₃OH during 3 h, followed by solid residue filtration, and then refluxed with CH₃OH during 15 minutes. After filtration, the combined liquid fractions were mixed, acidified with 2 M H₂SO₄ to pH 6, and evaporated to dryness in a rotating evaporator (Aircontrol, Spain). This residue was suspended in 100 mL H₂O and extracted with 100 mL CHCl₃ three times. The combined extracts were dried over Na₂SO₄, filtered, evaporated, and determined gravimetrically as suberin. Polar suberin monomers such as glycerol are not extracted with chloroform, and remain in the aqueous phase. The nonsaponifiable fraction (the desuberized fraction) was used for subsequent analyses. Klason lignin or acid-insoluble lignin were determined after acid hydrolysis with 72% H₂SO₄ at 30 °C during 2 h, dilution with water, and boiling for 4 h (TAPPI 222). The residue was washed with hot water, dried, and weighed as Klason (acid insoluble) lignin. The filtrate was used to determine acid soluble lignin by measuring the absorbance at 205 nm using a UV-vis spectrophotometer (Dinko, Spain). Klason lignin and acid-soluble lignin were combined to give the total lignin content.

Holocellulose was isolated from the desuberized fraction by delignification for 2 h using the acid chloride method (Wise et al. 1946). The holocellulose content was determined after measuring the Klason lignin content in holocellulose.

All measurements were reported as a percentage of the original sample.

Statistical analysis

Standard error was used to indicate the variability in the chemical composition data among the duplicates. Differences in the percentages of chemical components with respect to different areas of cork production, and bark layers were analyzed using the non-parametric Kruskal-Wallis test and the SPSS 15.0 software package.

RESULTS AND DISCUSSION

The average thickness of cork strips, back layers, and belly layers were similar in all samples because the separation of the three anatomical parts (back, cork, and belly) was based on the same criterion for cork punching from strips – the one required for cork stopper production (i.e. the thickness of the back should be higher than 10 mm and the upper belly at 3 mm) based on the final diameter of the stopper (24 mm) (Fig. 1).

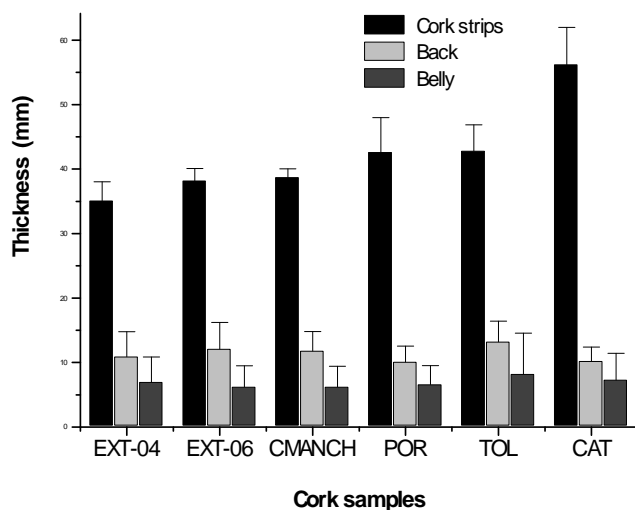


Fig. 1. Thickness of cork samples, depending on sample source and location within the bark layer

The cork strips used were cut with the same thickness – the one required for cork stopper production based on the final diameter of the stopper. The measurement of the thickness of each layer is important to maintain the distinction and not mix them. The greatest thickness was observed in the CAT sample (56.2 mm) because in Catalonia the stripping is carried out two years later than in other areas due to edafo-climatic conditions and tree density.

Results for chemical analysis of the three bark layers from the different production areas are shown in Table 2.

Table 2. Chemical Composition of *Quercus suber* L. of Three Bark Layers (belly, cork and back) from Different Production Areas. (Values in brackets are standard deviations of duplicate experiments.)

