# GC–MS Study of Volatiles of Normal and Microbiologically Attacked Cork from *Quercus suber* L.

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The volatile compounds of cork were studied by gas chromatography and combined gas chromatography–high-resolution mass spectrometry using simultaneous distillation–extraction to prepare the samples. To assess the origin of the volatiles, three different types of samples were analyzed: "normal", attacked by *Armillaria mellea*, and infested by molds. The study of the volatiles of these different types of corks allowed the identification of the chemical modifications which may occur in cork polymers. The cork attacked by *A. mellea* showed higher amounts of phenols, vanillin, benzaldehyde, benzyl alcohol, and chlorinated compounds than normal cork; this may indicate lignin degradation. The cork infested by molds contained higher levels of 3-methyl-1-butanol, 1-octen-3-ol, 1-octanol, 2-methylisoborneol, chlorinated compounds, and methyl ketones. These components resulting from microbial metabolism were also present in cork attacked by *A. mellea*. The use of cork attacked by *A. mellea* is not recommended in the manufacture of cork stoppers, since these types of cork have volatile compounds likely to cause off-flavors in wine. For the same reason it is important to reduce the likelihood of mold development during the standing period.

**Keywords:** *"Normal" cork; Armillaria mellea; molds; volatile components; musty and moldy odors*

## INTRODUCTION

Traditionally, cork stoppers have been used as closures for bottled champagne and other wines.

The "bouquet" of wine, caused by the action of several different flavor compounds on the sensory organs, is strongly influenced by the generation of undesirable aroma compounds during the production and storage of wine. In many cases, the resulting wine fault has a complex nature which is extremely difficult to determine.

The wine quality depends on proper conditioning. At this level the cork plays an important role because of its peculiar features: impermeability to air and liquids (preventing wine oxidation), ability to adhere to a glass surface, compressibility, resilience, and chemical inertness (Simpson and Amon, 1986).

In spite of the fact that the off-flavor in wine can have different sources, a specific problem associated with the use of cork stoppers is "cork taint". Within recent years several studies on cork taint have been published. Cork taint is usually associated with a musty, moldy aroma and taste (Simpson and Veitch, 1993). Some authors have expressed the view that there is confusion between cork taint and other off-flavors in wine, such as musty or moldy odors (Riboulet, 1989; Dubois and Rigaud, 1984; Maujean et al., 1985).

A large number of compounds have been reported in cork (Mazzoleni et al., 1994), some of them with musty and related odors, for example 2,4,6-trichloroanisole and 2,3,4,6-tetrachloroanisole (Wurding, 1975; Tanner and Zanner, 1978, 1983; Dubois and Rigaud, 1981; Rigaud et al., 1984; Maujean et al., 1985) and 1-octen-3-one, 1-octen-3-ol, geosmin, and 2-methylisoborneol (Amon et al., 1989). Other compounds with similar odor characteristics include guaiacol (Amon et al., 1989) and 1-octanol and 3-methyl-1-butanol (Kaminski et al., 1972).

The presence of molds on cork slabs is unavoidable since they are part of the specific microbiological flora of the tree (Lefebvre et al., 1983). After being stripped from the tree, the cork slabs are stored for some months in the forest to ripen. At the factory the slabs are boiled for about 1 h and, in general, left to stand in piles for 3 weeks to become flat. As soon as the water content is optimal, the slabs are ready to be processed to cork stoppers. It is mainly during this period of 3 weeks that molds may grow on the slabs. Microbial growth may generate metabolites that are able to contaminate wine in contact with this cork.

It is clear that the way to avoid the appearance of compounds arising from cork taint is to decrease the growth of microorganisms, especially molds. This can be done by appropriate storage procedures and reducing the time between the boiling of the cork slab and the actual manufacturing process of the stoppers. Nevertheless, because cork is a natural product, as pointed out above, even with the best possible care taken to ensure a short processing time, it is not always possible to avoid the presence of a some of the compounds arising from the previously existing microflora.

The growth of molds in cork slabs could promote substantial differences in the cork aroma, depending on the species growing. It is known that different species of *Penicillium* may lead to the production of different patterns of volatile secondary metabolites (Borjesson et al., 1993). Information concerning the identity of compounds with an earthy, mushroom-like quality that are produced by molds and mushrooms is generally limited to saturated or monounsaturated eight-carbon primary and secondary alcohols and the corresponding carbonyl compounds, which have been found in a large number of samples (Karahadian et al., 1985).

The cork can be attacked by fungi, such as *Armillaria mellea* (saprophytic basidiomycetes). The *A. mellea* living on the ground, decaying leaves, bark, wood, manure, etc., attacks the roots of the trees and kills their cortical tissue, growing up into the portions of the bark of the tree trunk and causing its death (Bessey, 1950).

