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STUDY OF CORK FROM *QUERCUS SUBER* L. WITH AND WITHOUT YELLOW SPOT: AROMATIC FRACTION AND CELLULAR STRUCTURE

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Abstract: The effect of the presence of yellow spot in cork from *Quercus suber* was studied in relation to volatile compounds and the influence on the cellular structure to better understand cork as a material. The volatile compounds from cork were analyzed using thermal desorption combined with gas chromatography (TD-GCMS). This methodology had identified 103 compounds in cork samples with and without yellow spot. All the samples showed compounds from different families according to its chemical structure such as aromatic compounds (36), carboxylic acids (11), aliphatic alcohols (9), alkanes (9), aliphatic aldehyde (8), chlorate compounds (5), ester (5), terpene (5), alkene (4), furane (4) and aliphatic ketone (1). Vanillin was the most abundant compound followed by isoeugenol. Chlorinated compounds such as 2,4,6-Trichlorophenol, 2, 4-Dichlorophenol and 2,4,6-Trichloroanisole have only been identified in cork with yellow spot. This study showed differences in aromatic cork profile according to the presence or absence of yellow spot. Cellular structure of cork seems to be influenced by the presence of yellow spot due to the appearance of changes in the level of cell corrugation and the degree of separation of the layers of cell wall. These results allow to expand the knowledge about changes produced in cork by the presence of yellow spot.

Keywords: *Quercus suber* L, cork, yellow spot, volatile compounds, cellular structure

INTRODUCTION

The cork oak (*Quercus suber* L.) is a dominant species of some agroforestry systems in Iberian Peninsula (Portugal and Spain, mainly). Cork oak landscape is integrated in one of the 34 "hotspots" of biodiversity worldwide because of it is the habitat for many species. Moreover, cork oak management is one of the best examples of sustainability due to its environmental, economic, and social functions. Nowadays, cork forest is economically sustained by the production of cork. It is a versatile raw material that be able to use in different applications due to its combination of properties. The cork oak forest supports hundreds of companies and employs thousands of people since it is the origin of highly valuable products such as cork stoppers.

Cork is mainly used to obtain cork stoppers or closures for still and sparkling wine due to its beneficial properties: compressibility, elasticity, impermeability to air and liquid and the capacity to adhere to a glass surface. These properties are the result of the combination of its chemical composition and cellular structure [1,2]. Because of these set of properties, cork is a material which has been used for multiple applications. The use of this material in different sectors contributes to the maintenance of cork oak landscapes biodiversity, ensures a significant retention of carbon, participates in the regulation of the hydrological cycle and help to prevent the advance of soil desertification.

Cork undergoes through different quality controls to ensure the smooth functioning of cork stoppers. Thus, the first step to high the cork adds value is classified it: cork suitable for cork stoppers production and cork not suitable. After extracting cork bark and before starting the phases of cork stoppers manufacturing, a visual check of cork slabs is done because occasionally, cork may include features of biological or external origin that impact the

quality to a degree that depends on their type and extent [3]. Fractures, inclusions, or stains such as yellow spot (YS) are some of them. In industrial manufacturing only the best quality slabs of cork are used, and every natural cork stopper produced is visually checked and graded into several visual classes. ISO 17727:2012 describes the quality control sampling plans for the receipt and shipping of ready-to-use cylindrical stoppers in semi-worked or finished cork used for still wines. The cork not suitable for cork stoppers production like cork with YS, can be the raw material in other cork sectors such as constructions due to its amazing properties [3]. So, the characterization of cork not suitable for cork stoppers production is an essential step to evaluate other future cork applications.

The presence of YS is a defect caused by the presence of *Armillaria mellea* or a basidiomycete that grows in ligninolytic materials [3-5]. In cork, this fungal attack causes the presence of regions that acquire a white-yellowish discoloration with a characteristic odour. Chemical and physical changes in cork with YS are also reported [6,7]. These changes could affect mechanical and structural properties of cork and, consequently, it may be closely related to the production of aromatic compounds [4, 7-9]. The effect of the presence of YS in chemical composition of cork is already described attributing lignin and polysaccharides content decreases: corks with YS contain a smaller amount of lignin and pectic polysaccharides that is responsible for changes in the cork cell wall [5,6]. These changes in the chemical composition are responsible for the differences in cellular structure between cork with and without YS (or standard cork): the cellular structure of cork with YS is composed by deformed and wrinkly cells with cell wall separation due to the degradation of the middle lamella pectic polysaccharides [5]. This fact contributes

to the availability of a nutrient source in the attacked cork that can be used by other microorganisms [5].

The study of the volatile compounds presents in attacked cork allows researchers to further the identification of chemical modifications that occurs in cork polymers. The degradation of lignin seems to be related to the presence of the largest concentration of phenols, vanillin, benzaldehyde, benzyl alcohol and chlorinated compounds [4]. Also, cork with YS contains other volatile components resulting from microbial metabolism with possible consequences in the cork aroma composition [10].