Cork samples/ Bark layers	Ash content (%)			Total Extractives content: Dichloromethane			Suberin (%)			Total lignin content: Klason lignin Soluble lignin (%)			Holocellulose content (%)		
	Belly	Cork	Back	Ethanol			Belly	Cork	Back	Belly	Cork	Back	Belly	Cork	Back
				Belly	Cork	Back									
EXT-4				6.8 (0.6)	5.2 (0.5)	1.8 (0.3)				14.3 (7.2)	13.4 (7.2)	17.4 (2.6)	7.3 (1.8)	15.1 (0.6)	33.6 (1)
				4.7 (0.7)	2.3 (0.5)	1.6 (0.1)				0.6 (0.2)	2.8 (0.5)	1.5 (0.5)			
	1.0 (0.0)	0.7 (0.0)	2.1 (0.4)	7.1 (0.2)	7.8 (0.1)	7.3 (0.9)	40.1 (5.7)	38.2 (0.4)	22.7 (2.5)						
EXT-6				2.3 (0.2)	2.6 (2.3)	1.2 (0.8)				30.1 (1.1)	24.3 (6.7)	25.6 (2.9)	2.3 (0.2)	7.1 (1.1)	10.1 (0.3)
				2.0 (0.2)	2.1 (1.4)	1.1 (0.3)				0.9 (0.3)	1.0 (0.1)	2.4 (0.5)			
	1.0 (0.0)	0.9 (0.0)	1.8 (0.0)	8.3 (0.1)	10.5 (2.6)	9.6 (1.0)	33.5 (3.1)	38.7 (0.1)	21.1 (0.5)						
CMANCH				0.2 (0.0)	4.0 (1.9)	4.8 (0.4)				15.4 (1.4)	21.4 (3.9)	23.1 (2.5)	6.6 (0.7)	10.3 (0.6)	12.6 (0.2)
				1.3 (1.4)	2.6 (0.6)	1.6 (0.2)				1.2 (0.0)	1.3 (0.7)	2.0 (0.0)			
	1.7 (1.3)	0.4 (0.0)	1.8 (0.4)	9.0 (2.2)	5.1 (0.7)	6.5 (0.6)	53.1 (1.9)	48.7 (0.6)	33.8 (1.1)						
POR				5.6 (0.1)	7.4 (2.7)	5.4 (0.2)				24.4 (3.0)	13.4 (1.1)	23.0 (1.1)	5.0 (0.2)	7.4 (1.3)	8.6 (0.2)
				2.1 (0.1)	2.7 (0.1)	1.6 (0.3)				1.3 (0.6)	1.2 (0.4)	0.8 (0.0)			
	0.8 (0.1)	0.5 (0.0)	1.9 (0.1)	8.7 (3.2)	9.7 (0.3)	4.9 (1.2)	44.1 (4.0)	41.3 (8.3)	40.7 (2.3)						
TOL				5.6 (0.3)	5.3 (0.2)	4.7 (0.4)				17.4 (2.6)	21.9 (1.5)	21.0 (1.2)	7.6 (1.2)	10.2 (0.6)	15.0 (0.3)
				1.9 (0.2)	2.7 (0.1)	2.1 (0.1)				0.7 (0.0)	0.9 (0.1)	2.4 (0.6)			
	1.5 (0.1)	1.3 (0.0)	1.9 (0.1)	12.1 (0.6)	3.1 (0.8)	6.9 (0.1)	38.3 (3.5)	39.1 (2.9)	32.0 (0.8)						
CAT				5.2 (0.8)	8.4 (2.6)	3.1 (0.3)				16.7 (0.3)	23.1 (7.5)	24.2 (3.0)	5.7 (0.3)	15.8 (4.9)	23.6 (1.6)
				2.0 (0.0)	3.5 (0.0)	0.6 (0.2)				0.7 (0.3)	1.1 (0.1)	1.3 (0.2)			
	1.4 (0.1)	1.3 (0.0)	3.3 (0.1)	6.0 (0.0)	5.9 (0.2)	3.7 (0.3)	45.5 (2.7)	34.4 (6.0)	30.6 (2.7)						

The ash content ranged from 0.4% (CMANCH cork) to 3.3% (CAT back). The back had the highest ash content of all samples. The amount of ashes in cork and belly were below 2%, which is in range of values reported in hardwoods from temperate zones and softwoods (Fengel and Wegener 1983).