This fungal attack causes chemical and physical changes in cork, such as modifications in its mechanical, structural, and optical properties and is potentially able to cause off-flavors in wine in contact with the cork.

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The study of the volatile components of "normal" cork and the modifications that it suffers by microbiological attack could also be of interest for the cork and wine industries if used as chemical fingerprints for classification of cork and eventually for identification of the origin of off-flavors.

In spite of the reports discussed above there has been no systematic study of the volatile compounds present in normal cork and those found in cork attacked by *A. mellea* and by molds. This paper reports an attempt to carry out such a study.

## EXPERIMENTAL PROCEDURES

**Materials.** Fifty-four standard compounds (see Table 1) were purchased from Fluka Chemie AG (Buchs, Switzerland), Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), and E. Merck (Darmstadt, Germany). Pentane analytical grade was also purchased from Merck and was bidistillated in glassware.

**Cork Samples.** Cork slabs, chosen randomly, without any apparent off-flavor or microbiological attack, were collected in the cork factory and were used to prepare the aliquots of normal cork (N). Cork slabs attacked by *A. mellea* (with white and brown marks) were collected to be used as cork with "mancha amarela" (MA, ("yellow stain"). Some slabs were stored in wet conditions for 2 months to promote the growth of molds (MO). No attempt was made to identify the species of molds present in cork samples. These cork slabs (MO) exhibited a strong musty and moldy aroma. None of the cork slabs used were submitted to any kind of previous treatment (i.e. boiling, washing and bleaching).

**Extraction of the Cork.** The samples of cork were submitted to a process of simultaneous distillation−extraction (SDE) in a modified Likens−Nickerson apparatus (Schultz et al., 1977). The capacities of the flasks were 3 L and 100 mL.

The cork was cut in pieces of about 2 cm × 2 cm; 90 g of cork pieces and 1 L of distilled water were spiked with internal standard (5.8 $\mu$L of ethyl pentanoate). These were placed in a Likens−Nickerson apparatus and extracted for 3 h with 60 mL of pentane. Three successive cork samples (total of 270 g of cork) were extracted with the original 60 mL of pentane.

The pentane extracts from cork were cooled to −10 °C to separate the frozen water from the organic phase by decantation. The excess of low-boiling solvent was removed by distillation using a Vigreux microfractionating column. The concentrate (about 800 $\mu$L) was stored in a glass screw-top vial at −10 °C.

**Gas Chromatography (GC).** Preliminary analyses by GC were carried out on a Konik 3000C gas chromatograph with a flame ionization detector (FID), equipped with a 25 m × 0.15 mm (i.d.) OV-17 fused silica capillary column. Injections were done in the splitless mode (0.7 min), sample size 3 $\mu$L. The injector and detector temperatures were 210 and 220 °C, respectively. The oven temperature was programmed from 35 to 200 °C at 2 °C/min. Helium was used as the carrier gas at an average linear velocity of 30 cm/s.

**Gas Chromatography−Mass Spectrometry (GC−MS).** Cork extracts were analyzed by GC−MS on a Konik 3000C gas chromatograph (with the same column used for the GC analyses) connected to a high-resolution mass spectrometer VG Autospec Q. Splitless injections was used. The oven temperature of the GC was programmed from 35 to 200 °C at 2 °C/min. Helium carrier gas had a column head pressure of 12 psi. The mass spectrometer was operated in the electron impact mode at 70 eV, scanning the range *m*/*z* 30−300 in a 1 s cycle.

Identification of components was achieved by comparison of the GC retention times and mass spectra with those, when available, of the pure standard compounds. All mass spectra were also compared with those of the data system library (NIHS Library) and other published spectra (*Eight Peak Index of Mass Spectra*, 1974).