Several compounds have been reported in cork samples such as alcohols, aldehydes, aliphatic hydrocarbons, ketones, esters, ethers, furans, among others [4,7, 11-15]. Some of them are associated with the presence of specific microflora on the tree and/or cork slabs [4, 16-18] and others are the result of reactions related to the reestructuracion of some macromolecular compounds such as lignin [4,7,8] and possibly also suberin [19, 20]. It seems that the identified compounds are different according to the cork sources for example between the case of cork bark compared to cork stoppers [12]. The study of the volatile compounds of standard and attacked cork using thermal desorption coupled to GCMS will bring another viewpoint to understand the changes brought about by the presence of this defect in cork.

The aim of this study was to compare the changes in aromatic profile and cellular structure between cork with and without YS. The results will allow us to better understand the relationship between the chemical composition of cork and cellular structure. As more as, the aromatic fraction of cork with YS would be used in other sectors such as cosmetic, food or pharmaceutics, increasing the added value of attacked cork.

MATERIALS AND METHODS

SAMPLE PREPARATION

The effect of the presence of YS in cork from *Quercus suber* L. was evaluated by analyzing ten slabs with YS and ten slabs without YS (or standard cork) from the same geographical origin (Figure 1). All samples were 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). The replicates were integrated to obtain 2 types of granulated cork that were used for the subsequent analyses. All samples were obtained from JVIGAS, S.A.

THERMAL DESORPTION EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (TD GC-MS) ANALYSIS

0.5 g of sample was placed in an empty stainless steel thermal desorption tube (6 mm O.D. × 90 mm long, 5 mm I.D., Markes International Limited, Pontyclun, UK) and introduced into a desorption unit (DU). Each tub was heated at a range of desorption temperature (from 80°C to 220°C) for 10 minutes. The extracted compounds were focused on a cold trap and were sent to the GC column following the methodology described by Jové et al., 2021 [13].

GC-MS was performed with an Agilent 6890N chromatograph equipped with a Gerstel MPS2 autosampler and coupled to an Agilent 5973N mass spectrometer. The separation was achieved using an HP-5MS column (30 m, 0.25 mm, 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA) and a GC oven program starting at 50°C (3 min), increased by 6°C min^{-1} to 325°C (held for 20 min). The carrier gas was helium (99.999 %) from Abello Linde (Barcelona, Spain) with a constant flow rate of 1 mL min^{-1}. The transfer line temperature was set at 300°C and the ion source temperature at 250°C. All mass spectra were acquired in the electron impact of 70 eV. The mass range was 35-600 m/z, with a scan rate of 6 scans s^{-1}. The analysis was performed in full scan mode. Extracted volatile organic compounds (VOCs) were identified by comparing the MS fragmentation of each compound in sample with the mass spectra present in the National Institute of Standards and Technology (NIST08) reference database version. The identified volatile compounds have a match quality greater or equal to 75 when compared the mass spectrum of those in the NIST08 spectrum library. The results were expressed as the percentage of the peak area of each extracted compound in relation to the sum of the total area for each sample.

SCANNING ELECTRON MICROSCOPY (SEM)

A granulometric fraction of 40 to 60 mesh from samples of cork with and without YS were cut using a sterile blade to obtain square chips. The SEM samples have been placed on a stub and evaporated carbon (Emitech, German, K950 turbo evaporator). Examinations were carried out with a scanning electron microscopy FE-SEM Hitachi, Japan, S-3900. Digital images were collected and processed by Quarz PCI program.

RESULTS AND DISCUSSION

The effect of the presence of YS in cork from *Quercus suber* was studied in relation to volatile compounds and cell wall structure. The volatile compounds of cork susceptible to be extracted with TD-GCMS in samples with and without YS are presented in Table 1. In this way, 103 compounds of different chemical natures were identified. The compounds were classified in 11 families: aromatic compounds

(36 compounds), carboxylic acids (11 compounds), aliphatic alcohols (9 compounds), alkanes (9 compounds), aliphatic aldehyde (8 compounds), chlorinated compounds (5 compounds), ester (5 compounds), terpene (5 compounds), alkene (4 compounds), furane (4 compounds) and aliphatic ketone (1 compounds). Most of them have been described previously in cork samples (Table 1) but not others because the extraction composition of cork varies depending on the methodology used [19]. Certain methodologies applied to study cork profile have been used solvents at various extraction phases to promote depolymerization phases and finally, to deal with the complexity of chemical components present into cork [14, 19, 37, 43]. The extraction with temperature may lead to compounds with a high volatility extraction instead of compounds with different polarities as in the case of use of solvents.