Cork extractives include n-alkanes, n-alkanols, waxes, triterpenes, fatty acids, glycerides, sterols, phenols, and polyphenols. They are classified in two groups: aliphatics or commonly named cork waxes that are solubilized with low-polarity solvents (e.g. hexane, dichloromethane, chloroform) and phenolics extracted by polar solvents (e.g. ethanol and water) (Pereira 2007).

In this study, the phenolic extractives (which ranged from 3.1% to 12.9%) were dominant over the aliphatic extractives (which ranged from 0.2% to 8.4%) in all samples.

Suberin is the primary structural component of the cork cells, representing around 50% of the total chemical composition. However, in the determination of suberin, only the long-chain fatty components were included, and the glycerol, which represents about 10% (Amaral et al. 2004), was excluded. The highest suberin contents – exhibiting percentages higher than 45% – were found in the belly and cork from Ciudad Real (CMANCH) and in belly from Girona (CAT); the lowest suberin contents were in the backs, particularly in samples from Extremadura. The suberin content in *Quercus suber* was found to be higher than in other species: 33% in *Pseudotsuga menziesii* (Graça and Pereira 2000), 28% in *Quercus cerris* (Sen et al. 2010), and 5% in *Calotropis procera* (Pereira 1988b).

With respect to the Klason lignin content, the sample with the highest content was EXT-6 (30.1%), and the samples with the lowest values were from EXT-4 and POR (13.4%). The percentages of soluble lignin were less than 3%. It was also observed that in most of the samples the total lignin content was higher in the back than in the belly and the cork.

The holocellulose content ranged from 2.3 to 33.6%, and appreciable differences could be observed among the three bark layers; an increase was observed following the order belly < cork < back. The holocellulose of cork represents a small fraction of the cork cell (in wood it corresponds to about 70%).

The averages of the percentages of the chemical components of the three layers are shown in Fig. 2. The average chemical components found in increasing order were suberin, ranging from 21.1% (EXT-6) to 53.1% (CMANCH); total lignin ranging from 16.7% (EXT-4) to 28.1 (EXT-6); holocellulose, from 6.5% (EXT-6) to 18.7% (EXT-4); total extractives, from 11.7% (CMANCH) to 16% (POR); and ash, from 1.1% (POR) to 2% (CAT).

Recent determinations of cork's chemical composition from samples with different geographical origins in Portugal (with a total of 58 cork samples) (Pereira, 2007) showed the following: total extractives 15.9% (range 10.7 to 29.7%); suberin 41.2% (range 22.1 to 51.7%); and lignin 20.4% (range rather low). These results are in the range to those obtained in the present work. A study on the chemical variation of cork from different provenances in Spain showed the following results (Conde et al. 1998): total extractives 18.4% (range 16.5 to 21%); lignin 22.7% (range 21.5 to 24.3%); and holocellulose 25.9% (15.3 to 34.3%). The suberin content determined in this work, due to the experimental procedure, cannot be used for comparison.