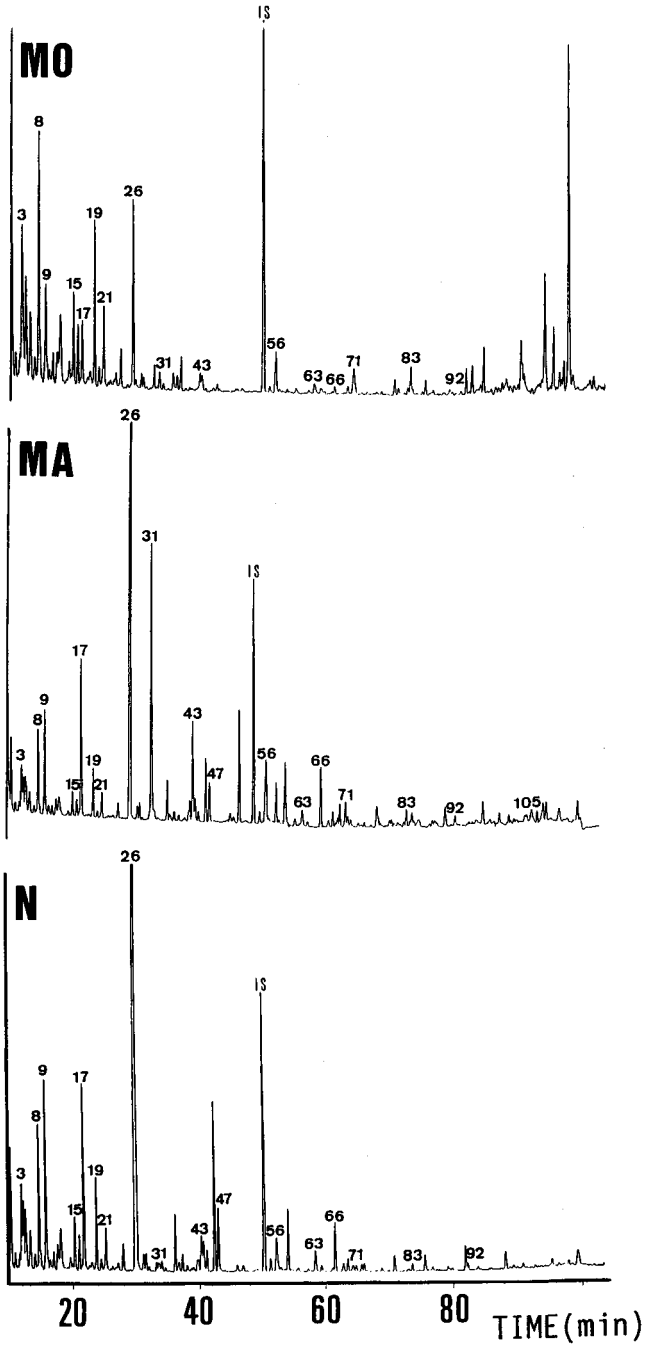


**Figure 1.** Total ion chromatograms of normal cork (N), of cork with mancha amarela (MA), and of cork attacked by molds (MO) (IS, internal standard). See Table 1 for peak assignments.

## RESULTS AND DISCUSSION

Figure 1 represents typical gas chromatograms of cork samples N, MA, and MO. Table 1 shows the compounds identified in the pentane extracts of the three kinds of samples. The data represent the average of 12 GC analyses for each sample (duplicates of 6 samples).

The volatile compounds found in cork extracts were grouped by chemical class. The major compounds in extracts were aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, alkanes, aromatic compounds, cycloalkanes, furans, terpenoids, chlorinated compounds, and lignin-related compounds.

The most important reaction producing volatile carbonyl compounds, such as ketones and aldehydes, is

degradation of fatty acids (lipid peroxidation). The waxlike fraction of cork includes the presence of saturated and unsaturated free fatty acids, such as araquidic, phellonic, oleic, and linoleic acids (Guillemonat, 1960). The suberin of cork contains large quantities of long-chain aliphatic acids ranging from $C_{16}$ to $C_{26}$ (Kolattukudy et al., 1982; Holloway, 1972); which are susceptible to degradation.

The degradation of fatty acids may occur either by autoxidation of fatty acids or by lipoxygenase activity. The autoxidation of unsaturated fatty acids promotes the formation of aldehydes and corresponding alcohols, ketones, alkanes, and alkenes. Several studies have reported that lipid oxidation causes changes in the flavor, color, and texture of certain products (Josephson et al., 1983, 1984; Hsieh and Kinsella, 1989; Milo and Grosch, 1993).

Autoxidation involves formation of volatile aldehydes. Thus, $C_5-C_{10}$ aliphatic aldehydes and 2-tridecenal could originate in cork by oxidation of fatty acids. Hexanal and nonanal are the main aliphatic aldehydes present in the cork extracts. Hexanal is a major lipid oxidation product of linoleic acid, and nonanal is the major lipid oxidation product of oleic acid (Belitz and Grosch, 1987). It was reported that these acids are present in the waxlike fraction of cork as free fatty acids, which may be more easily degraded than if they are present as suberin components. These types of aldehydes have exceptionally strong aromas, even if they are present in low amounts.

The four- and five-carbon alcohols were among the most common volatiles produced by 29 species of basidiomycetes (Borjesson et al., 1993). This is in agreement with results found in cork attacked by *A. mellea*, where 1-butanol, 3-methyl-1-butanol, and 1-pentanol were the most frequently found aliphatic alcohols in the cork samples studied.