Volatile compounds mentioned in table 1 could come from the depolymerization of cork components such as suberin and lignin [18] and/or by the effect of microbial degradation, mainly being fungus that can grow in cork planks [21-23]. Aromatic profile is quite different between standard cork and cork attacked by YS. Figure 1 shows the percentage of each family and the number of compounds in each one. Standard cork showed more extracted compounds (80 compounds) than cork with YS (69 compounds). In general, the presence of YS decreased the percentage of aromatic compounds and its number of extracted compounds compared with the cork without YS. Moreover, cork attacked by YS had highest percentages of alkenes, carboxylic acids, furanes, aliphatic aldehyde, aliphatic ketones and chlorinated compounds.

The presence of YS produces a decrease in the percentage of lignin related compounds and an increase of the percentage of compounds related to microorganisms' activity. Many compounds obtained from the degradation of lignin are part of the aromatic compound fraction. Vanillin or the aromatic compounds that has the greater impact on the overall aromatic profile of cork, were found in smallest percentages in cork with YS. Vanillin is widely used as flavoring agent and fragrance ingredient in food or cosmetic and, also it uses to produce other useful compounds such as veratraldehyde, protocatechualdehyde and their respective acids [24, 25]. The global market for vanillin is estimated as high as 16 thousand t/year. The production of pure natural vanillin is estimated around 40 t/year and the rest is not obtained from lignin-based vanillin [26]. The same applies to isoeugenol or the second most abundant aromatic compound. Acetophenone was only detected in cork with YS, and it is reported to be formed by microbial lignin degradation in cork (4). Other aromatic compounds such as Dimethoxyethyl phthalate, ethyl-vanillin or benzoic acid were present only in cork without YS

Cork with YS also showed a decrease in the percentage of aliphatic alcohols that are compounds mainly obtained by depolymerization or degradation of suberin and waxlike fraction. Suberin is a complex polymer of long aliphatic units [3, 27]. This is made up mostly of long chain hydroxycarboxylic acids [19] but also contains: fatty alcohols, fatty acids, ω-diacids and phenolic compounds [19,28]. Table 1 shows that the most abundant long chain fatty alcohols in both samples were doconasol and dotriacontanol. Hexadecanol and octadecanol were found in smaller percentages in samples with YS and eiconasol was only detected in YS cork. Carboxylic acids also are compounds mainly obtained by depolymerization or degradation of suberin and waxlike fraction. Cork waxlike fraction includes the presence of saturated and unsaturated fatty acids such as oleic and linoleic acid [29]. Linoleic acid was

detected in cork with YS in larger percentages and oleic acid was only detected in samples without YS. Contrary as in the case of aliphatic alcohols, the percentage of carboxylic acids is lower in standard cork.

Suberin and waxlike fraction were susceptible to degradation producing the corresponding aldehydes, alcohols, ketones, alkanes and alkenes [4, 14, 19]. This degradation may occur either by its autoxidation or by lipoxygenase activity [4]. Cork with YS showed higher percentages of aldehydes, ketones and alkenes than cork without YS. In the case of aliphatic aldehydes, pentanal, pentadecanal and tridecanal were only extracted from cork with YS and in high percentages. Microbial degradation of fatty alcohols also produces ketones such as nonadecanone, which was found in higher percentages in cork with YS than in cork without YS. In the case of ketones, samples with YS showed more variety of these compounds and higher percentages of pentatriacontene and eicosane, the dominant extracted ketones.

Furane compounds result from carbohydrate degradation and, sometimes, it could be an artifact originating from the applied methodology due to the application of temperature. Despite this, samples with YS exhibited a higher percentage of 6-Methoxy-3-methylbenzofuran than standard cork. Ester compounds were specially manifested in standard cork (Figure 1). The percentage of 2 ethylhexexyl salicylate or an ester of salicylic acid derived from benzoic acid, was lower in cork with YS when compared to standard cork. This could be because salicylic acid and other benzoic acid derivatives inhibited mycelial growth of some microorganisms [30]. It should be noted that benzoic acid was also detected in standard cork. More studies are necessary to verify if there is a relationship between the presence of benzoic acid and its derivatives and the presence of yellow stain on cork. On the other hand, salicylic acid is a regulatory compound of cells responsible of suberization process [31].