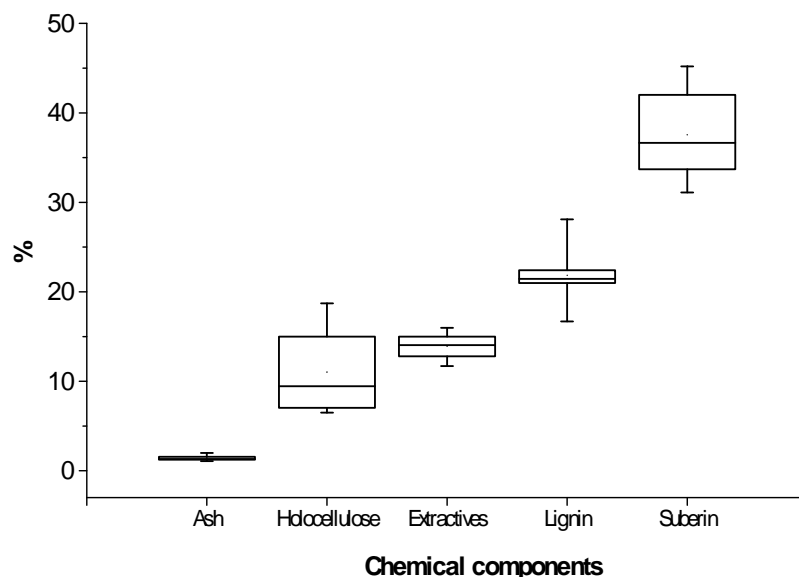


Fig. 2. Ranges of chemical component contents for the studied cork samples

A study on the chemical variation of cork plank, in which the scrape of the outermost back and cork were analyzed, showed the following results (Pereira 1987): ash 9.8% and 1.3%, respectively; total extractives 8% and 16%, respectively; suberin 4.3% and 39.4%, respectively; and lignin 32.5% and 33%, respectively. It is important to clarify that in our study the most crumbly surface was not analyzed, because this part is in direct contact with the environmental pollution. From the point of view of sorption studies it is obvious that the blanks should be free of contamination with interferences. Thus, compared with our study the higher variability in terms of ash, suberin, and extractives content is justified by the different criteria of separation of the three anatomical parts (back, cork, and belly).

In spite of the range of values found for each component, it is clear that cork's chemical composition is marked by a high suberisation degree (potato periderm has about 17% of suberin on a total dry basis) and by a lignin content similar to the values found in hardwoods. The suberin content in *Quercus suber* is higher than in other species: 33% in *Pseudotsuga menziesii* (Graça and Pereira 2000), 28% in *Quercus cerris* (Şen et al. 2010), and 5% in *Calotropis procera* (Pereira 1988b). Cellulose and hemicelluloses represent a small fraction of the cork cell wall (in wood they correspond to about 70%).

The results of the Kruskal-Wallis test indicate that there were no significant differences ($p > 0.05$) among the groups of suberin, lignin, holoallulose, and extractives with respect to the different areas of origin (Table 3). In contrast, there were significant differences ($p < 0.05$) between the groups of suberin and holoallulose with respect to the bark layer. In addition, suberin content in belly and cork was found to be higher than in the back, but in the case of holoallulose the opposite was true: the holoallulose content decreased from the back to the belly.

Table 3. Results of Kruskal-Wallis Test. (Groups significantly different ($p < 0.05$) are marked with an asterisk "**".)

Chemical Components	Production area		Bark layer	
	p- value	d.f. ^a	p- value	d.f. ^a
Suberin	0.077	5	.001*	2
Lignin	0.143	5	0.366	2
Holocellulose	0.065	5	.000*	2
Extractives	0.349	5	0.864	2

In the context of searching for high value-added applications of cork by-products, data obtained in this study can be helpful to optimize the efficiency of sorption based on the selection of the most suitable cork sample for the class of pollutants to be sorbed and to establish mathematical models to predict the adsorption capacity.

CONCLUSIONS

Characterization of the chemical composition of bark layers (belly, cork, and back) of cork strips, a by-product of the cork industry, from five Spanish samples and one Portuguese sample was performed for first time. Results obtained indicated that:

1. In all three bark layers suberin was the main chemical component, followed by lignin.
2. The suberin content was higher in the belly and cork than in the back; and holocellulose and lignin contents were higher in the back than in the cork and belly.
3. The major variability among samples was found to be for suberin and holocellulose.
4. No significant differences were found in the chemical composition with respect to the different production areas from which the samples were obtained.
5. Significant differences were found for suberin and holocellulose contents with respect to the bark layer, while no significant differences were found for lignin with respect to bark layer.
6. Given the heterogeneity of chemical compounds found among bark layers it is recommended for future sorption studies to separate the bark layers to ensure a good reproducibility of the results obtained.

The study presented here provides a basis for studies focused on understanding the role of the chemical components of cork in the sorption of organic pollutants (Olivella et al., submitted for publication b). The characterization of the bark layers help to optimize the efficiency of sorption based on the selection of the most suitable cork sample for the class of pollutants to be sorbed.

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