Cork MO shows smaller amounts of aliphatic aldehydes than corks N and MA, but the contrary is observed with aliphatic alcohols (higher levels in cork MO). Normal cork presented smaller amounts of aliphatic alcohols than microbially attacked corks (MO and MA). Alcohol dehydrogenases can reduce the aldehydes derived from fatty acids into the corresponding alcohols. Alcohol formation in plants and microorganisms is strongly favored by the equilibrium reaction between aldehyde and alcohol. Nevertheless, the enzyme specificity is highly variable. The oxidative cleavage of fatty acids produced a mixture of alcohols and aldehydes; in most cases the aldehydes predominate. It is possible that in cork MO the alcohol dehydrogenase reduces the aldehydes into the corresponding alcohols.

The microbial degradation of free fatty acids leads to the production of methyl ketones, which in part can be reduced to the corresponding secondary alcohols (Belitz and Grosch, 1987). It is known that a number of *Aspergillus* and *Penicillium* as well as several *Ascomycetes, Phycomycetes*, and *Fungi imperfecti*, which were found as natural microflora in cork slabs (Riboulet, 1982; Rossignol, 1984), are able to degrade fatty acids with short and medium chains to methyl ketones, such as 3-methyl-2-pentanone, 2-heptanone, 6-methyl-3,5-heptadien-2-one, 6-methyl-3,5-heptadien-2-one, 9-decen-2-one, 2-undecanone, and 6,10-dimethyl-5,9-undecadien-2-one. In agreement with this, the methyl ketones predominate in cork attacked by molds. The odor threshold values for methyl ketones are substantially higher than those for aldehydes.

Aromatic compounds were found in pentane extracts of all cork samples studied. Some claims have been made that certain alkylbenzenes contribute to musty aromas, but these claims are largely unsubstantiated and have not been analytically confirmed (Karahadian et al., 1985). Certains aromatic compounds, such as two dimethylbenzene isomers, 1,3,5-trimethylbenzene, and 1,2,4-trimethylbenzene, were present in higher amounts in cork MO, but others were present in larger quantities in corks N and MA. So, at this moment is not possible to establish a correct correlation between the origin of aromatic compounds and the microbial attack of cork.

The biochemical origin of the many cycloalkanes present is not well-known; however, their presence is specially manifest in cork infested by molds, which makes it possible to establish a correlation between the presence of those cycloalkanes and the microbiological activity. The same can be concluded about the presence of aliphatic alkanes, since these compounds predominate in cork MO. Different cycloalkanes have been found in many different samples, such as mango (MacLeod and Snyder, 1985), chickpea seed (Rembold et al., 1989), and pork meat (Rammarathnam et al., 1991). In these studies the formation mechanism of cycloalkanes is not explained at all.

The tremendous biosynthetic potentialities of plants are no better illustrated than by the group of natural products known as terpenoids. Terpene biosynthesis is carried out only by plants and some microorganisms (Belitz and Grosch, 1987). Cork samples in general show a variety of terpenoids, but these are more evident in cork attacked by *A. mellea*. This may be due to *A. mellea* metabolism or to the *A. mellea* attack on cork, which could help to set free the terpenoids from the cork structure. In general, these compouds generate a wide spectrum of aromas, mostly perceived as very pleasant. Some monoterpenes have a musty, moldy, or pungent note, such as 2-methylisoborneol, which is considered to contribute to the cork taint in wine (Amon et al., 1989). It appears only in corks MA and MO.

Phenolic acids and lignin are degraded thermally or decomposed by microorganisms to phenols, guaiacol, benzaldehyde, vanillin (Belitz and Grosch, 1987), and benzyl alcohol. These compounds are detected in cork extracts, being found in higher amounts in cork MA. The presence of these compounds is an indicator that the lignin was attacked by *A. mellea*. It is known that this fungi degraded lignin polymer, although the cork may be altered structurally (Hudson, 1986). The cork attacked by *A. mellea* has a musty and moldy odor when the slab is wet, although when the slab is dry ($a_w$ = 0.53), the cork has a sweet smell. The lignin building blocks are the phenylpropanes, coumaryl, coniferyl, and sinapyl alcohol; the lignin of different plants may contain different portions of these three building blocks (Hudson, 1986). Benzyl alcohol is a product from the phenylpropanoid metabolism (Buchbauer et al., 1993). The presence of this component and vanillin may cause the sweet and flowery notes observed in cork MA.