Certain chlorinated compounds such as cis-1-Chloro-9-octadecee and 1,2,4,5-Tetrachloroanisole (TeCP) were detected in both cork samples but, the other compounds from this family: 2,4-dichlorophenol (DCP), 2,4,6-Trichloroanisole (TCA) and 2,4,6-Trichlorophenol (TCP) were only detected in cork with YS as can be seen in Table 1. TeCA showed the highest percentage of chlorinated compounds, but it was found at highest levels in samples with YS. Surprisingly, samples of cork affected by YS had more content of TeCA than TCA. TCA is the main component responsible for cork taint in food and beverages. Its origin has been the subject of several publications [32-40] and it can originate from several sources. Certain publications exposed that TCP seems to be a possible precursor [21, 22, 33, 38]. The possible origins of TCP were also widely discussed, and one option is that TCP could be produced naturally from phenolic compounds of cork (or lignin related compounds) and then chlorinated [4]. Microbial growth of samples with YS could promote chemical modification in cork polymers which may contribute to the appearance of lignin degradation compounds that are later chlorinated [4, 32]. A study based on voltammetry assay showed the occurrence of an important peak that may be assigned to lignin related phenolics and which confirmed that lignin degradation may occur in cork with YS [7]. Assays with electronic tongues also found that the content of total phenolics in YS samples was approximately two times higher than in standard cork [8]. The decrease in lignin content has also appeared in the lower percentage of aromatic compounds observed in the sample with YS (Table 1) and in other studies that have described a decrease of lignin and polysaccharides and an increase of polar extractives in samples with YS [3,6].

Finally, the obtained results demonstrated that the used methodology is not useful to detect terpenes in cork probably due to its low volatility. One of the main terpenes present in cork is friedelin that was not found in this study. Terpenes are produced by plants. In cork, terpenes showed a great diversity in accordance with the origin of cork granules [14, 43, 44]. Thermal desorption extraction and GC-MS analysis allowed extraction and detection of tetraneurin A, copaene, emulphor, beta-Methylionone and squalene in small percentages.

The structure of the cork samples with and without YS were observed by scanning electron microscopy in the tangential and nontangential sections is shown in Fig. 3 and 4. In non-tangential section, the cork cells appear as rectangular shape that is like a brick wall (Fig. 3). Fig. 3 shows for comparative purposes scanning electron micrographs of standard cork (Figure 3a, b, c, d) and with YS (Figure 3e, f, g, h). Scanning electron microscopy of a transverse section of cork of both samples showing a regular radial alignment of rows, but there are differences between cork with and without YS. In samples of cork with YS, the cell wall undulations are more pronounced than in cork without YS (Fig 3b and 3f). Also, the pattern of undulations was not uniform because two, three or four corrugations per face could be seen. In both samples, scanning electron microscopy observations of cork cells showed deposits on the interior of the cells or remnants of a fungal hypha (Fig 3c and 3g). These deposits are also described for samples of *Quercus variabilis* [45]. Furthermore, a certain degradation degree at the middle of the lamella also appeared in samples with YS (Fig 3h).

The structure of the cork of *Quercus suber* with and without YS as observed by scanning electron microscopy in the non-tangential sections is shown in Fig. 4. In this case, the cells exhibit as hexagons that are arranged according to a honeycomb structure. Standard cork showed a regular structure with closed cells, as reported previously [3]. The cells of cork with YS seem to have lost a certain degree of well-defined prismatic form (Fig. 4a) compared to standard cork (Fig. 4e). As mentioned above, the observations showed the presence of hyphae across the cell wall in cork with YS (Fig. 4b). The fungi of the genus *Trichoderma* are closely associated with the YS [46]. These fungi are responsible for the degradation of the cell wall of cork cells, through the production of cellulosic enzymes and ligninolytic enzymes [47].

Normal cork also showed certain filaments that look like a hypha (Fig. 4f) because examined samples were raw cork (or cork that has not entered in the cork stopper production) but no case more evident than affected cork. Studies of cork using SEM have also shown the presence of numerous microorganisms, but a drastic decrease of its populations occurs during cork stopper production [48]. In non-tangential section samples, a separation at the middle of the lamella also appeared in samples with YS (Fig. 4c, 4d) but not in standard cork (Fig, 4g, 4h). The separation of cell wall was also observed in Fig. 5. Certain degree of separation in the middle lamella level also appeared in thermal treated standard cork because of cell wall degradation. Rocha et al, 1996 [4] reported that these changes were related to the degradation of some cork components such as lignin and pectic polysaccharides of cell wall [5]. Also, this change in cork cell wall was leaded to a relative accumulation of calcium in this region due to the presence of certain microorganisms [5].

Figure 6 shows scanning electron micrographs of the non-tangential section of cork from *Quercus suber* with YS, with the purpose of detailed the area near to the

hyphae's. As described previously, there is a strong cell wall undulation even to complete folding and with fracture in some areas. The cell wall undulation is also obtained after cork samples compression in the axial direction at strains (about 50% at left and 70% at right) [49]. This behavior is due to chemical structure of cork: suberin or a flexible macromolecule allows for cell wall undulation and lignin could help this deformation because of its inter-unit linkages (β-O-4 type). Even so, the aromatic fraction of lignin is related to compressive strength to the cell wall [49].

According to this, the relative proportions of chemical compounds of cork such as suberin and lignin in the cork could affect cork compression properties. In samples attacked by YS, may be done some chemical cork components degradation, causing these differences in cellular structure. This degradation process promotes the appearance of complete folding cell wall undulation and fractures. In some areas, a certain degree of a join among cell walls is appreciated

These results would be in accordance with aromatic profile of cork samples described above and with other studies that have showed a decrease of lignin and polysaccharides content in samples with YS compared to standard cork [3,6]. The comparison of cork aromatic profiles of samples with and without YS carried out in this study have showed differences in the percentage of certain families such as aromatic compounds and other related to the degradation or depolymerization of some cork polymers such as lignin. suberin or waxlike fraction. Overall, the effects associated with the presence of YS promote different chemical modification in cork polymers than standard cork producing cell wall disturbances such as changes in the level of cell corrugation, the degree of separation of the layers of cell wall and a join among cell walls.

CONCLUSIONS

The effect of the presence of yellow spots in samples of cork from *Quercus suber* was studied in relation to volatile compounds and the influence on the cellular structure. Thermal desorption extraction could be successfully used to obtain the aromatic profile of cork, allowing the extraction of 103 compounds. Aromatic compounds were the most abundant and heterogeneity family. The main difference in aromatic profile of standard and attacked cork with YS has been the percentage and type of some aromatic compounds related to lignin degradation such as vanillin and certain aliphatic alcohols and carboxylic acids that are mainly obtained by depolymerization or degradation of suberin and waxlike. Cork with YS also showed higher percentages of compounds obtained from suberin and waxlike fraction degradation by microbial degradation. These results showed that the microbial growth of samples with YS could promote different chemical modifications in cork polymers than standard cork obtaining other type of degradation products. The presence of chlorinated compounds is remarkable in cork with YS that contains TCA and TCP. Is not the case of samples without YS.

The observation of cork with YS at microscopic level showed changes in cell wall such as wrinkly cells, separation of the layers of cell wall, certain degree of disorganization of the cell structure and the presence of hyphae across the cell wall. These changes would be related to chemical modifications produced by the microbiota that is involved.

Compounds	(%)		Description	Ref.
	WITH	WITHOUT		
Aliphatic Alcohols				
Dotriacontanol	3,91	2,91	suberin monomer (long-chain fatty alcohol)	50
Docosanol (Behenic alcohol)	2,48	3,68	suberin monomer (long-chain fatty alcohol)	44, 51-54
Hexadecenol	1,23	0,10	suberin monomer (long-chain fatty alcohol)	51
1-Eicosanol	0,59		waxlike fraction	50
Octadecanol	0,31	2,48	waxlike fraction	50, 51
Nonadecatriene-5, 14-diol	0,08	0,11	long-chain fatty alcohol	
Hexadecanol	0,05	1,39	suberin monomer (long-chain fatty alcohol)	51
13-Heptadecyn-1-ol	0,03		long-chain fatty alcohol	50
Heptatriacotanol		0,04		
Aliphatic Aldehyde				
Pentanal	1,99		Fatty aldehyde	14 ,55
Pentadecanal	0,20		long-chain fatty aldehyde	
Tridecanal	0,08		long-chain fatty aldehyde	4,15, 20, 44, 55
Octadecenal	0,05	0,05	Fatty aldehyde	4, 15, 20, 42, 55
Hexanal		0,04	Fatty aldehyde	4, 14
Nonadienal		0,01	Fatty aldehyde	
Nonanal		0,01	Fatty aldehyde	14, 15, 42, 55
Tetradecanal		0,01	long-chain fatty aldehyde	14, 55
Aliphatic Ketones				
Nonadecanone	1,10	0,07		
Alkanes				
Hexadecane	0,21	0,18		41
Heptacosane	0,11	0,07		56
Docosane	0,08	0,07	Suberin related compound	51, 56
Eicosane	0,08		Suberin related compound	51
3-Methylheptadecane	0,03	0,03		4, 14
nonadecane		0,15	Suberin related compound	4, 20
Octadecane		0,28		
Tetracosane		0,04	Suberin related compound	51, 56
Tetradecane		0,08		41
Alkenes				
1-Eicosene	2,15	0,25	Suberin related compound	51
17-Pentatriacontene	1,84	0,52		
Octadecene	0,27		Suberin related compound	57
1, 19-Eicosadiene	0,23			51
Aromatic compound				
Vanillin	55,36	61,05	Lignin-Related Compound	4, 11, 20, 42, 43, 54, 58, 59, 60
Isoeugenol	7,07	6,73	Lignin-Related Compound	43, 59
2-Methoxy-4-vinylphenol	2,24	1,76	Lignin-Related Compound	43, 59