The methoxylated phenol derivatives, acetophenone, 4-methylacetophenone, 2-hydroxy-5-methylacetophenone, veratrole, resorcinol, and 2-methoxy-4-methyl-1-propylbenzene, are structurally related. These compounds predominate in cork MA, and it is possible to consider them as lignin-related compounds produced by the action of *A. mellea* on cork.

Guaiacol was identified as the major metabolite of a *Streptomyces* sp. growing on cork (Lefebvre and

**Table 1. Volatile Components Identified in Normal Cork (N), in Cork with Mancha Amarela (MA), and in Cork Attacked by Molds (MO), Grouped by Chemical Class**

| peak no. | compound | reliability of ID[a] | %[b] | | |
|---|---|---|---|---|---|
| | | | N | MA | MO |
| | | Aliphatic Alcohols | | | |
| 1 | 1-butanol | A, B, C | 0.47 | 0.33 | 0.88 |
| 15 | 3-methyl-1-butanol | A, B, C | 1.55 | 0.76 | 3.97 |
| 25 | 1-pentanol | A, B, C | ND[c] | ND | 0.12 |
| 27 | 1-octen-3-ol | A, B, C | ND | ND | 0.09 |
| 50 | 2-ethyl-1-hexanol | B, C | ND | 0.35 | 0.09 |
| 56 | 1-octanol | A, B, C | 1.57 | 3.29 | 1.93 |
| 98 | 1-undecen-4-ol | B | tr[d] | tr | 2.46 |
| 102 | 2,7-dimethyl-2,6-octadien-1-ol | B | 0.12 | 0.07 | 0.52 |
| 104 | 5,9-dimethyl-9-decen-3-ol | B | 0.06 | 0.11 | 0.21 |
| | | Aliphatic Aldehydes | | | |
| 2 | 3-methylbutanal | B, C | 0.38 | 0.36 | 0.75 |
| 10 | 2-methylpentanal | B, C | 0.33 | 0.38 | 0.37 |
| 17 | hexanal | A, B, C | 5.46 | 4.71 | 2.03 |
| 29 | heptanal | A, B, C | 0.58 | 0.67 | 0.57 |
| 40 | 2-heptenal | A, B | tr | 0.05 | 0.18 |
| 45 | octanal | A, B, C | 0.60 | 0.31 | 0.11 |
| 55 | 2-octenal | A, B | 0.33 | 0.44 | 0.42 |
| 58 | nonanal | A, B, C | 2.00 | 1.64 | tr |
| 68 | 2-nonenal | B, C | 0.37 | 0.51 | 0.23 |
| 71 | decanal | B, C | 0.22 | 0.63 | tr |
| 75 | 2,4-nonadienal | A, B, C | 0.53 | 0.65 | 0.14 |
| 81 | 2-decenal | B | ND | ND | 0.09 |
| 88 | 2,4-decadienal | A, B, C | 0.12 | tr | tr |
| 94 | 2-tridecenal | B | tr | ND | 0.34 |
| | | Aliphatic Ketones | | | |
| 12 | 3-methyl-2-pentanone | B, C | 0.41 | 0.61 | 1.70 |
| 28 | 2-heptanone | A, B, C | 0.50 | 0.53 | 0.74 |
| 44 | 6-methyl-5-hepten-2-one | A, B, C | 0.71 | 0.83 | 0.68 |
| 64 | 6-methyl-3,5-heptadien-2-one | B | 0.18 | 0.33 | 0.37 |
| 80 | 9-decen-2-one | B | ND | 0.12 | 1.08 |
| 90 | 2-undecanone | A, B, C | ND | ND | 0.19 |
| 97 | 6,10-dimethyl-5,9-undecadien-2-one | B | 0.46 | 0.89 | 0.49 |
| | | Alkanes | | | |
| 3 | 2-methylheptane | A, B, C | 2.05 | 1.56 | 8.26 |
| 5 | 3-methylheptane | A, B, C | 1.78 | 1.56 | 3.61 |
| 8 | octane | A, B, C | 4.51 | 2.95 | 8.30 |
| 11 | ?-dimethylheptane[e] | B, C | 0.98 | 0.27 | 0.92 |
| 14 | ?-ethylmethylhexane | B, C | 0.81 | tr | 0.81 |
| 16 | 3-methyloctane | B, C | 1.06 | 0.50 | 2.03 |
| 19 | nonane | A, B, C | 2.01 | 2.00 | 5.12 |
| | | Alkenes | | | |
| 49 | 3-ethyl-2-methyl-1,3-hexadiene | B | 0.20 | 0.06 | tr |
| 51 | 3,5-dimethyl-1,6-octadiene | B | 0.11 | 0.12 | 0.04 |
| | | Alkynes | | | |
| 57 | 1-octyne | B, C | 0.11 | 0.17 | tr |
| | | Aromatic Compounds | | | |
| 9 | toluene | A, B, C | 4.32 | 3.86 | 4.09 |
| 20 | 1,4-dimethylbenzene | A, B, C | 0.86 | 0.17 | 0.64 |
| 21 | 1,3-dimethylbenzene | A, B, C | 1.52 | 0.70 | 2.57 |
| 23 | 1,2-dimethylbenzene | A, B, C | 0.96 | 0.47 | 1.20 |
| 32 | propylbenzene | A, B, C | 0.27 | 0.30 | 0.04 |
| 33 | 1,3,5-trimethylbenzene | A, B, C | 0.23 | tr | 0.59 |
| 36 | 1,2,4-trimethylbenzene | A, B, C | 0.53 | tr | 1.04 |
| 37 | 1,2,3-trimethylbenzene | A, B, C | 0.15 | 0.26 | tr |
| 41 | 1-methyl-4-isopropylbenzene | B, C | tr | tr | tr |
| | | Cycloalkanes | | | |
| 6 | 1,3-dimethylcyclohexane | B, C | 1.32 | 0.57 | 2.23 |
| 7 | 1,2-dimethylcyclohexane | B, C | 0.44 | 0.28 | 0.89 |
| 13 | ethylcyclohexane | A, B, C | 1.92 | 1.23 | 3.70 |
| 18 | 1-ethyl-3-methylcyclohexane | B, C | tr | tr | tr |
| 22 | isopropylcyclohexane | B, C | 0.27 | tr | 0.42 |
| 24 | 1-ethyl-2-methylclohexane | B, C | tr | ND | tr |
| | | Furans | | | |
| 26 | furfural | A, B, C | 36.92 | 27.74 | 6.40 |
| 34 | 2-acetylfuran | A, B, C | 1.65 | 1.01 | 0.81 |
| 35 | 2-pentylfuran | B, C | 0.40 | 0.31 | 0.39 |
| 47 | 5-methylfurfural | A, B, C | 2.13 | 1.34 | 0.31 |
| 65 | 3,4-dimethyl-2,5-furandione | B | 0.11 | ND | tr |
| 86 | 5-methyl-2,5-dihydro-3(2*H*)-furanone | B | 0.12 | 0.16 | 0.22 |