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Acetovanillone	2,24	1,53	Lignin-Related Compound	43, 59
2,6-Diphenylphenol	1,48	1,07	Undefined phenolic source	4, 59
Coniferyl aldehyde	1,46	2,65	Lignin-Related Compound	42, 58, 59
Homovanillic acid	0,90	1,94	Lignin-Related Compound	
Eugenol	0,66	0,43	Lignin-Related Compound	41, 43, 59
Acetophenone	0,64		Lignin-Related Compound	4, 55
Benzene	0,43	0,66		
Guaiacylacetone	0,36	0,56	Lignin-Related Compound	
propanone	0,26	0,48		14
4-Hydroxy-2-methoxycinnamal-dehyde	0,18	0,20		
Phenol	0,11	0,23		4, 14
Cinnamic aldehyde	0,10	0,06	Lignin-Related Compound	50
Vanillic acid	0,10			43, 60
Isopropylnaphthalene	0,07	0,10		42, 55
1, 2-dimethoxybenzene (veratrole)	0,05	0,10	Lignin related compound	4, 14
2,4-Di-tert-butylphenol	0,01	0,11	Lignin related compound	4
Biphenyl	0,01	0,02	Suberin related compound	51
Isovanilline	0,01	0,03	Lignin related compound	
lapachol	0,01	0,02		
Syringaldehyde	0,01	0,01	Lignin-Related Compound	11, 41, 59
2-Methoxyhydroquinone		0,01		
4(1H)-Pyridone		0,04		59
4-Ethoxy-3-anisaldehyde (ethyl-vanillin)		0,05	Lignin-Related Compound	
4-Mercaptophenol		0,03	Lignin-Related Compound	4, 59
Benzoic acid		0,03	Lignin-Related C	4, 11, 20
Benzophenone		0,14		
Catechol		0,01	Lignin related compound	42
Dimethoxyethyl phthalate		0,19	Lignin-Related Compound	
Galaxolide		0,01	Lignin-Related Compound	
Hexyl cinnamic aldehyde		0,04		
Naphthalene		0,01	Lignin related compound	42, 55
Propiovanillone		0,01	Lignin-Related Compound	
Syringaldehyde acetate		0,03		4, 11, 20, 42, 43, 54, 58, 59, 60
Carboxylic acids				
linoleic acid	2,25	1,17	(Long fatty acid)	4
Erucic acid	0,44	0,19	(Long fatty acid)	
Nonanoic acid	0,17	0,19	suberin monomer (medium fatty acid)	43
Dodecanoic acid	0,14		(Medium fatty acid)	43
Octadecanoic acid	0,13	0,10		42
Eicosenoic acid	0,04	0,03	suberin monomer (long-chain fatty acid)	50, 54
9-Hexadecenoic acid	0,03			42
Octanoic acid	0,02		Fatty acids	42

Compound				
Decanoic acid	0,01		Fatty acids	
Nonadecenoic acid	0,01		Fatty acids	
Oleic Acid		0,02	Fatty acids	4
Chlorinated compounds				
1,2,4,5-Tetrachloroanisole	1,62	0,02		22
2,4,6-Trichlorophenol	0,06			61
2, 4-dichlorophenol	0,01			61
2,4,6-Trichloroanisole	0,01			61
cis-1-Chloro-9-octadecene	0,01	0,01		
Ester				
2-Ethylhexyl salicylate	0,38	0,96	Lignin related compound	
Azulene	0,02			
Aromandrene	0,01			
Homosalate		0,06	Lignin related compound	
Isopropyl myristate		0,33	Lignin related compound	
Furane				
6-Methoxy-3-methylbenzofuran	3,47	0,85		
2-Furancarboxamide	0,13			
5-Hydroxymethylfurfural		0,03		
Benzofuranone		0,04		
Terpene				
Tetraneurin A	0,03			
Copaene	0,02			14, 55
Emulphor		0,10		
beta-Methylionone		0,01		
Squalene		0,01		
Others				
Mellein	0,09	1,40		
2H-Pyran-2-one,5, 6-dihydro-6-pentyl- (styryldihydropyran)	0,04	1,25		
2-Methyladamantane		0,01		
4H-Pyran-4-one,2,3-dihydro-3,-5-dihydroxy-6-methyl-		0,04		
Î±-Amino-3'-hydroxy-4'-methoxyacetophenone		0,25		
Versalide		0,02		