**Table 1 (Continued)**

| peak no. | compound | reliability of ID[a] | %[b] N | %[b] MA | %[b] MO |
|---|---|---|---|---|---|
| | | Terpenoids | | | |
| 38 | camphene | A, B, C | ND | 0.19 | ND |
| 42 | 1,8-cineole | A, B, C | 0.33 | 0.91 | tr |
| 54 | geranial | B, C | ND | tr | tr |
| 62 | fenchyl alcohol | A, B, C | tr | 0.03 | tr |
| 66 | camphor | A, B, C | 1.61 | 2.01 | 0.96 |
| 70 | isoborneol | A, B, C | 0.19 | 0.69 | tr |
| 72 | a-terpineol | A, B, C | 0.23 | 0.23 | tr |
| 78 | geraniol | A, B, C | 0.12 | 0.20 | 0.19 |
| 82 | 2-methylisoborneol | B, C | ND | 0.06 | 0.17 |
| 83 | copaene | A, B, C | 0.52 | 0.55 | 0.51 |
| | | Phenols | | | |
| 61 | phenol | A, B, C | 0.07 | 0.37 | tr |
| 100 | 2,6-di-*tert*-butyl-4-methylphenol | B, C | 0.25 | 0.35 | ND |
| | | Chlorinated Compounds | | | |
| 48 | 1,4-dichlorobenzene | A, B, C | ND | 0.06 | 0.05 |
| 85 | 2,4,6-trichloroanisole | A, B | ND | 0.40 | 0.11 |
| 86 | 4,4-dichloro-1,1-biphenyl | B, C | ND | 0.22 | ND |
| 105 | ?-trichloro-1,1-biphenyl | B, C | ND | 0.61 | ND |
| 110 | ?-trichloro-1,1-biphenyl | B, C | ND | 0.11 | 0.07 |
| | | Lignin-Related Compounds | | | |
| 43 | benzaldehyde | A, B, C | 0.14 | 3.42 | 0.52 |
| 59 | phenylethanone (acetophenone) | A, B, C | 0.11 | 0.17 | 0.03 |
| 60 | benzenomethanol (benzyl alcohol) | A, B, C | 0.16 | 2.80 | tr |
| 63 | 4-methoxyphenol (guaiacol) | A, B, C | 0.83 | 0.94 | 0.61 |
| 67 | 1,2-dimethoxybenzene (veratrole) | A, B, C | 0.29 | 0.25 | tr |
| 69 | 1,3-dimethoxybenzene (resorcinol) | A, B, C | 0.21 | 0.16 | 1.56 |
| 73 | 2-methoxy-4-methyl-1-propylbenzene | B | tr | 0.15 | ND |
| 74 | 1-(4-methylphenyl)ethanone (4-methylacetophenone) | A, B, C | tr | tr | tr |
| 92 | 1-(2-hydroxy-5-methylphenyl)ethanone (2-hydroxy-5-methylacetophenone) | A, B | 0.26 | 0.80 | tr |
| 103 | 4-hydroxy-3-methoxybenzaldehyde (vanillin) | A, B, C | tr | 0.37 | tr |
| | | Others | | | |
| 4 | 1,1-diethoxyethane | B, C | 1.74 | 1.10 | tr |
| 31 | 2-butoxyethanol | B, C | 0.22 | 9.01 | 0.69 |
| 39 | ethyl hexanoate | A, B, C | ND | ND | 0.14 |
| 77 | benzothiazole | A, B, C | tr | tr | 0.24 |
| 89 | methyl 2-hydroxy-5-methylbenzoate | B, C | 0.07 | tr | tr |
| 109 | 3,8-dihydroxy-3,4-dihydronaphthalen-1-one | B | 0.12 | tr | ND |

[a] The reliability of the identification or structural proposal is indicated by the following: A, mass spectrum and retention time consistent with those of an authentic standard; B, structural proposals are given on the basis of mass spectral data (NIHS Library); C, mass spectrum consistent with spectra found in the literature. [b] Estimated concentrations for all compounds were made by peak area comparisons to the area of a known amount of internal standard (ethyl pentanoate) with no correction for individual detector response factors. [c] ND, not detected. [d] tr, peak area percent less than 0.02. [e] ?, unidentified isomer.

Riboulet, 1983), and it has also been considered to be responsible for cork taint in wine (Amon et al., 1989).

Various furan derivatives, such as furfural, 2-acetylfuran, 2-pentylfuran, 5-methylfurfural, 3,4-dimethyl-2,5-furandione, and 5-methyl-2,5-dihydro-3(2*H*)-furanone, have been found in cork extracts. These compounds result from carbohydrate degradation. Several furan derivatives, such as 2-pentylfuran, occur among the autoxidation products of linoleic acid (Belitz and Grosch, 1987). High accumulation of furaldehydes, such as furfural and 5-methylfurfural, indicates that reactions of Maillard type and acid-catalyzed sugar degradation may have taken place and may be a potential source of browning in stored foods and other natural products (Mansilla et al., 1992; Tu et al., 1992). 5-Methylfurfural is a decomposition product of hexoses, and furfural is the principal decomposition product of pentoses.

Chemical analysis has shown that corks MA and MO contain lower quantities of free sugars than normal cork (1.13% of free sugars in cork MA, 0.28% in cork MO, and 1.43% in normal cork) (S. Rocha, I. Delgadillo, and A. J. Ferrer Correia, unpublished results, 1993). We found that the simultaneous distillation−extraction method used to prepare the samples increased the furan formation. The amounts of furans found in cork samples are related to the level of free sugars. Figure 2 shows the increase of the amount of furfural, 5-methylfurfural, 1,8-cineole, benzaldehyde, 1-butanol, and hexanal as a function of the extraction time by SDE method. The level of furfural and 5-methylfurfural grows rapidly with the extraction time compared to the other compounds. Therefore, we consider that only the furfural and 5-methylfurfural levels are artifacts originating from free sugars in the cork when subjected to the Likens−Nickerson method (S. Rocha et al., unpublished results, 1994).

Buser et al. (1982) have reported 2,4,6-trichloroanisole (2,4,6-TCA) as the main component responsible for cork taint. 2,4,6-TCA cause strong "musty−moldy" taints in foods and beverages, even if it is present in low amounts. In wine the taste threshold of 2,4,6-TCA is only 0.01 $\mu$g/L (Whitfield et al., 1986). The origin of 2,4,6-TCA and related chlorinated compounds is not yet fully known. Buser et al. (1982) have affirmed that the occurrence of 2,4,6-TCA and related chlorinated com-

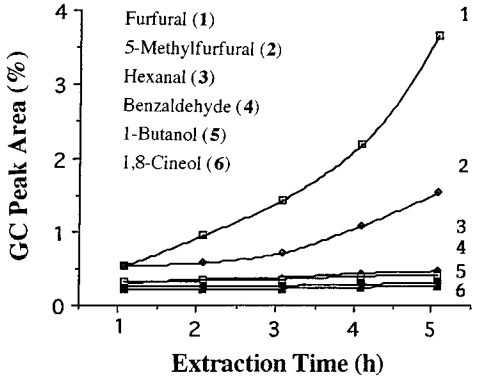


**Figure 2.** Variation of the amount of furfural, 5-methylfurfural, hexanal, benzaldehyde, 1-butanol, and 1,8-cineole as function of the extraction time by SDE method (in a modified Likens−Nickerson apparatus).