Table 1. Aromatic profile identified in cork with and without YS, grouped by chemical families. Results are presented in % of peak area of each compound in relation to total area of the sample.
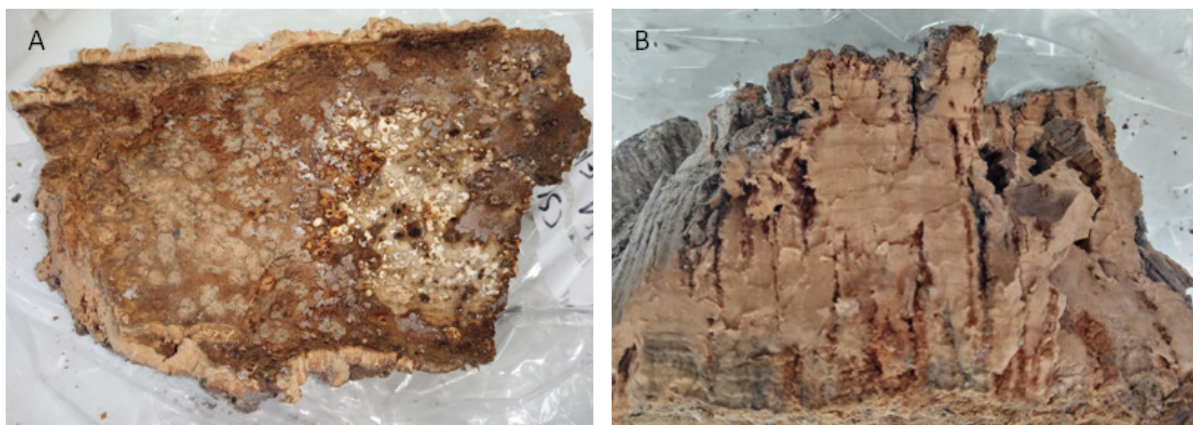
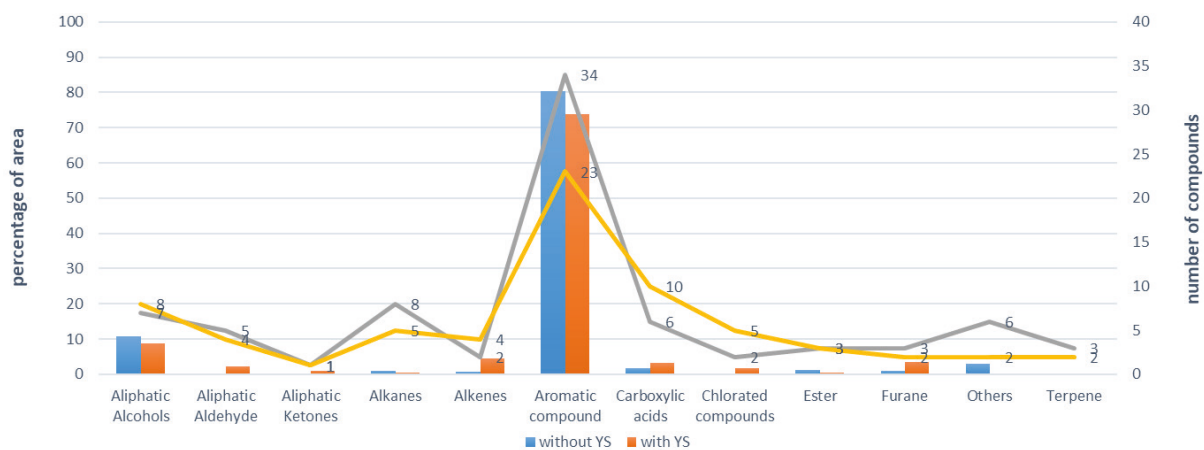**Figure 1.** Images of cork samples with (A) and without (B) yellow spot.

Figure 2. Percentage of different families of compounds extracted from cork without and with YS. Also showed the number of compounds count.
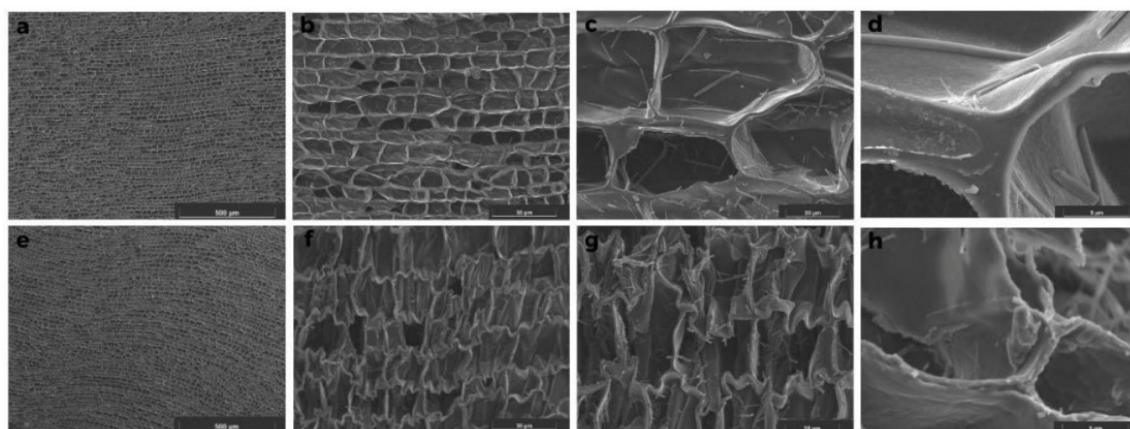