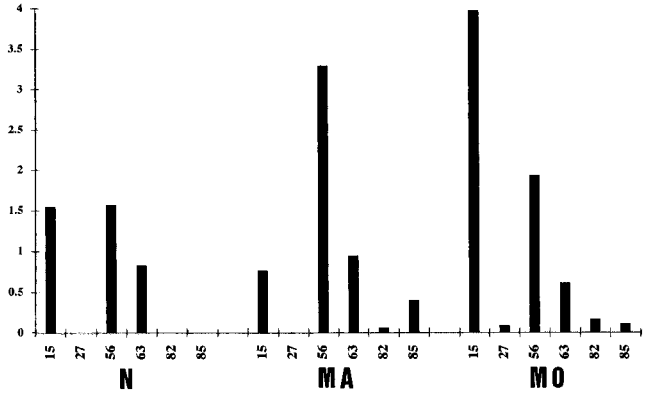


**Figure 3.** Profile of musty, moldy, or related odor compounds (3-methyl-1-butanol, 1-octen-3-ol, 1-octanol, 2-methylisoborneol, guaiacol, and 2,4,6-trichloroanisole) present in normal cork (N), in cork with mancha amarela (MA), and in cork attacked by molds (MO).

pounds could possibly arise from the chlorination of lignin-related components. The chloroanisoles would be formed by microbial methylation of corresponding chlorophenols (Tindale and Whitfield, 1989) initially present as lignin decomposition products. The normal cork did not present chlorinated compounds; only cork infested by molds and principally cork attacked by *A. mellea* have these compounds. This is in agreement with the results found in cork MA, which presented larger amounts of lignin-related derivatives, which eventually could be chlorinated microbiologically under certains conditions.

The presence of dichlorobiphenyl and trichlorobiphenyl in cork, to our knowledge, has not been reported before. Both of them were present in microbially damaged cork, e.g., cork attacked by molds and mainly cork attacked by *A. mellea*. Biphenyl structures constitute 10−25% of lignin natural polymers (Hudson, 1986). There is the possibility of microbial breakdown of the intact polymer and degradation to monomers, which could be subsequently chlorinated.

Cork MA shows higher amounts of 2-butoxyethanol when compared with normal cork or cork MO. The presence of this compound in cork was cited previously (Rigaud et al., 1984; Mazzoleni et al., 1994). Although no probable biosynthetic pathway was found, its formation appears to be related to microbiological activity.

The diversity of chemical structures suggests the involvement of numerous reactions in aroma formation, some of which may be produced by the normal metabolism of the plant and others by the microbiological flora.

Microbial growth can promote chemical modifications in cork polymers and produce metabolites which may contribute to the appearance of off-flavors in wine on contact with cork stopper. The mold growth was correlated with the presence of compounds with characteristic musty and moldy odors, e.g., 3-methyl-1-butanol, 1-octen-3-ol, 1-octanol, 2-methylisoborneol, and chlorinated compounds.

The components that have been reported as having musty and moldy odors and compounds considered to be responsible for cork taint in wine are present in Figure 3, including 3-methyl-1-butanol, 1-octen-3-ol, 1-octanol, 2-methylisoborneol, guaiacol, and 2,4,6-TCA. Normal cork shows the poorest volatile profile of compounds with musty and moldy flavors. Corks MA and MO show higher levels of the volatile compounds likely to cause off-flavors in wine, so these types of cork should be excluded from cork stopper production.

The pattern of volatiles of the specific type of chemical compounds like those associated with lignin degradation (lignin related compounds), microbial attack (aliphatic alcohols and aldehydes, methyl ketones, and chlorinated compounds), and off-flavor (Figure 3) could be used as an indicator for the quality of cork. The study of these components could be very important to establish a chemical fingerprint for identification of cork samples, which could be introduced as a quality control method in wineries and cork factories. Thus, as the specific aroma of the cork may be modified by microbial attack, so it is important to monitor the volatiles composition of cork to ensure cork stoppers of good quality.

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