Figure 3. Scanning electron micrographs of cork from *Quercus suber* in tangential section of standard cork (a, b, c, d) and with YS (e, f, g, h).
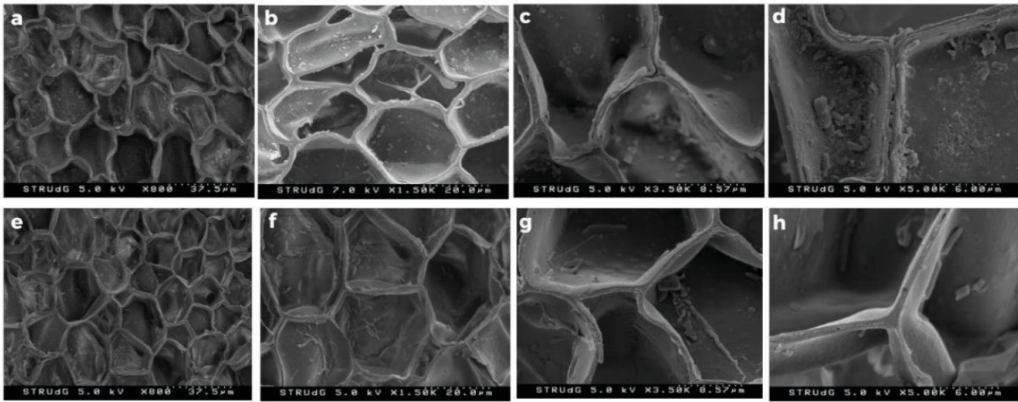
Figure 4. Scanning electron micrographs of cork from *Quercus suber* in non-tangential section of standard cork (a, b, c, d) and with YS (e, f, g, h).
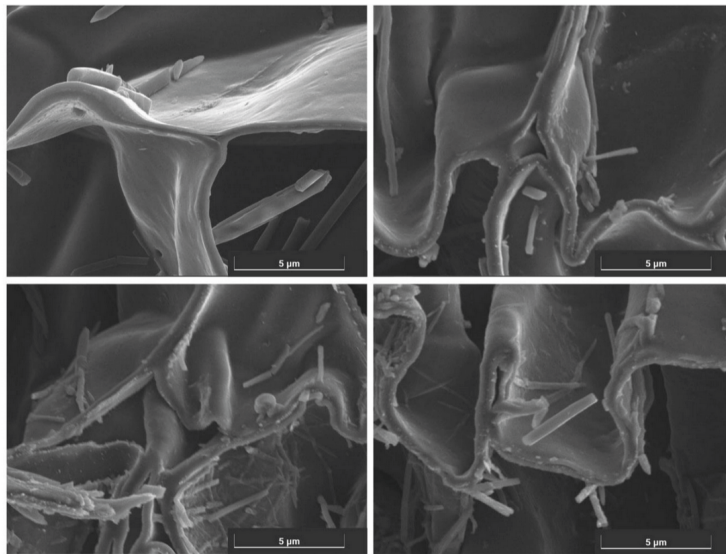

Figure 5. Scanning electron micrographs of cork from *Quercus suber* with YS in tangential section showing the cell wall separation at the middle of the lamella. Scale bar 5 μm.
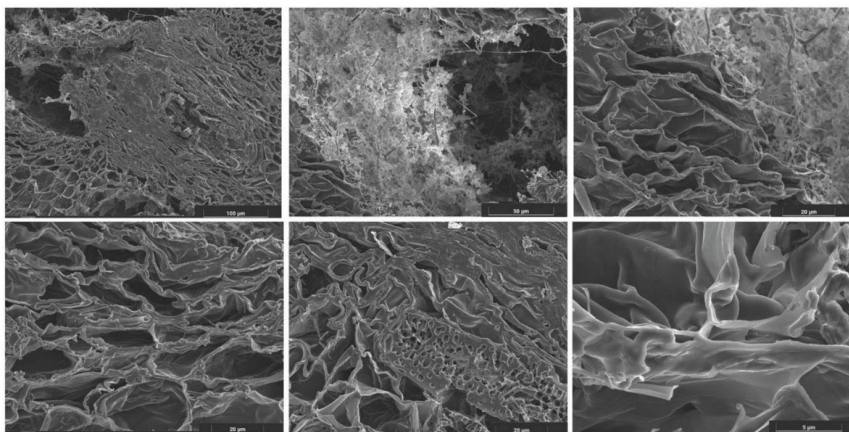

Figure 6. Scanning electron micrographs of cork from *Quercus suber* with YS in tangential section showing the areas near to hyphae's. Scale bar 100 μm, 50 μm, 20 μm and 5 μm.

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